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Chemical composition and antimicrobial activity of the essential oil from Hazomalania voyronii (Fam. Hernandiaceae) leaves

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Abstract

The yield, chemical composition and antimicrobial properties of the essential oil extracted from *Hazomalania voyronii* leaves were studied. The essential oils yield average is 0,22 %. Thirty-four components were identified by GC and GC/MS; bornyl acetate (30,74 %), α-pinene (23,54 %), camphor (17,27 %) and limonene (23,31 %) are the principal components of this oil. A strong inhibited activity against six microorganisms is obtained.

Keywords: *Hazomalania voyronii* leaves; Essential oil; Chemical composition; Antimicrobial properties

1. Introduction

The use of the antimicrobial properties of essential oils is not a recent practice. Ancient manuscripts already described numerous plant- and aroma-based preparations employed by priests and physicians.[1] Nowadays, essential oils are investigated on scientific and rational bases with the aim of developing novel products for medical, veterinary, and cosmetic applications. Indeed, several studies have demonstrated the antimicrobial, antiviral, and insecticidal activities of terpenoid compounds (Bakkali et al., 2008; Burt, 2004; Cowan, 1999; Dorman & Deans, 2000; Kalemba & Kunicka, 2003). [2-5]

However, a large number of biologically active substances such as alkaloids, phenols, terpenoids, steroids, and flavonoids remain largely unexplored [6]. It should also be noted that only a few medicinal plants have been subjected to comprehensive phytochemical and/or biological investigations [7]. Unfortunately, *Hazomalania voyronii* is a striking example of a forest species that has not escaped this trend [8]. These considerations have led us to explore the potential use of the essential oil from *H. voyronii* leaves as an affordable option for the treatment of various infections involving both fungal and bacterial pathogens.

Hazomalania voyronii (Hernandiaceae) is an endemic and endangered tree species of Madagascar, traditionally used in folk medicine, particularly in the SAVA and Analanjirofo regions [9]. In Malagasy traditional practices, its bark and leaves are employed as remedies against fever, malaria, fatigue, and infectious diseases, and are also valued for their tonic and aromatic properties [9]. To date, there are very few publications specifically addressing the antimicrobial essential oil

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from this species, despite its recognized traditional uses. A first preliminary investigation on the bioactivity of the essential oil extracted from the wood of *Hazomalania voyronii* has demonstrated its antimicrobial potential **[10]**.

The aim of the present study is to determine the chemical composition and to evaluate the antibacterial and antifungal activities of the essential oil extracted from the leaves of *Hazomalania voyronii*.

2. Materials and Methods

2.1. Plant material

Leaf samples of *Hazomalania voyronii* used in this study were collected in the SAVA region in June 2020. The plant material was shade-dried for seven days prior to analysis. This species was identified at the Department of Botany of Botanical and Zoological Park of Tsimbazaza (Antananarivo). A voucher specimen was deposited at the Analytical Chemistry and Formulation Laboratory, Faculty of Sciences, University of Antananarivo, for future references.

2.2. Essential oil extraction

The essential oils were extracted by hydrodistillation of ten samples using a Clevenger-type apparatus [10]. For each trial, 100 g of plant material were processed. The distillation lasted approximately 180 minutes after the appearance of the first drop of distillate at the outlet of the steam condensation tube. Three experiments were carried out for each sample. The essential oil was stored in the dark at 4 °C and dried over anhydrous sodium sulfate. To express the yields relative to the dry matter (in mL/100 g of dry material), the moisture content of the different samples was determined beforehand. The percentage of dry matter was estimated by drying 5 g of each sample for 4 hours in an oven at 102 °C.

2.3. Chromatographic analyses

For each sample, three chromatographic analyses were performed using a Hewlett-Packard GC system (HP 5890) equipped with a DB-5 fused silica capillary column (25 m × 0.25 mm i.d., 0.25 µm film thickness) and a flame ionization detector (FID) set at 260 °C. Nitrogen was used as the carrier gas (1 mL/min). The oven temperature was programmed from 50 °C to 250 °C at 4 °C/min. Injections were carried out in split mode.

Identification of components was based on their Kovats retention indices and by gas chromatography–mass spectrometry (GC–MS), carried out on an HP 5980 GC coupled with an HP 5772 MS. Electron impact ionization was applied at 70 eV, with helium as the carrier gas (2 mL/min). Compounds were identified by comparison of their mass spectra with the NIST 98 library (PDA). [12,13]

2.4. Microorganisms tested

In this study, the microorganisms selected were: *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Penicillium parasiticus*, and *Aspergillus niger*. These strains were chosen for their common occurrence as human or environmental pathogens and their relevance in evaluating antimicrobial activity [14]. The four bacterial strains were obtained from the American Type Culture Collection (ATCC) and maintained by subculturing on nutrient agar. The two fungal strains were cultured on Potato Dextrose Agar (PDA) under standard laboratory conditions [15].

2.5. Microbiological procedure

The essential oil was first emulsified using a 0.2% agar-agar solution [16,17], since it is not miscible with water or standard culture media. This allows a homogeneous distribution of the oil in the medium, maximizing contact between the microorganisms and the bioactive compounds. The essential oil was initially diluted 1:10 in the agar-agar solution. Aliquots of this dilution were then added to test tubes containing nutrient agar for bacteria and PDA for fungi. The media were subsequently sterilized, cooled to 45 °C, and poured into Petri dishes. The final concentrations of essential oil were 1:100, 1:250, 1:500, 1:1000, and 1:5000 (v/v). Control plates containing only the culture medium with 0.2% agar-agar solution were also prepared.

Inoculation was performed by streaking with a calibrated platinum loop to ensure a consistent volume of inoculum. Bacterial inocula were prepared from 24-hour cultures, while fungal inocula consisted of spore suspensions in physiological water obtained from 7-day-old PDA cultures. Incubation was carried out at 37 °C for 24 hours for bacteria and at 25 °C for 7 days for fungi. Each experiment was repeated three times to minimize experimental error.

3. Results and Discussion

3.1. Yield and chemical composition

The average yield of essential oil obtained from *Hazomalania voyronii* leaves was 0.22%. [18] This yield is lower than who observed a 0.70% yield after a 6-hour extraction of the leaves. The chemical composition of the essential oil is summarized in Table 1.

Table 1 Chemical composition of the essential oil from *Hazomalania voyronii* leaves

Compound	Kovats Retention Index (RI)	% Content
α-Thujene	931	2.26
α-Pinene	939	23.54
Camphene	953	2.59
β-Pinene	980	0.56
δ3-Carene	1011	0.02
α-Terpinene	1018	0.27
Limonene	1031	5.98
α-Campholenal	1125	0.31
(Z)-Limonene oxide	1134	0.29
(E)-Pinocarveol	1139	0.63
Camphor	1143	17.27
Isoborneol	1156	0.12
Borneol	1165	4.57
4-Terpineol	1177	0.71
Cymene-8-ol	1180	0.64
α-Terpineol	1189	0.40
Myrtenol	1194	0.46
Verbenone	1204	1.74
(E)-Carveol	1217	0.72
(Z)-Carveol	1229	0.10
Cis-Chrysanthenyl acetate	1262	0.04
Bornyl acetate	1285	30.74
Carvacrol	1298	0.03
α-Terpinyl acetate	1350	0.62
α-Longipinene	1351	0.04
α-Copaene	1376	0.06
β-Elemene	1433	<0.05
Germacrene D	1480	0.04
(E)-β-Guaiene	1500	0.13
γ-Cadinene	1513	0.14

Caryophyllene oxide	1581	0.23
Widdrol	1597	0.38
α-Humulene	1709	<0.05
Total	—	97.78

Thirty-four compounds were identified, representing approximately 98% of the total chemical composition of the essential oil. The major constituents were bornyl acetate (30.74%), α-pinene (23.54%), and camphor (17.27%), which together accounted for more than three-quarters of the total composition. Other components present in lower amounts included limonene (5.98%), borneol (4.57%), camphene (2.59%), and α-thujene (2.26%). Overall, the essential oil can be classified into four terpene classes: hydrocarbons (37.63%), esters (31.40%), ketones (19.01%), and alcohols (8.52%). These compounds confer a pleasant balsamic aroma and a yellowish color to the oil.

Satrani B et al. [18] reported that the essential oil from *Hazomalania voyronii* leaves was dominated by camphor (19.10%), bornyl acetate (16.50%), and borneol (9.60%). This chemical composition differs somewhat from that observed in our study. In particular, our essential oil exhibited a higher content of bornyl acetate (30.74%) and a lower content of borneol (4.57%). The same study also reported the presence of α-pinene and limonene, which were detected in our oil as well, albeit at higher percentages. However, the camphor content was comparable in both oils.

3.2. Antimicrobial tests

The results of the antibacterial and antifungal activities of the essential oil from *Hazomalania voyronii* leaves are presented in Table 2.

Table 2 Antibacterial and antifungal activities of the essential oil from *Hazomalania voyronii* leaves

Microorganism	**1/100**	**1/250**	**1/500**	**1/1000**	**1/5000**	**Control (T)**
Bacteria						
Escherichia coli	−	−	−	+	+	+
Bacillus subtilis	−	−	−	+	+	+
Staphylococcus aureus	−	−	−	−	−	+
Micrococcus luteus	−	−	−	−	+	+
Fungi						
Penicillium parasiticus	−	−	−	+	+	+
Aspergillus niger	−	−	−	+	+	+

T : Control; - : inhibition ; + : Growth.

The essential oil from *Hazomalania voyronii* leaves has demonstrated significant inhibitory activity against the microorganisms tested in vitro. However, the sensitivity of different germs varied based on their nature.

Staphylococcus aureus showed a more marked sensitivity to the essential oil than *Escherichia coli*, which aligns with observations from several studies [19, 20]. Additionally, *Escherichia coli* and *Bacillus subtilis* showed similar vulnerability to this essential oil, corroborating the results reported by Satrani et al. [21].

Both molds tested, *Penicillium parasiticus* and *Aspergillus niger*, were inhibited at a concentration of 1/500 V/V, which is comparable to the results obtained by Satrani et al. [21].

3.3. Antimicrobial Mechanisms

The observed antimicrobial activity can be attributed to the essential oil's chemical composition. Alpha-pinene, present at 23.54%, is known for its inhibitory properties [22]. A study by Angioni et al. [23] also highlighted the inhibitory activity against *Candida albicans* and *Staphylococcus aureus* by the essential oil of *Hazomalania voyronii*, which was characterized by a high alpha-pinene content (approximately 86%).

Furthermore, camphor (17.27%) and borneol (4.57%), also present in this essential oil, are compounds with known antimicrobial activities [24]. Felice et al. [25] concluded that camphor and its derivatives, as well as borneol, can be considered antimicrobial constituents of *Achillea teretifolia* essential oil. Bioassays conducted on essential oils rich in borneol have proven that this compound has potent antimicrobial power [26].

Additionally, terpenic alcohols such as trans-pinocarveol, 4-terpineol, cymene-8-ol, alpha-terpineol, and trans-carveol, which make up about 9% of this oil, are also recognized for their strong antimicrobial properties. This is due to their high-water solubility, which gives them a great ability to penetrate the cell walls of bacteria and fungi [27].

3.4. Additional Contributors and Synergistic Effects

Esters, particularly bornyl acetate (30.74%), can also contribute to the observed antibacterial and antifungal effects. Tzakou et al. [28] reported that the essential oils of two *Thymus longicaulis* chemotypes, one rich in geranyl acetate and the other in alpha-terpenyl acetate, have significant antimicrobial activity. The synergistic effect among all these chemical constituents may also account for this activity [29].

4. Conclusion

This work focuses on determining the yield, chemical composition, and antibacterial and antifungal properties of the essential oil extracted from *Hazomalania voyronii* leaves. The average yield of the essential oil is 0.22%. Chemical analyses, using GC and GC/MS, identified approximately 98% of the total volatile compounds in this oil. Bornyl acetate (30.74%), α-pinene (23.54%), camphor (17.27%), and limonene (23.31%) are the main compounds identified among the thirty-four characterized. The results obtained in this study show that the essential oil from *Hazomalania voyronii* leaves exhibits significant inhibitory activity, in vitro, on the four bacteria and two fungi tested.

The antimicrobial activity of this essential oil is primarily due to its richness in the following constituents: α-pinene, camphor, borneol, and esters. All these compounds are well known for their antimicrobial properties. The highlighted antibacterial and antifungal performance warrants further, more detailed study in order to consider potential applications for this essential oil.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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