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Aroma characterization of a new essential oil chemotype from Vepris madagascarica (Baill.) H. Perrier RUTACEAE growing in the North Eastern coast of Madagascar

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Abstract – The aim of this work is to report the chemical composition and the aroma profile of a new chemotype of Vepris madagascarica essential oil. In-depth study of the intraspecific and interspecific variability of Madagascan Vepris spp essential oils was also carried out using multivariate analysis. Odor activity values of the compounds and their contributions to the whole essential oil smell were studied in order to determine the odor profile. For essential oil quality control purposes, a comparison with the odor profiles of two other locally growing species, with a high likelihood of use as an adulterant was also performed: Cymbopogon citratus and Cymbopogon flexuosus. While the Vepris madagascarica essential oils previously described by other authors are rich in estragole and anethole, this new chemotype contains 39.98 % of citral, 20% of citronellol and 10.81% of myrcene. It was proven in this study that intraspecific variation of the essential oil composition was more significant than interspecific variation within the Madagascan Vepris species. Two other species of Madagascan Vepris which are V. macrophylla and V. leandriana also have the chemotype citral / citronellol. The perfumery radar method has shown that Citrus is the dominant family odor for this V. madagascarica essential oil. This is a common point with the two Cymbopogon species. However, a strong floral scent distinguishes the essential oil of V. madagascarica. This different smell allows a particular criterion for the quality control of this essential oil.

Keywords— Vepris madagascarica – Cymbopogon citratus – Cymbopogon flexuosus – Odor Activity Value – Perfumery radar

I. INTRODUCTION

Madagascar is known for its exceptional biodiversity: 93% of its trees are endemic [1] and Madagascan plants exhibit a wide diversity in their natural chemical compositions. New species and new plant chemical profiles are still discovered recently. Among new findings published in the year 2021, 5 species of traveller's palm [2], 11 ebony tree species [3], 6 coffee species [4] and 5 orchids [5] were described from Madagascan flora. This richness of nature is a source of new components of industrial, commercial and scientific value [6].

Genera *Vepris* Comm. ex A. Juss. (RUTACEAE-TODDALIEAE), comprises 93 accepted species, and among them 23 are endemic to Madagascar [7]. *Vepris madagascarica* is an endemic species of the North region of the Island [8]. Formerly named *Pelea madagascarica*, this species has gained the interest of researchers since its discovery by botanists in the early 1900 because of the essence which confers its particular odor [9], [10], [11]. However, its essential oil composition shows a wide variability as reported by different authors [12]. This work also reports another new chemotype of *Vepris madagascarica* essential oil.

Intraspecific and interspecific differences between *Vepris spp*. essential oils from Madagascar are investigated using chemometric techniques in order to point out their variability and to study if any compound may be used as specific identification criteria. Correct determination of discrimination criteria allows the design of an appropriate method of quality assessment of plant extracts and essential oils [13]. Chemometric modeling of chromatographic data by using Principal Component Analysis is widely used to characterize the variability of essential oil chemotypes [14]. This method has been chosen in this study to compare essential oils from different *Vepris* species, and to evaluate the possibility of any discrimination criterion.

As products of high commercial values, essential oils require appropriate quality insurance procedures in order to prevent adulteration. Rapid detection methods of counterfeit are necessary for quality assessment. Sensory analysis is a frequently used technique of essential oil quality control [15]. For that purpose, human olfactive discrimination tests were performed between *Vepris madagascarica* essential oil and other commonly commercialized essential oils which are extracted from plants of different botanical family, but yet presenting similarity in their major components.

II. Materials and methods

2.1. Plant material

Leaves of *Vepris madagascarica* were collected in the Region of Analanjirofo, North Eastern Madagascar in September 2021, and voucher specimen is deposited in the Botanic Research Department of the Homeopharma Pharmaceutical Company, Antananarivo, Madagascar.

2.2. Essential oil isolation and physical properties

Essential oil was extracted by steam distillation of fresh plant materials using a traditional stainless steel alembic during 3 hours. Floral water was separated to the essential oil using Florentine vase. The oil yield was expressed on a fresh weight basis w/w. Extraction was repeated 5 times. Relative density was calculated using a calibrated pycnometer with distilled water at 20°C. Refraction Index was measured using an Abbe apparatus at 20°C. Miscibility with ethanol was measured as the volume of ethanol required to dissolve 1 volume of essential oil for which the mixture becomes clear. These analyses were performed in triplicate, and the mean value was considered.

2.3. Aroma characterization

2.3.1. Analytical Gas Chromatography

Analytical Gas Chromatography was performed on a SHIMADZU GC-14A apparatus equipped with a BP5 capillary column 5% Phenyl / 95% Dimethylpolysiloxan (30m x 0,32mm x 0,5µm) and a Flame Ionization Detector. The oven temperature was programmed from 60°C to 230°C at a rate of 3°C/min. Injection temperature was 250°C and detection temperature was 280°C. Carrier gas was Nitrogen at a flow rate of 3ml/min. The injected volume was 1µl. Relative percentages of the essential oil constituents were calculated according to peak area proportions. Compounds were identified by using external reference samples, by matching with laboratory computer database, and by comparing with the literature [16].

2.3.2. Chemometric modeling

Chemometric modeling was performed by using PAST 4.03 software for Multivariate Analysis. This chemotype of *Vepris madagascarica* essential oil was compared to the literature data for other essential oils of Madagascan *Vepris* species which are: an another chemotype of *V.madagascarica* essential oil [12], *V. elliotii* [17], *V. heterophylla* [18], *V. leandriana* [19], *V. unifoliolata* [20] and *V. macrophylla* [21].

2.3.3. Odor profiling

The Perfumery Radar 1.0 methodology was used to describe the odor profile [22], [23]. Essential oil components were classified into primary, secondary and tertiary olfactory families according to literature [24], [25], [26]. The Odor Activity Value

(OAV) of each compound was calculated by dividing its concentration with its sensory threshold. Quantitative analysis of components was carried out according to the method described by Costa *et al*, 2010 [27], allowing the determination of the concentration. Threshold values were obtained from the literature and from web databases [28], [29], [30] [31], [32]. Only compounds with Odor Activity Values ≥1 were taken into account. The odor value of each olfactory family was then calculated considering each compound's classification with weighing criteria as proposed by Teixeira *et al*, 2010 [22]. The odor profile for this *Vepris madagascarica* essential oil was compared to those for two essential oils having similar dominant compounds from locally growing plants: *Cymbopogon flexuosus* (Nees) Stapf POACEAE and *Cymbopogon citratus* (DC) POACEAE.

2.3.4. Sensory discrimination test

In order to allow the full capacity of human sensory organs and neurons in recognizing odors, and to avoid any bias due to the use of descriptors [33], the discrimination test was conducted on the basis of the method used by Poivret et al., 2018 [34] with modifications. The panelists were asked to discriminate samples of essential oil of *V. madagascarica* and essential oil of *Cymbopogon flexuosus* (Nees) Stapf, without making use of any verbal descriptor. However, they were free to express any spontaneous remark if they felt the need to do so. A simple question of preference between the two essential oils was also asked. The same procedure was performed for *Cymbopogon citratus* (DC) essential oil. 25 people including professional and non-professional on quality control of essential oils were chosen to perform the test. Results were computed with JASP software for descriptive analysis.

III. Results and Discussion

3.1. Physical properties

The essential oil was collected as a yellow mobile liquid. Yields ranged from 0.95% to 1.15% with an average value of 1.03%. The relative density at 20°C, d^{20}_{20} was 0.879 and the refraction index at 20°C n_D^{20} was 1.475. For 70° ethanol 5 volumes were necessary to obtain a clear solution for the dissolution of *V. madagascarica* essential oil at an ambient temperature of 25°C. For ethanol 90°, 1.5 volumes were needed.

3.2. Essential oil composition

97% of the essential oil constituents were identified. The two isomers of citral constitute the main compounds of the essential oil with relative percentages of 17.37% for neral and 22.61% for geranial. Citronellol has a relative percentage of 20%. Hydrocarbon monoterpenes are 13.71% and oxygenated monoterpenes are 83.29%. Details are shown in table 1.

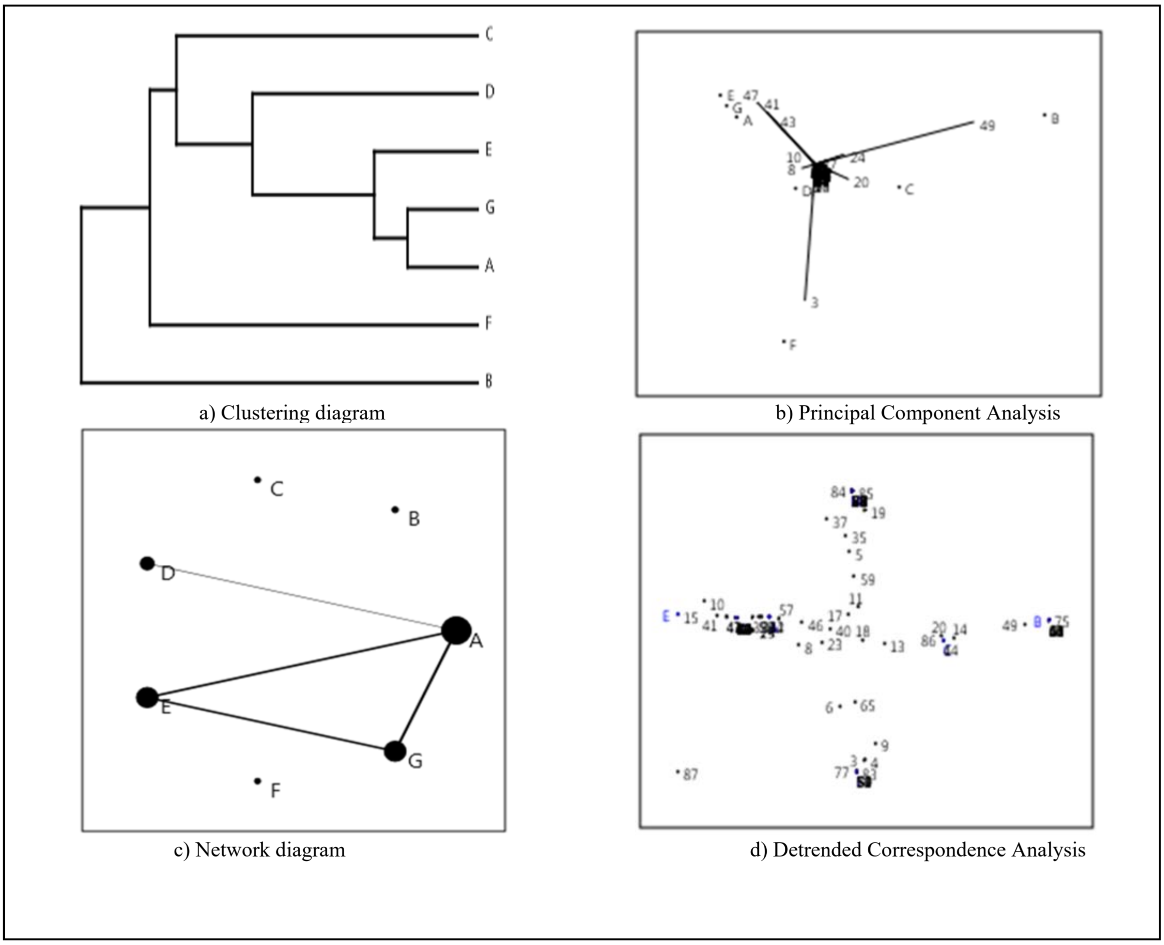
TABLE I. COMPOSITION OF *Vepris madagascarica* ESSENTIAL OIL

Compounds	Retention Index	% Area	Response Factor	Concentration %
Sabinene	975	0.55	1	0.44
Beta-Pinene	977	0.73	1	0.58
Myrcene	989	10.81	1	8.63
Limonene	1029	0.97	1	0.77
Z-Beta-ocimene	1039	0.65	1	0.52
Linalool	1100	1.68	1.3	1.74
Trans pinocarveol	1141	0.47	1.3	0.49
Citronellal	1154	0.99	1.3	1.03
Pinocarvone	1161	0.67	1.3	0.69
Delta-Terpineol	1165	0.89	1.3	0.92

Terpinen-4-ol	1177	0.55	1.3	0.57
Citronellol	1228	20.00	1.3	20.76
Nerol	1229	6.19	1.3	6.42
Neral	1242	17.37	1.3	18.03
Geraniol	1254	0.98	1.3	1.02
Geranial	1270	22.61	1.3	23.47
Citronellyl acetate	1352	3.62	1.6	4.62
Neryl acetate	1362	4.27	1.6	5.45
Geranyl acetate	1379	3.00	1.6	3.83
TOTAL		97.00		100

3.3. Chemometric modeling

The 7 madagascan *Vepris spp* essential oils show 5 different chemotypes. For the first group citral and citronellol are the characteristic compounds. This *V. madagascarica* essential oil chemotype belongs to this group, together with *V. macrophylla* and *V. leandriana*. Clustering analysis shows that *V. macrophylla* essential oil is more closely related to V. madagascarica. For *V. leandriana*, citronellyl acetate, neryl acetate and geranyl acetate are absent. The network diagram (figure 1) shows that there is no correlation at all between the two *V. madagascarica* essential oils. The other *V. madagascarica* essential oil which was previously studied by RABEHAJA et al., 2013 [12] is characterized by E-Anethole. This confirms the wide variation of the essential oil chemotypes of this species that was previously described by this author. Here it can be said that intraspecific variation of the essential oil compositions is more significant than the interspecific difference. The 3 other *Vepris* essential oils have distinct chemotypes with terpinolene and E-Anethole as characteristic compounds for *V. elliotii*, Alpha-pinene and Alpha phellandrene for *V. unifoliolata*, sabinene, geijerene and elemol for *V. heterophylla*.



a) Clustering diagram
b) Principal Component Analysis
c) Network diagram
d) Detrended Correspondence Analysis

A: V. *madagascarica* new chemotype
B: V. *madagascarica* [17]
C: *V. elliotii* [18]
D: *V. heterophylla* [19]
E: *V. leandriana* [20]
F: *V. unifoliolata* [21]
G: *V.* macrophylla [22]

3: Alpha pinene
5: Sabinene
8: Myrcene
9: Alpha phellandrene
10: Alpha terpinene
20: Terpinolene
24: Estragole

26: Geijerene
41: Citronellol
43: Neral
46: Geraniol
47: Geranial
49: E-Anethole
56: Citronellyl acetate

57: Neryl acetate
58: Geranyl acetate
59: Beta elemene
74: Elemol
77: Germacrene-D-4-ol

Fig. 1. Chemometric modeling of the Madagascan *Vepris spp* essential oils

3.4. Odor profiling

Analysis of the Odor Activity Values (OAV) of the components of this new chemotype of *Vepris madagascarica* essential oil is shown in table 2. Geranial and neral are the compounds attributing the main odor of this essential oil. Citronellol and myrcene also bring floral note for its smell. The OAV for the different family odors corresponding to this *Vepris madagascarica* essential oil were compared to those for the two *Cymbopogon* essential oils *C. citratus* and *C. flexuosus*. The Perfumery radars for the 3 essential oils show a common dominant odor of Citrus (figure 2). This odor is the mostly pronounced for *Cymbopogon flexuosus* essential oil. Further analysis of the three major dominant family odors Citrus, Floral and Green shows that the two *Cymbopogon spp* essential oils have a more pronounced Green note (figure 3). This new chemotype of *Vepris madagascarica* essential oil is distinguishable by a pronounced floral note. Detrended correspondence analysis of the compositions shows that the existence of citronellol, citronellyl acetate, neryl acetate, and to a lesser extent, geranyl acetate, would contribute to this singularity of the *V. madagascarica* essential oil (figure 4). The odor activity values of the components of the two essential oils of *Cymbopogon spp* are presented in table 3.

TABLE II. ODOR ACTIVITY VALUES OF *Vepris madagascarica* ESSENTIAL OIL COMPONENTS AND CLASSIFICATION TO OLFACTORY FAMILIES

Compounds	**OTV [ppm]**	**OAV per Compound**	**Olfactory families**					
			Primary		***Secondary***		***Tertiary***	
			Family 1	***OAV per Family***	***Family 2***	***OAV per Family***	***Family 3***	***OAV per Family***
Myrcene	0.100	86.30	Floral	86.30				
Limonene	0.200	3.87	Citrus	3.87				
Z-beta-ocimene	0.030	15.26	Herbaceous	15.25				
Linalool	0.0074	235.63	Floral	164.94	Citrus	70.69		
Citronellal	0.030	34.25	Citrus	34.24				
Citronellol	0.040	518.95	Floral	518.95				
Nerol	0.300	21.42	Floral	14.99	Citrus	6.42		
Neral	0.030	600.94	Citrus	420.66	Green	180.28		
Geraniol	0.040	25.43	Floral	24.43				
Geranial	0.032	733.33	Citrus	733.34				
Citronellyl acetate	1.000	4.62	Citrus	2.77	Fruity	1.39	Floral	0.46
Neryl acetate	1.205	4.53	Floral	4.53				
Geranyl acetate	0.150	25.55	Floral	17.88	Herbaceous	7.66		

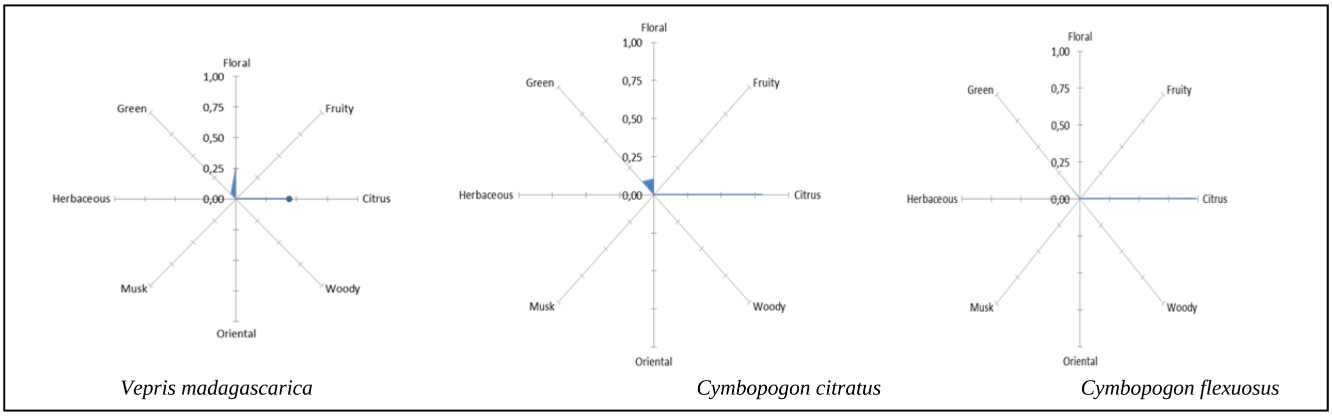


Fig. 2. Perfumery radars of the three essential oils having the same major components

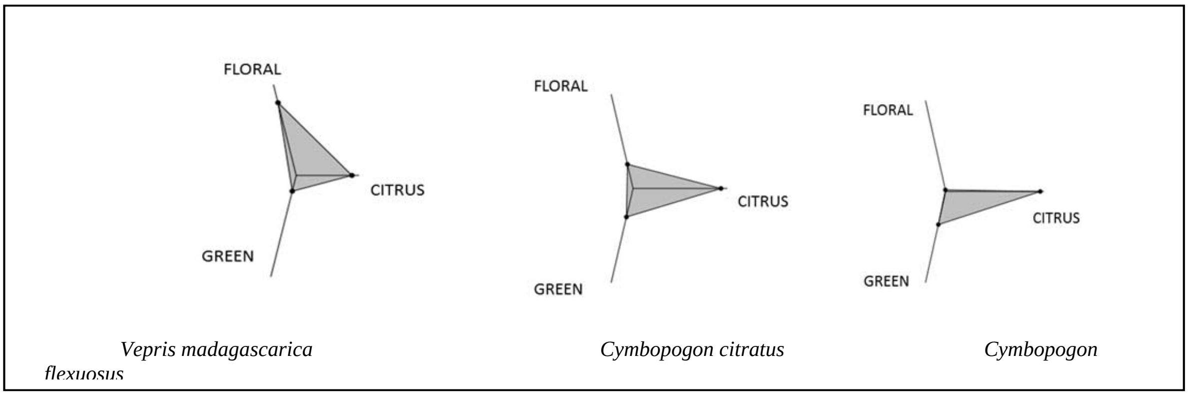
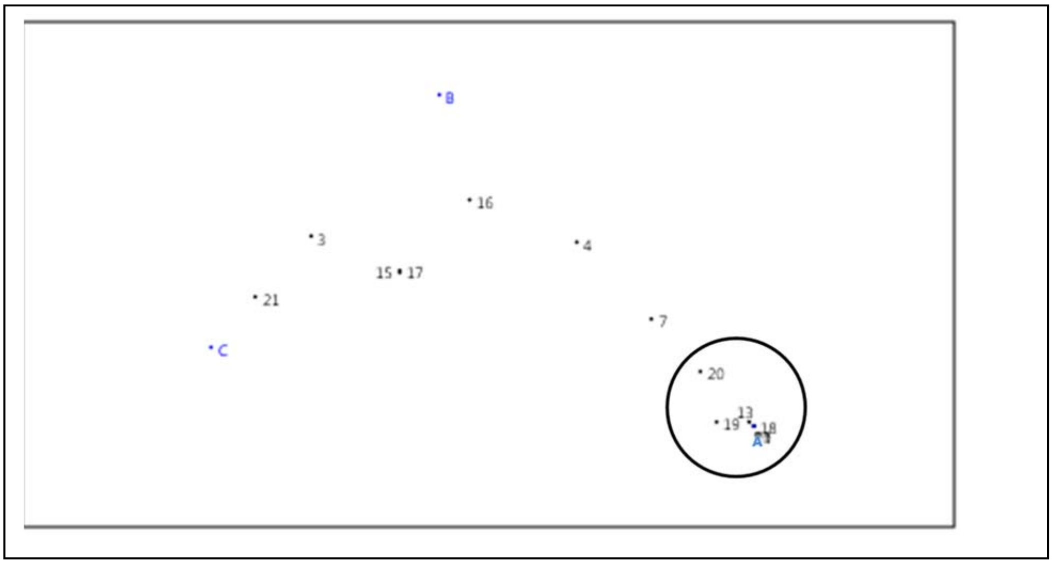


Fig. 3. Analysis of the dominant family odors



A: *Vepris madagascarica* new chemotype

B: *Cymbopogon citratus*

C: *Cymbopogon flexuosus*

3: 6-Methylhept-5-en-2-one

4: Myrcene

7: Linalool

13: Citronellol

15: Neral

16: Geraniol

17: Geranial

18: Citronellyl acetate

19: Neryl acetate

20: Geranyl acetate

21: Beta caryophyllene

Fig. 4. Detrended correspondence analysis of the 3 essential oil compositions

TABLE III. ODOR ACTIVITY VALUES OF THE COMPONENTS OF THE TWO ESSENTIAL OILS OF *Cymbopogon spp*

Compounds	***Cymbopogon citratus***			***Cymbopogon flexuosus***		
	Concentration %	***OTV [ppm]***	***OAV***	***Concentration %***	***OTV [ppm]***	***OAV***
Myrcene	11.64	0.1	116.41	0.08	0.1	0.84
6-methyl-5-hepten-2-one	0.88	0.16	5.523	1.094	0.16	6.84
Linalool	0.88	0.0074	119.42			
Beta caryophyllene	0.17	0.15	1.13	0.67	0.15	4.49
Citronellol	0.33	0.04	8.28			
Neral	34.68	0.03	1156.14	41.70	0.03	1390.08
Geraniol	3.42	0.04	85.61	0.77	0.04	19.15
Geranial	47.17	0.032	1473.945	55.27	0.032	1727.34
Neryl acetate				0.40	0.15	2.69
Geranyl acetate	0.82	0.15	5.44			

3.5. Sensory discrimination test

More than 70% of the panelists could discriminate the odor of *Vepris madagascarica* essential oil from those of the two *Cymbopogon* species as shown in the pie chart of the figure 5. The *C. citratus* essential oil smell is the mostly preferred, followed by *V. madagascarica* and finally *C. flexuosus*. Spontaneous explanations from the panelists describe a high intensity for the smell of *C. flexuosus* essential oil, which would make of it, the least preferred one. This description corresponds to the values of the odor activity of the Citrus note. For *C. flexuosus*, it is 2 706, which was considered as too intense. For *C. citratus* and *V. madagascarica*, the values are 2 321 and 1 272 respectively. It can be inferred that the panelists are sensitive to the Citrus note, and that they refer to the dominant odor to state their preferences even though the question of odor intensity was not at all the subject of this study. In other words, odor intensity can be considered to some extent, as one of the criteria that could influence the subjects 'preferences about odor. The professionals of essential oil quality control affirmed recognizing a floral note for the *V. madagascarica* essential oil, even if they were neither asked to give any description nor trained with the odor profile of this essential oil. All these data indicate that there is a possibility to detect an adulteration by the two easily affordable *Cymbopogon* *spp* essential oils using sensory criteria for quality control.

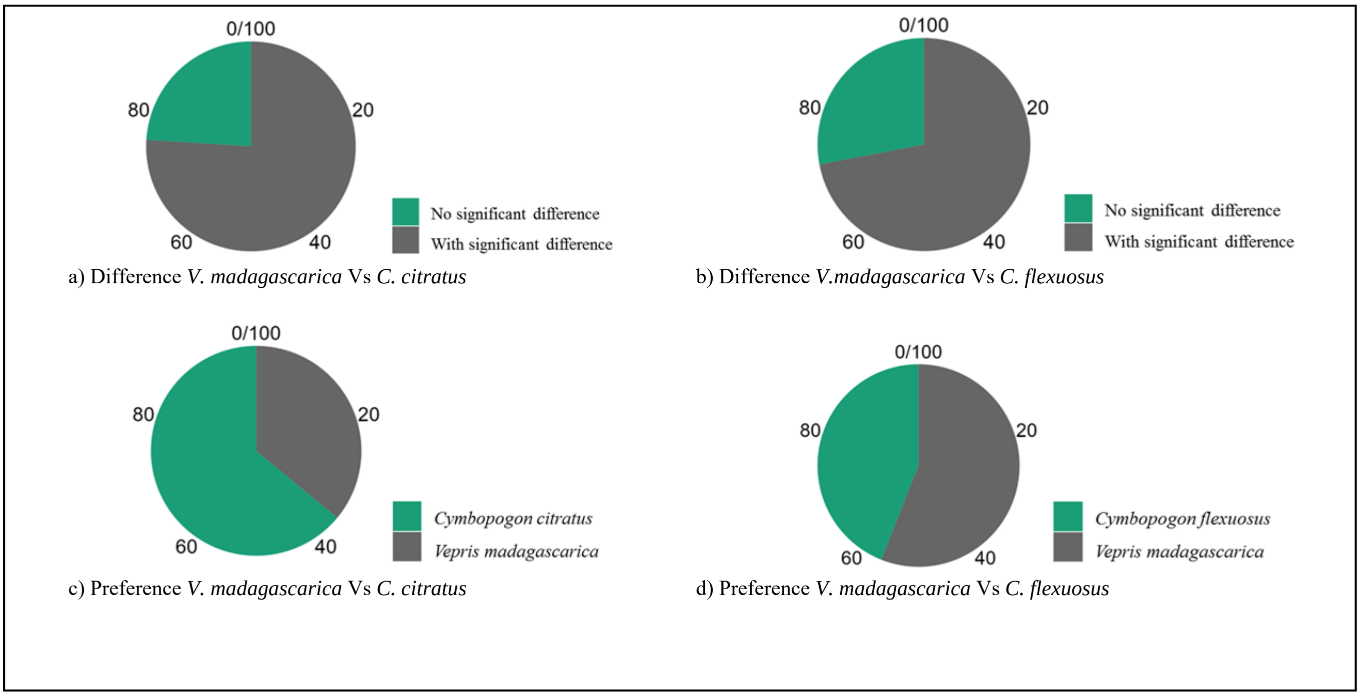


Fig. 5. Sensory discrimination test for the three essential oils.

3.6. Discussion

The dominance of Floral and Citrus smells would make of this new essential chemotype of *V. madagascarica* a suitable ingredient for feminine as well as for unisex perfume, if referred to the analysis made by Teixeira et al, 2014 [35]; whereas the two *Cymbopogon spp* species have a greater tendency for unisex perfume mainly.

Citral, the major compound of this essential oil has a good antinociceptive activity [36], [37]. It is effective for acute as well as for chronic pain [38]. Citronellol also has a pain relieving property [39]. Myrcene synergizes the activities of the other terpenes, enhances their absorption and increases their transport to the brain. So myrcene, is not only analgesic, but it also intensifies the analgesic activities of the other constituents [40]. Other known important activities of this essential oil major compounds are antibacterial, antifungal and insect repellent.

Chemical variability of aromatic plants is a widespread phenomenon and clear relationship to possible causes is often not well established. Among possible factors affecting this polymorphism are soil hydrology, soil pH, climate and in particular, the microclimate [41]. This plant grows in a region, which is classified as “Key Biodiversity Area” [42]. This particular environment would be favorable to the development of components that are different from essential oils of the same species; however a further study would be necessary to investigate the influence of the growing site to the essential composition of this plant.

IV. Conclusion

The essential oil of *Vepris madagascarica* shows a wide variability. Three chemotypes have been discovered to date: Estragole chemotype, Anethole chemotype and, Citral/ Citronellol/ Myrcene chemotype for this new one. All these plants grow in the North and in the North Easter regions of Madagascar. These regions are all under a humid tropical climate. A more in-depth study would be necessary to clarify if this variation is of genetic origin, or due to the microclimate. Understanding these variations is important to have stability in the exploitation of the essential oil. Each of these chemotypes gives specific and characteristic smell, and their industrial applications are very different.

V. Acknowledgment

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References

[1] E Beech,.et al. , Rivers, M., Rabarimanarivo, M., Ravololomanana, N., Manjato, N., Lantoarisoa, F., Andriambololonera, S., Ramandimbisoa, B., Ralimanana, H., Rakotoarisoa, S., Razanajatovo , H., Razafiniary, V., Andriamanohera, A., Randrianasolo, V., Rakotonasolo, F., Rakotoarisoa, A., Randriamamonjy, N., Rajaovelona, L., Rakotomalala, N., Randriamboavonjy, T., Rajaonah, M., Rabehevitra, D., Ramarosandratana, A.V., Rakotoarinivo, M., B.H. Ravaomanalina and Jeannoda, V. (2021). The Red List of Trees of Madagascar.

[2] Haevermans, T., Hladik, A., Hladik, C. M., Razanatsoa, J., Haevermans, A., Jeannoda, V., & Blanc, P. (2021). Description of five new species of the Madagascan flagship plant genus Ravenala (Strelitziaceae). Scientific reports, 11(1), 1-15.

[3] Schatz, G. E., Lowry II, P. P., & Rakouth, H. N. (2021). Taxonomic Studies of Diospyros (Ebenaceae) from the Malagasy Region. VIII. New Species from the Littoral Forests of Eastern Madagascar. Novon: A Journal for Botanical Nomenclature, 29(1), 159-187.

[4] Davis, A. P., & Rakotonasolo, F. (2021). Six new species of coffee (Coffea) from northern Madagascar. Kew Bulletin, 76(3), 497-511.

[5] Hermans, J., Rajaovelona, L., & Cribb, P. (2021). New species in Orchidaceae from Madagascar. Kew Bulletin, 76(1), 39-56.

[6] Upadhyay, S. K., & Singh, S. P. (Eds.). (2021). Bioprospecting of Plant Biodiversity for Industrial Molecules. Wiley.

[7] Langat, M., Kami, T., & Cheek, M. (2021). Chemistry, Taxonomy and Ecology of the potentially chimpanzee-dispersed Vepris teva sp. nov.(Rutaceae) of coastal thicket in the Congo Republic. bioRxiv.

[8] Schmelzer, GH. & Gurib-Fakim, A. (Eds). (2013). Plant Ressources of Tropical Africa 11(2). Medicinal plants 2. PROTA Foundation, Wageningen, Netherlands/CTA, Wageningen, Netherlands. 384 pp.

[9] HECKEL, E., (1911). Sur une plante nouvelle à essence anisée. C. R. Ac. Se, T. CLII, 565.

[10] Juillet, A., (1912). Etude du Pelea madagascarica. Annales du Museé Colonial de Marseille, sér. 2, 10, 175.

[11] John, H. St., (1937). Notulae Systematicae. Herbier du Museum de Paris 6, 3, 125.

[12] Rabehaja, D. J., Ihandriharison, H., Ramanoelina, P. A., Ratsimamanga-Urverg, S., Bighelli, A., Casanova, J., & Tomi, F. (2013). Leaf oil from Vepris madagascarica (Rutaceae), source of (E)-anethole. Natural Product Communications, 8(8), 1934578X1300800835

[13] Kharbach M, Marmouzi I, El Jemli M, Bouklouze A, Heyden YV, Recent advances in untargeted and targeted approaches applied in herbal-extracts and essential-oils fingerprinting - A review, Journal of Pharmaceutical and Biomedical Analysis (2019), doi: https://doi.org/10.1016/j.jpba.2019.112849

[14] Vella, F. M., Calandrelli, R., Cautela, D., Fiume, I., Pocsfalvi, G., & Laratta, B. (2020). Chemometric screening of fourteen essential oils for their composition and biological properties. Molecules, 25(21), 5126.

[15] Rasekh, M., Karami, H., Wilson, A. D., & Gancarz, M. Classification and identification of essential oils from herbs and fruits based on a MOS electronic-nose technology. Chemosensors 2021; 9: 142. https://doi.org/10.3390/chemosensors9060142

[16] Babushok, V. I., Linstrom, P. J., & Zenkevich, I. G. (2011). Retention indices for frequently reported compounds of plant essential oils. Journal of Physical and Chemical Reference Data, 40(4), 043101. https://doi.org/10.1063/1.3653552

[17] Poitou, F., Masotti, V., Viano, J., Gaydou, E. M., Andriamahavo, N. R., Mamitiana, A., ... & Andriantsiferana, M. (1995). Chemical compositon of Vepris elliotii essential oil. Journal of Essential Oil Research, 7(4), 447-449.

[18] Moulis, C., Fouraste, I., Keita, A., & Bessiere, J. M. (1994). Composition of the leaf essential oil from vepris heterophylla R. let. Flavour and fragrance journal, 9(1), 35-37.

[19] Rakotondraibe, L. H., Rakotovao, M., Ramanandraibe, V., Ravaonindrina, N., Frappier, F., & Brouard, J. P. (2001). Composition and antimicrobial activity of leaf oils of Vepris leandriana H. Perr. Journal of Essential Oil Research, 13(6), 467-469.

[20] Garcia, G., Charmillon, J. M., Roux, E., Sutour, S., Rakotozafy, J. B., Désiré, O., ... & Rabehaja, D. J. (2017). Chemical composition of leaf and bark essential oils of Vepris unifoliolata from Madagascar. Journal of EssEntial oil rEsEarch, 29(3), 214-220.
[21] Maggi, F., Fortuné Randriana, R., Rasoanaivo, P., Nicoletti, M., Quassinti, L., Bramucci, M., ... & Vittori, S. (2013). Chemical composition and in vitro biological activities of the essential oil of Vepris macrophylla (Baker) I. Verd. endemic to Madagascar. Chemistry & biodiversity, 10(3), 356-366.
[22] Gonçalves, D., Costa, P., Rodrigues, C. E., & Rodrigues, A. E. (2017). Fractionation of acid lime essential oil using ethanol/water mixtures: Effect of the process on the aroma profile. The Journal of Chemical Thermodynamics, 108, 118-126.
[23] Teixeira, M. A., Rodríguez, O., & Rodrigues, A. E. (2010). Perfumery radar: A predictive tool for perfume family classification. Industrial & engineering chemistry research, 49(22), 11764-11777.
[24] Sharmeen, J. B., Mahomoodally, F. M., Zengin, G., & Maggi, F. (2021). Essential oils as natural sources of fragrance compounds for cosmetics and cosmeceuticals. Molecules, 26(3), 666.
[25] Buettner, A. (Ed.). (2017). Springer handbook of odor (p. 1100). Berlin/Heidelberg, Germany: Springer, https://doi.org/10.1007/978-3-319-26932-0
[26] Burdock, G.A. (2009). Fenaroli's Handbook of Flavor Ingredients (6th ed.). CRC Press. https://doi.org/10.1201/9781439847503
[27] Costa, R., Pizzimenti, F., Marotta, F., Dugo, P., Santi, L., & Mondello, L. (2010). Volatiles from steam-distilled leaves of some plant species from Madagascar and New Zealand and evaluation of their biological activity. Natural Product Communications, 5(11), 1934578X1000501123.
[28] MOOKDASANIT, J., TAMURA, H., YOSHIZAWA, T., TOKUNAGA, T., & NAKANISHI, K. (2003). Trace volatile components in essential oil of Citrus sudachi by means of modified solvent extraction method. Food Science and Technology Research, 9(1), 54-61.
[29] Tamura, H., BOONBUMRUNG, S., Yoshizawa, T., & VARANYANOND, W. (2001). The volatile constituents in the peel and pulp of a green Thai mango, Khieo Sawoei cultivar (Mangifera indica L.). Food Science and Technology Research, 7(1), 72-77.
[30] Miyazawa, M., Nakashima, Y., Nakahashi, H., Hara, N., Nakagawa, H., Usami, A., & Chavasiri, W. (2015). Volatile compounds with characteristic odor of essential oil from Magnolia obovata leaves by hydrodistillation and solvent-assisted flavor evaporation. Journal of Oleo Science, 64(9), 999-1007.
[31] Burdock, G.A. (2004). Fenaroli's Handbook of Flavor Ingredients (5th ed.). CRC Press. https://doi.org/10.1201/9781420037876
[32] Tamura, H., Hata, Y., Chida, M., & Yamashita, K. (2008). Picking aroma character compounds in Citrus limon oils by using odor thresholds in aroma mixtures. Food Flavor: Chemistry, Sensory Evaluation, and Biological Activity (Tamura H, Ebeler SE, Kubota K, Takeoka GR, eds), 229-242.
[33] Poivet, E., Peterlin, Z., Tahirova, N., Xu, L., Altomare, C., Paria, A., ... & Firestein, S. (2016). Applying medicinal chemistry strategies to understand odorant discrimination. Nature communications, 7(1), 1-9.
[34] Poivet, E., Tahirova, N., Peterlin, Z., Xu, L., Zou, D. J., Acree, T., & Firestein, S. (2018). Functional odor classification through a medicinal chemistry approach. Science Advances, 4(2), eaao6086.
[35] Teixeira, M. A., Barrault, L., Rodríguez, O., Carvalho, C. C., & Rodrigues, A. E. (2014). Perfumery radar 2.0: A step toward fragrance design and classification. Industrial & Engineering Chemistry Research, 53(21), 8890-8912.
[36] Mota, C. M., Rodrigues-Santos, C., Carolino, R. O., Anselmo-Franci, J. A., & Branco, L. G. (2020). Citral-induced analgesia is associated with increased spinal serotonin, reduced spinal nociceptive signaling, and reduced systemic oxidative stress in arthritis. Journal of Ethnopharmacology, 250, 112486.
[37] Quintans-Júnior, L. J., Guimarães, A. G., Santana, M. T. D., Araújo, B. E., Moreira, F. V., Bonjardim, L. R., ... & Santos, M. R. (2011). Citral reduces nociceptive and inflammatory response in rodents. Revista Brasileira de Farmacognosia, 21(3), 497-502.
[38] Nishijima, C. M., Ganev, E. G., Mazzardo-Martins, L., Martins, D. F., Rocha, L. R., Santos, A. R., & Hiruma-Lima, C. A. (2014). Citral: a monoterpene with prophylactic and therapeutic anti-nociceptive effects in experimental models of acute and chronic pain. European Journal of Pharmacology, 736, 16-25.

[39] Santos, P. L., Matos, J. P. S., Picot, L., Almeida, J. R., Quintans, J. S., & Quintans-Júnior, L. J. (2019). Citronellol, a monoterpene alcohol with promising pharmacological activities-A systematic review. Food and Chemical Toxicology, 123, 459-469.
[40] Surendran, S., Qassadi, F., Surendran, G., Lilley, D., & Heinrich, M. (2021). Myrcene—What Are the Potential Health Benefits of This Flavouring and Aroma Agent?. Frontiers in nutrition, 400.
[41] Barra, A. (2009). Factors affecting chemical variability of essential oils: a review of recent developments. Natural product communications, 4(8), 1934578X0900400827.
[42] Conservation International - Madagascar (2014). Ecosystem profile Madagascar and Indian ocean islands.