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ISOLATION AND CHARACTERIZATION OF STIGMASTEROL AND B-SITOSTEROL FROM ETHYL ACETATE EXCTRACT FROM *GNIDIA DAPHNIFOLIA* L.F STEMS (THYMELACEAE): A NATIVE PLANT OF MADAGASCAR

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ABSTRACT

Photochemical screening of ethyl acetate extract of *Gnidia daphnifolia* Lf stems indicated the presence of steroids and triterpene. Column chromatography of the crude extracts (AcOEt) lead to a number of fractions. Purification of ethyl acetate extract fraction resulted in the isolation of mixture of two sterols namely Stigmasterol and β-Sitosterol. The isolation compounds afforded white crystalline powder which was characterized on the 1D RMN (^{1}H and DEPT) and 2D RMN (HSQC, COSY and HMBC) spectral and compared with their literature data.

KEYSWORDS: *Gnidia daphnifolia* Lf, RMN, stem, Thymelaceae.

I. INTRODUCTION

The genus *Gnidia* is among of the flowering plants of Thymelaceae family. It includes about 140 to 160 species which 20 species are endemic to Madagascar.[1] The species of the genus *Gnidia* is specifically characterized by its fibrous stem. Most of species have a leaves arranged alternately and some have opposite leaves.[2,3]

Gnidia daphnifolia L.f belongs the genus *Gnidia*. It is an endemic plant of Madagascar. *G.daphnifolia* L.f is a tree about two-meter-tall, with a twigs glabrescent, reddish brown outer bark and a hairy stems. Its flowers comprise about ten stamens in two whorls of uneven length, superior ovary, and the fruits are oblong.[4]

This plant has therapeutic virtues. The decoction of the whole plant is traditionally used to treat diarrhea and dysentery. The decoction of the bark's stem is used to fight the fatigue and the treatment of the yellow fever or hepatitis, the decoction of leafy stem is presumed.

This plant has fibrous stems and this fiber is used for ropes. It is also used for the manufacture of Antaimoro paper.[5,6]

The purpose of this study is to extract, isolate, identify and characterize by spectroscopic method the bioactive principles from the stem of *Gnidia daphnifolia* L.f.

II. METHODOLOGY

Several methods were applied in this study such as collection, identification, preparation of samples, extraction, chromatographic separation and isolation, and spectroscopic characterization.

II.1 Collection, identification and preparation of plant materials

Plant specimens were collected in Ramena, Region of Diana, Madagascar, in May 2008. This plant was taxonomically identified by Jacqueline RAZANANTSOA, botanist at Biological and Zoological Park of Tsimbazaza, Antananarivo. The stems of plant were dried at room temperature, sheltered from the sun, and powdered into a fine powder by blender.

II.2 Extraction

The powdered stem (300g) of *Gnidia daphnifolia* L.f was defatted with hexane (40°C) in a Soxhlet extractor for six hours. The solvent was recovered under pressure to obtain the extract which was labeled as hexane extract (M_1) and was left it dry under room temperature after the solvent are evaporated. If the resulting marc was dried, it was extracted successively with ethyl acetate with polarity superior (reflux extraction). After the supernatant was filtered and the solvent was evaporated, the ethyl acetate extract (M_2) was obtained. The resulting marc was air dried at room temperature and exhaustively extracted with Methanol: maceration during seven days and repeated in two operations. After filtering and evaporating, the extract obtained was labeled as methanol extract (M_3).

II.3 Tests for triterpenoid and steroid

Before doing the test, hydroethanolic extract from *Gnidia daphnifolia* L.f stem was depigmented. Several tests are carried out such test of Lieberman Burchard, test of Salkowski and test de Badjet Kedde to highlight the presence of triterpene and steroid.[7]

The presence of other chemical families was also tested.

II.4 Chromatographic separation, isolation and purification

The ethyl acetate extract of *Gnidia daphnifolia* L.f stem (2.75g) was powdered. The column chromatographic (CC) was used to fractionate its compounds using solvent gradient from 100% Hexane to 100% MeOH.

The eluates were collected in numbered bottles and they were followed by chromatographic analyzes (CCM) and then the compounds that had the same profile were assembled.

For the isolation and purification, different technic were applied. The fractions combined were analyzed by CCM to verify its purity. Then, micro-column chromatographic and washing with appropriate solvent was carried out to have pure products.

II.5 Spectroscopic characterization

Structural elucidation of pure compounds was based on in-depth analysis NMR spectrums. To illustrate, the 1D-NMR such ^{1}H-NMR provides the information about the types of ^{1}H (-CH-, -CH_2- or –CH_3. The ^{13}C-NMR and DEPT give information about the number of Carbon and its types. There is also 2D-NMR, like HSQC and HMBC; they respectively show the correlation between H and its C-holder and long-distance correlation separated from two or three bond.

III. RESULTS AND DISCUSSIONS

After the extraction by solvents of increasing polarity from the plants powder, the mass yields and characteristic of each extract are summarized in Table 1. The abundance of yield of M_3 extract indicates that *Gnidia daphnifolia* L.f stems are rich in polar compounds.

The phytochemical screening of the hydroethanolic extract gave a positive test to Lieberman Burchard and Salkowski which they respectively indicate, the presence of triterpenoids and Steroids. But, it gave a negative test to Badjet Kedde.

The separation of ethyl acetate extract was provided nine fractions (F_1-F_9) after the CCM analysis. The compound GD-M_{11} was isolated in the first fraction (F_1) that eluted with solvent system (Hexane/Ethyl acetate-9/1). This compound is white crystalline substance and Rf value 0.52 (Hexane/Ethyl acetate-8/2), 0.66 with eluent Hexane/Ethyl acetate-9/1).

This one was analyzed by NMR using $CDCl_3$ as solvent. Five spectrums were recorded such as ^{1}H NMR, DEPT, COSY, HSQC and HMBC. The ^{1}H NMR spectrum of GD-M_{11} (figure 1) varied between 0.70 to 5.05 ppm. This spectrum divides into two distinct areas. The first area shows the presence of high intensity peaks at 0.70 to 2.31 ppm. According to the literature, the presence of the peaks forests between this interval indicates that the molecule in question should be a terpenoid or steroid.[8] So, the peaks at δ 0.70, 0.83, 0.85, 0.88, 0.95 and 1.03 ppm were attributed of six methyl groups (-CH_3). The other signals are assigned to the methylene (-CH_2-) and methine (-CH-) groups. The second area is the functional grouping zone which varies between 3.00 to 6.00 ppm. The peak coming out at 3.5 ppm corresponds with a proton of methine group that it bounds to a hydroxyl group (-C**H**-OH). According to the literature, the signal was appeared at δ 5.38 ppm as a single of doublet was attributed to a proton of ethylenic methine group (-C=C**H**-).[9]

The DEPT spectrum (figure 2) informs the types and number of the methine, methylene and methyl groups. The peaks at δ 128.2, 129.3 and 121.7 ppm correspond to ethylenic methine (-CH=). So, the signals are oriented at the bottom are the methine and methyl groups; and the other peaks are attributed to methylene group. According

to this spectrum, the product contains at least 28 Carbons including 11-CH-, 11-CH_2- and 6-CH_3. The difference between the peaks intensity at δ 128.2, 129.3 and 121.7 ppm should be indicated that the compound in question is a mixture of two molecules.

The HSQC spectrum shows the correlation between a proton and its holder Carbon (figure 3). On the one hand, the peak coming out at 138.2 and 128.3 ppm corresponds of the methine group, but these types of Carbon don't show any correlation in the HSQC spectrum. On the other hand, the presence of proton-proton correlation between H5.20 and H5.10 carried respectively by C138.2 and C129.3 in the COSY spectrum (figure 4) confirms that GP-M_{11} is a mixture of molecules. According to the literature, this correlation indicates the presence of Stigmasterol.[10]

The HMBC spectrum analysis can find the correlations that are established between a proton and Carbons (figure 5). The correlations tasks of a proton with a carbon at α and β are intense.

According to the literature, Stigmasterol and beta-Sitosterol are always a mixture. It is very difficult to obtain one of these compounds in pure state, because the only difference between these two compounds is the presence of C22=C23 double bond in Stigmasterol (figure 6) and C22-C23 single bond in beta-Sitosterol (figure 6).[11,12]

Therefore, the compound GP-M_{11} is a mixture of Stigmasterol and beta-Sitosterol. Due the following HMBC spectrum, the correlation with C33.9 indicates that beta-Sitosterol is the majority product.

Chemical shift values of all protons and carbons were assigned on the basic of ^{1}H, DEPT, HSQC, COSY and HMBC NMR spectrums compared with those of the literature were given in the Table 1.

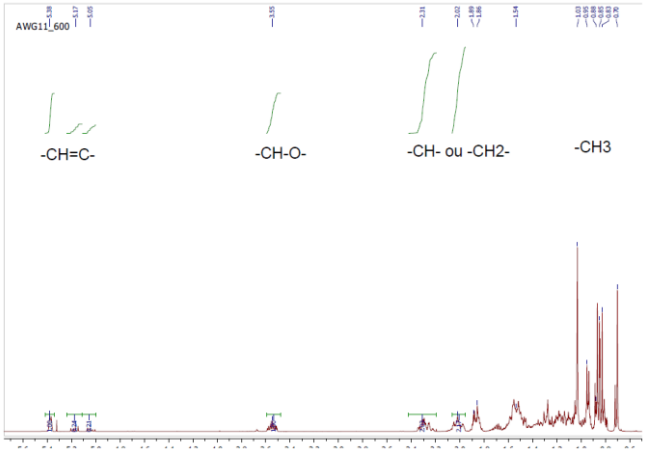


Figure 1: ^{1}H Spectrum of GP-M_{11}.

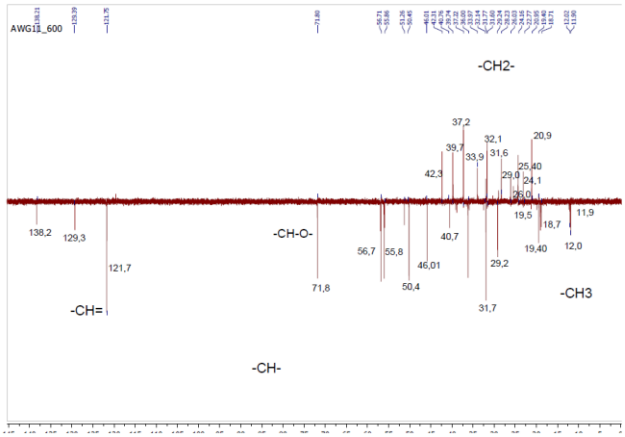


Figure 2: DEPT Spectrum of GP-M_{11}.

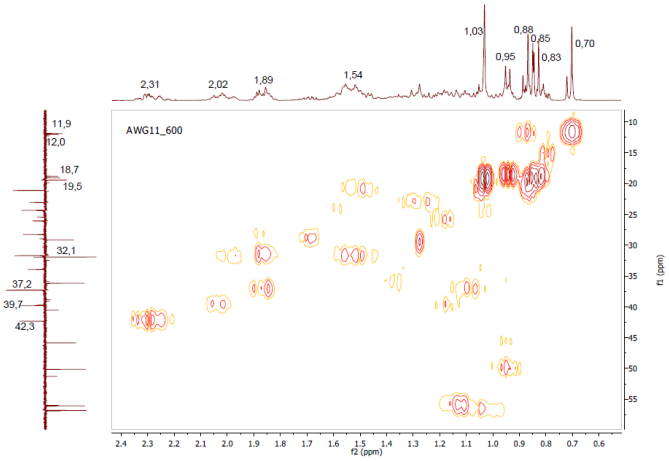


Figure 3: HSQC Spectrum of GP-M_{11}.

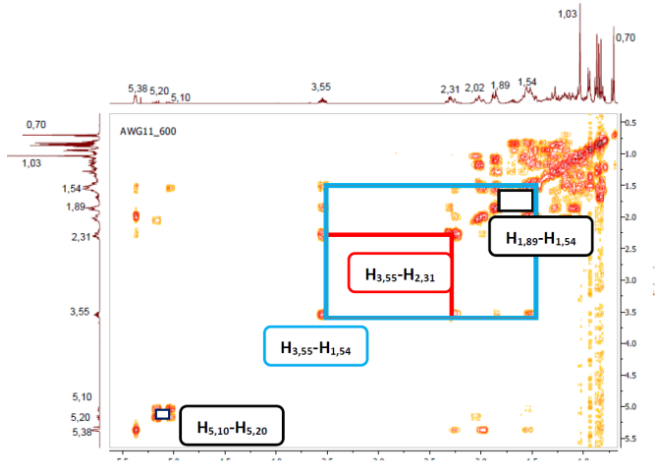


Figure 4: COSY Spectrum of GP-M_{11}.

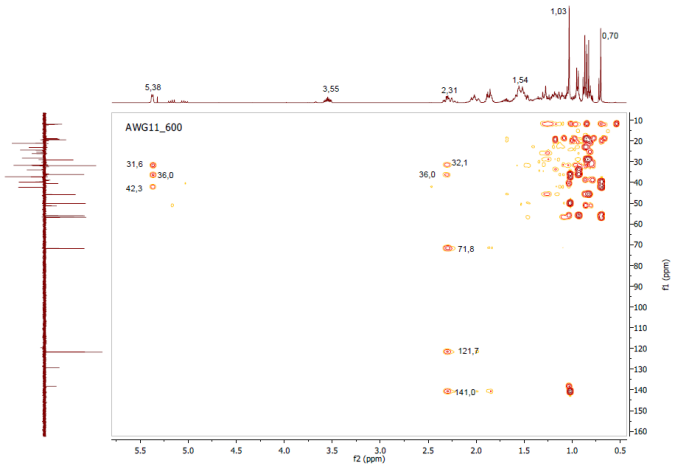
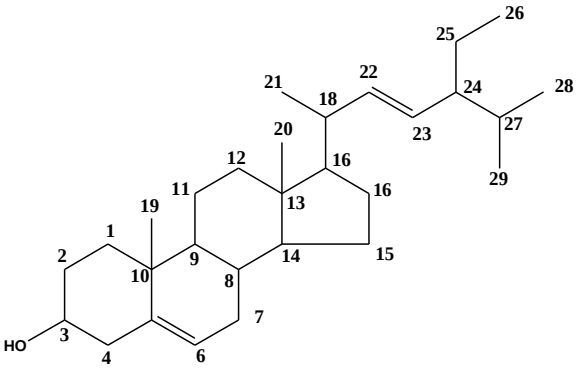


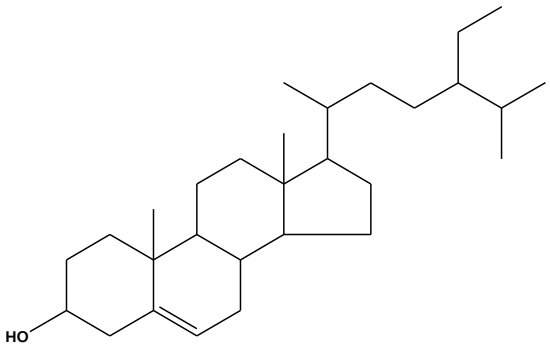
Figure 5: HMBC Spectrum of GP-M_{11}.

IV. CONCLUSION

According to the result above, the isolated compound from stem extract of *Gnidia daphnifolia* L.f is a mixture of two molecules. The structures of these molecules were identified as Stigmasterol and β-Sitosterol, obtained on the basic of spectroscopic methods analysis and by comparing their chemical shift value reported in the literature.



Stigmastérol



B-Sitostérol

Figure 6: Chemical structure of β-Sitosterol and Stigmasterol.

Table 1: ^{1}H and ^{13}C NMR chemical shift values for Stigmasterol recorded in CDCl3 (600MHz) compared with those the literature.

Position	^{13}C NMR experimental	^{13}C NMR literature [13]	^{1}H NMR experimental	^{1}H NMR literature [13]	Nature of Carbon
1	37,2	37,5			$-CH_2-$
2	32,1	31,9			$-CH_2-$
3	71,8	72,0	3,55 (tdd, 1H)	3,53 (tdd, 1H)	-CH-
4	42,3	42,5			$-CH_2-$
5	141,0	140,9	5,38 (sd, 1H)	5,36 (t, 1H)	-C=C-
6	121,7	121,9			-C=CH-
7	31,6	32,1			$-CH_2-$
8	31,7	32,1			-CH-
9	50,4	50,3			-CH-
10	36,0	36,7			-C-
11	20,9	21,3			$-CH_2-$
12	39,7	39,9			$-CH_2-$
13	40,7	42,6			-C-
14	56,7	56,9			-CH-
15	25,4	26,3			$-CH_2-$
16	29,0	28,5			$-CH_2-$
17	55,8	56,3			-CH-
18	11,9	12,0	1,03 (s, 3H)	1,01(s, 3H)	$-CH_3$
19	19,5	19,0	0,70 (s, 3H)	0,68 (s, 3H)	$-CH_3$
20	37,2	36,3			-CH-
21	18,7	19,2	0,95 (d, 3H)	0,93 (d, 3H)	$-CH_3$
22	33,9	34,2			$-CH_2-$
23	26,0	26,3			$-CH_2-$
24	46,0	46,1			-CH-
25	24,1	23,3			-CH-
26	12,0	12,2	0,88 (t, 3H)	0,84 (t, 3H)	$-CH_3$
27	29,2	29,4			$-CH_3$
28	19,4	20,1	0,85 (d, 3H)	0,83 (d, 3H)	$-CH_2-$
29	18,7	19,6	0,83 (d, 3H)	0,81 (d, 3H)	$-CH_3$

Chemical shift values are in ppm

REFERENCES

1. **Boiteau P. and Allonge-Boiteau L.**, (2001), Dictionnaire des noms malgaches de végétaux, Index des noms scientifiques avec leurs équivalents malgaches (4), Editions Alizieu, Grenobles, France, 80-81.
2. **Beamont A.J. and *al*** (2001), Patterns of diversity among involural bracts, influorescences and flowers in *Gnidia (Thymelacea)*, ***Systematics and Geography of Plants, 71(2):*** 419.
3. **Beamont A.J and *al.***, (2009), *Gnidia (Thymelacae*) is not momoleptic: toxanomic implications for *Thymelaoidaea* and a partial new generic taxonomy for *Gnidia*, ***Botanical Journal of the Linnen Society, 160(4*)**: 402.

4. **Leandri J.,** (1995), Thymelacées (Thymelacaea): Flore de Madagascar et des Comores (Plantes Vasculaires), ***Famille 146***, Paris, France, 40.
5. **Nicolas J.P.** (2012), Plantes médicinales de Nord de Madagascar: Ethnobotanique Antakarana et informations scientifiques, ISBN : 978-2-9543726-0-0, ***Editions Jardin du Monde 15***, Madagascar, 130.
6. **Rogers Z.S. and Spencer M.A.** (2009), Typification of Plants Names in *Thymelacaea*, ***Ann. Missouri Bot Gard, 96*****:** 324-368.
7. **Fong H.H.S. Tin-Wa and Farnsworth N.R**. (1997), Phytochemical Screening, Review: College of Pharmacy, University of Illinois, Chicago (USA), 275-277.
8. **Daouda N'Diaye, Mbaye Diagne Mbaye, Abdoulaye Gassama, Catherine Lavaud and Serge Pilard**, (2017) « Détermination structurale de triterpenoïdes isolés des feuilles de *Combretum glutinosium* Perr. Ex DC (*Combretaceae)* », ***International Journal of Biological Chemical Sciences, 11(1)*****:** 488-498.
9. **Shashi B., Ashko K., Nandy and Gita R**. (1992) "Review article number 67: Triterpenoid", *Phytochemistry, 31(7):* 2199-2249.
10. **Luhata L. P., and Munkombwe M.N**. (2015)" Isolation and characterization of Stigmastérol and β-sitosterol from *Odontonema Strictum* (Acanthaceae), ***Journal of Innovations in Pharmaceuticals and Biological Sciences, (2):*** ISSN: 2349-2759, 88-96.
11. **Pollock, J.R.A, Stevem R.S** (1965), Dictionary of organic compounds**,** ***Eyre and spottiswoode (Publishers) Ltd., 4(5):*** 5.
12. **Anjoo Kamboj and Ajay Kumar Saluja**, (2011), Isolation of stigmasterol and b-sitosterol from petroleum ether extract of aerial parts of Ageratum conyzoides (Asteraceae), ***International Journal of Pharmacy and Pharmaceutical Sciences, 3:*** 94-96.
13. **Venkata Sai P.C., & Indra P.,** (2012), "Isolation of Stigmasterol et β-sitosterol of Dichloromethane extract of *Rubus suavissimus*", ***International Current Pharmaceutical Journal, 1(9):*** 239-242.