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FELLOWSHIP FINAL REPORT

Phytochemical study of plants of cosmetic interest

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REPORT INFO

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ABSTRACT

Ravenala or Traveller's Tree (STRELITZIACEAE) and Litchi (SAPINDACEAE) are two species widely distributed in Madagascar. Particularly, on the one hand, the Litchi fruit is exploited by the local agri-food industry and offers valuable co-products. On the other hand, some part of Ravenala enters the preparation of traditional remedy used by the Malagasy. In this research project focused on the valorisation of plant and co-product, our strategy is to characterize active compounds. Our study consists of developing analytical analysis methods and performing biological tests to identify the active compounds. Since the Institute of Organic and Analytical Chemistry - ICOA UMR7311 is at the cutting edge of technology and has internationally renowned expertise in natural substances for cosmetic uses. Our cooperation can easily meet our objectives. Thus, we would have found in plants widely distributed in Madagascar biosourced ingredients.

1- Introduction

The cosmetic industry consistently seeks novel, environmentally-friendly ingredients. The utilization of industrial by-products emerges as one the solutions, providing interest natural substances like phenolic compounds with a wide range of biological activities such as antioxidants, photoprotectors, antimicrobial agents [1], [2].

Madagascar's biodiversity is characterized by one of the highest levels of endemism in the world, with over 90% of its fauna and flora being unique to the region [3]. With a potential represented by more than 12000 species of vascular plants currently described, it comes as no surprise that the Malagasy flora provides a wide variety of medicinal plants. However, the information available on the pharmaceutical properties of Malagasy flora is far from exhaustive [4], [5].

The emblematic traveler's tree of Madagascar is a monospecific genus within the STRELITZIACEAE. Until very recently, this endemic genus was known to consist of a single species: *Ravenala madagascariensis* Sonn., cultivated as an ornamental plant. The plant is immediately recognizable by its enormous fan-shaped leaves resembling banana trees and is referred to locally by several vernacular names such as "ravinala". A 2021 article in Nature formally describes five new species and sets the application of the name *R. madagascariensis* to populations growing on the east coast of Madagascar [6]. Different parts of the plant are currently used by the population as human food, utensils and tools, and the construction of houses [7].

Litchi chinensis Sonn., commonly known as litchi, is an exotic tropical fruit from the SAPINDACEAE, originally native to China. Widely distributed in tropical and subtropical regions worldwide [8], including places like Madagascar. This fruit is renowned for its numerous health benefits. Extensive research has highlighted the pharmacological effects of litchi flowers and pulp, showcasing their analgesic, hepatoprotective, antioxidant, cardiovascular, and anti-inflammatory properties. Extracts from litchi seed and

pericarp have demonstrated antibacterial, antifungal, and inhibiting cell proliferation [8]. Additionally, chemical investigation have identified flavonoids, steroids, terpenes, and phenols as phytochemical constituents present in litchi [8], [9].

In this initiative, our goal is to increase in value two species, one widely distributed (*Ravenala madagascariensis* Sonn.) and the other as a co-product of agri-food transformation (*Litchi chinensis* Sonn.), by exploring their phytochemical potential applied to the field of cosmetics.

2- Experimental details

a. Plant material and sample preparation

Samples of Litchi and Ravenala were collected to Madagascar region in 2023 following Table 1.

Table 1 : Information on collected materials and sample preparation.

	Litchi	**Ravenala**
Collection location	- Fruit (Seeds, epicarps) : Antsiranana market (supply Sambava region) - Leaves : Sakaramy village, Antsiranana	- Fruit (seeds and blue arils) : Mahanoro, Tamatave - Inflorescence : Mahanoro, Tamatave
Collection date	- Fruit : January 2023 - leaves: February 9, 2023	- Fruit (Seeds et blue arils) : January 23, 2023 - Inflorescence : January 30,2023
Grinding method	- Seeds : coffee grinder - Epicarps : coffee grinder - Leaves : grinder	- Seeds : coffee grinder - Blue arils: not crushed - Inflorescence : coffee grinder

One gramme of crushed dried of each parts were extracted in 20 mL of ethanol (70%) during 40 min using ultrasound assisted extraction and it's completed with 12h of maceration. The supernatant was recovered and centrifuged 10 min at 10000 g before evaporation of the solvent under nitrogen flow to obtain the dry crude extract.

b. Materials

The following reagents were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France): 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic acidmonosodium salt hydrate (97%), copper (II) chloride (99.99%), sodium acetate (≥99%), 2,2′-azobis(2-methylpropionamidine) dihydrochloride granular (97%), trolox (≥98%), ferric chloride (97%), neocuproine (≥98%), glacial acetic acid, formic acid, ammonium acetate and ferric chloride hexahydrate.

Ultrapure water was produced with the PurelabFlex system from Veolia (Wissous, France). Ethanol and acetonitrile were of HPLC analytical grade and were obtained from SDS Carlo Erba (Val-de-Reuil, France). Formic acid was provided by Sigma-Aldrich (Saint Quentin Fallavier, France).

c. UHPLC/HRMS/MS

Ultra-high performance liquid chromatography was performed using an Ultimate 3000 RSLC system (Thermo Fisher Scientific Inc., MA, USA) consisting of a binary pump, an online vacuum degasser, an autosampler and a column compartment. Separation of extract was achieved on a sphynx C18 column (150 mm × 4.6 mm, 1.8 µm). Mobile phase C was water, mobile phase D was acetonitrile. The flow rate was 1 mL/min, and the gradient profile was 5 to 50% D for 0–15 min, 50% D for 15–20 min, 50 to 100 % D for 20–35 min, 100 % D for 35–40 min, 100 to 5 % D for 40–41 min and 5% D in 41–50 min. The injection volume was 0.6 µL. The equilibration time between two injections was 5 min.

UHPLC was coupled with mass spectrometry detection performed on a maXis UHR-Q-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The instrument was used in negative electrospray ionization (ESI-) mode. The capillary voltage was maintained at −4 kV, the gas flow to the nebulizer was set at 2 bar, the drying temperature was heated at 200 °C and the drying gas flow was 10.5 L/min.

Mass spectra were recorded in the data dependent acquisition (DDA) mode with an m/z range of 50–1650 for MS spectra and

an m/z range of 230–660 for MS^2 spectra. The collision-induced dissociation (CID) energy was applied at 30 eV. Two precursor ions with intensities higher than 1000 au were selected per fragmentation cycle among the most intense ions to be fragmented.

Data were analyzed using Bruker Data Analysis 4.0 software.

d. In-vitro antioxidant capability

The antioxidant capacity was assessed through DPPH, CUPRAC, and Iron (II) chelating assays. Measurement of absorbance was employed to determine this capacity, with various samples (solvent for the blank, trolox for the positive control, crude extract, or fractions) being tested. The colour change in the solution served as an indicator of reactions.

Evaluation of biological activity was performed in 96-well plates; each sample was deposited at 3 cascading concentrations. Ethanol was used to dilute the samples and for the negative control. Trolox was the positive control and was evaluated at 10 cascading concentrations ranging from 0.01 mg/mL to 5 mg/mL. Stock solutions of crude extract were at 10 mg/mL, thus after dilution, the concentrations of 0.01, 0.02, 1 and 5 mg/mL were evaluated. All the assays were done in triplicate (n=3).

DPPH radical scavenging activity assay

DPPH assays were performed by readjusting the method described by Lee et al., 1998. Briefly, 10 µL of sample were mixed with 190 µL of DPPH reagent at 60 µM in ethanol and incubated 30 min in the dark at room temperature. The absorbance was recorded using a microplate reader (MULTISCAN GO, ThermoFisher, USA) at 516 nm.

CUPRAC (Cupric ion reducing antioxidant activity) assay

CUPRAC assays were carried out according to the method described by Apak et al., 2004 with slight modifications. In short, 10 µL of sample were mixed with 190 µL of CUPRAC reagent prepared by combining 10 mM Cu (II) with 7.5 mM Neocuproinein ethanol and 1 M acetate ammonium buffer in a *1:1:1 (v/v/v)* ratio. Finally, the mixture was incubated 30 min in the dark at room temperature and the absorbance was measured at 450 nm using a microplate reader.

Iron (II) chelating assay

Iron (II) chelating assays was evaluated using a method described by Dinis et al., 1994 with some modifications. The reagent was prepared by combining 1 mM $FeCl_3$ and 0.3 mM ferrozine at a *1:1 (v/v)* ratio. The total volume of the samples or the blank (10 µL) was mixed with 190 µL of reagent and incubated for 30 min in the dark at room temperature. The absorbance was measured at 562 nm.

3- Results and discussion

The outcomes of our investigations can be succinctly summarized into two components: chemistry and biological activity.

a. Chemistry

Extraction yield defined as the mass of dry crude extract on the mass of dry raw material was presented in table 2.

Table 2: Extraction yield of Litchi and Ravenala

Plants	Parts of plant	Crude extract (mg)	Extraction yield (%)
Litchi	Seeds (LG)	154	15,4
	Epicaps (LP)	54	5,4
	Leaves (LF)	-	-
Ravenala	Seeds (RG)	134	13,4
	Blue arils (RA)	48	4,8
	Inflorescence (RFl)	48	4,8

The Litchi leaf extract appeared oily, although its mass was not measured.

The analysis of the extracts using HPTLC was conducted to observe the profile of polyphenols, lipids, sugars, and amino acids. The base polyphenol profil of Litchi and Ravenala HPTLC was exhibited as follow (Figure 1 and 2).

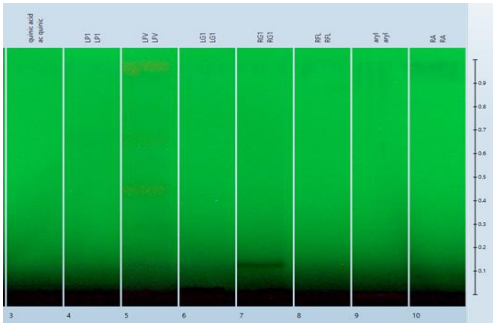

Figure 1 : Polyphenol profil of Litchi and Ravenala HPTLC, PEG

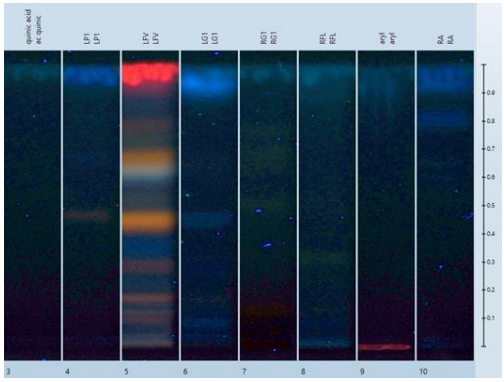

Figure 2 : Polyphenol profil of Litchi and Ravenala HPTLC, PEG

The base peak of the analysis by UHPLC/HRMS of the Litchi extract is presented in Figure 3.

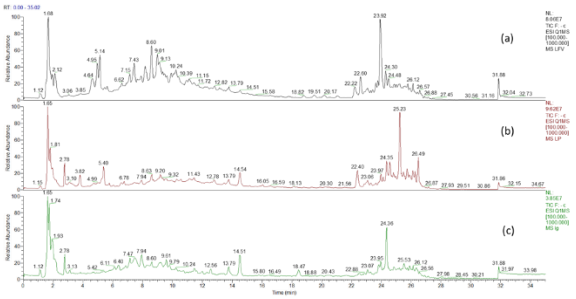

Figure 3 : Litchi. (a) Leaves extract, (b) Epicarp extract, (c) Seeds extract.

The base peak of the analysis by UHPLC/HRMS of the Ravenala extract is presented in Figure 4.

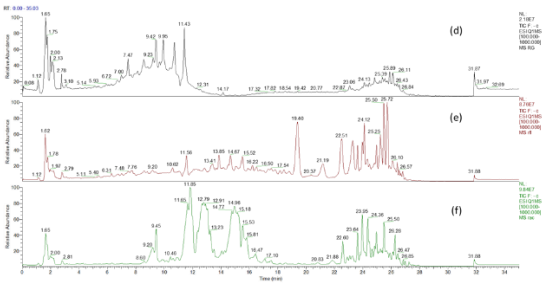

Figure 4 : Ravenala. (d) seeds extract, (e) Inflorescences extract, (f) Blue aril extract.

The analysis of mass spectra requires in-depth investigation. This step will be carried out later in order to establish the molecular profiles of each extract.

b. Biological activity

The antioxidant capacity was assessed through DPPH, CUPRAC, and Iron (II) chelating assays. We conducted tests on various dilutions of the extracts, but the results were inconclusive. Specifically, we observed the formation of precipitate, which interfered with the measurements.

4- Conclusion

The various analyzes indicate that extracts from different parts of plants are significantly rich in molecules, which paves the way for the identification of promising compounds that can be developed for cosmetic applications. These discoveries suggest an interesting potential for the selection of beneficial molecules and their exploitation in the field of cosmetics.

5- Perspectives of future collaborations with the host laboratory

The future collaborations with the host laboratory include promoting student exchange, co-authoring publications and applying collaboration projects. We are committed to advancing technical development by securing access to state-of-the-art technology. Additionally, our efforts aim to provide the host laboratory with access to exceptional plant materials, enabling the exploration of samples rich in valuable natural compounds, such as polyphenol for applications in well-being and human health.

Furthermore, we will continue our collaboration on the study of Glucosinolates

from Malagasy species. This project, developed in partnership with the host laboratory (ICOA, France), my university (UNA, Madagascar), and the University of Split (Croatia), will strengthen our joint research efforts.

6- Articles published in the framework of the fellowship

During the 6 months stay we presented the workin progress at the following event:

- 3rd International Congress Natural productsand sustainable development-Health, Food and Cosmetic Applications. May 25-27, 2023, Rabat, Morocco.(Oral Communication)
- LE STUDIUM Thursday - Interdisciplinary monthly seminar : Potential of natural products in cosmetics : sourcing, analytical chemistry and biological assays.6th July 2023, Tours, France. (Oral Communication)

Furthermore, the stay during the fellowship program allowed with LE STUDIUM support organized an international Conference: “Plant rediscovery with advanced tools for well-being applications” is the 137th in a series of LE STUDIUM Conferences. This event gathering internationally renowned researchers to a cross-disciplinary to discuss 4 main topics : Ethic Sourcing of natural products by ethnobotanical / Plant valorization / Bioactivity and Eco-process / Phytochemistry. October 9-11, 2023, Orléans, France.

7- Acknowledgements

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8- References

[1] A. M. Martins et J. M. Marto, « A sustainable life cycle for cosmetics: From design and development to post-use phase », *Sustain. Chem. Pharm.*, vol. 35, p. 101178, oct. 2023, doi: 10.1016/j.scp.2023.101178.

[2] A. Nunes, J. Marto, L. Gonçalves, A. M. Martins, C. Fraga, et H. M. Ribeiro, « Potential therapeutic of olive oil industry by-products in skin health: a review », *Int. J. Food Sci. Technol.*, vol. 57, nº 1, p. 173-187, 2022, doi: https://doi.org/10.1111/ijfs.15384.

[3] A. Antonelli *et al.*, « Madagascar’s extraordinary biodiversity: Evolution, distribution, and use », *Science*, vol. 378, nº 6623, p. eabf0869, déc. 2022, doi: 10.1126/science.abf0869.

[4] P. Rasoanaivo, « Rain Forests of Madagascar: Sources of Industrial and Medicinal Plants », *Ambio*, vol. 19, nº 8, p. 421-424, 1990.

[5] I. Riondato *et al.*, « First ethnobotanical inventory and phytochemical analysis of plant species used by indigenous people living in the Maromizaha forest, Madagascar », *J. Ethnopharmacol.*, vol. 232, p. 73-89, mars 2019, doi: 10.1016/j.jep.2018.12.002.

[6] T. Haevermans *et al.*, « Description of five new species of the Madagascan flagship plant genus Ravenala (Strelitziaceae) », *Sci. Rep.*, vol. 11, nº 1, Art. nº 1, nov. 2021, doi: 10.1038/s41598-021-01161-1.

[7] N. Rakotoarivelo *et al.*, « Ethnobotanical and economic value of Ravenala madagascariensis Sonn. in Eastern Madagascar », *J. Ethnobiol. Ethnomedicine*, vol. 10, p. 57, juill. 2014, doi: 10.1186/1746-4269-10-57.

[8] A. M. Miranda-Hernández *et al.*, « Characterization by HPLC–ESI–MS2 of native and oxidized procyanidins from litchi (Litchi chinensis) pericarp », *Food Chem.*, vol. 291, p. 126-131, sept. 2019, doi: 10.1016/j.foodchem.2019.04.020.

[9] L. Wang, G. Lou, Z. Ma, et X. Liu, « Chemical constituents with antioxidant activities from litchi (Litchi chinensis Sonn.) seeds », *Food Chem.*, vol. 126, nº 3, p. 1081-1087, juin 2011, doi: 10.1016/j.foodchem.2010.11.133.

[10] S. K. Lee *et al.*, « Evaluation of the antioxidant potential of natural products », *Comb. Chem. High Throughput Screen.*, vol. 1, nº 1, p. 35-46, avr. 1998.

[11] R. Apak, K. Güçlü, M. Ozyürek, et S. E. Karademir, « Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method », *J. Agric. Food Chem.*, vol. 52, nº 26, p. 7970-7981, déc. 2004, doi: 10.1021/jf048741x.

[12] T. C. P. Dinis, V. M. C. Madeira, et L. M. Almeida, « Action of Phenolic Derivatives (Acetaminophen, Salicylate, and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Peroxidation and as Peroxyl Radical Scavengers », *Arch. Biochem. Biophys.*, vol. 315, nº 1, p. 161-169, nov. 1994, doi: 10.1006/abbi.1994.1485.