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Chemical composition and antimicrobial properties of the essential oil from the leaves of *Helichrysum ibityense* R.Vig. & Humbert (Asteraceae)

Zara Nomentsoa Razafiarimanga, Lovarintsoa Judicael, Randriamampianina, Hanitra Ranjana Randrianarivo, Saïd M'bae Sadam, Danielle Aurore Doll Rakoto and Victor Louis Jeannoda *

Laboratory of Applied Biochemistry to Medical Sciences, Fundamental and Applied Biochemistry Department, Faculty of Sciences, University of Antananarivo, P. O. Box 906, Antananarivo 101, Madagascar.

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Abstract

The present work aimed to study the composition and antimicrobial properties of the essential oil of *Helichrysum ibityense* leaves (HIEO). HIEO was extracted by hydrodistillation from fresh leaves with a yield of 1.9%. It had a relative density of 0.9247 at 20℃, a refractive index of 1.4706, an optical rotation of -0°33, an acid index of 2.10 and an ester index of 54. Gas chromatography analysis/flame ionisation detection of HIEO had identified 17 components, representing more than 99.54% of the overall composition of the HIEO including 1.8-cineole (69.46%) the major component, α-terpinene (4.62%), β-caryophyllene (4.39%), α-pinene (4.13%), β-pinene (2.96%), p-cymene (2.39%) and γ-terpinene (2.16%). The HIEO antimicrobial activity was tested against nine pathogenic bacteria including *Staphylococcus aureus, Staphylococcus pneumoniae, Streptococcus pyogenes, Clostridium perfringens, Bacillus cereus, Pseudomonas aeruginosa, Enterobacter cloacae, Enterobacter aerogenes, Yersinia enterocolitica,* and *Candida albicans* using the Disc diffusion and the Microdilution assays. HIEO exhibited broad activity spectrum and high microbial activity with inhibition zones (IZ) ranging from 12 to 35 mm. On *Bacillus cereus*, the most sensitive bacterium, its Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were 9.37 mg/ml and 13.22 mg/ml respectively. With an MBC/MIC ratio of 1.41, HIEO exerted a bactericidal action on *Bacillus cereus*. When administered orally to mice at 1000 mg/kg body weight, HIEO caused symptoms of intoxication which disappeared after 24 h. These preliminary results revealed HIEO could be used to treat different infectious diseases if its safety will be confirmed.

Keywords: *Helichrysum ibityense*; Asteraceae; Essential oil; Chemical compounds; Antimicrobial properties; Toxicity

1. Introduction

The history of aromatic medicinal plants is associated with the evolution of civilizations. In all regions of the world, the history of peoples shows that these plants have always played an important role in the treatment of many diseases [1]. They are currently used in medicine, perfumery, cosmetics and culinary flavoring [2].

Essential oils are increasingly used in primary health care today. Studies have shown that aromatic plants are a reservoir of new remedies [3, 4]. They are considered as sources of raw materials for the discovery of new molecules necessary for the development of future drugs [5].

Since the ban on antibiotics as a growth promoter in livestock farming in 2006, the search for alternatives such as plant extracts and in particular essential oils (EO) is undergoing significant development [6]. EOs applications are vast,

* Corresponding author: Victor Louis Jeannoda
Laboratory of Applied Biochemistry to Medical Sciences, Fundamental and Applied Biochemistry Department, Faculty of Sciences, University of Antananarivo, P. O. Box 906, Antananarivo 101, Madagascar.

affecting well-being, health and the environment. The antimicrobial activity of plant extracts and EOs has been known for many years [7, 8, 9]. It is possible to sanitize the atmosphere with an essential oil ionizer. True aromatic aerosols are thus formed, ionized, creating ionic nascent oxygen, which is highly bactericidal, acaricidal and fungistatic, and without any toxicity for humans at the doses used [10, 11].

In Madagascar, where access to health care remains difficult for most of the population, traditional medicine is still widely used thanks to the therapeutic potentials of many plants. The diversity of aromatic plants endemic to Madagascar shows the real potential of its flora. Among them, the genus *Helichrysum* is well represented with its 115 endemic species. Traditional therapeutic uses of some *Helichrysum* species have been reported. For example, according to Benelli *et al*., [12], the juice from crushes *Helichrysum faradifani* leaves is used for healing wounds and as disinfectant for the treatment of opened boils and wounds and the leaf decoction is used in the treatment of syphilis, diarrhea, cough and headache. *H. gymnocephalum* is used to treat a variety of diseases, including stomach aches, headaches, typhoid, herpes, pyrosis, amenorrhea, dysmenorrhea, ulcers, goiter, rickets, gout, rheumatism etc. [13]. *H. bracteiferum* is considered as a stimulating plant and its ashes are used as a topical on excavated syphilitic wounds [13].

Some Malagasy *Helichrysum* species including *H. faradifani* [12, 14, 15], *H. bracteiferum* [16, 17], *H. benthamii, H. dubardii, H. indutum, H. bojerianum, H. diotoides* and *H. hirtum* [18], *H. cordifolium, H. hypnoides, H. rusillonii* [19], *H. gymnocephalum* and *H. selaginifolium* [17], *H. aphelexioides, H. triplinerve, H. achyroclinoides, H. lecomtei, H. retrorsum* [20] were already studied. However, works focused on the chemical composition of essential oils. Only a few species have been studied in terms of biological activity: *H. gymnocephalum* (Antiplasmodial and antioxydant activies, cytotoxicity) [21, 22], *H. faradifani* (Insecticidal activity) [12], *H. bracteiferum* and *H. lavanduloides* (Antibacterial activities) [22].

H. ibityense was chosen as the subject of this work mainly because no studies on it other than botanical ones have been reported so far. The fact that this plant has the same vernacular name ("Rambiazina") as *H. gymnocephalum* and *H. bracteiferum*, well-known for their therapeutic virtues in traditional Malagasy medicine, suggested that it may have the same therapeutic properties as these two medicinal plants. Indeed, the data we collected during our fieldwork on traditional therapeutic uses of *H. ibityense* were reminiscent of some of those known for *H. gymnocephalum and H. bracteiferum* especially their antibacterial properties. Thus, the main objectives of our investigations were to determine the chemical composition of the essential oil extracted from the fresh leaves of *H. ibityense* and evaluate its antimicrobial activities.

2. Material and methods

2.1. Materials

2.1.1. Plant material

The *Helichrysum* genus belongs to the Asteraceae family and has more than 400 species mostly found in Australia, southern Africa and Madagascar. *Helichrysum ibityense* is one of the hundred and fifteen (115) *Helichrysum* species endemic to Madagascar. This plant is a shrub from 1 to 4 m high (Figure 1) [23, 24].

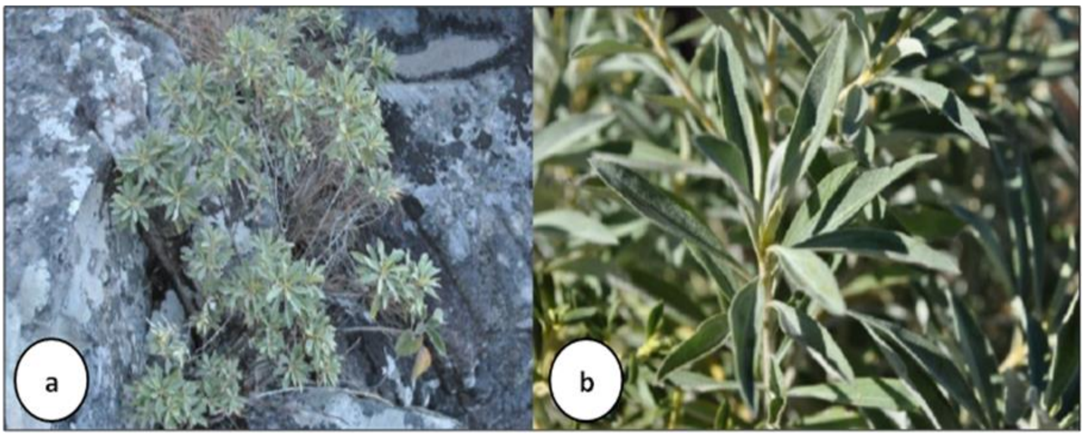

Figure 1 *Helichrysum ibityense*: a) the whole plant and b) leaves

It grows abundantly in the highlands of Madagascar. It is found in the Andringitra forest, Marojejy forest corridor, South Anjanaharibe and North Tsaratanana, Manongarivo, Tsaratanana and in the Mount Ibity. The plant samples were collected in June 2020 on the Mount Ibity (20°03’ 56, 7’’ S, 047° 00’ 02, 2’’ E) in the Vakinankaratra region.

2.1.2. Microbial strains

The microbial strains used are pathogens commonly sought in medical and food microbiological analysis and/or control. They include Gram (-) and Gram (+) bacteria and a yeast (Table 1).

Table 1 List of microbial strains used

Germ-Tests	**Gram**	**Reference**
Staphylococcus aureus	+	ATCC 6538
Staphylococcus pneumoniae	+	ATCC 6505
Streptococcus pyogenes	+	ATCC 19615
Clostridium perfringens	+	ATCC 13124
Bacillus cereus	+	ATCC 14579
Pseudomonas aeruginosa	-	ATCC 10145
Enterobacter cloacae	-	ATCC 13047
Enterobacter aerogenes	-	ATCC 13048
Yersinia enterocolitica	-	ATCC 23715
Candida albicans		ATCC 10321

2.1.3. Animals

OF-1 strain Albino mice (*Mus musculus*), weighing 25 ±2 g, were provided by the Pasteur Institute of Madagascar (IPM) breeding farm.

2.2. Methods

2.2.1. Extraction of the essential oil of Helichrysum ibityense (HIEO)

The extraction of the essential oil (EO) from the fresh leaves was carried out by hydrodistillation using a Clevenger type apparatus [25].

2.2.2. Physico-chemical characterization of HIEO

The concentration of the pure EO was calculated from its relative density. The physico-chemical parameters to be determined and the references used are presented in Table 2.

Table 2 Parameters to determine and the standards used

Parameters	**Standards used**
Relative density	AFNOR, NF-T 75-111
Refraction index	AFNOR, NF-T 75-112
Rotation power	AFNOR, NF-T 75-13
Acid index	AFNOR, NF-T 75-103
Ester index	AFNOR, NF-T75-104

2.2.3. Essential oil analysis

The chemical composition of HIEO was determined by gas chromatography/flame ionization detection (CPG/FID) [26].

The oil was analysed using a SHIMADZU GC 14-A chromatograph equipped with a TRACSIL TR-WAX fused silica (polydimethylsiloxane) capillary column (30 mm × 0.32 mm × 0.25 µm) and a flame ionization detector. The sample volume of 25 µl was diluted in 0.5 ml of isooctane. The carrier gas used was nitrogen (N_2).

The peaks obtained were identified using AMDIS software Version 2.69 (Automated Mass Spectral Deconvolution and Identification System).

2.2.4. Assessment of antimicrobial activity

All the methods used for antimicrobial assay were detailed in our previous paper [27, 28].

Antimicrobial susceptibility testing

The sensitivity of microorganisms to the essential oil was determined by the agar diffusion method or aromatogram. Sterile paper disks (6 mm in diameter BioMérieux ®, REF 549916) were soaked with pure essential oil and placed on the surface of the inoculated Mueller-Hinton Agar (Scharlau®). The Petri dishes were incubated at 37℃ for 24 h and the Inhibition Zones (IZ) were measured. The sensitivity to the essential oil was classified according to the IZ diameter as: not sensitive (-) for IZ ≤ 8 mm; sensitive (+) for 9 ≥ IZ ≤ 14 mm; very sensitive (++) for 15 ≥ IZ ≤ 19 mm and extremely sensitive (+++) for IZ ≥ 20 mm [29].

Antibiotic and antifungal used as references in this study were respectively Neomycin 30 µg/disc and Miconazole 500 µg/disc.

MIC and MBC determination

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by microdilution method [30]. The standards used to interpret MIC results were those of Dalmarco *et al.* [31]. The essential oil type of action is bactericidal when the ratio MBC/MIC is ≤4 or bacteriostatic when MBC/MIC is >4 [32, 33, 34].

2.2.5. Toxicity determination

A volume of 0.3 mL of HIOE per 25 ± 2 g of body weight was administered to mice by oral route by means of an intubation cannula with a curved distal. Two batches of 5 male mice were used. The mice were observed for 24 h.

3. Results

3.1. Extraction yield and physico-chemical parameters

The extraction yield of HIEO was 1.9%. The values of the physico-chemical parameters determined are presented in Table 4.

Table 4 Physico-chemical indexes of HIEO

Relative density	Refractive index	Rotation power	Acid index	Ester index
0.9247 ± 0.0001	1.4706 ± 0.0002	−0°.33 ± 0.17	2.10 ± 0.14	54 ± 2.47

3.2. Chemical composition

GC-FID analysis of HIEO identified 17 constituents representing approximately 99.54% of the overall composition (Figure 2 and Table 5). 1.8-cineole (69.46%) was the main constituent and the predominant components were α-terpinene (4.62%), β-caryophyllene (4.39%), α-pinene (4.13%) and, β-pinene (2.96%), p-cymene (2.39%) and γ-terpinene (2.16%).

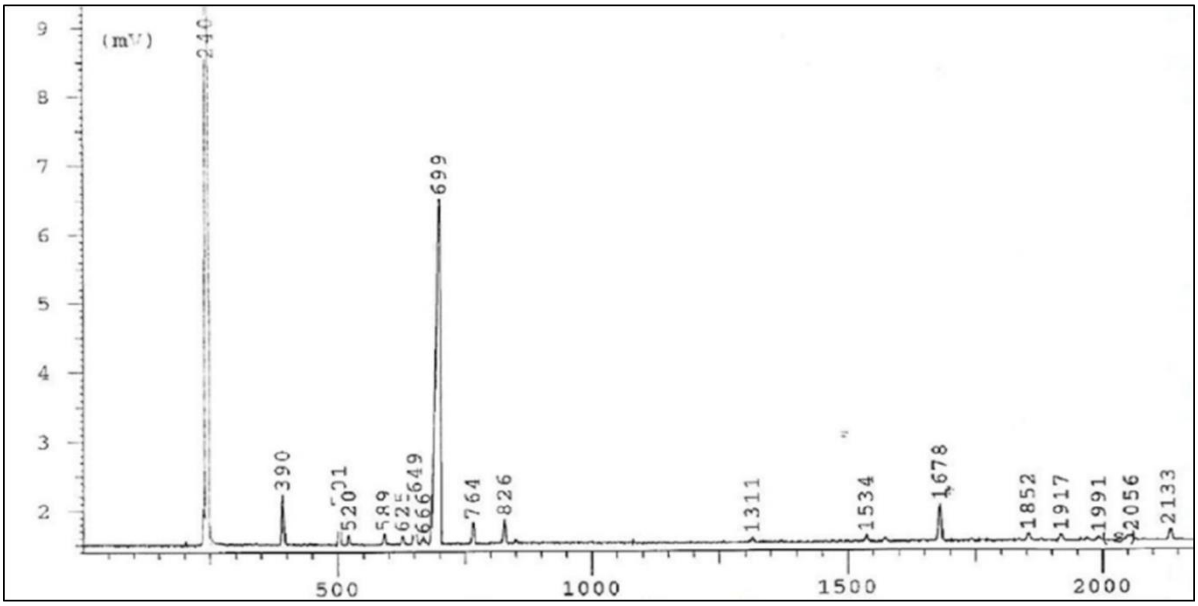

Figure 2 Chromatographic profile of HIEO

Table 5 Major components in HIEO

Pic n°	Retention times	Components	Relative rates (%)
1	390	α-pinene	4.13
2	501	β-pinene	2.96
3	520	sabinene	0.90
4	589	β-myrcene	1.09
5	625	α-phellandrene	0.91
6	649	α-terpinene	4.62
7	666	limonene	0.92
8	699	1.8-cineole	69.46
9	764	γ-terpinene	2.16
10	1311	p-cymene	2.39
11	1534	linalol	0.68
12	1678	β-caryophyllene	4.39
13	1852	aromadendrene	1.01
14	1917	α-humulene	0.80
15	1991	α-terpineol	0.50
16	2056	β-selinene	1.06
17	2133	γ-cardinene	1.56

3.3. Antibacterial activity of HIEO

3.3.1. Aromatogram

The HIEO antimicrobial activity was tested at 923 mg/ml against nine strains of bacteria and one yeast using the disc method on solid medium. HIEO IZs on the germs tested are shown in Table 6.

Table 6 Qualitative assessment of HIEO antibacterial efficacy by disc diffusion method

Strains	Inhibition zone (mm)			
	IZ (9.23 mg/disk)	Sensitivity	Neomycin (30 µg/disk)	Miconazole (500 µg /disk)
Staphylococcus aureus	12	+	18	
Staphylococcus pneumoniae	16	++	19	
Streptococcus pyogenes	14	+	24	
Clostridium perfringens	10	+	22	
Bacillus cereus	35	+++	21	
Pseudomonas aeruginosa	12	+	18	
Enterobacter cloacae	13	+	27	
Enterobacter aerogenes	11	+	19	
Yersinia enterocolitica	14	+	20	
Candida albicans	20	+++		25

+++: Extremely sensitive; ++: Highly sensitive; +: Sensitive

According to the standard used by Ponce *et al*. [29]. HIEO was active on all (10) microbial strains tested with IZ ranging from 10 mm (*Clostridium perfringens*) to 35 mm (*Bacillus cereus*).

3.3.2. HIEO MIC and MBC on Bacillus cereus

The MIC and MBC of HIEO were determined on the most sensitive strain (*Bacillus cereus*) in the aromatogram. HIEO was tested at various concentrations (7.21 to 14.42 mg/ml). The results are shown in Table 7.

Table 7 Determination of the HIEO MIC and MBC on *Bacillus cereus*

Wells number	1	2	3	4	5	6	7	8	9	10
Concentrations (mg/ml)	14.42	13.22	12.14	11.13	10.21	9.37	8.59	7.89	7.23	7.21
Turbidity	-	-	-	-	-	-	+	+	+	+
Results		MBC				MIC				

(-): Clear medium; (+): Turbid medium

The HIEO MIC was 9.37 mg/ml and the MBC were 13.22 mg/ml (Table 6). The MBC/MIC ratio was less than 4 (1.41), which demonstrated that HIEO had a bactericidal action on *Bacillus cereus*.

3.3.3. HIEO toxicity

After orally administration of three doses of HIEO (100 mg/kg, 500 mg/kg and 1000 mg/kg), some symptoms were observed including abdominal contortion, piloerection, weakening of the animal, convulsions and difficulty in moving. The mice recovered gradually and after 24 hours no mortality was observed.

4. Discussion

For the same plant, the chemical compounds present in an essential oil are not constant. They can vary greatly depending on the biotope (sunshine, climate, soil composition, altitude, etc.), the part of the plant (leaves, bark, flowers, fruit, etc.) and the harvesting period [35, 36] which can often correspond to specific vegetative stages of the plant. Therefore, their pharmacological properties could also vary greatly.

In this study, the HIOE analysis was performed on leaves collected at the same location (Mount Ibity) in June when the plant was not bearing flowers or fruits. Therefore, as stated above, the chemical composition might be different if HIEO was extracted from plant material collected at other times.

The yields of essential oils are extremely variable depending on the plants considered, but they are generally very low, below 1% [37]. That of HIEO is well over 1 (1.9%) whereas that of several Malagasy *Helichrysum* EOs are below 1%: 0.4% for *H. gymnocephalum* EO [16]; 0.01, 0.012, 0.03, 0.07, and 0.16 for *H. selaginifolium, H. hypnoides, H. cordifolium, H. faradifani* and *H. bracteiferum* respectively [17].

Seventeen (17) compounds were detected in HIOE. Compared to the number of compounds found in other Malagasy *Helichrysum* EOs, this value was relatively low: 23 for *H. gymnocephalum* [19]; 43 for *H. bracteiferum* [16]), 47 for *H. faradifani* [14]. However, as mentioned above, this number could change significantly at other times of the harvest.

The knowledge of physical and chemical indices is important because it allows the characterization and identification of an essential oil [38]. In addition, it makes it possible to decide their utilization in eating, pharmaceuticals and industrial making [39]).

The density of EO is very often lower than 1 (density of water) and varies according to their chemical composition: the value of 0.92 can be considered as an average. The HIEO density was 0.924. The AFNOR standards (2005) recommends a density between 0.906 for low quality oils and 0.990 for very high-quality oils and set a density of 0.925 below which the oil is considered to be quality III [40].

Refractive index is a physical constant which is used frequently to test the purity of oils [39]). The lower the refractive index**,** the better the quality of the essential oil. According to the AFNOR 2005 standards, the refractive index of an essential oil should be between 1.495 and 1.513, 1.495 for high quality oils and 1.513 for lower quality oils [40]. With a refractive index of 1.470, HIEO can be considered to be of good quality in terms of purity. According to Kanko *et al.* [41], the low refractive index of essential oils (1.4710 to 1.4880) indicate their low refraction of light, which could favour their use in cosmetic products.

The rotation power rotation power of HIEO was **-** 0°33. It was different from that of other Malagasy species which was **-** 7°50 for *H. gymnocephalum*, + 9°10 for *H. lavanduloides* and + 9°30 for *H. bracteiferum* [16].

The acid index should be as small as possible and acid index of less than 2 is an indicator of a good conservation of the oil [40]. The HIEO acid index (2.10) was significantly lower than those of leaves EOs from *Chromolaena odorata* (15.62) and *Pimenta racemosa* (7.39) but higher than that of *Cananga odorata* EO (0.421) [40]. The low acidity of oils is considered as neutralized and safe for making skin care products whereas high acidity of oils may be harmful for skin. According to Kumar [39] with an acid value of 1.12 *Kaempferia galanga* oil has an exceptional shelf life and edibility.

The HIEO ester index (54) is significantly higher than that of *Chromolaena odorata* (21) and for *Pimenta racemosa* (33.75) [43], but by far inferior to that of *Kaempferia galanga* rhizomes EO (189.65) [39] and *Cananga odorata* flower EO (350.6) [40]. The higher the ester index, the better the quality of an EO [40].

As reported by Rabehaja *et al*. [18], Malagasy *Helichrysum* EOs exhibited an important chemical variability. The major components of EOs vary according to the species: for examples, α-pinene for *H. benthamii, caryophyllene* for *H. indutum* and *H. bojerianum* [18] and 1.8-cineole for *H. dubardii* [18], *H. gymnocephalum* and *H. bracteiferum* [17].

There are large differences between the proportions of the major components in HIOE and those in other Malagasy species. As examples, 1.8-cineole: 69.5% vs 7.1% for *H. selaginifolium* [17] and 0.7% for *H. faradifani* [15]; β-caryophyllene: 4.39% vs 46.4% for *H. cordifolium* [19]; β-pinene: 2.96% vs 38.2% for *H. selaginifolium* [17]; limonene: 0.92% vs 5% for *H. bracteiferum* and p-cymene: 2.39% vs 4.3% for *H. gymnocephalum* and sabinene*:* 0.90% vs 9.8% for *H. hypnoides.*

The composition of HIEO was largely dominated by 1.8-cineole (69.46%). High contents of this compound were also found in endemic species of Madagascar including *H. bracteiferum* EO (27.3%) and *H. gymnocephalum* EO (59.7%) [17]. In contrast, the European species *H. stoechas* and *H. italicum* EOs do not contain it [44, 45]. Concerning the HIEO other major components, the β-pinene and β-caryophyllene contents were low (2.96% and 4.39% respectively) compared to β-pinene (38.2%) for the *H. selaginifolium* EO [19] and β-caryophyllene (46.4%) for *H. cordiofolium* EO [17].

Antimicrobial activity of a given essential oil may depend on one or two of the major constituents only that make up the entire oil but the interactions between these and minor constituents in the oils are also important [46].

Like the EOs of other Malagasy species of *Helichrysum*, (*H. lavanduloides*, *H. bracteiferum* and *H. gymnocephalum*) [22], HIEO also exhibited antibacterial activities. It was active on all the tested microbial strains. *Bacillus cereus* and *Candida albicans* were extremely sensitive with IZ of 35 mm and 20 mm respectively. Investigations of the HIEO antimicrobial properties should be extended to other pathogens.

As mentioned above, 1.8-cineole was the main component of HIEO and given its various known pharmacological properties, it probably played a determining role in the antibacterial activity of HIEO. Indeed, this compound has anti-viral properties (Anti-infectious bronchitis virus or anti-IVB) and is a potential source of anti-IVB ingredients for the pharmaceutical industry [47]. In Germany, 1.8-cineole is an authorized medicinal product, formulated in soluble capsules indicated for acute and chronic bronchitis, sinusitis and respiratory infections [48]. In Madagascar, the EOs of Ravintsara (*Cinnamomum camphora*) and Mandravasarotra (*Cinnamosma fragrans*), rich in 1.8-cineole (50%), are used to treat different types of infections: viral and bacterial respiratory tract infections, urinary tract infections, gynaecological infections, oral infections, parasitic infections etc. The contribution of other components known for their antibacterial activities and present in HIEO such as β-carophyllene, β-pinene, p-cymene and limonene could not be excluded.

The antimicrobial activity of essential oils is due to their solubility in the phospholipid bilayer of cell membranes of bacteria and mitochondria, resulting in loss of membrane integrity and increased permeability. This could result in the death of bacterial cell due to leakage of critical molecules and ions from the bacterial cell to a great extent [46].

Since 1.8-cineole is present in large amount in HIEO and known to be toxic at high doses, a preliminary acute toxicity test was carried out on mice to assess the toxicity of HIEO. Indeed, 1.8-cineole is a powerful neurotoxicant: in humans, oral ingestion of doses from 10 to 30 ml of essential oil with 70% 1.8-cineole is potentially fatal [42]. In mice it has a relatively high oral LD_{50} of 3849 mg/kg (low toxicity), but at high doses it causes pathological changes in the liver and kidney after 30 days of treatment [49]. At 1000 mg/kg body weight, HIEO did not kill the mice but caused disturbing symptoms such as convulsions and movement difficulties which disappeared after 24 hours. Therefore, in depth toxicological investigations will be necessary to better understand the toxicity of HIEO and to determine the conditions of its possible safe uses.

5. Conclusion

The chemical composition and physicochemical characteristics of the essential oil of *H. ibityense* leaves are well established. The first data on its antibacterial activity have been acquired. Thanks to its rapid growth and the quality of its oil, *H. ibityense* constitutes a potential source of interesting and accessible therapeutic molecules. These results contribute to the knowledge of the endemic *Helichrysum* of Madagascar.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interests.

Statement of ethical approval

All the tests on animals were approved and in line with the standard established by Ethics Committee of the IPM

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