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Determination of the chemical composition and evaluation of the antimicrobial and antioxidant potential of the essential oil from *Cryptocarya ovalifolia* van der Werff leaves (Lauraceae)

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Abstract

The present work aims to evaluate the chemical composition, the antimicrobial and antioxidant properties of the essential oil from *Cryptocarya ovalifolia* van der Werff (COEO) leaves collected in Mandraka forest. COEO was extracted by steam distillation from fresh leaves, with a yield of 0.7%. COEO appeared as a clear liquid, yellow, with a pleasant odour and relative density of 0.8943 at 20 °C, a refractive index of 1.4746, an optical rotation of – 31°13, an acid index of 0.638 and an ester index of 6.375. A total of 31 constituents accounting for 99.98% of the total essential oil content were identified by gas chromatography/mass spectrometry. The main component was sabinene (30.65%) and the major components (>5%) were limonene (17.33%), methyleugenol (14%) and α-pinene (8.33%). The antibacterial activity was tested on nine pathogenic microorganisms including four Gram positive bacteria, four Gram negative bacteria and one yeast using the disk diffusion and the microdilution assays. COEO was active on 4 strains with inhibition zone diameter (IZD) ranging from 8.7 mm (*Bacillus cereus*) to 12.7 mm (*Clostridium perfringens*). Minimum Inhibitory Concentration (MIC) varied from 1.12 to 2.23 mg/ml and Minimum Bactericidal Concentration (MBC) was 8.93 mg/ml for all strains. COEO had bacteriostatic action on *Streptococcus pneumoniae*, *Clostridium perfringens*, *Bacillus cereus* and *Candida albicans* (MBC/MIC>4) and bactericidal action on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Vibrio fischeri* (MBC/MIC≤4). COEO exhibited radical scavenging activity against DPPH with an IC_{50} of 123.29 mg/ml. By oral route, its LD_{50} was 1.9/Kg body weight.

Keywords: *Cryptocarya*; Lauraceae; Essential oil; Chemical composition; Antimicrobial properties; Antioxidant activity

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1. Introduction

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

The rising interest in essential oils is reflected in the extensive research being carried out around the world on aromatic plants. The essential oils (EO) have great therapeutic power and interesting biological activities such as antibacterial, antifungal, larvicidal, insecticidal and antioxidant properties [3].

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 2500 species in about 55 genera [4].

The genus *Cryptocarya*, one of the larger genera of Lauraceae, comprises 365 known species of evergreen and aromatic trees and shrubs that are distributed throughout tropical and subtropical climates [5].

In Madagascar, the genus *Cryptocarya* is well represented with its more than 20 endemic species [6]. It has a wide distribution, growing in all humid and subhumid evergreen forest on the middle and upper slopes of the East between 900 to 1500 m altitude. The genus *Cryptocarya* has a wide range of pharmacological activities, such as anti-inflammatory, cytotoxicity, antimicrobial, anti-insect, anticancer and antioxidative activities [7] and several species have been used as traditional herbal medicines [8]. *Cryptocarya ovalifolia* has not yet been the subject of study other than botanical. An ethnobotanical survey in the region where the harvest was made, indicated that the plant is only used in carpentry.

The main objectives of this study were to determine the composition and physicochemical characteristics of *Cryptocarya ovalifolia* van der Werff leaf essential oil (COEO) and to explore its potential antibacterial and antioxidant activities and toxicity.

2. Material and methods

2.1. Materials

2.1.1. Plant material

Cryptocarya ovalifolia whose vernacular name is « Tavolomanitra » [6] is a tree 9 to 15 m high. The plant leaves were collected in Mandraka forest (21°22’12.4’’S, 047°49’59.7’’E, altitude 1355 m) located at 70 km from Antananarivo in June 2022. At the date of collection, the plant was in vegetative phase, without flowers or fruits. It was identified at the Tsimbazaza Botanical and Zoological Park, where a herbarium was deposited and compared with the voucher specimen No. 440 established by Randrianaivo A. in July, 1996.

2.1.2. Microbial strains

The microbial strains used were pathogens commonly sought in medical microbiological analysis and/or control. They included 4 Gram (–), 4 Gram (+) and one yeast (Table 1).

Table 1 List of microbial strains used

Germ-tests	Gram	Reference
Staphylococcus aureus	+	ATCC 6538
Staphylococcus 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
Candida albicans		ATCC 10321

2.1.3. Animals

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

2.2. Methods

2.2.1. Extraction of the essential oil

The extraction of the essential oil from the fresh leaves of *Cryptocarya ovalifolia* (COEO) was carried out by steam distillation [9].

2.2.2. Physico-chemical characterization

The concentration of the pure oil 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 be determined 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-T 75-104

2.2.3. Composition analysis

The chemical composition of the essential oil was determined by gas chromatography/mass spectrometry (GS/MS) [10]. The sample was analysed using a THERMO chromatograph (TRACE Network mass selective detector). It is equipped with a DBWAX column (0.25 mm x 30 m x 0.25 µm). Column temperature was programmed from 50 °C to 250 °C. Injector and detector temperatures were set at 280 °C. Helium was the carrier gas used, with a flow rate of 1 ml/min, the volume of sample injected being 1 µl. The peaks obtained were identified using AMDIS software Version 2.69 (Automated Mass Spectral Deconvolution and Identification System).

2.2.4. Antimicrobial activity assessment

All methods used for antimicrobial assay were detailed in our previous papers [11, 12]

The sensitivity of the microorganisms to the essential oil was determined by agar diffusion method or aromatogram. Sterile paper disks (6 mm diameter BioMérieux®, REF 549916) on which 10 µl of pure essential oil (893 mg/ml) have been deposited were placed on the surface of the inoculated Mueller-Hinton Agar medium (Scharlau®). The Petri dishes were incubated at 37 °C for 24 h and the inhibition zone diameters (IZD) were measured. The sensitivity to the essential oil was classified according to the IZD as: not sensitive (–) for IZD ≤ 8 mm; sensitive (+) for 9 mm ≤ IZD ≤14 mm; very sensitive (++) for 15 mm ≤ IZD ≤ 19 mm and extremely sensitive (+++) for IZD ≥ 20 mm [13].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by microdilution method [14]. The essential oil type of action is bactericidal when the ratio MBC/MIC is $\underline{\leq}$ 4 or bacteriostatic when MBC/MIC is > 4 [15, 16, 17].

Experiments were performed in triplicate.

2.2.5. Antioxidant activity determination

The antioxidant capacity was evaluated by the method using free radical scavenging against DPPH (2,2-Diphenyl-1-Pycryl Hydrazyl). The method was detailed in a previous paper [18]. The IC_{50} value represents the concentration of antioxidant required to reduce the absorbance by 50%: it was determined graphically.

2.2.6. Toxicity determination

A volume of 0.3 ml of COEO per 25 g of body weight was administered to mice by oral route by means of an intubation cannula with a curved distal. The acute toxicity indexes (LD_0, LD_{50} and LD_{100}) of COEO in mice were determined by Reed and Muench method (1938) [19]. Four 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 COEO was 0.7%. The values of the physico-chemical parameters determined are presented in Table 3.

Table 3 Physico-chemical parameters of COEO at 20° C

Relative density	Refractive index	Optical rotation	Acid index	Ester index
0.8943	1.4746	– 31°13	0.638	6.375

3.2. Chemical composition

The GC-MS analysis of the COEO identified 31 constituents representing approximatively 99.981% of the overall composition (Figure 1 and Table 4). Sabinene (30.65%) is the main constituent and major compounds with levels >5% were limonene (17.33%), methyl eugenol (14%) and α-pinene (8.33%) α-phellandrene).

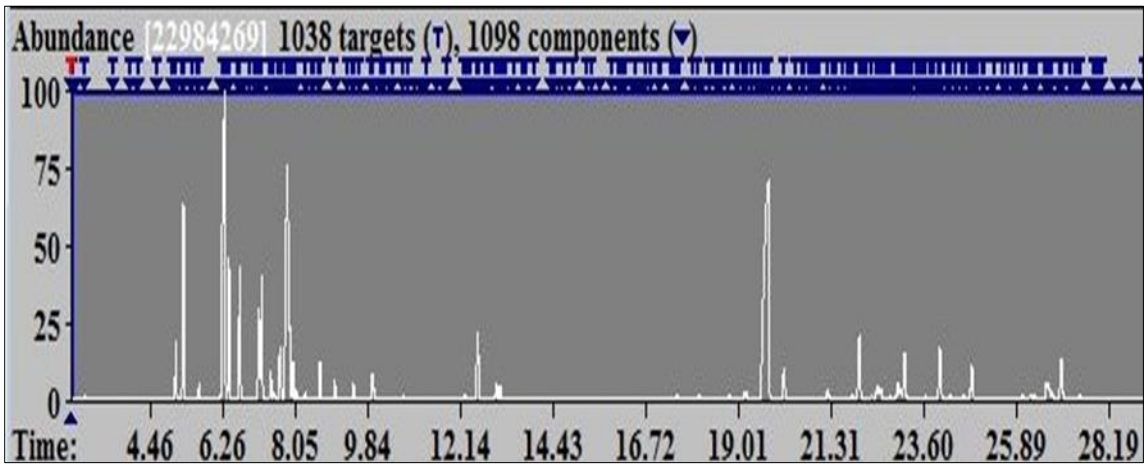

Figure 1 Chromatographic profile of COEO

Table 4 Major components in COEO

Peak number	Retention time (min)	Components	Relative rate (%)
1	5.0994	α-thujene	1.08
2	5.2905	α-pinene	8.33
3	5.6816	camphene	0.355
4	6.2875	sabinene	30.65
5	6.4130	β-pinene	2.79
6	6.6866	myrcene	3.09
7	7.1613	α-phellandrene	4.38
8	7.2286	δ-3-carene	2.74
9	7.4485	terpinolene	1.04
10	7.6773	para-cymene	1.34
11	7.8741	limonene	17.33
12	7.9995	cis-ocimene	0.732
13	8.3061	trans-ocimene	0.131
14	8.6672	γ-terpinene	0.894
15	9.0358	cis-hydrate de sabinene	0.429
16	9.9724	linalool	0.522
17	12.5748	terpinolene-4-ol	1.92
18	13.0362	α-terpineol	0.425
19	13.1281	cis-anethole	0.315
20	19.7661	methyl eugenol	14
21	20.1501	β-caryophyllene	0.953
22	21.2363	α-humulene	0.287
23	21.8497	copahu 5	0.148
24	22.0177	germacrene-D	2.12
25	22.5486	α-muurolene	0.273
26	22.9755	γ-cadinene	0.518
27	23.1401	δ-cadinene	1.35
28	24.2492	γ-gurjunene	0.0306
29	24.5999	palustrol	0.0734
30	24.8015	vetiver-3	1.04
31	27.0122	X*	0.695

X*: Component found in essential oil of *Cymbopogon citratus* (Java lemongrass essence)

3.3. Antibacterial activity of COEO

3.3.1. Aromatogram

The COEO antimicrobial activity was tested at 893 mg/ml against nine strains of bacteria and one yeast using the disk method on solid medium. COEO IZDs on the germs tested are shown in Table 5.

Table 5 Antimicrobial activity of COEO

Strains	Inhibition zone diameter (mm) Average ± SD	Sensitivity
Staphylococcus aureus	11.7 ± 0.6	+
Streptococcus pneumoniae	7.7 ± 0.6	–
Clostridium perfringens	12.7 ± 0.6	+
Bacillus cereus	8.7 ± 1.2	+
Pseudomonas aeruginosa	8 ± 0.0	–
Escherichia coli	9.7 ± 1.2	+
Salmonella typhi	7.7 ± 0.6	–
Vibrio fischeri	7.3 ± 0.6	–
Candida albicans	7.7 ± 0.6	–

IZD: Inhibition zone diameters; SD: standard deviation; +: Sensitive; –: not sensitive,

According to the standard used by Ponce *et al.* [13], COEO was active on 4 microbial strains tested with IZD ranging from 8.7 mm (*Bacillus cereus*) to 12.7 mm (*Clostridium perfringens*).

3.3.2. COEO MIC and MBC

The MIC and MBC of COEO were determined on all strains by microdilution method. Results are summarized in Table 6.

Table 6 Determination of the COEO MIC and MBC

Strains	MIC	MBC	MBC/MIC
Staphylococcus aureus	2.23	8.93	4
Streptococcus pneumoniae	1.12	8.93	7.97
Clostridium perfringens	1.12	8.93	7.97
Bacillus cereus	1.12	8.93	7.97
Pseudomonas aeruginosa	2.23	8.93	4
Escherichia coli	2.23	8.93	4
Salmonella typhi	2.23	8.93	4
Vibrio fischeri	2.23	8.93	4
Candida albicans	1.12	8.93	7.97

MICs were 1.12 mg/ml on *Streptococcus pneumoniae, Clostridium perfringens, Bacillus cereus* and *Candida albicans* and 2.23 mg/ml on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi* and *Vibrio fischeri*. MBC was 8.93 mg/ml on all microorganisms tested.

COEO had bacteriostatic action on *Streptococcus pneumonia*, *Clostridium perfringens*, *Bacillus cereus* and *Candida albicans* (MBC/MIC > 4) and bactericidal action on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi* and *Vibrio fischeri* (MBC/MIC ≤ 4).

3.4. COEO antioxidant activity

The activity of the oil were tested by the DPPH method at various concentrations.

Results enable to draw the graphs showing the variation of the percentage of inhibition according to the concentration of the extract I% = f (C) (Figure 2).

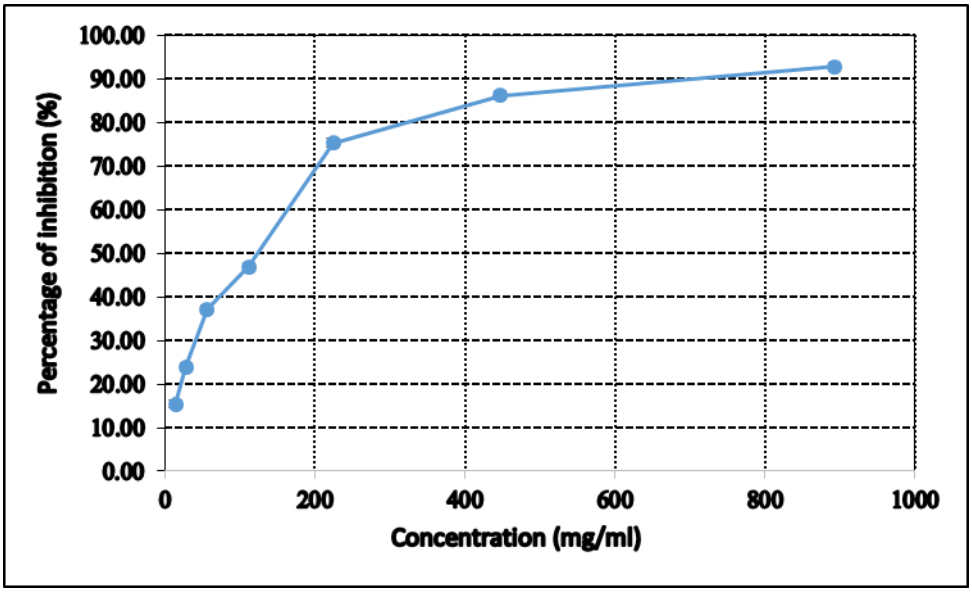

Figure 2 Percentage of DPPH inhibition according to COEO concentration

Scavenging activity against DPPH increased with concentrations. The IC_{50} for COEO was 123.29 mg/ml.

3.5. COEO toxicity

The acute toxicity indexes (LD_0, LD_{50} and LD_{100}) of COEO in mice by the oral route are presented in Table 7. Four increasing doses were used (0.67 g/Kg; 1.34 g/Kg; 2.68 g/Kg; 5.36 g/Kg).

Table 7 Oral acute toxicity of COEO on mice

	COEO (g/Kg body weight)
LD_0	0.67
LD_{50}	1.9
LD_{100}	5.36

LD = Lethal Dose

By oral route, at the doses tested corresponding to LD_0, LD_{50} and LD_{100}, the symptoms were the same, but their intensity and duration as well as the mortality rate were dose dependent. They included enophthalmia, palpebral ptosis, nervous disorders (ataxia, loss of reflex, analgesia and anesthesia). At the LD_0 dose, the symptoms gradually decreased after 5 minutes, disappeared after 1 h and the animals have fully recovered after 24 h, whereas at the LD_{100} dose, the symptoms intensified and all animals died after 2 to 4 h.

4. Discussion

The chemical composition of essential oils depends not only on the plant species but also on its variety, the part collected, origin, climate, preparation and other factors [20]. It is clear that this has a major impact on the biological properties of the oil. It should be therefore remembered that the plant material used in this study consisted of fresh leaves from a single tree growing at altitude 1355 m, harvested during the vegetative phase in June, a month in Madagascar's southern winter.

Under these conditions, the extraction yield of COEO by steam distillation was 0.7%. Depending on the plant, yields of essential oils vary widely but are generally less than 1% [21]. The COEO yield (0.7%) was high compared to essential

oils from other *Cryptocarya* species such leaf essential oils of *Cryptocarya impressa* (0.35%), *Cryptocarya infectoria* (0.34%) and *Cryptocarya rugulosa* (0.38%) [22].

COEO was clear and yellow with a pleasant odour. It was a light oil with a relative density of 0.8943, less dense than the essential oils from *Cryptocarya crassifolia* (0.928) and *Cryptocarya thouvenotii* (0.916) [23]: a density value around 0.92 is typically considered an average [1]. It was levogyre (– 31°13). Its refractive index was low (1.4746), lower than that of *Cryptocarya crassifolia* (1.497) and *Cryptocarya thouvenotii* (1.495) [23] an essential oil with a refractive index between 1.4710 and 1.4880 reflects little light, making it ideal for use in cosmetic products [24]. Its low acid index (0.638), well below 2, is an indicator of good conservation of the oil [25]: in comparison, the acid index of *Cryptocarya crassif*olia and *Cryptocarya thouvenotii* was 0.79 and 1.79 respectively [23]. The ester index of COEO (6.375) was very low compared to the EO of *Cryptocarya crassifolia* (228.06) and *Cryptocarya thouvenotii* (210.83) [23]. The higher the ester index, the better the quality of an EO [25].

The knowledge of physical and chemical indices is important because it allows the characterization and identification of an essential oil [26]. In addition, it enables to decide its utilization in eating, pharmaceutical and industrial making [27].

Thirty-one (31) components representing 99.98% of the overall composition were detected in COEO. The monoterpenes and their derivatives were dominant and represented 64.504% of the COEO components with 19.351% of oxygenated monoterpenoids. About 35.478% of the components have been identified as sesquiterpenes, including 9.676%.

Sabinene (30.65%) was by far the main component. Limonene (17.33%), methyl eugenol (14%) and α-pinene (8.33%) were major components but others of oxygenated sesquiterpenoids. such as α-phellandrene (4.38%), myrcene (3.09%), β-pinene (2.79%), δ-3-carene (2.74%) and germacrene-D (2.12%) were not quantitatively insignificant.

Some components are common to the essential oils of various *Cryptocarya* species, but in different percentages (Table 8). For example, sabinene, limonene, methyl eugenol and α-pinene which are major constituents of COEO. However, other major components present in other *Cryptocarya* species such as methyl chavicol in *C. Agathophylla* (>90%) [28], viridiflorol (8.5%) and (E)-β-bergamotene (15.6%) in *C. alba* [29], were not detected in COEO.

Table 8 Quantitative variation in the content (%) of essential oil components* in the leaves of some *Cryptocarya* species

Major components in COEO	***C. ovalifolia* (COEO)**	***C. agathophylla* [28]**	***C. aschersoniana* [30]**	***C. botelhensis* [31]**	***C. saligna* [31]**
Sabinene	30.65	25-34	nd	nd	5.6
Limonene	17.33	nd	42.3	0.2	2.6
Methyl eugenol	14	72-80	nd	nd	nd
α-pinene	8.33	nd	nd	22.7	4.3

* Components with percentage concentrations greater than or equal to 5.0% are displayed; nd: not detected

Sabinene has been found in different plant essential oils [32]. It is an important component of culinary spices for their distinctive aroma [33], as well as for perfume additives and fine chemicals. It has antifungal [34] and anti-inflammatory [35] activities and plays a role in the pharmaceutical industry. The other major components of COEO have well-known properties, many of which are already being exploited in various industries. Methyleugenol is used as a flavoring agent in various food products and as a fragrance in perfumes, creams, lotions, detergents and soaps [36]. Limonene is mainly used in the flavour and fragrance industries, as a solvent, and in the manufacture of polymers and adhesives [37, 38] and it can be effectively used for treating various ailments and diseases [39]. Alpha-pinene possess antibacterial and antifungal effects and is an effective natural anti-inflammatory agent [40].

Several species of *Cryptocarya* (Lauraceae) are traditionally used in folk medicines for the treatment of various diseases and disorders in tropical and subtropical countries. The essential oil extracted from the leaves and barks of *Cryptocarya* species are reported to possess antimicrobial, insecticidal, antioxidant, and antitumoral activities [41]. The major components of COEO include several compounds known for their biological properties, such as antimicrobial and antioxidant activities. These two properties were therefore investigated for COEO.

Against the micro-organisms tested in this study, despite the presence of significant amounts of various compounds known for their antimicrobial activity, such as limonene [42] α-pinene and β-pinene [43], alpha-phellandrene [44] and germacrene [45] the values obtained were relatively low. In addition, the two methods used did not produce the same results for certain germs. For example, *Staphylococcus aureus* and *Escherichia coli* were sensitive with agar diffusion method (solid medium) but insensitive with microdilution method (liquid medium). Conversely, *Streptococcus pneumoniae* and *Candida albicans*, which were insensitive in solid medium, were sensitive in liquid medium. The existence of positive or negative interactions between certain components of COEO, which vary depending on the method used and the germ tested, could be one explanation for these results. According to Touma *et al*., [46], the biological effect of an essential oil is the result of the synergy of all the molecules it contains, and it is possible that the activity of the main component may be modulated by other minor molecules.

The antioxidant power of COEO, measured by its reduction capacity of DPPH, was IC_{50} = 123.29 mg/ml. It is much weaker than that of EOs leaves from *Cryptocarya amygdalina* (IC_{50} = 6.97 µg/mL) [41] and *Cryptocarya alba* (IC_{50} = 492.7 µg/mL) [46, 47]. It was reported that the antioxidant properties of monoterpene in essential oils have been referred by several authors and it is the same of some oxygenated monoterpenes such as α-terpineol and linalool which are mainly responsible for the antioxidant potential of plant oils. The monoterpenes and their derivatives were dominant components in COEO (64.504%) but α-terpineol and linalool levels were too low, 0.425% and 0.522% respectively. Negative interactions between different compounds in COEO could not be excluded.

A preliminary study of the potential toxicity of COEO has been carried out. COEO had oral LD_{50} of 1.9 g/Kg body weight. These values were relatively high which meant that COEO was slightly toxic [48]. However, other toxicological studies such as subchronic and chronic toxicity, impacts on major physiological functions (cardiac, renal and hepatic) etc., will still be needed to better determine the acceptable conditions for the possible use of this EO.

The analysis of essential oils extracted from different parts of the plant collected at different seasons should be carried out to better assess COEO's antimicrobial and antioxidant potential.

5. Conclusion

The chemical composition and physicochemical characteristics of the essential oil of *Cryptocarya ovalifolia* leaves collected in June were well established. *Cryptocarya ovalifolia* could be an interesting alternative for the production of sabinene, limonene, methyl eugenol and α-pinene.

Moderate or low antimicrobial and antioxidant activity has been demonstrated.

Work is underway to continue studying the essential oils extracted from the leaves and other parts of the plant collected throughout the year, in order to determine the best period for the desired activities.

These results contribute to the knowledge of the endemic *Cryptocaria* of Madagascar, especially those of the Mandraka forest and Malagasy aromatic plants.

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 Pasteur Institute of Madagascar.

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