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# CHEMICAL CONSTITUENTS OF *RAZAFIMANDIMBISONIA MINOR* (BAILL.) KAINUL & B. BREMER, AERIAL PARTS, RUBIACEAE OF MADAGASCAR

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**ABSTRACT**

Traditional medicine uses the aerial parts of *Razafimandimbisonia minor* (Rubiaceae), a species endemic to Madagascar, to treat liver disorders and fatigue, diseases associated with oxidative stress. A phytochemical analysis of this plant made to isolate, by chromatography, six compounds: vanillin, ursolic acid, and oleanic acid were identified in the leaf extracts, while stigmasterol, (-) - epicatechin, and fraxin were extracted from the stems. These compounds are isolated for the first time from the aerial parts of this plant. Their structure was confirmed by NMR spectroscopy and compared to existing data in the literature. According to previous research, some of these compounds have antioxidant properties.

**KEYWORDS**: *Razafimandimbisonia minor,* endemic, Rubiaceae.

## 1. INTRODUCTION

The Rubiaceae family is the fourth largest among the Angiosperms in terms of diversity, comprising some 637 genera and 13,000 species.[1] It is abundant and ubiquitous in tropical wet and dry forests, accounting for 7-9% of Madagascar's phytodiversity. The percentage of endemism is high: 98% of species and 30% of genera of the Rubiaceae of Madagascar are found nowhere else.[2] The plant genus *Razafimandimbisonia*, endemic to Madagascar, was described for the first time in 2009. This plant genus belongs to the subfamily Ixoroideae, tribe Alberteae, Rubiaceae family.[3] It contains five species, including *Razafimandimbisonia minor (syn Alberta minor)* distributed in the subhumid and mountain forest, from 500 m to 2499 m altitude, but it has not been reported in the western part of the country.[3] The vernacular names of this plant is Hazomborondreo, Voamalitony, and Malambovony.[4,5] The decoction of branches is used for the treatment of liver disorders and fatigue.[5] The present study aims to isolate and structurally elucidate what this plant constitutes. To the best of our knowledge, this is the first report about the chemical study of this plant.

## 2. MATERIALS AND METHODS

### 2.1. General

TLC was performed on aluminum silica gel 60 F254 (Merck) plates (0.2 mm layer thickness). Spots were visualized using a UV lamp (254 and 366 nm) and spraying with vanillin-sulfuric acid reagent. Column chromatography was performed on silica gel 60 (6.3-20 µm) (Merck, Darmstadt, Germany). NMR spectra were recorded with a Brüker AV-400 with a cryoprobe for $^{1}H$, $^{13}C$. Chemical shift values are in δ (ppm) using the peak signals of the solvents $CDCl_3$ (δ-H = 7.26 ppm and δ-C = 77.16 ppm) and $CD_3OD$ (δ-H = 4.87 ppm and δ-C = 49.00 ppm) as reference, and coupling constants are reported in Hz.

### 2.2. Plant material

The aerial parts of *Razafimandimbisonia minor*, (Baill.) Kainul. & B. Bremer (Rubiaceae) were collected in October 2014, in Andaingomadinika in the Moramanga district of the Alaotra Mangoro region. The plant was identified by Dr Stephan Richard RAKOTONANDRASANA, botanist at National Centre for Applied Pharmaceutical Research (CNARP). A voucher specimen, referenced ROL 734, was deposited in the herbarium of CNARP.

### 2.3. Extraction and Isolation

A total of (400 g) dried leaves were extracted by maceration with 1L of absolute methanol for 72h at room temperature, with repeated agitation and filtered. The filtrate was dried using rotary evaporator at temperature of 40° C. The methanol extract collected was weighed

and preserved (40.91 g).The methanol extract (40 g) was dissolved in 300 mL distilled water and partitioned gradiently into hexane and ethyl acetate (3 x 800 mL each) to obtain three fractions and dried. A phytochemical screening was performed to determine the main chemical families in the plant.[6,7]

8g of EtOAc was applied to a silica gel column with Hexane/ $CH_2Cl_2$ and $CH_2Cl_2$/MeOH as an eluent of increasing polarity afforded 20 fractions (LF1→ LF20). Fractions were monitored by TLC, and similar fractions were combined. Fractions LF8, LF9, and LF12 (800 mg, 510 mg, and 180 mg, respectively) were chromatographed on silica gel.

Purification of LF8 using the eluent mixture of $CH_2Cl_2$/MeOH in increasing polarity led to the isolation of compound P1 (4.1 mg). The fraction LF9 was purified by chromatography on a column of silica gel and eluted with a gradient consisting of a mixture of Hexane/AcOEt which was made to isolate compound P2 (20 mg). And the purification of LF12 following the Hexan/$CH_2Cl_2$ led to the isolation of a mixture of compounds P2 and P3 (25.3 mg).

The powdered stems of *Razafimandimbisonia minor* (400 g) were extracted with 1L of a $CH_2Cl_2$/MeOH mixture in the proportion of 20/80 (v/v) for 72h at room temperature. The filtrate was recovered by Buchner filtration and evaporated using the rotary evaporator. The crude extract collected was weighed and preserved (26.61 g).

A part of the crude extract (26 g) was applied to a silica gel column with Hexane/ $CH_2Cl_2$ and EtOAc/MeOH as binary mixtures of increasing polarity afforded 10 fractions (ST1→ ST10). Further purification of ST1 was performed on silica gel eluted with Hexane/$CH_2Cl_2$ step gradients to obtain compound P4 (5.4 mg). ST4 was purified using a gradient of Hexane / EtOAc to obtained compound P5 (13.4 mg). Compound 6 (10.1 mg) was obtained from the purification of ST8 a silica gel column using the mixture $CH_2Cl_2$/MeOH/Water as eluant.

## 3. RESULTS AND DISCUSSIONS

Phytochemical screening revealed the presence of various chemical classes in plant extracts. The stem extract of *Razafimandimbisonia minor* is particularly rich in phenolic compounds (coumarins, flavonoids, and tannins), steroids and cardenolides. As for the leaves, they are rich in coumarins, polyphenols, terpenoids (triterpens, steroids), and saponins. Indeed, this family is distinguished by the synthesis of terpenes, flavonoids, other phenolic derivatives, iridoids, and coumarins, particularly for their pharmacological potential.[8,1]

The structures of the isolated compounds were determined by analysis of their spectroscopic data. Through 1D NMR spectra and comparison with the literature, they are recognized respectively as vanillin,[9] ursolic acid,[10,11] and oleanic acid [11] are identified from the leaf extract (Figure 1); stigmasterol,[12] (-) - epicatechin,[13] and fraxin[14] are identified from the stem extract (Figure 2).

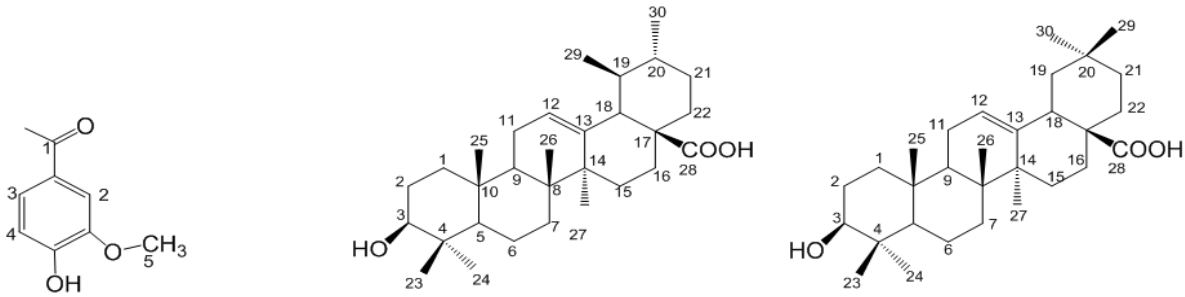


**Fig. 1: Structure of the compounds isolated from the leaf extract.**

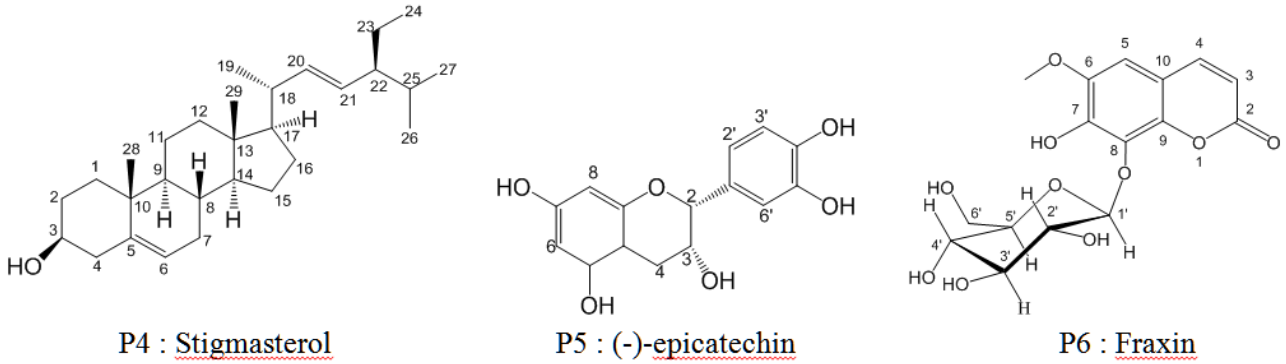


**Fig. 2: Structure of the compound isolated from the stem extract.**

**Vanillin 1:** White solid: $^{1}$H-NMR (300 MHz in $CD_3OD$): δ (ppm) 9.77 (1H, s, CHO), 7.47 (1H, s, H-2), 7.45 (1H, d, J = 7.2 Hz, H-6), 6.98 (1H, d, J = 8.1 Hz, H-5), 3.94 (1H, s, $OCH_3$).

**Ursolic acid 2**: White solid: $^{1}$H-NMR (400 MHz in $CD_3OD$): δ (ppm) 5.25 (1H, m, H-12), 3.17 (1H, dd, J = 11.0, 4.7 Hz, H-3), 2.21 (1H, d, J = 11.4 Hz, H-18), 1.97 (2H, dd, J = 8.8, 3.5 Hz, H-15), 1.70 (1H, m, H-22), 1.67 (1H, dd, J = 13.9, 6.8, H-6), 1.60 (2H, m, H-16), 1.38 (3H, m, H-19), 1.18 (1H, m, H-7), 1.14 (3H, s, H-27 ), 0.98 (1H, m, H-20), 0.91 (3H, d, d, J = 6.5 Hz H-29), 0.87 (3H, m, H-26), 0.80 (3H, m, H-30).

$^{13}$C NMR (100 MHz in $CD_3OD$) : δ (ppm) 181.40 (C-28), 139.42 (C-13), 126.70 (C-12), 79.49 (C-3), 56.53 (C-5), 54.15 (C-18), 43.03 (C-14), 40.57 (C-20), 40.20 (C-19), 39.79(C-4, C-8), 39.63 (C-1), 37.89 (C-10), 34.12 (C-7), 31.55 (C-21) , 29.00 (C-23), 28.56 (C-2) , 27.68 (C-15), 25.10 (C-27), 24.15(C-16) , 23.88 (C-11), 21.35 (C-30), 19.26 (C-6), 17.59 (C-29) , 17.43 (C-24) , 15.81 (C-25, C-26).

**Mixture of ursolic acid 2 and oleanolic acid 3**: White solid: $^{1}$H-NMR (400 MHz in $CD_3OD$): **ursolic acid 2:** δ (ppm) 5.30 (1H, s, H-12), 3.22 (1H, dd, J = 8.6, 4.0 Hz, H-3), 2.03 (1H, d, J = 4.4 Hz, H-18), 1.08 (3H, s, H-27), 0.99 (3H,s, H-26), 0.95 (3H, s, H-25), 0.93 (3H, d, J = 4.3 Hz, H-30), 0.87 (3H, d, J = 6.4 Hz, H-29), 0.79 (6H, d, J = 3.91 Hz, H-23, H-24).

**oleanolic acid 3:** δ (ppm) 5.25 (1H, m, H-12), 3.22 (1H, dd, J = 8.6, 4.0 Hz, H-3), 2,20 (1H, d, J = 4.4 Hz, H-18), 1.25 (3H, m, H-27), 0.99 (3H,s, H-26), 0.95(3H,s, H-25), 0.93 (6H, d, J = 4.3 Hz, H-29, H-30), 0.79 (3H, d, J = 3.5 Hz, H-23), 0.77 (3H, s, H-24).

**Stigmasterol 4**: White crystal: $^{1}$H-NMR (300 MHz in $CDCl_3$): δ (ppm) 5.34 (1H, d, J = 2.5 Hz, H-6), 5.14 (1H, dd, J = 15.2 Hz, J= 8.6 Hz, H-21), 5.02 (1H, dd, J = 15.2, 8.6 Hz, H-20), 3,53 (1H, m, H-3), 1.01 (3H, d, J = 7.6 Hz, H-29), 0.88 (3H, m, H-24), 0,85 (3H, d, J = 6.5 Hz, H-26), 0.84 (3H, d, J = 3.8 Hz, H-19), 0.82 (3H, d, J = 6.5 Hz, H-27), 0.70 (3H, d, J = 7.3 Hz, H-28).

**(-) - Epicatechin 5**: Brown amorphous solid: $^{1}$H-NMR (400 MHz in $CD_3OD$): δ (ppm) 6.99 (1H, d, J = 1.9 Hz, H-2'), 6.81 (1H, dd, J = 8.4, 1.8 Hz, H-6'), 6.78 (1H, d, J = 8.1 Hz, H-5'), 5.95 (1H, d, J = 2.3 Hz, H-8), 5.93 (1H, d, J = 2.3 Hz, H-6), 4.84 (1H, s, H-2), 4.20 (1H, m, H-3), 2.87 (1H, dd, J = 2.6, 16.82, H-4b) , 2.78 (1H, dd, J = 2.5, 16.82, H-4a).

$^{13}$C NMR (75 MHz in $CD_3OD$) : δ (ppm) 158.60 (C-7), 158.26 (C-5), 157.96 (C-9), 146.53 (C-3'), 146.37 (C-4'), 132.86 (C-1'), 119.94 (C-6'), 116.44 (C-5'), 115.88 (C-2'), 100.62 (C-10), 96.92 (C-6), 96.43 (C-8), 80.46 (C-2), 68.07 (C-3), 29.85 (C-4).

**Fraxin 6:** Yellow solid: $^{1}$H NMR (400 MHz in $CD_3OD$): δ (ppm) 7.91 (1H, d, J = 9.5 Hz, H-4), 7.01 (1H, s, H-5), 6.29 (1H,d, J = 9.4 Hz, H-3), 5.00 (1H, d, J = 7.8 Hz, H-1'), 3.92 (3H, s, O-CH3).

$^{13}$C NMR (75 MHz in $CD_3OD$) : δ (ppm) 162.16 (C-2), 147.49 (C-7), 146.17 (C-4), 145.10 (C-6), 144.55 (C-8), 131.80 (C-9), 117.81 (CH, C-3), 113.79 (C-10), 104.68 (C-5, C-1'), 77.05 (C-3'), 76.42 (C-5'), 74.09,(C-2'), 69.53 (C-4'), 60.81 (CH2, C-6'), 55.51 (C-6-O-CH3).

Oxidative stress is linked to numerous diseases such as liver damage due to the oxidation of biomolecules as lipids.[15] Many studies have been conducted in recent years due to interest in natural sources of antioxidant compounds for the treatment of oxidative stress. [16] Several species of the Rubiaceae family have shown antioxidant activity.[17] According to different studies, ursolic acid, frequently present in Rubiaceae, is used in various traditional medicines.[1] This compound has antioxidant properties, as confirmed by the work of Do Nascimento and Hung.[18] Furthermore, Lin and Abd El's research has highlighted the importance of (-) - epicatechin for human health, in particularly the ability to reduce the risks of diabetes, cardiovascular diseases and protection of oxidative stress.[19] Additionally, the antioxidant capacity of fraxin was demonstrated by Whang and co-workers.[20] The richness of this plant in secondary metabolites could justify the traditional use.

## 4. CONCLUSION

The results of this study contribute to confirming the traditional use of *Razafimandimbisonia minor* (Rubiaceae). This is the first report about the chemical study of this plant. The leaves are mainly rich in triterpenoids, while the stems contain sterols and phenolic compound, all known for their biological activities. This plant has an particularly interesting for the research of antioxydant activity.

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