Lipophilic Flavonoid Profile as Marker of Differentiation Between two Species of Malagasy *Helichrysum* Sharing the Same Vernacular Name, Rambiazina

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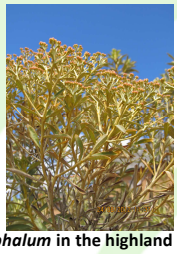
The genus *Helichrysum* includes some 500 species originating from warm areas, especially in Europe and Africa. In Madagascar, 115 different species of the genus *Helichrysum*, all endemic to the island, have been identified by Humbert (Humbert, 1962). Among these, one representative species, *H. gymnocephalum* (DC.) Humbert, which name derived from the greek 'gymnos', naked and 'kephale', head (Syn. = *Stenocline incana* Baker, *S. gymnocephale* De Candolle) is called « immortelle à tête nue », Rambiaze (French names) and Rambiazina (Merina, Madagascar). It shares common vernacular name, Rambiazina, with another Malagasy *Helichrysum*, *Helichrysum bracteiferum* (DC.) Humbert.
Philippe Rasoanaivo and Philippe De La Gorce, from « Institut Malgache de Recherches Appliquées », mentioned that although they are commonly known under the same vernacular name, they can be distinguished through the size of their leaves: *H. bracteiferum*, with small leaves, is called the male « Lahy », while *H. gymnocephalum*, with larger leaves, is named the female « Vavy » (Rasoanaivo, 1998). The size of leaves is a faintly discriminatory morphologic criteria during harvest of wild specimens. Taking together the wide phenotypic variability described for *Helichrysum* species, as well as intra- and interspecific variabilities, it seemed important to study and describe the less common, *H. gymnocephalum*, in terms of botanical description, biotope, traditional uses and lipophilic flavonoid composition, for which we have shown that the profile was a selective criterion between both species.

Plant collection and extraction

33 batches of aerial parts of *Helichrysum gymnocephalum* and *H. bracteiferum* were collected at different growth stages (before flowering, early flowering, complete flowering, post-flowering and vegetative stage), at different periods of the year, and in 3 different sites around Tananarive (Table 1). The aerial parts were dried under sun exposure during 5 to 7 days. After ethanolic extraction, their flavonoid HPLC and LC-MS profile were analyzed and compared.

Picture 1: macroscopic aspects of the two dried species



Picture 2: plant of *H. gymnocephalum* in the highland

SITES	Region	Geographic coordinates	Altitude (m)	Soil type	Mean annual pluviometry (mm)
Ambatomanga	Vakinankaratra	18°96'67"	1 463,00	ferruginous brown yellow (sloping floor)	1 500,00
Talata volonondry	Analamanga	18°75'00"	1 480,00	ferruginous brown red (sloping floor)	1 430,00
Imerintsiatosika	Itasy	18°58'59"	1 301,00	ferruginous brown red (sloping floor)	1 200,00

Table 1: features of the harvest localisations

Flavonoids purification and quantification

An aliquote of an *H. gymnocephalum* extract batch was submitted to a silica gel column. After elution with heptane, heptane / acetone (75:25, 50:50 and 25:75), acetone and methanol, we obtained 8 fractions which were combined according to HPLC analysis.
Fractions 2, 3 and 4 were further fractionated by semi-preparative HPLC using a Symmetry Shield RP-18 column 19x300 mm, 7µm, eluted with water / acetonitrile (50:50 to 10:90, 10mL/min, v/v), leading to the isolation of Alpinetin (**1**), Pinobanksin (**2**), 8-hydroxygalangin (**3**), Pinocembrin (**4**), Acetylpinobanksin (**5**), Trihydroxychalcone (**6**), Galangin (**7**), Cardamonin (**8**) and a new terpenylated flavonoid called Gymnochalcone (**9**). The chemical structures of isolated flavonoids were determined by mass spectrometry and NMR (Fig.1). Purified compounds are used to determinate the flavonoid content of each extract.

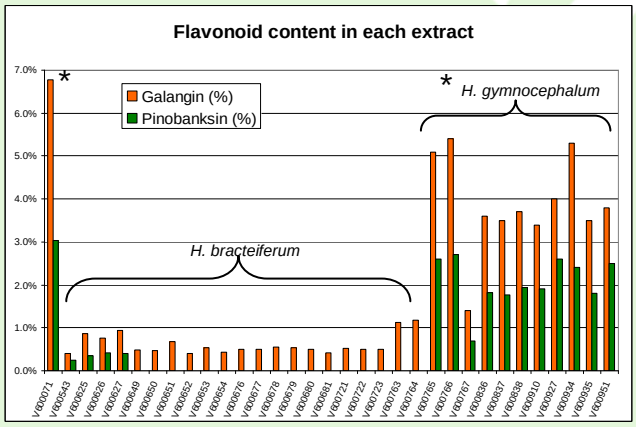
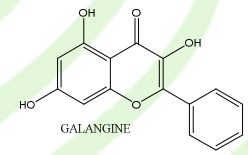
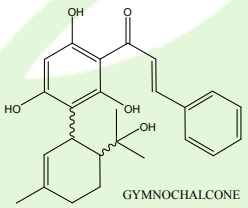


Figure 3: Galangin and pinobanksin content in each ethanolic extracts

Ground notes

The female Rambiazina (*Helichrysum gymnocephalum*) has quite large and elongated leaves compared to the male Rambiazina (*Helichrysum bracteiferum*) (Pict. 1 and 2). We also note the three main characteristic nervures on each limb.
Biotope: The female Rambiazina grows on rocks, stony grounds, mountainsides from an altitude of 1250 to 1500 m, in the region of Madagascan highlands (Analamanga, Itasy, Vakinankaratra, Mania).
This woody plant thus likes fairly moist grounds of mountainside, area more or less sunny, ferralitic soils, more or less lumpy. It also likes cracks between the rocks. We rarely see it in the lowlands. We hardly ever see it together with the male Rambiazina.





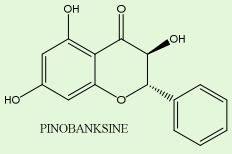


Figure 1: chemical structures of 3 isolated flavonoids

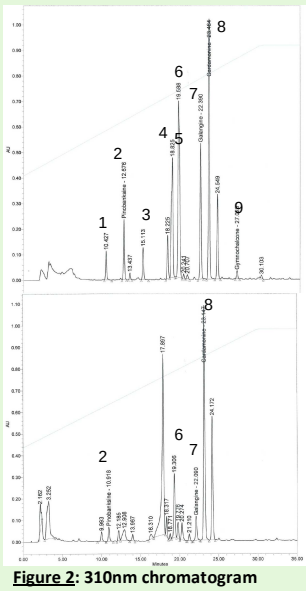


Figure 2: 310nm chromatogram comparison between *H. gymnocephalum* and *H. bracteiferum* (H_2O/Acetonitrile 60:40 to 10:90 in 30', Symmetry Shield RP18 250x4.6 mm, 5µm)

The main differences between *H. gymnocephalum* and *H. bracteiferum* are the absence of Alpinetin (**1**), 8-hydroxygalangin (**3**), Pinocembrin (**4**), Acetylpinobanksin (**5**), and Gymnochalcone (**9**) in *H. bracteiferum* (Fig. 2) as well as the drastic decrease of both Pinobanksin and Galangin (Fig.3). These molecules could therefore be good markers of differentiation between both species. The HPLC profile helps us to rapidly distinguish the species we work on, thus ensuring a correct identification for a future development.

This work shows that the analysis of lipophilic flavonoids could help to differentiate both Rambiazina species, when morphological identification is unclear. It highlights the importance of the multidisciplinary approach, involving botanists, chemists and ethnopharmacologists.
The high content of these lipophilic flavonoids in *H. gymnocephalum* may explain its extensive use in ethnomedicine, for treatment of respiratory disease or of rheumatic complains. The valorisation of the endemic plant taxa may be a way to stop or, at least slow down, the threat of extinction caused by man made changes in the natural environment (deforestation) which have drastically accelerated in Madagascar during the last decades.

Humbert H., Flore de Madagascar et des Comores, Tome II 189e famille – Composées. Typographie Firmin-Didot et Cie, 56, rue Jacob, PARIS. 1962.
Rasoanaivo P., De La Gorce P., 1998, Essential oils of economic value in Madagascar: Present state of knowledge, Herbalgram, 43: 31-59.