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Antioxidant and antimicrobial biological activities of bioactive molecules in Ravintsara bark: *Cinnamomum camphora (L.) J.Presl*. case study from Tsimbazaza Botanical and Zoological Park

Fanoina Ny Riana RAZAFINDRAKOTO[1,*], Rokiman LETSARA [1,2], Rigobert ANDRIANANTENAINA[3], Baholy Robijaona RAHELIVOLOLONA[4]

1 - Doctor, Chemical and Industrial Process Engineering, Ecole Supérieure Polytechnique d'Antananarivo - Ecole Doctorale Génie des Procédés des Systèmes Industriels Agricoles et Alimentaires, BP 1500, 101 Antananarivo, Madagascar
2 - Doctor, Tsimbazaza Botanical and Zoological Park, 101 Antananarivo, Madagascar
3 - Doctor, Centre National de Recherches sur l'Environnement, 101 Antananarivo, Madagascar
4 - Professeur Titulaire, Mention Génie des Procédés Chimiques et Industriels, Ecole Supérieure Polytechnique d'Antananarivo - Ecole Doctorale Génie des Procédés des Systèmes Industriels Agricoles et Alimentaires, BP 1500, 101 Antananarivo, Madagascar

*Corresponding author : Fanoina Ny Riana Razafindrakoto
Address: Ecole Supérieure Polytechnique d'Antananarivo - Ecole Doctorale Génie des Procédés des Systèmes Industriels Agricoles et Alimentaires, BP 1500, 101 Antananarivo, Madagascar
E-mail: fannyrazafindrakoto93@gmail.com
Telephone: +261 34 15 318 89

ABSTRACT

Cinnamomum camphora (L.) J. Presl., commonly referred to as Camphor tree, is native to Southeast Asia and has a long history of medicinal use. A comprehensive research effort has been conducted on the biological activities of the essential oil extracted from the bark of the plant at the Tsimbazaza Botanical and Zoological Park (PBZT), in order to contribute to the conservation of natural resources. The essential oil of the bark of *Cinnamomum camphora*, has a specific chemotype. The TGC analyses showed that the major compounds are monoterpenes: 1.8-cineole (34,0%), α-terpineol (10,1%), terpinen-4-ol (4,0%), camphor (25,0%), sabinene (2,1%), limonen (3,0%) and safrol (3,6%) phenyl propanoide. The essential oil of the bark of *Cinnamomum camphora* has antioxidant properties due the high presence of camphor, α-terpineol, terpinen-4-ol which act by neutralizing free radicals. However, it is important to note that camphor exhibits high toxicity at elevated concentrations, necessitating careful use and dilution control. Furthermore, the synergy of 1,8 cineole, camphor, α -terpineol, terpinen-4-ol and limonen contribut to the antimicrobial activities that can inhibit the growth of pathogens. In vitro tests have demonstrated its effectiveness against many strains of pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*, as well as against fungi such as *Candida albicans*. The use of *Cinnamomum camphora* bark essential oil from the PBZT provides an opportunity for research and education on the unique biological properties of this essential oil.

Keywords: *Cinnamomum camphora*, Tsimbazaza Botanical and Zoological Park, essential oil, biological activity, antioxidant, antimicrobial, chemotype, toxicity

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1. INTRODUCTION

Health products containing camphor or eucalyptus oils are frequently used in everyday life without medical prescription as a remedy for coughs and colds [5][6]. In recent decades, cases of side-effects have been observed in young children who have accidentally swallowed products containing camphor not intended for oral use, and have been reported in Canada and around the world. The widespread use of medicinal plants raises issues of biodiversity protection and the reforestation of certain species, given the context of climate change. For this reason, we focused our research on the biological antioxidant and antimicrobial activities of bioactive molecules in the bark of Ravintsara, *Cinnamomum camphora* (L.) J. Presl. Belonging to the Lauracea family. Our studies concern the Tsimbazaza Botanical and Zoological Park in Antananarivo-Madagascar. This is a site that is very popular, especially with young children. With this in mind, we set out to study the bark *of Cinnamomum camphora,* given that it is in this aerial part that most of the camphor is found, hence the name camphora. To do this, a physico-chemical study and several in vitro analysis were carried out: GPC analysis to determine the chemical compositions, various biological analyses to determine the antimicrobial and antioxidant activity and finally the determination of the percentage of inhibition in vivo of the lethal doses [10] necessary to know the limit of the toxicity of this essential oil in order to contribute to the warning of the abusive use of this plant in the daily life of the Malagasy.

2. MATERIALS AND METHODS

In this part of the study, the plant used was *Cinnamomum camphora,* an introduced plant. The barks were collected at the Tsimbazaza Botanical and Zoological Park in Antananarivo, Madagascar.

Part used: fresh bark of the plant

Extraction method used: Hydrodistillation, using the Clevenger type essencier to obtain the light type essential oil according to AFNOR standards [1] [2].

Determination of organoleptic characteristics

Determination of physical and chemical parameters

Specific gravity at 20°C : $d_{20}^{20} = \frac{m_2 - m_0}{m_1 - m_0}$; Index of refraction : $n_D^t = n_D^{t'} + 0.0004\,(t' - t)$

Rotating power : $[\propto] = \frac{\alpha_D^t}{C} \times 100$; Acid value : $IA = \frac{5.61\,V}{m}$;

Ester index : $IE = \frac{28.05}{m} \times (v_0 - v_1)$

Analysis by gas chromatography :

Used to identify the chemical composition of the essential oil (APPENDIX IV)

Determination of some biological activities of *Cinnamomum camphora* EO

The antioxidant activity of *Cinnamomum camphora essential* oil [5][7][8].

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reduction test: Qualitative TLC test (APPENDIX IV)

The semi-purified components are then identified by GPC after analysis by isolation of the DPPH radical on TLC (APPENDIX IV).

Percentage inhibition of the DPPH free radical is determined (P%):

P% = ((A white – A sample) / A white) x 100

Inhibitory concentration at 50% (IC50) :

P(%) = f (C).

The antimicrobial activity of Cinnamomum camphora essential oil

The antimicrobial properties of the EO were evaluated on 8 reference microorganisms, including 3 Gram-positive strains (APPENDIX IV).

The sensitivity of the various strains to the EO studied was evaluated on the basis of the diameter of the inhibition halos in accordance with the classification in Table I. Tests were carried out to evaluate the effect of *Cinnamomum camphora* essential oil on the development of bacteria, fungi and moulds. These tests determine its antimicrobial potential.

Evaluation of the toxicity of Cinnamomum camphora essential oil. *In vivo* tests were carried out to assess the potential toxicity of *Cinnamomum camphora* essential oil, (ANNEX IV)

Determination of the LC50 value: (ANNEX IV)

Determination of the LD50 : DL50 = DL100 - (Σ (a x b) / n) (ANNEXE IV)

3. RESULTS

Organoleptic test results:

Colour: Light green**; Odour:** Intense aromatic; **Taste:** Pungent; **Consistency:** Clear mobile

Physical characteristics of the EO extracted from the bark of *Cinnamomum camphora* :

Extraction yield: 0.38% (with: (M (plant matter= 500g; M(EO)= 1.92g)

Low relative density: 0.93 at 20°; Refractive index at 20°: 1.4750; Rotatory power at 24°C: +0.2°(C° :1 mg/ml)

Chemical characteristics of EO extracted from the bark of *Cinnamomum camphora*

Acid number (AI): 3.37 ; Ester number (EI): 84.15

The results of GPC analysis of *Cinnamomum camphora* bark EO, with a chemical content of 85.8%, are shown in Figure I. Figure II shows the diagram of the chromatographic spectrum of this extract with a content of 85.8% of the essential oil of *Cinnamomum camphora* bark.

Chemotypes: 1,8-cineole (34%), Camphor (25%), α-terpineol (10%), terpinen-4-ol (4%),

Other chemical compounds above 1%: : α-pinene (1,5%), β–pinene (1,2%), sabinene (2,1%), β-myrcene (1,4%), limonene (3%), safrol (3,6%)

Results of the antioxidant activity of Cinnamomum camphora bark EO

Results of qualitative tests on TLC by DPPH trapping: using a staining dish to carry out capillary migration of the various chemical compounds in relation to the eluent. Revelation with DPPH on a TLC plate with the samples to be analysed showed yellow spots with ascorbic acid as the control. As shown in Figure III. Once the presence of antioxidant compounds had been identified by TLC, a separation procedure for these molecules revealed by DPPH was carried out using n-hexane as the solvent. With the aim of carrying out a GPC analysis to assess the activity of semi-purified bioactive molecules with antioxidant properties, enabling these molecules to be identified, Figure IV shows the chemical composition by GPC of the TLC fractionation of a total of 77%.

The GPC results shown in Figure IV, by fractionation of the semi-purified compounds of *Cinnamomum camphora* with an antioxidant activity of 77%, show that The chemotypes: camphor (32.8%), terpinen-4-ol (24%), α-terpineol (10.2%) and a new constituent which appears after radical reactions, γ-curcumene (7%), Other antioxidant organic compounds greater than 1%: 1,8-cineole (1.2%), eugenol (1.8%). Figure V shows the results of the fractionation chromatographic spectra of the semi-purified compound of *Cinnamomum camphora* with an antioxidant activity of 77%

The Scavenger effect of the DPPH radical. Determination of the percentage inhibition of the DPPH free radical in (P%) and the Inhibition Concentration IC50 of *Cinnamomum camphora*: 113 mg/ml, with 99% pure ascorbic acid control 0.05 mg/ml, as seen in the graph in Figure VI.

Results of the antimicrobial activity of *Cinnamomum camphora* bark EO. The results of the qualitative evaluation of antibacterial activity are presented in Figure VII for the aromatogram test. Diffusion in a solid medium was used to determine the spectrum of activity and to assess the MIC of the sample. Of the nine strains tested, *Streptococcus pneumoniae* was found to be extremely sensitive (18mm); the rest were moderately sensitive with halo diameters ranging from 6mm to 9mm.

Results of the evaluation of the LC_{50} and LD_{50} toxicity of *Cinnamomum camphora* bark EO

The results of the clinical signs observed on *Culex quinquefaciatus* LC_{50} larvae are given in Figure VIII, which shows the percentage mortality of larvae as a function of the concentration (ppm) of *Cinnamomum camphora* bark EO. The effect of *Cinnamomum camphora* bark essential oil on larval stage 4 is shown in Figure IX.

Results of clinical signs observed on LD_{50} mice:

After administration of EO diluted with distilled water to mice that were fasted for 24 hours. We were able to observe that some mice had difficulty breathing, others convulsed while some had epileptic seizures and that subsequently they all had delays in their actions and reactions. Within 2 hours, the mice showed signs of physical weakness and locomotion, and subsequently did not survive the reaction to the interaction of the bioactive EO molecules. Results in Figure X show that crude extracts are active *in vivo* at doses of less than 500 mg/Kg body weight, and show that extracts administered as a single dose exerted their toxic action over a period ranging from 2 h to 24 h post-injection, depending on the clinical signs observed.

4. ANALYSIS OF RESULTS

The essential oil of *Cinnamomum camphora* bark specifically and predominantly contains the following chemical complexes:

Mono-terpene oxygenates: 1.8-cineole (34.0%), α-terpineol (10.0%), the component that gives it its expectorant, respiratory oxygenation, immunostimulant, antiviral, anti-angiogenic (*leukaemic cell*) properties, acting on rhinitis and sinusitis, but with a risk of drug interaction, either orally, set at 50-500 mg/kg, relatively high in mice LD_{50} = 3,849 mg/kg. Camphor (25.0%), mono-terpene ketones acting as a natural antiseptic, to treat rheumatism and aches and pains, it is also an antispasmodic, analgesic, anti-inflammatory and stimulant, but represents a real danger because it is a compound that is neurotoxic, exciting, causing seizures and

convulsions. Sabinene (2.1%), mono-terpene carbides: anti-Helicobacter pylori; antioxidant; prevents peroxide formation Limonene (3.0%), mono-terpene carbides: antiseptic, antiviral Terpinen-4-ol (4.0%), a mono-terpene alcohol Safrol (3.6%) phenylpropanoid, suspected carcinogen.

The essential oil of *Cinnamomum camphora* bark has significant antioxidant activity. This is due to the presence of various compounds such as camphor (32.8%), terpinen-4-ol (24%), α-terpineol (10.2%) and a new constituent that appears after radical reactions -curcumene (7%), as well as other organic compounds such as 1,8-cineole (1.2%) and eugenol (1.8%). These compound were identified by DPPH revelation of the semi-purified compounds, using the qualitative TLC method. The percentage of inhibition concentrations of 113mg/ml essential oil compared to 99% purified ascorbic acid is significant. It can therefore be said that the essential oil of *Cinnamomum camphora* bark has an interesting antioxidant activity.

The active ingredient essential oil of *Cinnamomum camphora* bark exerted significant antimicrobial activity on the strains tested. The extremely sensitive bacteria were *Streptococcus pneumoniae* (18mm) and then *Bacillus cereus* (9mm) to the action of the essential oil of the bark, which is extremely sensitive to Gram (+) bacteria. The halo of inhibition (< 8mm) of Gram (-) bacteria indicates resistance to essential oils.

Interpretation of the LC50 result: in terms of the LC50 larvicidal effect, *Cinnamomum camphora* bark EO is more effective at 22.90ppm. This result is due to the significant presence of limonene (3.0%), a member of the monoterpene family known for its insecticidal activities, in the composition of the EO.

Concerning the interpretation of the LD_{50} result: A dose of less than 500 mg/kg already causes mortality in mice. The LD50 would therefore be lower than this dose. Analysis of the results obtained in single-dose acute oral toxicity tests indicates a dose-dependent effect on mice. The maximum tolerated dose (MTD) is considerably lower than 500 mg/kg and could therefore potentially be used experimentally in a sub-acute or chronic toxicity study. After calculation in relation to the relative density of *Cinnamomum camphora* EO at 20°C, the LD_{50} acute toxicity limit for *Cinnamomum camphora* bark EO must not exceed 3712.8mg/kg: LD_{50}<3.7g/Kg. Thus, the value of the lethal dose 50 below 500 mg/kg body weight in mice enables the extracts to be categorised as a toxic substance. Furthermore, for the LD_{50} value, a person would have to receive at least a quantity in a single dose to cover the same symptoms of intoxication.

5. CONCLUSIONS

According to this study, we were able to identify the physico-chemical characteristics, chemical content and certain biological activities of the essential oil of the bark of the *Cinnamomum camphora* species found in our research area, the Tsimbazaza Botanical and Zoological Park. Our first hypothesis was "the use of this plant in everyday life without medical prescription or regulation in force" and our second hypothesis was "the strong possibility of the presence of camphor in this plant causing neurotoxic effects, or even convulsions, what about the bark? The case of the Tsimbazaza Botanical and Zoological Park". According to *in vitro* tests for bioactive molecules, the majority of elements in the oil are mono-terpene compounds 1.8-cineole (34.0%), camphor (25.0%) and the use of a phenyl-propanoid, safrol (3.6%), which has been suspected of carcinogenic activity in the literature. The search for antioxidant activity was positive, thanks to the presence of the following chemical constituents: camphor (32.8%), terpinen-4-ol (24%), α-terpineol (10.2%), γ-curcumene (7%), 1,8-cineole (1.2%), eugenol (1.8%), which were detected following GPC identification of the fractionations of semi-purified compounds in the tests via DPPH revelation on TLC. As far as antimicrobial activity is concerned, the essential oil of *Cinnamomum camphora* bark is highly sensitive to the gram-positive germ *Streptococcus pneumoniae* at 18mm, which explains why the plant is used as a very good inhalant. At a certain point, according to traditional uses, it is able to solve lung-related problems. This prompted us to carry out an *in vivo* acute toxicity test on *Swiss mice (Mus musculus*). We observed that a few hours after oral administration of the EO, the mice began to show signs of agitation and emotional disturbance, followed by loss of memory and reaction, which weakened them. There was mortality, with a rate of 100% for the animals studied with LD_{50}<3.7g/Kg. We also carried out in vivo studies on the larvae of (*Culex quinquefaciatus*), the results of which led us to put forwards the hypothesis that the EO from the bark of *Cinnamomum camphora* of the PBZT species has a good efficacy on the larvicidal effect of LC_{50} at 22.9 ppm. . This repellent activity has been verified by the presence of remarkable mono-terpene compound in the composition of the oil, in particular limonene (3.0%), which is already known to have this activity. Consequently, we can deduce that it is strongly inadvisable to use the essential oil of *Cinnamomum camphora* bark from the Tsimbazaza Botanical and Zoological Park orally or internally. It is also not recommended for external use, given the high dose of camphor present in the oil, which could cause irritation or burns.

6. BIBLIOGRAPHIC REFERENCES

[1]. **AFNOR, (1986).** Recueil des Normes Françaises « HE », AFNOR. Paris. 57p.

[2]. **AFNOR, (2000)** : Huiles essentielles. Ed. PARA Graphic. Tome 1 -

Échantillonnage et méthode d'analyse 471 P. Tome 2 - Volume 1 Monographie relative aux huiles essentielles 323 P. Tome 2 - Volume 2 Monographie relative aux HES 663 P.

[3]. **Andrianantenaina R. (2017).** Caractérisation en vue de la valorisation de la plante aromatique, *Ocotea auriculiformis* Kost. (Lauraceae), endémique de Madagascar 86-136

[4]. **Boyd, W.C. (1966).** Fundamentals of Immunology. 4ème Ed. New York: Wiley and Sons Editors; 503p.

[5]. **Bruneton J. (1993).** Pharmacognosie, phytochimie des plantes médicinales. Edition. Technique et documentaire, 3eme édition. 484-634 p.

[6]. **Hemingway J., Field L., Vontas J. (2002).** An overview of insecticide resistance. Science 298(5591) : 96-7.

[7]. **Hodge, H.C. & Sterner, J.H., (1943).** Determination of substances acute toxicity by LDB50B. Amer. Industrial Hyg. Assoc. 10: 93.

[8]. **Hooser S.B. March (1990)** D-limonene, linalool, and crude citrus oil extracts - Veterinary Clinics of North America: Small Animal Practice -, Vol 20, No 2, 383-385.

[9]. **The Book of Flavonoids. (2021)** Chao-Hui Feng, PhD (Editor) , Juan Francisco García Martín, PhD (Editor)., Books, Chemistry, Food Science and Technology, Nova, Plant Science Research and Practices, Science and Technology. Chapter 3. Evaluation of the Antioxidant and Larvicidal Potential of Phenolic and Flavonoid Compounds from Different Extracts of the Flowers of *Matricaria recutita* L. DOI: https://doi.org/10.52305/WGCH6543.

[10]. **The Book of Flavonoids. (2021)** Chao-Hui Feng, PhD (Editor) , Juan Francisco García Martín, PhD (Editor). Books, Chemistry, Food Science and Technology, Nova, Plant Science Research and Practices, Science and Technology. Chapter 1. Sample Pre-Treatment and Flavonoids Analytical Methodologies for the Quality Control of Foods and Pharmaceutical Matrices. DOI: https://doi.org/10.52305/WGCH6543. AISBN: 978-1-68507-376-3

[11]. **Journal of Alternative Medicine Research Volume 14 Issue 3. (2021).** Joav Merrick (Director, National Institute of Child Health and Human Development, Israel). ISSN: 1939-5868. Frequency: Quarterly. Binding: Softcover. Size: 8.5 x 11, Double column

[12]. **Revision of Genus *Alysicarpus* Neck ex Desv. (Fabaceae) in India: A Monographic Approach. (2021).** Dileep Pokle, PhD (Author) Biology, Books, Botany, Life Sciences, Nova, Plant Science Research and Practices, Science and Technology. ISBN: 978-1-53619-390-9. BISAC: SCI011000

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APPENDIX I: List of tables

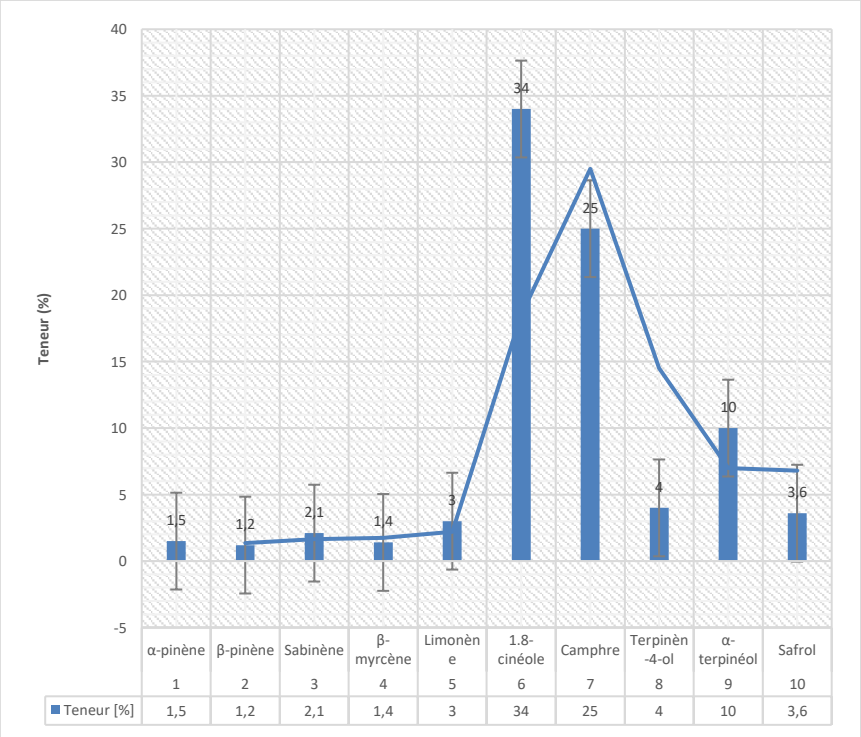
Table I: Classification of the sensitivitý of strains

Degré de sensibilité des germes	Diamètre du Halo d'inhibition (x)
Insensible ou résistante	X < 8 mm
Sensible	9 mm < X < 14 mm
Très sensible	15 mm < X < 19 mm
Extrêmement sensible	19mm < X

(Source: Andrianantenaina R. (2017). [3])

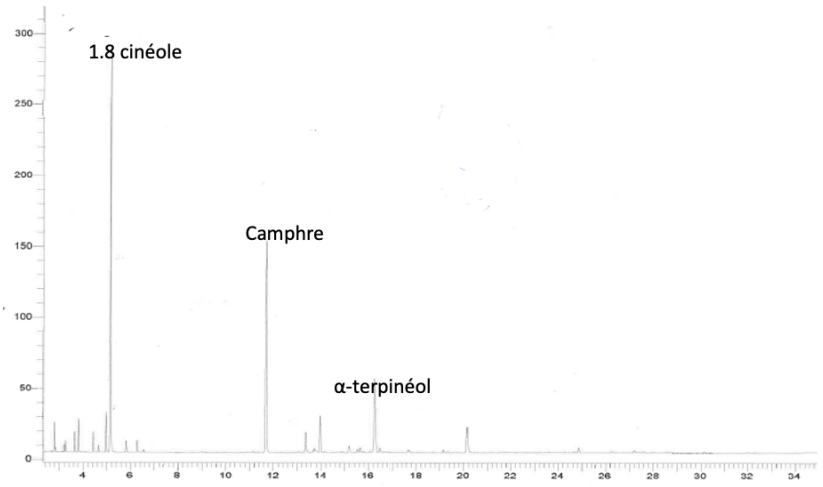
APPENDIX II: List of Figures

Graph I: Chemical composition of EO extracted (85.8%) from *Cinnamomum camphora* bark



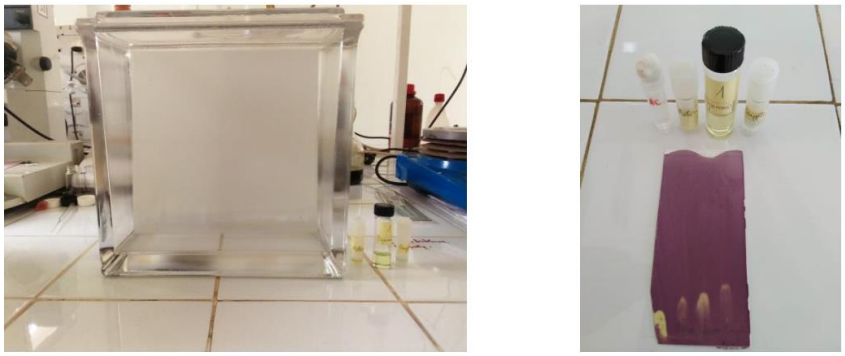
(Source: Author)

Graph II: Chromatographic spectrum of the essential oil of *Cinnamomum camphora* bark.



(Source: Author)

Graph III: Qualitative test by DPPH revelation on a TLC plate (a) in the TLC cuvette and (b) the reaction after DPPH revelation on a TLC plate with the samples to be analysed and an ascorbic acid control.



(a) (b)

(Source: Author)

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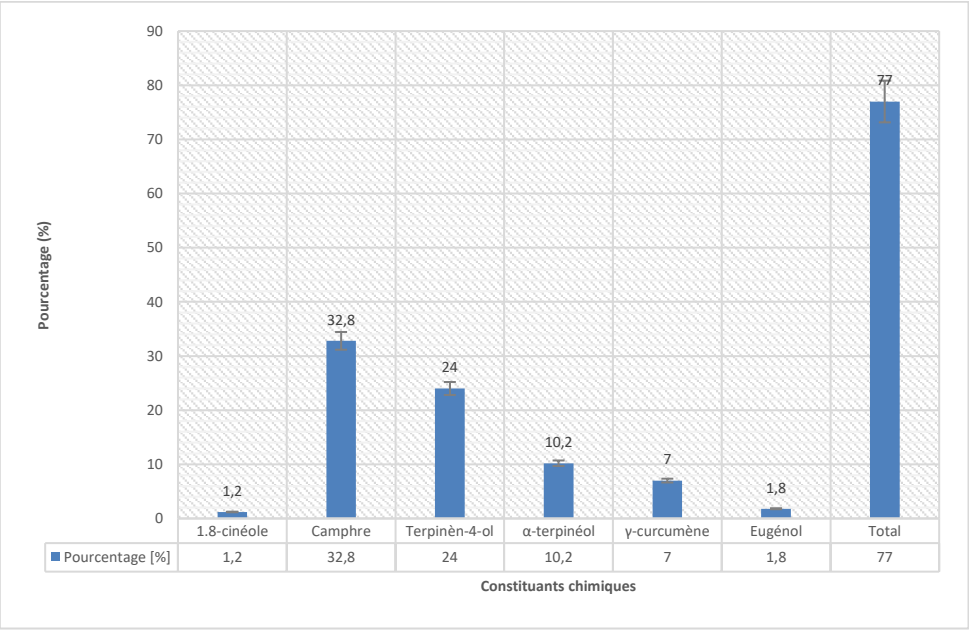
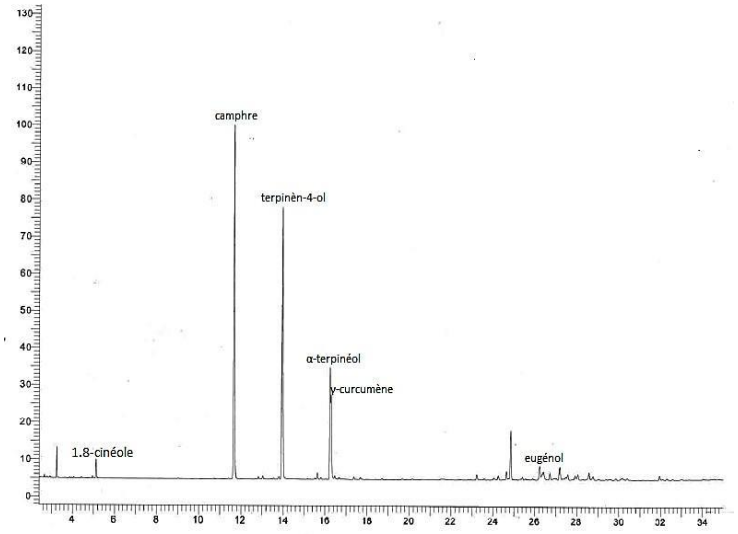
Graph IV: Composition of bioactive molecules with antioxidant activity by fractionation of semi-purified compounds revealed by TLC on DPPH of *Cinnamomum camphora* bark EO.



(Source: Author)

Graph V: Fractionation chromatographic spectra of the semi-purified compound of *Cinnamomum camphora* with antioxidant activity at a content of 77%,



(Source: Author)

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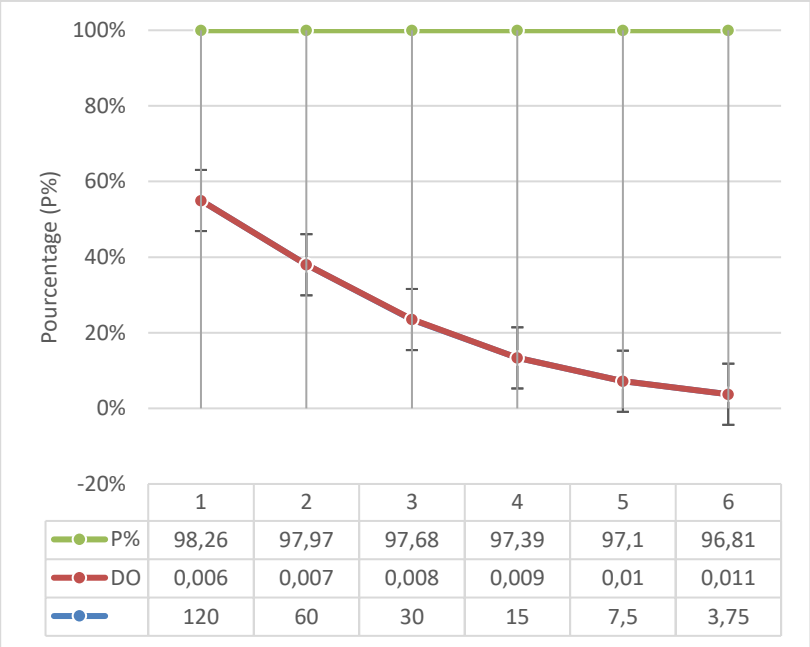
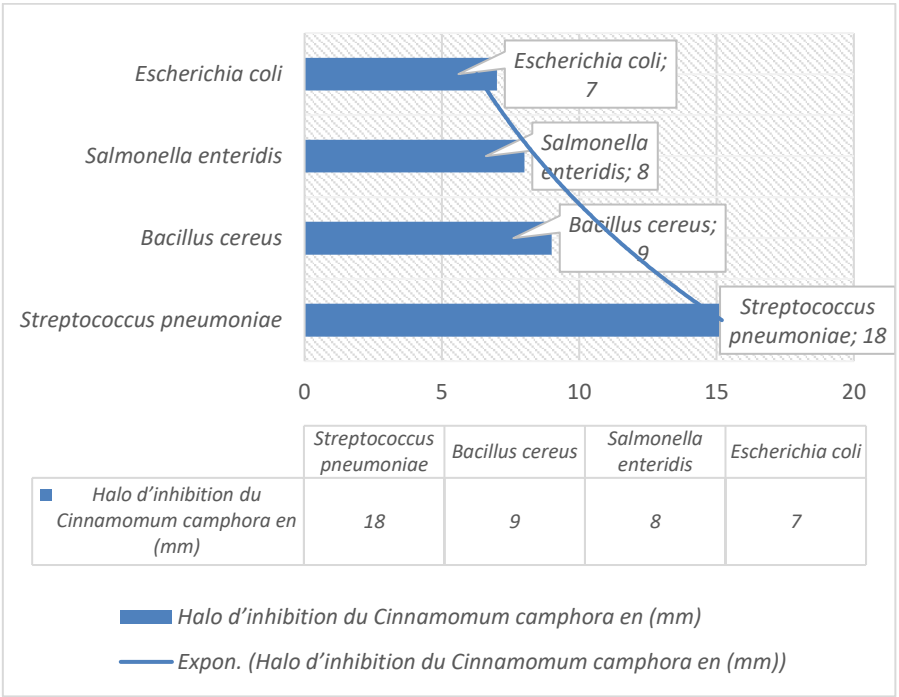
Graph VI: *Scavenger* effect of the DPPH radical with ascorbic acid as a control



	1	2	3	4	5	6
P%	98,26	97,97	97,68	97,39	97,1	96,81
DO	0,006	0,007	0,008	0,009	0,01	0,011
	120	60	30	15	7,5	3,75

(Source: Author)

Graph VII: Aromatogram test results for *Cinnamomum camphora* bark essential oil



	Streptococcus pneumoniae	Bacillus cereus	Salmonella enteridis	Escherichia coli
Halo d'inhibition du Cinnamomum camphora en (mm)	18	9	8	7

Halo d'inhibition du Cinnamomum camphora en (mm)
Expon. (Halo d'inhibition du Cinnamomum camphora en (mm))

(Source: Author)

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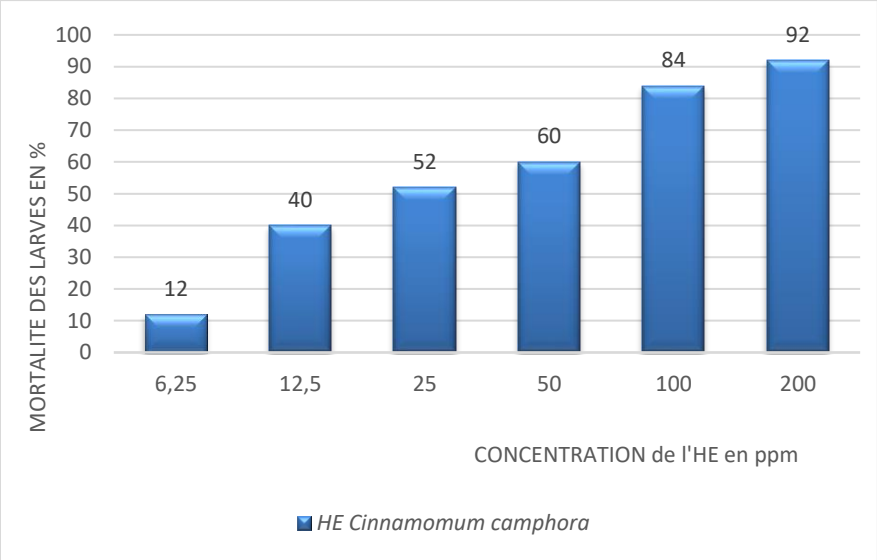
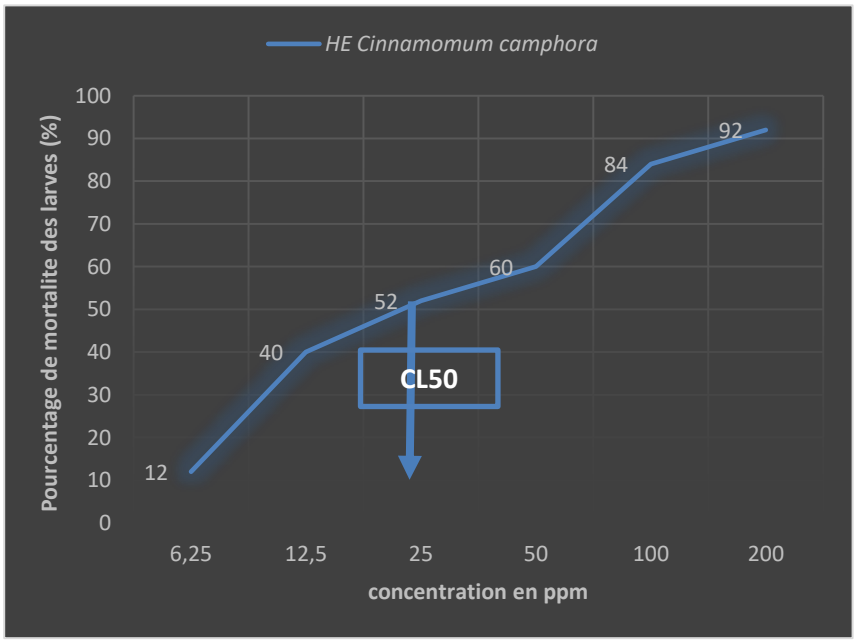
Graph VIII: Mortality (%) of *Culex quinquefaciatus* larvae as a function of concentration (ppm) of *Cinnamomum camphora* bark EO (blue)



(Source: Author)

Graph IX: Larvicidal effect of *Cinnamomum camphora* EOs at larval stage 4



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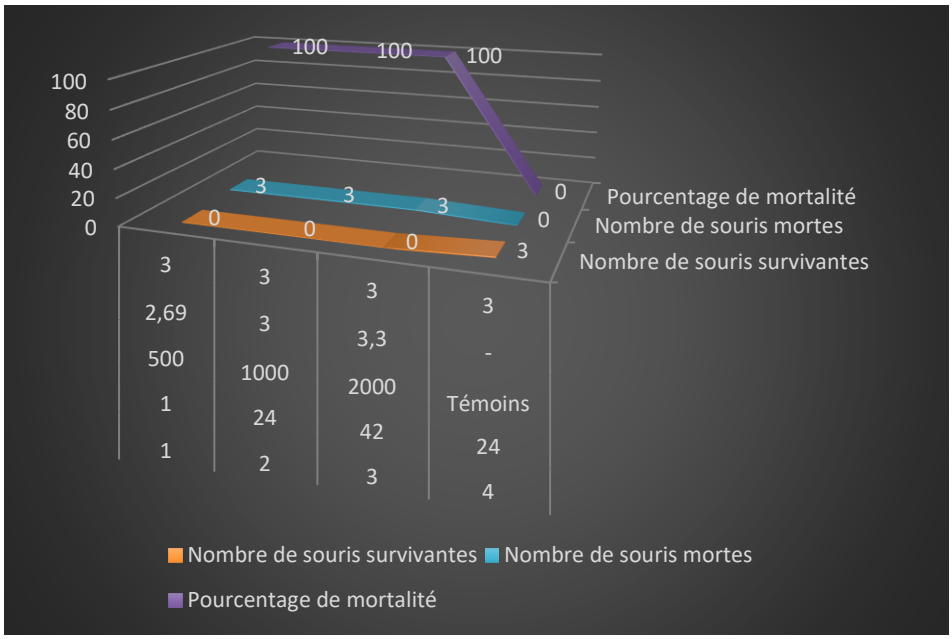
Graph X: Results of the effect of gavage with *Cinnamomum camphora* bark essential oil on mouse mortality.



(Source: Author)

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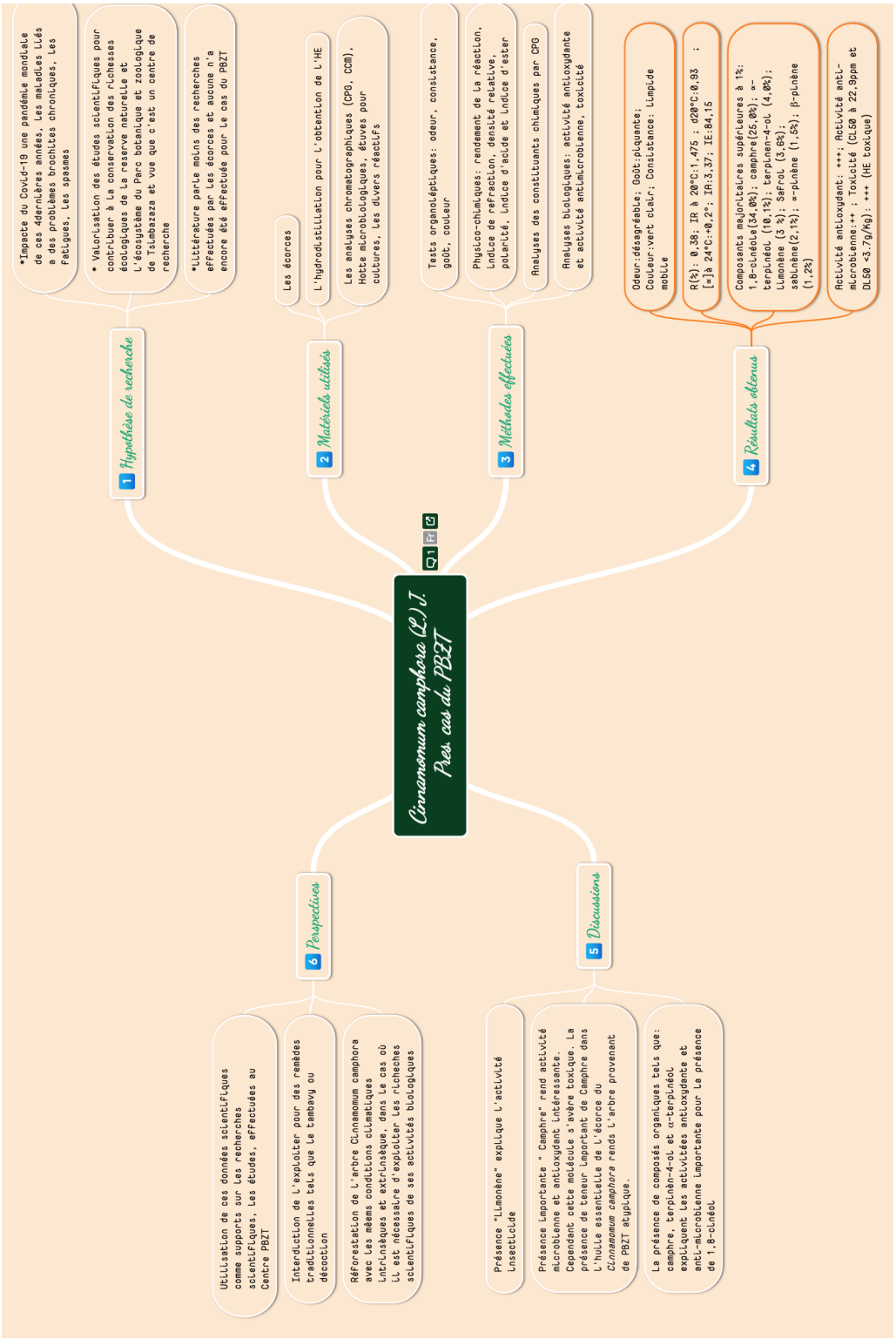
APPENDIX III: Conceptual map summarising research into the antioxidant and antimicrobial biological activities of bioactive molecules in Ravintsara bark: *Cinnamomum camphora* (L.) J.Presl. case of the Tsimbazaza Botanical and Zoological Park.



(Source: Author)

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APPENDIX IV: EXPERIMENTAL PROCEDURES

Analysis by gas chromatography

Operating conditions: GC: Clarus 580 PE with automatic injector; Column: ELITE-WAX (30m*0.32mm*025 mm); Oven: 50°C to 245°C (5°C/min); Detector: FID; Carrier gas: Hydrogen, pressure: 0.33 bar (4.8psi); Injection: split mode (1/75); Integration: percentage area - threshold: 0.02%.

Determination of some biological activities of *Cinnamomum camphora* EO

Antioxidant activity of *Cinnamomum camphora* essential oil[5][7][8].

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reduction test: Qualitative TLC test: this assesses the sample's ability to reduce the DPPH radical, which is an indicator of antioxidant activity. Firstly, a qualitative test which consists of testing on a Thin Layer Chromatography (TLC) plate which was subsequently developed with a solvent mixture (v/v). The DPPH radical is a violet-coloured radical, and by adding an eluent preparation (Hexane/ methane/ ED).

The semi-purified components were then identified by GPC after analysis by isolation of the DPPH radical on TLC. Using the solvent n-hexane as the operating condition. The operating conditions will be the same as for the essential oils of Cinnamomum camphora bark.

Determination of the percentage of DPPH free radical inhibition (P%):

P% = ((A blank - A sample) / A blank) x 100

With :

A-blank: Absorbance of the methanolic solution of DPPH (0.004%) and

A-sample: Absorbance of test sample.

Inhibitory concentration 50% (IC_{50}) :

P(%) = f (C).

The IC_{50} (inhibitory concentration) value waś defined as the concentration of each of the essential oils that causes the loss of 50% of the activitý of DPPH.

The antimicrobial activity of essential oil *Cinnamomum camphora* essential oil.

The antimicrobial property of EO waś evaluated on 8 reference microorganisms including 3 Gram-positive strains: *Streptococcus pneumoniae* (ATCC 6301), *Bacillus cereus* (ATCC 13061), *Staphylococcus aureus* (ATCC 11632); 4 Gram-negative strains: *Escherichia coli* (ATCC 70032), *Salmonella enteridis* (ATCC 13076),

Enterobacter cloacae (ATCC 700323), *Pseudomonas aerigunosa* (ATCC 9207) and on one yeast: *Candida albicans.* It waś tested using the solid medium diffusion method (agar) or disc method [8].

Operating conditions : Two sterile discs 6 mm in diameter soaked with 10µl of essential oil is deposited on the surface of *Muëller Hinton* agar medium previously flooded with the inoculum of 106 CFU/ml of germ. After 24h of incubation at a temperature of 37°C, the diameters of the zones of inhibition (mm) weré measured, including the diameter of the discs.

The sensitivitý of the different strains towards the HE studied is evaluated according to the diameter of the inhibition halos according to the classification in Table I. Tests are carried out to evaluate the effect of *Cinnamomum camphora* essential oil on the growth of bacteria, fungi and moulds. These tests determine its antimicrobial potential.

Assessing the toxicity of *Cinnamomum camphora* essential oil. *In vivo* tests are carried out to assess the potential toxicity of *Cinnamomum camphora* essential oil, including cytotoxicity and genotoxicity tests on cells or living organisms. Assessment of toxicity on the larvicidal activity of *Cinnamomum camphora* essential oil. We collected larvae of mosquitoes *(Culex quinquefasciatus*), which are vectors of disease, in their natural habitat in the Tsimbazaza Botanical and Zoological Park (PBZT) (S18o55'56", E 047o31'34", Alt. 1276m), which were used for the sensitivity test.

The LC50 value is determined [9]. The LC50 (24 h), or the concentration that kills 50% of the animals tested in 24 h, is determined by the method using an experimental curve giving the variations in the percentage of mortality, at 24 h, as a function of increasing doses of EO. The clinical signs observed during the effect of essential oil gavage on mouse mortality. During these investigations on the mice (24 h), various signs of toxicity were noted. *Cinnamomum camphora* bark EO was administered at doses ranging from 1,000 to 8,000 mg/kg, which could have an impact on mouse mortality.

To determine the LD_{50}: $LD_{50} = LD_{100} - (\Sigma (a \times b) / n)$

The LD_{50} values for HEs in mice were 2818,38 mg/Kg and 1380.38 mg/Kg respectively, depending on the toxicity rating [10]. The acute toxicity indices can be deduced from the projections made on the curve in [10]. The values found are then confirmed by the calculation method of [9] according to [10].

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