BIoEx - Journal
Britain International of
EXACT Sciences Journal
Publish: | January - May - September |
E-ISSN: 2686-1208
P-ISSN: 2686-1216
http://biarjournal.com/index.php/bioex

Valorization of aromatic and Medicinal Plants of Ranomafana, District Ifanadiana, Region Vatovavy Fitovinany: Case of Vepris ampody and Vepris sp

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Abstract:

For the conservation of the biodiversity of Ranomafana, the valorization of the aromatic and medicinal plants of Ranomafana is an alternative. Two plants of the RUTACEAE family were chosen Vepris ampody and Vepris sp. For this a multidisciplinary approach was adopted: an ethnobotanical survey for the traditional use, ecology of the plant in order to identify environmental conditions favorable to the development of the species, phytochemical screening and identification of the essential oils of the leaves, isolation of mycoendophytes and identification of the fatty acids resulting from the extracts of fermentation. Vepris ampody is used in traditional medicine, and the decoction of leaves and bark is used in case of fever, malaria, fatigue, and muscle pain. The leaves are rich in alkaloids and the essential oils in sabinene. Four strains have been isolated. After fermentation, these four strains produce fatty acids, mostly unsaturated. Vepri ssp. has no medicinal use, it is used to ward off a spell. The leaves are rich in alkaloids. The essential oil is rich in linalool. The perspectives of the study concern the popularization of culture and the identification of alkaloids.

Keywords:
Ranomafana, Ecology, mycoendophytes, essential oil, fatty acid

I. Introduction

The island of Madagascar has developed a unique fauna and flora, and the rate of endemism is extremely high. About 85% of the plant species and 90% of the animal species of Madagascar are not found in any other region of the world. Biodiversity is the raw material that ensures the survival of their population. Plants and their products are among the most used materials (Raharinirina, 2009). Ranomafana National Park, declared a UNESCO World Heritage Site (Froling, 2014) is one of the exceptional ecosystems existing in the country. In addition, Ranomafana abounds in aromatic and medicinal plants little exploited. Some are used in traditional medicine. This makes that the access of the population to natural resources is very limited. Despite this, the restriction on this wealth has not prevented deforestation and bushfires. Income-generating activities as alternatives for the population are therefore important to limit the loss of biodiversity and contribute to local development. Some plants secrete volatile and odorous substances. Apart from odorant molecules, molecules from the fermentation of mycoendophytes can also be identified (Strobel, 2003; Zareb, 2014).

Among the aromatic and medicinal plants of Ranomafana, the species of the family RUTACEAE, the species Vepris can be proposed. Indeed, species of Vepris such as Vepris heterophylla, Vepris ampody, Vepris fitoravina have been studied either for the production of essential oil or for the production of alkaloids. The identified alkaloids have many medicinal uses (Palmer, 2014; Kamden, 2017; Talla, 2015; Rabehaja, 2013). In contrast, aside from the essential oil of Citrus species, few essential oils of Vepris are found in the market. Therefore, Vepris species have real potential for aromatherapeutic and phytotherapeutic uses. In addition, the development of para pharmaceutical products such as balms, essential oils, and aromatherapeutic products is an asset for the community of Ranomafana since the problems related to COVID.19 Among the species of Vepris identified in Ranomafana, two endemic species Vepris ampody and Vepris sp. were selected. The theme of this study is: "Valorization of aromatic and medicinal plants of Ranomafana, Ifanadiana District, Vatovavy-Fitovinany Region: the case of Vepris ampody and Vepris sp." The studies were divided into three parts.

Ethnobotanical surveys provide information on the medicinal and traditional use of plants. The ecology of the plant allows it to situate the plant in its ecosystem. As the exploitation of plants within the National Park is forbidden, the collection of raw materials will be done outside the Park. It would be necessary to increase the number of feet. The ecological parameters of the plant would allow us to know the conditions of cultivation of the plant. Chemical and biotechnological studies include the identification of essential oil components, isolation of mycoendophytes, and identification of fatty acid components of ferments.

II. Material and Methods

2.1 Ethnobotanical surveys

In order to valorize the studied species, surveys were conducted to know their therapeutic uses and their importance. The surveys were carried out in the form of closed, semi-open or open questions following a previously established survey form. The questionnaires were sent to members of the FIMARA association (Razanaka, 1995).

2.2 Plant ecologies

The study of the habitat of the plants is essential for the valuation of a plant. It is a question of determining the favorable or unfavorable factors for the growth and development of the selected species. This consists of the study of the availability of raw material in case of exploitation and valorization of the species.

For the ecological studies, the site of the collection is Talatakely. Measurements of ecological parameters (Diameter at Breast Height, Total Height, Height of the Fut) were carried out during the cold season of 2018. The methods used were taken from the phytosociological survey methods described by Braun-Blanquet (Braun-Blanket, 1975). This section aims to describe and understand the current ecological status of the target species.

Thus, the strategies and action plans of the stakeholders in the sector should consider these factors as a priority in order to guarantee better performance and sustainability of reforestation regarding the variables (Eröling, 2014)..

2.3 Estimating the Abundance of each Species in a Sub-population

Adult individuals within the plots were counted. The total number of mature individuals in a population is estimated. From the estimated abundance in a subpopulation and the total number of subpopulations, the relative abundance in a subpopulation is given by the formula:

N = A x D

N number of mature individuals in a subpopulation, A: area suitable for the species estimated from the survey plots,
D: density (total number of individuals per unit area) in the subpopulation.

2.4 Study of Natural Regeneration

The natural regeneration is studied by calculating the ratio between the number of regenerated individuals (diameter < 5cm) and those of the seedlings (diameter > 5 cm). This ratio expresses the regeneration rate of the species. An individual is a seeder when he is capable of ensuring the survival of the present and future generations. In addition, any individual with a DBH ≥ 10cm is considered a seedling, a non-seedling individual has a DBH ≤ 3cm, and an individual at a height < 1.5m belongs to seedlings and young individuals in the regeneration stage. The regeneration rate is calculated by

TR= IR/IS ×100
IR : number of regenerated individuals
IS : number of seed individuals
TR : regeneration rate
Si TR < 100 : low regeneration rate
Si 100 < TR < 1 000 : average regeneration rate
Si TR > 1 000 : high regeneration potential
(Razanaka, 1995).

2.5 Preparation of Plant Extracts

The leaves of the species Vepris ampody and Vepris sp. of the family RUTACEAE were collected outside Ranomafana National Park by members of the association FIMARA in the villages Vohiparara, Torotosy, Mangevo. The dried leaf samples were finely ground. Then, 10 mg of leaves of RUTACEAE species were soaked in 50 ml of organic solvent: methanol, dichloromethane, hexane, and acetone, separately. The extracts were filtered through a Whatman paper. The filtered extracts are dried. The concentrated extracts are put in test tubes. Phytochemical screenings following Fong et al. were performed on both extracts. The various phytochemical screening tests revealed the chemical families in the leaves (Thilagavathi, 2015b).

2.6 Extraction of the Essential Oil by Hydrodistillation

The fresh leaves are crushed. They are placed in a flask with water and a few pieces of a pumice stone to ensure the mixing of the solution. The water brought to a boil carries with it the aromatic molecules. When passing through a cooler, the vapors are condensed and the essential oil separates by density difference. After letting the contents rest for a few seconds, it is possible to completely eliminate the aromatic water. The apparatus used is of the Clevenger type (Thilagavathi, 2015a).

2.7 Isolation and Fermentation of Endophytes

The collection sites are outside the Ranomafanad National Park in the villages of Vohiparara, Torotosy, and Mangevo. The materials used are pruning shears, and cooler containing ice packs. Alcohol 70° is used to clean the secateurs.

The old organs are privileged. For this purpose, branches of healthy and mature appearance are collected. The sectioned samples are put in autoclavable bags and stored in a cooler. They are transported to the Laboratory of Molecular Biology, University of Fianarantsoa where they are put in culture within 24 hours.

2.8 Surface Sterilization and Culture Protocol

The type of sterilization used is surface sterilization. The parts put under culture (leaves, roots, bark) are rinsed under tap water for about ten minutes to get rid of impurities and surface debris. Then they are immersed in 70% ethanol for 1 minute, then in sodium hypochlorite (NaOCl) (3%) for 4 minutes. In a sterile medium, the cultured parts are then put back in 70% ethanol for 30 seconds. They are rinsed three times with sterile bidistilled water for 1 minute each time and dried on sterile filter paper (Zareb, 2014).

2.9 Microdissection

One leaf is segmented horizontally into three. Three Petri dishes are provided for the three segments. For each segment, fragments of about 5mm*5mm were cultured. For the other parts, sections of 5mm*5mm are taken (Zerroug, 2011).

2.10 Culture and Incubation

The culture media usually used for fungi are favorable for the isolation of endophytic fungi and for subculturing toward identification. The most commonly used is agar extract. Elimination of bacteria by antibiotics is necessary for some host tissues. Often, fast-growing fungi complicate the development of slow-growing fungi (Parra, 2005).

In this study, the culture medium is semi-solid called PotatoCarrot Agar (PCA). The antibiotics used are amoxicillin, cyclosporin, and gentamycin. In order to avoid bacterial growth, Petri dishes containing agar medium are autoclaved at 121°C for 15 minutes, and aseptically supplemented with 150 mg/l of three antibiotics (amoxicillin, cyclosporin, gentamycin) to inhibit bacterial growth. Host tissue segments are placed on the agar medium surface in serial order so that the effects of position and distribution can be determined. Sections placed in each plate are 6 to 8 segments per plat and are incubated at 25°C for hyphal development (Papagianni, 2004).

L'effet de la température d'incubation, le cycle de lumière sur l'émergence des endophytes sont inconnus. Cependant ces facteurs peuvent influencer la sporulation et les caractères utilisés pour différencier les espèces. Les conditions optimales d'incubation varient en fonction de l'origine des tissus hôtes. Les températures d'incubations doivent refléter les conditions naturelles. Habituellement elle varie de 18 à 25°C (Strőbel, 2003).

2.11 Purification, Isolation of Endophytes

Pure cultures are obtained by transferring tips of the hyphae into new Petri dishes with a semi-solid Potato Carrot Agar PCA culture medium. All isolates are incubated at 25°C to induce sporulation of the isolates and periodically examined.

Isolated strains are periodically examined and identified on the basis of morphology and structural characteristics (i.e., colony appearance). Colony descriptions are made macroscopically by observation of colony characteristics such as growth rate, color, and colony morphology. Colonies with the same morphologies are collected. Pure strains are then preserved in deionized water at -20°C before fermentation (Zareb, 2014).

2.12 Fermentation and Extraction of Fermentation Products

Fermentation is done in two steps. The fungi are grown on a liquid culture medium called Potato Dextrose Broth (PDB) at 25°C for five days. Two to three pieces (0.5 cm x 0.5 cm) of each strain are inoculated into 500 ml Erlenmeyer flasks containing 300 ml of PDB (potato dextrose broth) and the operation is repeated twice for strain enrichment. Pellets are formed. They are then incubated in solid media composed of barley, wheat, and minerals MnCl2 and

peptone. This change of medium stimulates the stress of the strains and favors the production of secondary metabolites.

The cultures are then incubated at 25°C for three to five weeks. In the end, the content is extracted by adding solvent to the ferments. The extraction is done cold by maceration with a mixture of dichloromethane and methanol (3:1, v/v). After evaporation to dryness, the extract is obtained.

2.13 Separation and Identification of Fatty Acids by GPC

Fatty acid samples were methylated with ethanolic potassium hydroxide solution to make them volatile. The analyses were performed with the laboratory Santé, Analyse, Innovation (SAI) Chimie, Aix-en-Provence, France.

For the identification of methyl esters, the analyses are performed with a control mixture of fatty acids for peak identification, esterification, TGWAX 60m column, ID: 0.32mm. Program: 180° at 230°C (2°/mn), inj: 0.3µl. The calculation of Equivalent Chain Length confirms the proposed identification (Velosaotsy, 2005).

2.14 Separation of Essential Oils by GPC

The analysis was done at the Institut Malgache de Recherches Appliqués (IMRA), Department of Phytochemistry and Quality Control - Laboratory of Quality Control and Standardization of Drugs. The identification of the different constituents of essential oils is done on the basis of their retention indices. For the analyses carried out at IMRA, the operating conditions are the following: GC: Trace 1300, automatic injection AI 1310, column UBWAX (30m*0.32mm*0.5µm), oven: 50°C to 250°C (5°C/mn); detector: FID; carrier gas: hydrogen at 0.50Bar; split mode injection; integration: percentage of threshold area: 0.01% (Rabehaja, 2013).

III. Research Methods

3.1 Vepris Ampody

In the case of Vepris ampody, the leaves were studied.

3.2 Ethnobotanical Uses

The bark is used for the fermentation of 'toakagasy' which is used in circumcision rites as a euphoriant, aphrodisiac, and stimulant. The decoction of the leaves and bark is used as a tonic in case of fever. They can also relieve symptoms of malaria, fatigue, and uscle pain.

Les feuilles sont utilisées pour soigner les plaies, blessures et brûlures. Les feuilles fraiches sont pilées puis mises sur les plaies pendant trois jours. La décoction des feuilles est indiquée contre la diarrhée.

3.3 Ecological Parameters

For the inventories, the survey site is one of the sites within Ranomafana National Park named Talatakely. Three survey plots were chosen.

Table 1. Geographical coordinates of the Talatakely plots

Plot	Longitude EAST	Latitude SOUTH	Altitude (m)	Distance% to RN 25 (m)
P1	045°25'22.4"	21°15'50.7"	1020	1015
P2	045°25'20.1"	12°15'50.2"	1011	970
P3	043°25'24.3"	21°15'49.2"	980	920

According to the inventories, 30 species have been identified, and 24 are endemic. The most numerous families are LAURACEAE and CLUSIACEAE. The most encountered genus are *Cryptocarya* and *Weinmania*. In the survey site, Talatakely, 19 plants were found. The plants could be divided into three classes according to their total height and diameter at chest height. The distribution is as follows:

Class 1: HT [9;11[, DBH [6; 11[
Class 2: HT[11;15[, DBH[5;20[
Class 3: HT [15;19[, DBH [5;20[

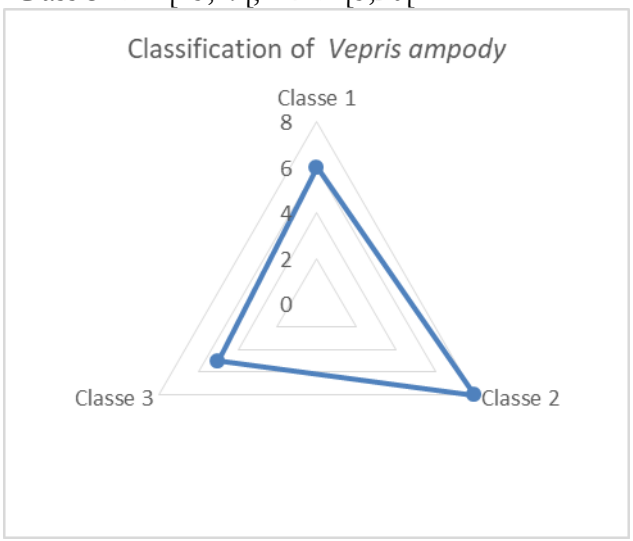

Figure 1. *The three classes of Vepris ampody on the Talatakely site*

Class 1 includes six (6) individuals. Class 2 includes eight (8) individuals and class 3 includes five (5) individuals.

Among the 19 plants recorded, 13 plants are seedlings, 06 plants are adults. No seedlings were found. Most of the plants were found on the upper slopes. Relative abundance, regeneration rate and density on the three plots were evaluated.

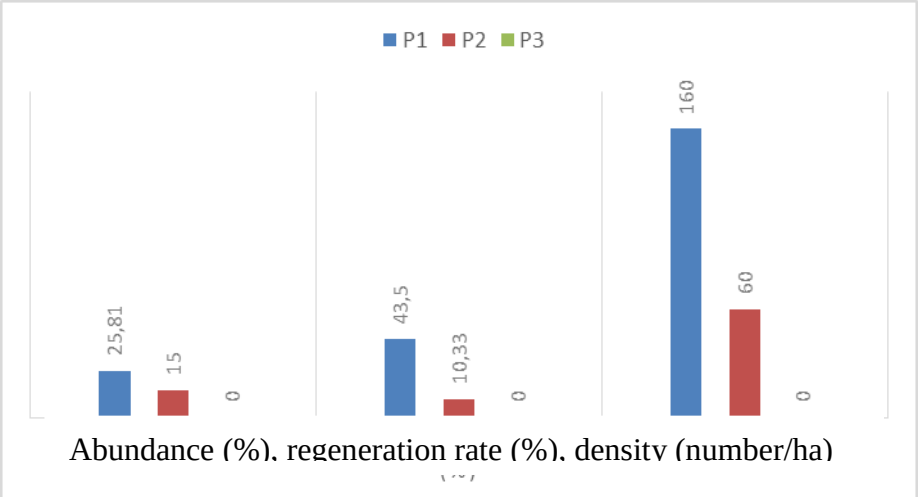

Figure 2. *Ecological parameters of Veprisampody at the Talatakely site*

The feet are abundant on Placeau 2 which is at an altitude of 1020m.

3.4 Chemical and Pharmacological Properties of Leaves

The chemical families in the leaves have a relationship with these properties. They have been evaluated according to the abundance of their reactions.

Table 2. Chemical Families in Leaves

Chemical families	Observation	Interpretation
Alkaloïd	+++	Very abundant
Polysaccharide	++	Abundant
Flavone	++	Abundant
Saponin	+	Low

Alkaloids are abundant in the leaves of *Vepris ampody*.

Table 3. Physicochemical Characteristics of the Essential Oil

Color	Odor	Viscosity	Yield (%)	Density (g/ml)	Type of oil
Brown	Pungent	Low viscous	0.44	0.87	Light oil

The essential oil obtained is then analyzed by gas chromatography. This analysis allowed knowing the components and their content.

Table 4. Constituents of the Essential Oil of Leaves

Constituents	Content
α-pinene	4.3
β-pinene	0.2
Sabinène	39.8
γ-terpinene	2.2
Terpinolene	4.3
Linalol	2.6
Terpinen-4-ol	2.7
Elemol	2.9
Unidentified	9.6
Unidentified	4.5

The essential oil is **chemotypesabinene, monoterpene-bicyclic**.

Several parts of the plant were put on agar culture: leaf, root, and stem. Cultures of mycoendophyte strains were performed on leaves, barks and flowering tops of *Vepris ampody*. The strains were isolated from the leaves. No strains were isolated from bark and roots.

Table 5. Isolated strains from *Vepris ampody*

Code	Part used	Evaluation of growth (mm/day)	Color	Edge	Density of micelles	Air micelle	Form	Elevation
Va L1	leaf	2,5	top: white back: pink	serrated	little	compact	circular	plane
VaL2	leaf	2,5	grey	entire	compact	little	circular	plane
VaL3	leaf	10	grey	filamentous	compact	compact	circular	high
VaL7(D)	leaf	10	white	filamentous	medium	medium	circular	plane
VaL7	leaf	10	white	filamentous	little	little	circular	plane
VaL9	leaf	10	white inside with green edge and green spore	filamentous	medium	little	circular	plane

The isolated strains, VaL1, VaL7, VaL7(D), VaL9, VaL4 were fermented. From the fermented solutions, the fatty acid compositions were evaluated.

The fatty acids isolated from VaL9 are rich in methyl palmitate.

Table 6. Fatty acids identified from VaL9 fermentation extract

N°peak	T_R (mn)	LCE	Content	Allocation	Usual nomenclature	Official IUPAC nomenclature
1	5.05	15.972	17.8	C16 :0	Palmitate	Methyl Hexanoate
2			<0.1	C16 :1	Palmitoleate	Methyl Hexadecenoate
3	8.09	18.031	1.5	C18 : 0	Stearate	Methyl Octadecanoate
4	8.54	18.268	17.5	C18 :1	Oleate	Methyl Octadecenoate
5	9.57	18.765	56.0	C18 :2	Linoleate	Methyl Octadecadienoate
6	1.97	19.362	5.0	C18 :3	Linolenate	Methyl Octadecatrienoate
7	12.61	19.970	1.0	C20 :0	Arachidate	Methyl Eicosanoate
8	13.13	20.147	1,3	C20 :1	Eicosenoate	Methyl Eicosenoate
9			<0.1	C22 :0	Behenate	Methyl Docosanoate

The majority product is methyl oleate. The fatty acids contained in the VaL9 fermentation extract were categorized as.

Table 7. Classification of fatty acids of VaL9

Type of fatty acid	Content (%)
Long-chain saturated fatty acids (SFA)	20.3
Monounsaturated fatty acids (MUFA)	18.8
Polyunsaturated fatty acids (PUFA)	61.0

The oil is rich in polyunsaturated fatty acids. However, the content of saturated fatty acids is high. The fatty acid composition of the VaL7 extract was evaluated.

Table 8. Identified fatty acids of the fermentation extract VaL7

N°peak	T_R (mn)	LCE	Content	Allocation	Usual nomenclature	Official IUPAC nomenclature
1	5.03	15.881	21.3	C16 :0	palmitate	Methyl Hexanoate
2	5.37	16.184	18.0	C16 :1	palmitoleate	Methyl Hexadecenoate
3	8.10	18.089	9.0	C18 : 0	stearate	Methyl Octadecanoate
4	8.53	18.329	25.8	C18 :1	oleate	Methyl Octadecenoate
5	9.50	18.828	16.2	C18 :2	linoleate	Methyl Octadecadienoate
6	10.93	19.478	0.6	C18 :3	linolenate	Methyl Octadecatrienoate
7	12.58	20.129	0.1	C20 :0	arachidate	Methyl Eicosanoate
8	13.16	20.338	7.4	C20 :1	eicosenoate	Methyl Eicosenoate
9	18.29	21.864	0.7	C22 :0	behenate	Methyl Docosanoate

The majority product is methyl linoleate. The identified fatty acids of VaL7 were categorized as.

Table 9. Classification of fatty acids of VaL7

Type of fatty acid	Content (%)
Long-chain saturated fatty acids (SFA)	30.91
Monounsaturated fatty acids (MUFA)	51.20
Polyunsaturated fatty acids (PUFA)	17.16

The oil is rich in monounsaturated fatty acids. However, the monounsaturated fatty acid content is very high. The fatty acid composition of the fermented VaL1 extract was evaluated.

Table 10. Identified fatty acids of the Val1 fermentation extract

N°peak	T_R (mn)	LCE	Content	Allocation	Usual nomenclature	Official IUPAC nomenclature
1	4.96	15.982	14.50	C16 :0	palmitate	Methyl Hexanoate
2			<0.10	C16 :1	palmitoleate	Methyl Hexadecenoate
3	7.98	18.052	3.00	C18 : 0	stearate	Methyl Octadecanoate
4	8.45	18.301	37.30	C18 :1	oleate	Methyl Octadecenoate
5	9.42	18.774	41.70	C18 :2	linoleate	Methyl Octadecadienoate

6	10.82	19.377	2.60	C18 :3	linolenate	Methyl Octadecatrienoate
7	12.44	19.984	0.33	C20 :0	arachidate	Methyl Eicosanoate
8	12.97	20.165	0.67	C20 :1	eicosenoate	Methyl Eicosenoate
9			<0.10	C22 :0	behenate	Methyl Docosanoate

The majority product is methyl linoleate. The fatty acids of the VaL1 fermentation extract are categorized as.

Table 11. Classification of fatty acids of VaL1

Type of fatty acids	Teneur(%)
Long-chain saturated fatty acids (SFA)	17.83
Monounsaturated fatty acids (MUFA))	37.97
Polyunsaturated fatty acids (PUFA)	44.30

The oil is rich in polyunsaturated fatty acids. However, the unsaturated fatty acid content is very high. The fatty acid composition of the VaL7(D) extract is presented.

Table 12. Fatty acids identified from VaL7D fermentation extract

N°peak	T_R (mn)	LCE	Content	Allocation	Usual nomenclature	Official IUPAC nomenclature
1	5.05	15.972	30.00	C16 :0	palmitate	Methyl Hexanoate
2			3.10	C16 :1	palmitoleate	Methyl Hexadecenoate
3	8.09	18.031	10.40	C18 : 0	stearate	Methyl Octadecanoate
4	8.54	18.268	23.30	C18 :1	oleate	Methyl Octadecenoate
5	9.57	18.765	28.40	C18 :2	linoleate	Methyl Octadecadienoate
6	10.97	19.362	1.80	C18 :3	linolenate	Methyl Octadecatrienoate
7	12.61	19.970	0.79	C20 :0	arachidate	Methyl Eicosanoate
8	13.13	20.147	1.10	C20 :1	eicosenoate	Methyl Eicosenoate
9			1.00	C22 :0	behenate	Methyl Docosanoate

The main product is palmitate. It is the only ferment rich in palmitate, a saturated fatty acid. The identified fatty acids have been categorized.

Table 13. Classification of fatty acids of VaL7(D)

Type of fatty acids	Teneur(%)
Long-chain saturated fatty acids (SFA)	42.19
Monounsaturated fatty acids (MUFA))	27.50
Polyunsaturated fatty acids (PUFA)	30.20

The endophytes isolated from *Vepris ampody* produce, in the majority, polyunsaturated fatty acids.

***Vepris sp.* (Mahavalia)**

The species studied has not yet been described. Its vernacular name is "mahavalia". The leaves have been studied.

3.5 Ethnobotanical uses

The plant is used by the *'ombiasa'* to protect themselves against witchcraft and evil spells. Etymologically, the term means, the action of returning the favor, to avenge an insult, an affront thus who can return the favor. This name is given to several plants of which one drinks the infusion when one was the victim of an affront by pronouncing imprecations against the author of the insult. This practice is supposed to turn the spell against him.

3.6 Ecological parameters

For the inventories, the survey site is one of the sites within Ranomafana National Park named Talatakely. The table represents the coordinates of the three survey plots.

Table 14. Plot Coordinates

Plot	Longitude EAST	Latitude SOUTH	Altitude (m)	Distance (m) % to the national road
P1	047°25'24.4"	21°15'52.2"	965	700
P2	047°25'27.5"	21°25'27.5"	1016	1000
P3	047°25'29.5"	21°25'29.5"	1020	1200

On the survey site, 39 species were recorded, and 35 species are endemic including 36 genera and 25 families. The most frequently encountered families are LAURACEAE, RUBIACEAE, and MYRTACEAE. The most common genera are *Cryptocaria* and *Weinmania.*

On the survey site, twelve (12) individuals were counted. They can be divided into 3 classes according to the total height (HT) and the diameter at breast height (DBH):

Class 1: HT [6 ; 8[, DBH [5; 11[

Class 2: HT [8; 10[, DBH [6; 10[

Class 3: HT [10;15[, DBH [6;113]

Class 1 includes 2 individuals; class 2 includes 5 individuals and class 3, 5 individuals

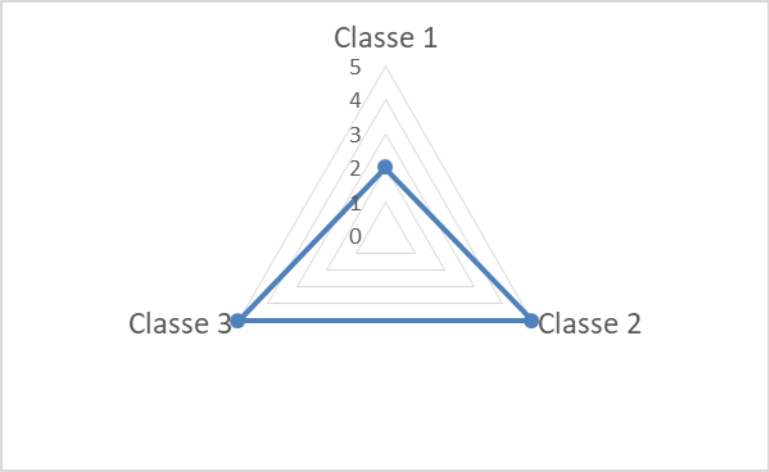

Figure 3. *The three classes of Vepris sp. at the Talatakely site*

Twelve plants were collected at the survey site. Feet are most abundant on the lower slope. Individuals with a total height of more than 8m and a diameter at breast height of more than 6cm are more abundant.

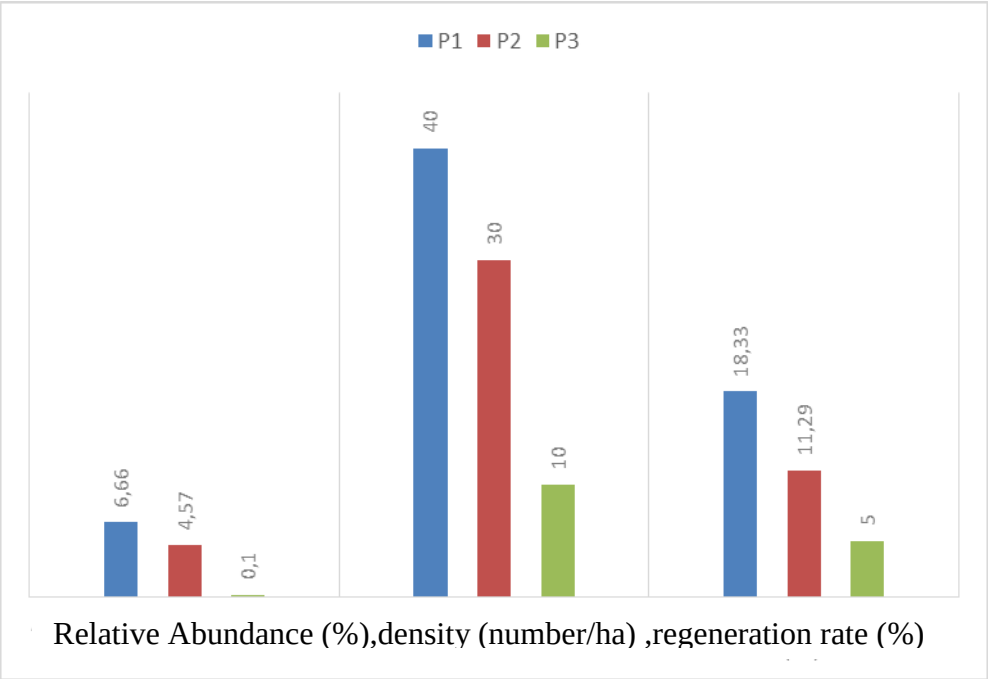

Figure 4. *Ecological parameters of Vepri ssp. at the Talatakely site*

The feet develop better at an altitude of 965m, at low slope. In Ranomafana, this species can develop at high, as well as at low altitude. On the other hand, the feet develop better near the water points and at low altitude.

3.7 Chemical families in leaves

The chemical families in the leaves are not related to traditional uses. The abundance of chemical families in the leaves was appreciated.

Table 15. Chemical families in leaves

Chemical families	Observation	Interpretation
Alkaloïd	+++	Very abundant
Polysaccharide	++	Abundant
Flavone	++	Abundant
Saponin	+	Low

As in the case of Vepris ampody, the alkaloid family is abundant.

3.8 Physicochemical properties of the essential oil of the leaves

The extraction of essential oil from the leaves was carried out during the dry and cold period. The properties of the essential oil were estimated.

Table 16. Organoleptic and physical properties of the essential oil

Properties	Color	Odor	Yield (%)	Density (g/ml)	Type of oil
Mahavalia EO	Brown	Lemon	0.41	0.83	Light oil

The smell of the oil is quite similar to that of Citrus aurantium. The chemical composition of the essential oil was acquired after analysis in gas chromatography.

Table 17. Chemical composition of the essential oil of petitgrainVepris sp

Constituents	Content (%)
α-Pinene	2.5
β-Pinene	2.2
Sabinene	4.1
Myrcene	1.2
Camphene	2.3
β-Phelandrene	2.1
Limonene	12.1
Linalool	36.2
(Z)-anethole	2.2
(E)-anethole	2.82

The essential oil of Vepris sp. leaf has a "linalool" chemotype.

3.9 Discussion

Based on the results of the phytochemical screenings, the identified chemical families of the two RUTACEAE species are similar. The traditional use of Vepris ampody is justified by the chemical families present. However, in the case of Vepris sp. the use is not related to the chemical families present.

The ecology of the two species is different, Vepris ampody grows better on upper slopes while Vepris sp. grows better on lower slopes near water points.

To get an idea of the particularity of the essential oils of the species of Vepris, comparisons were made.

Table 18. Comparison of the major components of the species of genus Vepris

Composés	*V. heterophilla* (%)	*V. leandriana* (%)	*V. lanceolata* (%)	*V. madagascarica* (%)	*V. ampody* (%)	*V. sp (Mahavalia)* (%)
α-pinene	0.1	0.20	8.20	1.2	4.3	2.5
β-pinene	0.3	3.02	1.50	0.1	0.2	2.2
Sabinene	14	32.60	27.60	tr	39.8	4.1
γ-terpinene	1.3	0.80	2.60	-	2.2	-
terpinolene	1.6	-	5.30	-	4.3	-

Limonene	4.3	4.20	-	0.1	-	12.1
Linalool	0.9	8.02	3.00	0.2	2.6	36.2
E-anethole	-	20.80	-.	78.2		2.82
Z-anethole	-	14.60	-	0.5		2.2
Terpinen-4-ol	-	-	6.52	-	2.7	-
Elemol	14.37	-	4.15	-	2.9	-
β-ocimene	14	-	-	1.1		-
Guaiol	12.85	-	-	-		-
Estragol				15.6		-

Comparing the composition of Vepris essential oils, the typical components of Vepris essential oils are α-pinene, sabinene, linalool, and E-anethole. These molecules are used in aromatherapy.

The four strains of Vepris ampody produce fatty acids with different contents. The categories of fatty acids from these strains were compared.

Table 19. Comparison of fatty acid contents of Vepris ampody ferments

Type of fatty acids	**Content (%)**			
Sample	**VaL9**	**VaL1**	**VaL7**	**VaL7D**
Long-chain saturated fatty acid (SFA)	20,3	17,83	30,91	42,19
Monounsaturated fatty acid (MUFA)	18,8	37,97	51,2	27,5
Polyunsaturated fatty acid (PUFA)	61	44,3	17,16	30,2

Even when growing in the same micro-ecosystem, the strains of microfungi produce different types of fatty acids. This diversity provides the plant with protection against predators. On a large scale, these fatty acids can be used in aromatherapy or natural

IV. Conclusion

In conclusion, this study was carried out in order to propose alternatives for the conservation of the biodiversity of Ranomafana.. Two plants of the RUTACEAE family were chosen: Vepris ampody and Vepris sp. These two plants are endemic. For the valuation of these plants, information on the use of the plant was obtained by ethnobotanical surveys, the ecology of the plant was evaluated according to the phytosociological methods recommended by Braun-Blanket in order to know the conditions of the culture of the species and finally chemical studies made it possible to highlight that the alkaloids are in majority for the two species. The essential oil of Vepris ampody is chemotype sabinene and Vepris sp. is chemotype linalool. The fatty acids obtained from the extracts of Vepris ampody fermentations are quite rich in polyunsaturated fatty acids. As a prospect, the study of other

species of Vepris and the identification of alkaloids are planned as well as the setting in a nursery of the two species.

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