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Chemical composition and antibacterial activities of the essential oils from *Ocotea zahamenensis* Van Der Werff (Lauraceae)

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Abstract

The present work aimed to study the composition and antibacterial properties of the essential oils (EO) of *Ocotea zahamenensis* leaves (LEO), stem (SEO) and root (REO) barks from two harvest periods (March and June). All EOs were extracted by hydrodistillation from fresh plant parts with yields up to 4.5%. They are colourless, clear, with a strong odour, heavy, levogyre, with a low acid index and an ester index up to 14.89. Gas chromatography/flame ionisation detection analysis of these EOs identified 5 to 12 components representing 96.06 to 99.96% of the overall composition. Safrole was by far the most predominant constituent with contents ranging from 77.45% (SEO, June) to 97.05% (REO, March). The antibacterial activity was tested against eight pathogenic bacteria including 4 Gram (-) and 4 Gram (+) using microdilution assays. With Minimum Inhibitory Concentration (MIC) values of less than 1 mg/mL, all EOs showed antibacterial activity which varied according to the strain. There was not much difference between the activities of March and June Eos, and in both cases SEO were slightly more effective than LEO and REO. All EOs had bacteriostatic action on *Bacillus cereus* and *Vibrio fischeri* and bactericidal on almost other strains. When administered orally to mice at 0.5 mg/kg body weight, all EOs caused symptoms of intoxication. Their LD_{50} varied from 1.019 to 2.73 g/kg body weight. These EOs could be a new source of safrole and could be used for various purposes with further toxicological studies.

Keywords: *Ocotea zahamenensis*; Lauraceae; Essential oil; Chemical composition; Safrole; Antibacterial properties

1. Introduction

Plant oils have many exploitable properties that allow them to be used in a wide variety of fields [1]. Their uses are linked to their various recognised biological activities. They are widely used in aromatherapy, pharmacy, perfumery and cosmetics [2] and are also of growing interest to industry especially the food industry [1].

The growing interest in essential oils is reflected in the extensive research being carried out around the world on aromatic plants. Lauraceae is one of the plant families that are good producers of essential oils and is relatively important from an economic point of view. It includes almost 2,500 species in about 55 genera [https://www.plantes-botanique.org/famille_lauraceae].

The genus *Ocotea*, one of the larger genera of Lauraceae [3], comprises 350 known species of evergreen and aromatic trees and shrubs that are distributed throughout tropical and subtropical climates. In Madagascar, the genus *Ocotea* is well represented with its 34 endemic species [3]. It has a wide distribution, growing in all humid and subhumid

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evergreen forests on the middle and upper slopes of the east from Ranomafana to Zahamena between 900 and 1500 m altitude. The genus *Ocotea* has a wide range of pharmacological activities, such as anti-inflammatory, cytotoxicity, antimicrobial, larvicidal, and antiproliferative activities and several species have been used as traditional herbal medicines [4].

Within the framework of our research programme entitled "Knowledge and valorisation of little or unknown useful plants of the Mandraka forest" we have undertaken preliminary investigations on some Malagasy *Ocotea* species including *O. madagascariensis* [5], *O. laevis* [6], *O. cymosa* [7, 8], *O. zahamenensis* [9, 10], *O. auriculiformis* [11], *O. macrocarpa* [12] and *O. racemosa* [13]. In the present paper we report on the more extensive and unpublished work on the EOs of *O. zahamenensis*. This species was chosen because, although it is known by the local population as an aromatic plant, it is only used as construction wood, firewood or to make charcoal according to the field surveys we conducted. However, the first results on the properties of its EOs were promising.

Thus, the main objectives of our research on *O. zahamenensis* were to determine the composition and physicochemical characteristics of essential oils extracted from different parts of the plant (leaves, stem and root barks) at different periods and to explore their potential antibacterial activities and toxicity.

2. Material and methods

2.1. Materials

2.1.1. Plant material

Ocotea zahamenensis whose vernacular name is « Varongy ravimanga » is a tree 9 to 15 m high (Figure 1), flowering in February and fruiting from May to October [3].

The plant samples were collected in the Mandraka forest (18°52'05.4''S, 47° 53'53.7''E, altitude 1412 m) located 70 km from Antananarivo in June 2021. The plant was identified by comparison of an herbarium made from the collected material with the voucher specimen n°12895 of the Botanical and Zoological Park of Tsimbazaza (Antananarivo) made by Van Der Werff.

Figure 1 *O. zahamenensis* a) the whole plant and b) leaves **Source**: The authors

2.1.2. Microbial strains

The microbial strains used are pathogens commonly sought in medical and food microbiological analysis and/or control. They include 4 Gram (-) and 4 Gram (+) bacteria (Table 1).

Table 1 List of bacterial strains used

Germ-Tests	Gram	Reference
Staphylococcus aureus	+	ATCC 6538
Streptococcus pneumoniae	+	ATCC 6505
Clostridium perfringens	+	ATCC 13124
Bacillus cereus	+	ATCC 14579
Pseudomonas aeruginosa	-	ATCC 10145
Escherichia coli	-	NTCC 11954
Salmonella typhi	-	ATCC 14028
Vibrio fischeri	-	ATCC 49387

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 EOs

The extraction of the essential oils was carried out by hydrodistillation using a Clevenger type apparatus.

2.2.2. Physico-chemical characterisation of EOs

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
Optical rotation	AFNOR, NF-T 75-13
Acid index	AFNOR, NF-T 75-103
Ester index	AFNOR, NF-T75-104

2.2.3. EOs analysis

The chemical composition of the EO_S was determined by gas chromatography/flame ionisation detection (GPC/FID) [14]. The EOs analysis was carried out using a SHIMADZU GC 14-A chromatograph equipped with a TRACSIL TR-WAX fused silica (polydimethylsiloxane) capillary column BP5 (30 m × 0.32 mm × 0.25 µm) and a flame ionisation detector. 25 µl of sample were diluted in 0.5 mL of isooctane; the carrier gas used was nitrogen (N_2). The proportion of each compound was given by the peak areas.

2.2.4. Assessment of antimicrobial activity

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by microdilution method [15]. The standards used to interpret MIC results were those of Dalmarco *et al.* [16]: for crude extracts and fractions, a MIC lower than 100 µg/mL was considered as an excellent effect, from 100 to 500 µg/mL as moderate, from 500 to 1000 µg/mL as weak and over 1000 µg/mL as inactive. The essential oil type of action is bactericidal when the ratio MBC/MIC is ≤4 or bacteriostatic when MBC/MIC is >4 [17] [18] [19].

2.2.5. Toxicity determination

A volume of 0.3 mL of EO 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 yields of *O. zahamenensis* EOs and the physico-chemical parameters are presented in Table 3.

Table 3 Extraction yields and physico-chemical indexes of *O. zahamenensis* EOs

Collection period	EOs	yield	Density	Refractive index	Optical rotation	Acid index	Ester index
March	LEO	3.3%	1.1025±0.0001	1.5369±0.0002	-0°30±0°17	0.56±0.11	6.41±0.10
	SEO	3%	1.0927±0.0001	1.5354±0.0002	-022±0°17	1.10±0.11	26.58±3.76
	REO	2.8%	1.1039±0.0001	1.5373±0.0002	-0°41±0°17	1.11±0.11	18.44±4.25
June	LEO	2.7%	1.0898±0.0001	1.5344±0.0002	-0°70±0°17	0.27±0.02	5.99±0.10
	SEO	4.5%	1.0518±0.0001	1.5256±0.0002	-0°26±0°17	0.48±0.02	14.89±0.71
	REO	2.4%	1.0834±0.0001	1.5332±0.0002	-0°70±0°17	0.62±0.02	4.26±0.25

3.2. Chemical composition

As shown in Table 4 and Figures 2, 07, 05 and 06 main components representing approximately 96.06%, 99.27% and 99.97% of the overall composition respectively were identified in the March LEO, SEO and REO. For the June LEO, SEO and REO, 07, 12 and 05 main components representing 99.96%, 99.12% and 99.49% of the overall composition respectively were identified. Safrole was by far the predominant component of all EOs, regardless of the source organ and the time of harvest: its content varied from 77.45% (SEO, June) to 97.05% (LEO, March).

Table 4 Relative rates (%) of the major compounds detected in the essential oils from leaves, stem and root barks collected in March and June 2021

Components	LEO		SEO		REO	
	March	June	March	June	March	June
α-pinene	0.74	0.50	0.47	2.52	-	0.73
α-pinene	1.13	0.92	-	0.21	-	-
δ-3-carene	-	0.51	2.23	10.37	0.80	3.18
α-phellandrene	-	-	-	1.19	-	0.27
β-myrcene	0.98	0.36	-	0.46	-	-
α-terpinene	-	-	-	0.35	-	-
limonene	-	-	-	0.22	-	-
β-ocimene	-	-	-	0.36	-	-
δ-terpinene	-	-	-	0.32	-	-
α-terpinolene	0.67	1.03	1.23	5.45	0.44	1.70
δ-cadinène	-	-	-	-	0.47	-
safrole	91.27	96.32	94.11	77.45	97.05	93.61
eugenol	0.86	0.32	1.23	0.22	0.79	-
Diehyl phatalate	0.41	-	-	-	0.42	-
Total	96.06	99.96	99.27	99.12	99.97	99.49

LEO: Leaf EO; SEO: stem bark EO; REO: Root bark EO; - : not detected

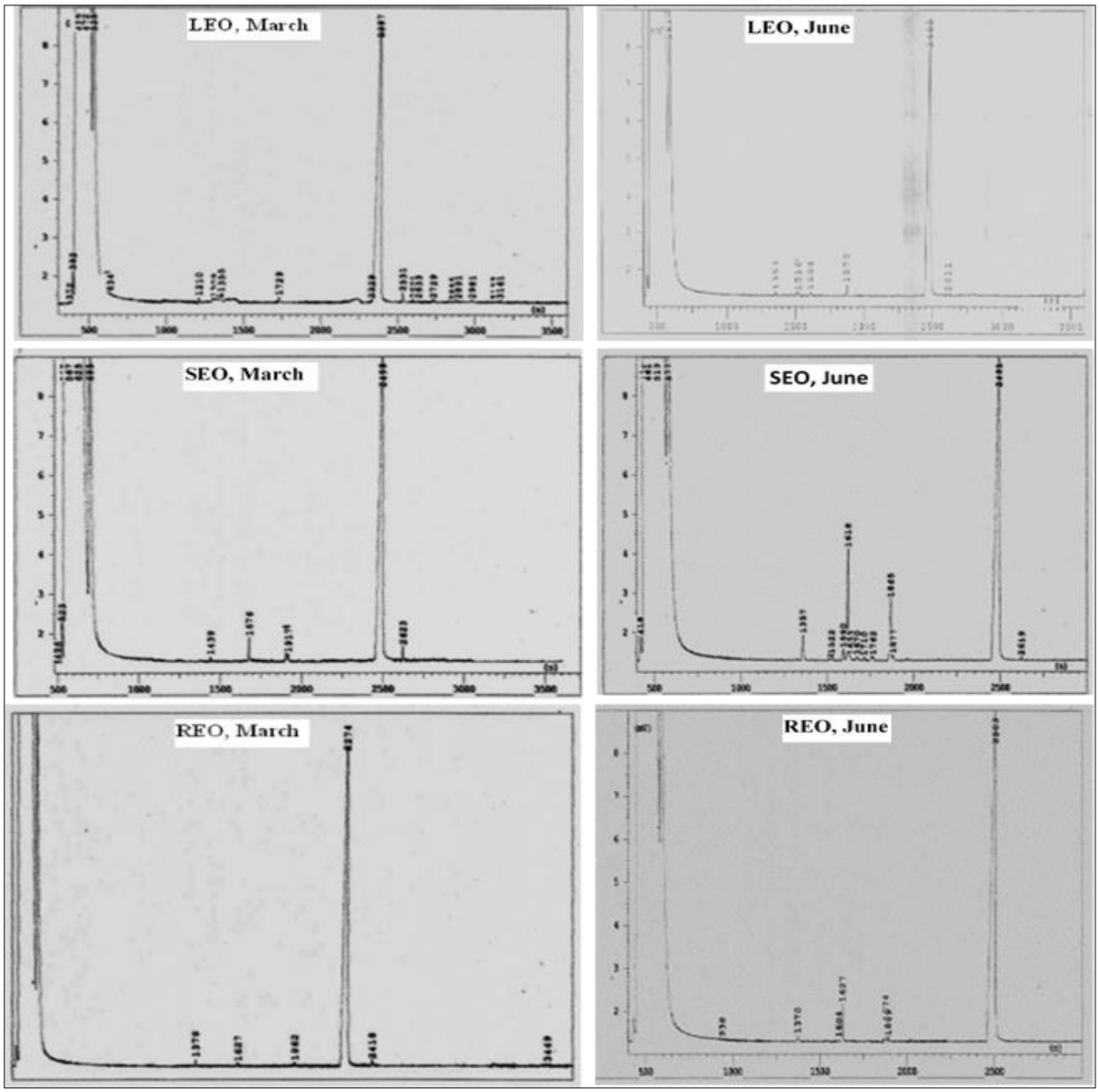

Figure 2 Chromatographic profiles of LEO, SEO and REO extracted from plant materials collected in March and June

3.3. Antibacterial activity of *O. zahamenensis* EOs

Table 6 MIC, MBC and MBC/MCI of *O. zahamenensis* Eos from March

	MIC (mg/mL)			MBC (mg/mL)			MBC/MIC		
	LEO	**SEO**	**REO**	**LEO**	**SEO**	**REO**	**LEO**	**SEO**	**REO**
Staphylococcus aureus	0.526	0.270	0.266	1.052	0.270	0.266	2	1	1
Streptococcus pneumoniae	0.526	0.541	0.533	1.052	1.083	1.067	2	2	2
Clostridium perfringens	0.657	0.270	0.533	1.052	1.083	1.067	1.6	4	2
Bacillus cereus	0.065	0.067	0.133	1.052	1.083	1.067	16	16	8
Escherichia coli	0.526	0.270	0.066	1.052	0.541	0.266	2	2	4
Pseudomonas aeruginosa	0.526	0.541	0.533	1.052	0.541	0.533	2	1	1
Salmonella typhi	0.526	0.541	0.533	1.052	1.083	1.067	2	2	2
Vibrio fischeri	0.131	0.067	0.133	1.052	1.083	1.067	8	16	8

MIC, MBC and the ratio MBC/MIC are presented in Tables 6 and 7.

LEO, SEO and REO from March and June showed bacterial activity against all strains tested (MIC < 1 mg/mL). MBCs values ranged from 0.266 mg/mL to 1.083 mg/mL for March EOs and from 0.270 to 1.088 mg/mL for June Eos. Almost all the EOs had bactericidal action (MBC/MIC ≤ 4) on all strains except *Bacillus cereus* and *Vibrio fischeri* on which their action was bacteriostatic (MBC/M I C > 4).

Table 7 MIC, MBC and MBC/MIC of *O. zahamenensis* EOs from June

	MIC (mg/mL)			MBC (mg/mL)			MBC/MIC		
	LEO	**SEO**	**REO**	**LEO**	**SEO**	**REO**	**LEO**	**SEO**	**REO**
Staphylococcus aureus	0.544	0.262	0.270	1.088	0.262	0.270	2	1	1
Streptococcus pneumoniae	0.544	0.524	0.541	1.088	1.049	1.081	2	2	1.9
Clostridium perfringens	0.544	0.262	0.541	1.088	1.049	1.081	2	4	1.9
Bacillus cereus	0.068	0.065	0.135	1.088	1.049	1.081	16	15	7.9
Escherichia coli	0.544	0.262	0.067	0.544	0.525	0.270	1	2	3.9
Pseudomonas aeruginosa	0.544	0.524	0.541	0.544	0.524	0.541	1	1	1
Salmonella typhi	0.544	0.524	0.541	1.088	1.049	1.081	2	2	1.9
Vibrio fischeri	0.045	0.045	0.067	0.270	0.270	0.270	6	6	4.02

3.4. Toxicity of *O. zahamenensis* EOs

After oral administration of the LD_{100} dose to mice, the same symptoms were observed with all EOs: a succession of symptoms suggestive of nervous system damage including itchy muzzle, immobility, enophthalmos, ataxia and violent clonic convulsions until death which occurred after about 1 h. With the LD_0 dose, the same symptoms were observed except for convulsions and progressive remission was observed from the 4 th hour onwards and no death was observed after 24 hours. The acute toxicity indexes (LD_0, LD_{50} and LD_{100}) are shown in Table 8.

Table 8 Oral acute toxicity indexes on mice in g/kg weight of *O. zahamenensis* EOs from March and June

EOs	**LD**	**LEO**	**SEO**	**REO**
From March	LD0	0.5	0.5	0.5
	LD100	1.9201	1.0368	0.7320
	LD50	1.0655	0.7328	0.5858
from June	LD0	0.544	0.525	0.540
	LD100	3.264	3.1497	3.254
	LD50	2.35	2.73	1.019

The LD50 of EOs from March ranged from 0.58 g/kg (REO) to 1.065 g/kg (LEO) and those of EOs from June from 1.09 g/kg (SEO) to 2.73 g/kg (REO).

4. Discussion

The *O. zahamenensis* EOs analysis was performed on fresh plant materials collected at the same location (Mandraka) in March and June. At these two periods the plant was not bearing flowers or fruits.

According to Laguerre [20], the yields of essential oils are extremely variable depending on the plants considered, but they are generally very low, below 1%. The EOs of *O. zahamenensis* were obtained with yields significantly higher than 1%: from 2.4% (REO, June) to 4.5% (SEO, June).

The number of compounds detected in the essential oils of the 3 plant parts studied was different. For the June EOs, SEO contained 12 compared to 7 and 5 in LEO and REO. Furthermore, the contents of common compounds were very different: the contents of α-pinene, δ-3-carene and α-terpinolene in SEO were significantly higher than those in LEO and REO. However, its content of safrole, the major common component, was significantly lower than that of LEO and REO. For the March EOs, differences between the three parts of the plant also existed but they were less important than those observed with the June EO.

All the *O. zahamenensis* EOs were clear, colourless with a strong odour and denser (density >1.09) than water.

The refractive index the *O. zahamenensis* EOs was about 1.52. 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 [21].

All *O. zahamenensis* EOs had negative optical rotation values which means that they were all levogyres.

The acid index of all the *O. zahamenensis* EOs was lower than 1. 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 [21]: 4.26 (REO) to 14.89 (SEO) in EOs from June.

The ester index of *O. zahamenensis* EOs ranged from 6.41 (LEO) to 26.58 (SEO) in EOs from March and from 4.26 (REO) to 14.89 (SEO) in EOs from June. Those values were by far lower than those of *Helichrysum ibityense* leaves (54) [22], *Kaempferia galanga* rhizomes EO (189.65) [23] and *Cananga odorata* flower EO (350.6) [21].

According to Chaverri and Cicció [24], the majority of the oils from the genus *Ocotea* of South America are characterized by the presence of phenylpropanoids like safrole. All the *O. zahamenensis* EOs were largely dominated by safrole with contents ranging from 77.45% (LEO, June) to 97.05% (REO, March). However, in other sympatric *Ocotea* from Mandraka, the predominant constituent was not the same in the EOs of different parts of the same plant (Table 9). For example, for *P. auriculiformis*, α-humulene and α-pinene were the predominant constituents in the leaves and stem bark respectively [11]. Safrole was not detected in the EOs of these other *Ocotea* species and conversely, the major constituents present in these other species such as α-humulene, limomene and β-elemol were not also detected in *O. zahamenensis* EOs. In addition, the predominant constituents of EO from different parts of these other species were not the same: for example, in *O. auriculiformis* α-humulene for leaves and α-pinene for stem bark.

Table 9 The major components of the *Ocotea* Eos from Mandraka forest

***Ocotea* species**	**EO from**	**α-pinene**	**β-pinene**	**limonene**	**α-humulène**	**safrole**	**β-elemol**
O. zahamenensis	LEO March	0.74	1.13	-	-	91.27	-
	LEO June	0.50	0.92	-	-	96.32	-
	SEO March	0.47	-	-	-	94.11	-
	SEO June	2.52	0.21	0.22	-	77.45	-
O. auriculiformis [11]	leaf	6.4	8.5	-	42.6	-	-
	Stem bark	23.54	12.29	2.64	0.34	-	-
O. cymosa [7] [8]	leaf	23.78	8.88	16.54	1.35	-	-
	Stem bark	0.592	0.253	6.88	-	-	-
O. laevis [6]	leaf	11.08	14.81	1.24	2.42	-	5.46
	Stem bark	4.13	2.25	6.79	-	-	1.45
O racemosa [13]	Leaf	13.49	11.51	27.93	4,68	-	-
O. macrocarpa [12]	Stem bark	0.22	0.02	0.8	1.68	-	20.37

-: not detected

High levels of safrole were also found in other aromatic plants EOs but those of the *O. zahamenensis* EOs were significantely higher (Table 10). However, this compound is lacking in some species such as *Ocotea brenesii* [24].

Table 10 Safrole contents in different plant species Eos

Plants	Plant parts	Safrole (%)
Ocotea zahamenensis	Leaf	91.27 to 96.32
	Stem bark	77.45 to 94.11
	Root bark	97.05 to 99.49
Ocotea odorifera [25]	Leaf	42
Sassafras albidum [26]	Root bark	85
Cinamommun camphora [27]	Fruit	29
Piper divaricatum [28]	Leaf	98
	Stem	83
	Fruit	87

Safrole has potential antidiabetic, antioxidant, antimicrobial, and anticancer activities [29]. Thanks to its aroma and fragrance, it is used as flavouring agent in food and beverages [30] and in household products such as floor waxes, polishes, soaps, detergents and cleaning agents [31]. In addition, it has insecticidal activity [26, 27].

There is no consensus on the standard scale for interpreting antimicrobial activity of natural products [32]. According to the scale of [16] used in this work, all EOs from March and June exhibited antibacterial activities (MCI < 1 mg/mL) but their efficiency depended on the strain. There was not much difference between the activities of March and June EOs, and in both cases SEO was slightly more effective than LEO and REO. An excellent effect (MCI < 0.1 mg/mL) was registered for LEO, SEO and REO on *V. fischeri*, for LEO, SEO on *B. subtilis* and for REO on *E. coli*. On the other strains, the antibacterial activity of all EOs was moderate or weak. With standards used by other authors, plant extracts with MIC values higher than 500 µg/ml [33] and even much higher than 1000 µg/ml [34, 35, 36] were classified as having strong antimicrobial activity. Therefore, all *O. zahamenensis* EOs exhibited strong antibacterial against all the bacteria tested. All EOs showed a bacteriostatic action (MBC/MIC > 2) on B*acillus. cereus* and *Vibrio fischeri* and a bactericidal action (MBC/MIC ≤ 2) on almost all other strains.

The antibacterial 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 [2]. The antibacterial activity of a given essential oil may depend on one or two of the main constituents of the oil: safrole for *O. zahamenenesis* EOs. However, the contribution of minor components with known antibacterial properties such as α-pinene, β-pinene and α-terpinolene should not be excluded.

According to a literature review on its toxicity [37], safrole was classified as being a moderately toxic compound. While its acute toxicity was associated with neurological dysfunction, the subacute and chronic toxicities are linked with damage to the liver and other tissues, and the induction of hepatic carcinomas. Its oral LD_{50} for rats was 1950 mg/kg body weight, with major symptoms being depression, ataxia, and diarrhea, with death occurring within 4 hours to 5 days. As this compound was present in large quantities in all *O. zahamenensis* EOs, an acute toxicity study of all these EOs was carried out on mice. By the oral route, all EOs had LD_{50} ranging from 1.019 and 2.73 g/kg, LD_{100} about 3.1 g/kg and LD_0 about 0.52 mg/kg. There was no significant difference between the toxicity of EOs from different parts of the plant. However, with the exception of REO, EOs from March were twice as toxic as those from June. Therefore, further toxicological studies, e.g. on the effects of doses below the LD_0, the subchronic and chronic toxicity, the impacts on major physiological functions (cardiac, renal and hepatic), etc., will still be needed to determine the acceptable conditions of use of these EOs.

The exploration of other biological properties of the plant's EOs is underway and their study on samples collected at other phenological stages of the plant is planned.

5. Conclusion

The chemical composition and physicochemical characteristics of the essential oils of *O. zahamenensis* parts were well established. All EOs of the plant parts have shown antibacterial activity and their toxicity has been assessed. *O. zahamenensis* could be an interesting alternative for the production of safrole. These results contribute to the knowledge of the endemic *Ocotea* of Madagascar, especially those of the Mandraka forest.

Compliance with ethical standards

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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 Pasteur Institute of Madagascar.

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