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Essential Oil of *Hyptis suaveolens* (L.) Poit: A comprehensive study of its chemical and biological properties for therapeutic and industrial applications

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Abstract

The essential oil from the leaves of *Hyptis suaveolens* was extracted through steam distillation, yielding an average of 0.08% with a density of 0.8. The chemical composition was determined using Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS), identifying 24 compounds, predominantly sesquiterpenes. Antimicrobial activity tests were conducted using the microdilution method to evaluate the efficacy of the oil against 14 bacterial strains. The essential oil was mainly composed of β-Caryophyllene (33.9%), Germacrene D (25.4%), α-Humulene (8.3%), and Germacrene B (8.2%). Antibacterial tests revealed bactericidal activity against most of the tested strains, with Minimum Inhibitory Concentration (MIC) values ranging from 0.3 to 12.2 mg/mL and slightly higher Minimum Bactericidal Concentrations (MBC). The essential oil of *Hyptis suaveolens* shows significant antibacterial activity, making it a potential preservative agent for the food industry. Further studies on its antifungal, anticancer activities, and other physical parameters such as refractive index are suggested to explore its full potential.

Keywords: Hyptis suaveolens; Essential oil; Antibacterial activity; Sesquiterpenes

1. Introduction

Medicinal plants have always held a central role in traditional healthcare systems worldwide [1]. They are a valuable source of bioactive compounds, particularly secondary metabolites, which possess a wide diversity of chemical structures and biological activities, making them ideal candidates for the development of new pharmacological treatments. The World Health Organization (WHO) estimates that about 80% of the population in developing countries continues to rely on plant-based medicines for primary healthcare, highlighting their importance in these regions [2]. This growing interest in medicinal plants and their modern applications is reflected in scientific research, where they are studied for their therapeutic potential [3, 1]. Among these plants, aromatic plants occupy a special place, especially in the field of aromatherapy. This practice uses essential oils, extracted from these plants, for their benefits on physical and mental health. Aromatherapy was popularized by René-Maurice Gattefossé, a chemist and perfumer, who, after suffering severe burns from a laboratory explosion, used lavender essential oil to relieve his pain and observed rapid healing [4, 5, 6]. This event marked the beginning of numerous studies on the therapeutic properties of essential oils. Today, essential oils attract the attention of scientists due to their antimicrobial, antifungal, and antioxidant properties [7]. They are also widely used in the food industry to extend the shelf life of products by reducing oxidation and limiting microbial growth [8]. Public interest in these natural products, perceived as less harmful than synthetic chemicals, also

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contributes to their growing popularity. Essential oils are now readily available in various retail outlets such as pharmacies and cosmetic stores [9]. However, despite their many advantages, essential oils are not without risks. Improper use or excessive doses can lead to toxic effects, particularly on the skin, respiratory system, or internal organs. Therefore, it is crucial to have a thorough understanding of their properties to use them safely [10]. This study continues the research on aromatic plants and specifically aims to investigate the essential oil of *Hyptis suaveolens*, a plant from the Lamiaceae family. The goal is to extract essential oils from the leaves of this plant, determine their chemical composition using analytical methods such as Gas Chromatography (GC), Mass Spectrometry (MS), and Nuclear Magnetic Resonance (NMR), and evaluate their antibacterial activities. This study could open new perspectives for the use of this essential oil in therapeutic and industrial applications.

2. Material and methods

2.1. Material

2.1.1. Plant material

The collection of *Hyptis suaveolens* leaves took place in June 2021 in Daloa, Côte d'Ivoire. After harvesting, the leaves were carefully cleaned to remove any impurities, then placed in bags and transported to the chemistry laboratory. Prior to analysis, an extraction of the essential oils was performed.

2.1.2. Laboratory materials and equipment

The equipment used included a Clevenger-type apparatus for steam distillation, gas chromatography (GC), and GC coupled with mass spectrometry for the analysis of the essential oil.

2.1.3. Reagents and chemical products

The reagents used included DMSO (Dimethyl Sulfoxide), sterile distilled water, Mueller Hinton Agar (MHA), and Mueller Hinton Broth (MHB).

2.2. Methods

2.2.1. Essential Oil Extraction of Hyptis suaveolens

The essential oils (EO) of *Hyptis suaveolens* were extracted by steam distillation using a Clevenger-type apparatus. The weighed plant material was placed on a grid inside the distillation unit (pressure cooker) containing water, which was heated. The grid ensured separation between the plant material and the water at the bottom of the unit. The steam generated passed through the plant material, carrying the volatile compounds into a vertical tube, and then into a condenser where condensation occurred. Each extraction operation lasted 3 hours. Afterward, the essential oil sample was stored in a refrigerator.

2.2.2. Essential Oil Yield Calculation Method

The essential oil yield is the ratio between the mass of the essential oil obtained and the mass of the plant material used for extraction. Expressed as a percentage, the essential oil yield is determined using the following formula:

$$Rd = \frac{M_{OE}}{M_{plant}} X\ 100 \qquad \text{……(Equation1)}$$

Where:
Rd: Yield
M_{EO}: Mass of essential oil
M_{plant}: Mass of plant material

2.2.3. Determination of Density

To determine the density of the essential oil, the ratio between a certain volume of the essential oil and the mass of that same volume is calculated using the following formula:

$$d = \frac{\rho_{EO}}{\rho_{water}} \qquad \text{……..(Equation 2)}$$

Where:
ρ_{EO}: Density of essential oil (g/mL)
ρ_{water}: Density of water (g/mL)

2.2.4. Concentration Calculation Method

The essential oil solution was prepared by diluting the oil in DMSO. This allowed the concentration of the essential oil solution to be calculated using the following formula:

$$C = nd \quad \text{.....(Equation 3)}$$

Where:
C: Concentration in mg/mL
n: Dilution factor
d: Density of essential oil

2.2.5. Determination of Minimum Inhibitory Concentration (MIC)

From an 18-hour bacterial culture on Mueller Hinton Agar (MHA), a 3-hour broth culture was prepared and inoculated into 10 mL of Mueller Hinton Broth (MHB) using 0.3 mL for Gram-positive bacteria and 0.1 mL for Gram-negative bacteria. Then, 1.8 mL of this inoculum was distributed into hemolysis tubes, followed by the addition of 0.2 mL of the pre-diluted essential oil solution, starting from the lowest to the highest concentration. Two control solutions without essential oils were prepared: one with sterile distilled water and the other with DMSO. The tubes were manually shaken and incubated at 37 °C for 24 hours. After incubation, the results were read by visual comparison with the controls, and the MIC was determined as the lowest concentration of essential oil at which no visible turbidity was observed.

2.2.6. Determination of Minimum Bactericidal Concentration (MBC)

For the determination of the MBC, successive dilutions from 10^{-1} to 10^{-5} were performed starting from 1 mL of the initial inoculum. On Plate A, a 5 cm streak inoculation was performed using a calibrated loop (2 mm diameter), and the plate was incubated at 37°C (or at 30°C for *Staphylococcus aureus*) for 18 hours. On Plate B, streak inoculation was also performed using the contents of the tubes where no visible bacterial growth was observed, followed by incubation under the same conditions. On Plate C, a count was performed for the control tube containing DMSO, also incubated at 37°C (or 30°C for *Staphylococcus aureus*) for 18 hours. The MBC was determined as the lowest concentration of essential oil where 10^{-4} or 0.01% surviving bacteria were observed. The MBC was read by comparing the results of Plate B with those of Plates A and C [11].

2.2.7. Essential Oil Analysis Methods

The essential oil sample obtained was analyzed at the Chemistry and Biomass Laboratory of the University of Corsica (France) using two main methods. First, gas chromatography (GC) was performed to separate the different volatile compounds present in the sample. A more detailed analysis was then carried out using gas chromatography coupled with mass spectrometry (GC-MS), which allowed the identification and quantification of compounds based on their spectral characteristics, providing a precise analysis of the chemical composition of the essential oil.

Gas Chromatography (GC) Analysis

Gas chromatographic analyses were performed using a Perkin-Elmer Clarus 500 system equipped with a split injector, two columns (50 m x 0.22 mm i.d.; film thickness: 0.25 µm), non-polar (BP-1, polymethylsiloxane) and polar (BP-20, polyethylene glycol), and two flame ionization detectors. The operating conditions were as follows: carrier gas: helium; column head pressure: 20 psi; injector and detector temperatures: 250°C; temperature program: from 60°C to 220°C (80 min) at 2°C/min, with a 20-minute hold at 220°C; injection: split mode with a 1/60 split ratio.

Gas Chromatography Coupled with Mass Spectrometry (GC-MS)

This method involves injecting the essential oil solution into a chromatographic column connected in series with a mass spectrometer. Upon exiting the column, each solute enters the ionization source of the spectrometer. The molecules are subjected to an electron beam, fragmenting them into multiple parts. The positively charged fragments pass through a magnetic field, which alters their movement into a curvilinear trajectory. Heavier fragments follow longer trajectories, allowing the fragments to be received and analyzed sequentially. Two types of information are accessible: the exact molecular mass of the compound and structural information based on the observed fragmentations. The spectra are then recorded and compared to reference spectra for compound identification.

3. Results

3.1. Physicochemical Characteristics of Essential Oils

The density of the essential oil extracted from *Hyptis suaveolens* was measured to be 0.8. The essential oil yields (EO) were calculated using equation 1 (Table 1), which determines the amount of oil extracted based on the initial mass of the plant used for extraction, expressing the efficiency of the extraction process as a percentage relative to the raw plant material.

Table 1 Yields of *Hyptis suaveolens* Essential Oil

Extraction No.	Mass of Fresh Plant (g)	Extraction Duration (hours)	Mass of EO (g)	Yield (Rd) (%)
1	3279.30	2,30	1,137	0,034
2	3758.74	2,30	3,090	0,082
3	4087.49	3	4,110	0,101
4	4801.24	3	5,111	0,106
5	3521.97	3	2,146	0,061
Average				**0.08±0.03**

3.2. Chemical Composition of the Essential Oil

The analysis of the chemical composition of *Hyptis suaveolens* essential oil identified twenty-four (24) compounds with concentrations equal to or greater than 0.2% (Table 2).

Table 2 Chemical Composition of *Hyptis suaveolens*

N°	Identified Compounds	Content (%)
1	β-Pinene	0.5
2	Limonene	0.2
3	Thymol	0.6
4	Delta Elemene	0.2
5	β-bourbonene	0.7
6	α-Copaene	2.9
7	β-Elmene	2.8
8	β-Caryophyllene	33.9
9	α-trans-bergamotene	1.0
10	demethoxy-agératochromene	2.9
11	Germacrene D	25.4
12	α-Humulene	8.3
13	Trans β-Farnesene	0.3
14	Bicyclogermacrene	0.7
15	α-trans-Bergamotene	1.0
16	β-Copaene	0.2
17	Germacrene- A	1.8
18	δ-Cadinene	1.6

19	β-sesquiphellandrene	0.2
20	Germacrene- B	8.2
21	Oxyde de Caryophyllene	1.3
22	Spathulenol	0.2
23	1,2-Epoxyde humulene	0.3
24	Phytol	4.3
Total		96.6
Hydrocarbon monoterpenes		0.7
Oxygenated monoterpenes		0.6
Hydrocarbon sesquiterpenes		89.2
Oxygenated sesquiterpenes		1.8
Diterpenes		4.3
Total		96.6

Among the groups of terpenoid compounds identified, sesquiterpenes account for 91%, of which 89.2% are hydrogenated and 1.8% are oxygenated, while monoterpenes make up 1.3% (0.7% hydrogenated and 0.6% oxygenated) and diterpenes represent 4.3%. Among the sesquiterpenes, hydrogenated sesquiterpenes form the dominant class with a rate of 89.2% (Figure 1).

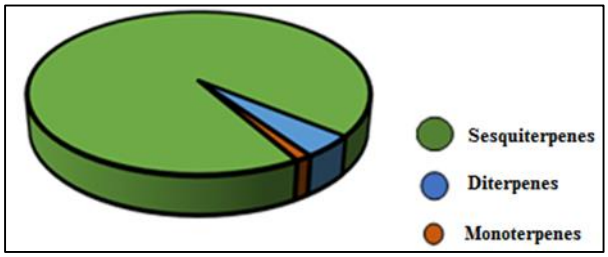

Figure 1 Proportion of Compounds Identified in the EO of *Hyptis suaveolens*

The major compounds of the essential oil from *Hyptis suaveolens* leaves are β-Caryophyllene, Germacrene D, α-Humulene, and Germacrene B (Figure 2).

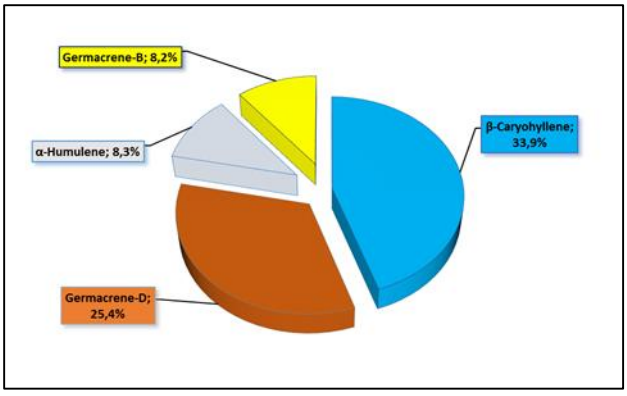

Figure 2 Proportion of Major Compounds

3.3. Antibacterial Tests

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the essential oil extracted from *Hyptis suaveolens* leaves were determined using the broth dilution method. The results are shown in

(Table 3). The MIC values ranged from 0.3 to 12.2 mg/mL. The MBC values were slightly higher than the MIC values. The MBC/MIC ratio was also calculated to highlight the antibacterial effect of the tested oils. According to AFNOR standards, if the MBC/MIC ratio is ≥ 4, the antibiotic is bacteriostatic, and if MBC/MIC ≤ 4, the antibiotic is bactericidal.

Table 3 Minimum Inhibitory and Bactericidal Concentrations

Bacterial Strains	MIC (mg/ mg.mL^{-1})	MBC (mg.mL^{-1})	MBC/MIC (mg.mL^{-1})	Effet
Acinetobacter baumanii	1.02	5.02	4.92	Bactériostatique
Citrobacter freundii	1.53	2.3	1.50	Bactéricide
Citrobacter koseri	12.2	12.5	1.02	Bactéricide
Enterobacter aerogenes	0.76	1.2	1.58	Bactéricide
Enterobacter agglomerans	0.32	0.75	2.34	Bactéricide
Enterobacter cloacae	1.53	2.2	1.44	Bactéricide
Escherichia coli K1	0.3	0.37	1.23	Bactéricide
Klebsiella ozaenae	1.53	3.01	1.97	Bactéricide
Klebsiella pneumoniae	0.49	0.7	1.43	Bactéricide
Proteus sp	1.53	3.01	1.97	Bactéricide
Pseudomonas aeruginosa	6.12	7.01	1.15	Bactéricide
Salmonella sp	0.38	1.5	3.95	Bactéricide
Serratia marcescens	1.45	1.92	1.32	Bactéricide
Staphylococcus aureus	0.98	1.5	1.53	Bactéricide

4. Discussion

The essential oil obtained from *Hyptis suaveolens* presented a light-yellow color with an average extraction yield of 0.08%. Compared to literature data, this yield is similar to that observed in the Eastern (Dimbokro) and Central (Toumodi) regions of Côte d'Ivoire [12]. However, it is lower than the yields reported in other studies, which are 0.1% and 0.22% respectively [13, 14]. It is important to note that essential oil yields from aromatic plants are strongly influenced by factors such as soil type, regional climate, edaphic factors, the condition of the plant organs (fresh or dry), and especially the extraction method. It has been reported that the essential oil yield of *Hyptis suaveolens* depends on the age, maturity, and type of plant organ. According to literature, the chemical study of *Hyptis suaveolens* has been the subject of many studies [15], most of which focus on its essential oils [16]. For the analysis of the chemical composition of *Hyptis suaveolens* oil, gas chromatography coupled with mass spectrometry was used. The results showed that the oil from the leaves of this plant is composed of 96.6% terpene derivatives, with 91% sesquiterpenes, 4.3% diterpenes, and 1.3% monoterpenes. Regarding the identification of the different terpene groups in our essential oil, it is consistent with the results obtained in Northern Côte d'Ivoire, specifically in Séguéla, Korhogo, and Kaniasso [12]. The presence of these terpene derivatives could explain the strong biological activity. Sesquiterpenes, in particular, are known to be key components in essential oils [17]. Our essential oil is predominantly composed of sesquiterpenes, with major constituents including β-Caryophyllene (33.9%), Germacrene D (25.4%), Germacrene B (8.2%), and monoterpenes such as α-Humulene (8.3%). This result is consistent with findings from Mali [16] and Togo [18], where β-Caryophyllene was also identified as a major constituent. However, our results differ from those obtained in the Netherlands Antilles [19], where 1,8-cineole (35.9%) and sabinene (12%) were identified as the main constituents. In Burkina Faso, the presence of limonene, α-thujene, α-pinene, β-phellandrene, and β-caryophyllene has also been reported in this oil [20]. Comparing the major compounds in our oil with those reported in the literature, we find that β-caryophyllene and germacrene D, the dominant components of our sample, are also present in several previous studies. The observed chemical composition differences between the essential oils of the studied plant and those reported by other authors could be attributed to various ecological factors, such as temperature, relative humidity, sunlight exposure, and soil type [21]. With the exception of *Citrobacter koseri* and *Pseudomonas aeruginosa*, all bacteria tested, including *Acinetobacter baumanii*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Proteus sp.*, *Salmonella sp.*, *Serratia marcescens*, and *Staphylococcus aureus*, were

sensitive to the *Hyptis suaveolens* oil (MIC < 1.54 mg/mL and MBC < 5.03 mg/mL). For MIC values higher than 5.03 mg/mL, *Citrobacter koseri* and *Pseudomonas aeruginosa* were less sensitive than the other bacteria. The antibacterial effect of the oil decreased (MIC > 6 mg/mL) in the case of *Citrobacter koseri* and *Pseudomonas aeruginosa*. An antibiotic is considered bactericidal for a bacterium if the MBC/MIC ratio is between 0 mg/mL and 4 mg/mL, and bacteriostatic if this ratio is greater than or equal to 4 mg/mL. We observed that the *Hyptis suaveolens* oil exhibits good activity against all tested bacterial strains, with a primarily bactericidal effect. This strong efficacy is likely due to the presence of certain volatile components, increasing the concentration and, consequently, the antibacterial activity. The variability in the MIC values across bacterial strains can be primarily explained by the chemical composition of the essential oil studied. Indeed, it has been shown that the antimicrobial activity of an essential oil is often linked to its major compounds [22]. In our study, the oil was characterized by the dominance of hydrocarbon compounds such as β-caryophyllene (33.9%), germacrene D (25.4%), α-humulene (8.3%), and germacrene B (8.2%). The low minimum inhibitory concentration (MIC < 1.54%) observed in bacteria such as *Acinetobacter baumanii*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Proteus sp.*, *Salmonella sp.*, *Serratia marcescens*, and *Staphylococcus aureus* may be due to the presence of oxygenated compounds, which exhibit strong activity against the tested bacteria. This aligns with the literature, as it is known that terpene aldehydes and terpene alcohols, particularly formaldehydes, have strong activity against bacteria. These carbonyl compounds create an attractive arrangement, which could explain their activity. Such components interfere with biological processes involving electron transfer [4]. Despite the high levels of β-caryophyllene (33.9%) and germacrene D (25.4%), *Pseudomonas aeruginosa* is resistant to the essential oil. This bacterium is known for its resistance to many antimicrobial agents, as it can form a biofilm, a complex organization composed of different layers where bacteria are in specific physiological conditions [23].

5. Conclusion

This study focused on the chemical and biological investigation of the essential oil of *Hyptis suaveolens*, a plant belonging to the Lamiaceae family. The essential oils from the leaves of *Hyptis suaveolens* were obtained by steam distillation, yielding 0.08%, with a density of 0.8. These values are close to those reported in some previous studies. The chemical composition of the isolated oil was determined using chromatographic methods (GC and GC-MS). These techniques revealed that the studied oil is primarily composed of compounds of the chemotype: β-Caryophyllene, Germacrene D, α-Humulene, and Germacrene B. Antimicrobial activity tests were conducted to evaluate the activity of *Hyptis suaveolens* leaf oil against 14 bacterial strains using the microdilution method. The results obtained through this method allowed the determination of MIC and MBC values. The essential oil demonstrated bactericidal activity against the tested bacteria. Our results suggest that the studied essential oil could be considered as a preservative agent for the food industry, capable of preventing bacterial growth. In the future, it would be interesting to conduct further biological activity tests, such as antifungal and anticancer activities, as well as to investigate other physical parameters, such as the refractive index of *Hyptis suaveolens* oil.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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