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SECONDARY METABOLITES ISOLATED FROM *SMILAX ANCEPS*

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ABSTRACT

Two steroids, Stigmasterol and Sitosterol-β-D-glucoside, and five flavonoids, Kaempferol, Afzelin, Astragalin, Quercetin and Quercitrin were isolated from the ethyl acetate extract of *Smilax anceps* (Smilacaceae) using combinations of column and thin-layer-chromatographic methods. The structures of the compounds were elucidated on the basis of NMR experiments and literature data.

KEWORDS: Smilax anceps, flavonoids, steroids, NMR.

1- INTRODUCTION

Smilax anceps (Smilacaceae) is widely used in Madagascar and appreciated by its anti-inflammatory property.[1] According to the local people, the leaves are used as infusion and cataplasm to treat several diseases as headaches, wounds, bleeding. Studies conducted on the genus Smilax revealed the presence of steroids and flavonoids.[2] The roots, stems and leaves of Smilax anceps are source of steroids and polyphenols which were identified from phytochemical screening.

According to available literature, no phytochemical research work has been carried out on this plant. However, our previous work published about the screening phytochemical and mineral analysis.[3] [4] This paper deals with the isolation and the structural elucidation of two steroids and five flavonoids.

2- MATERIALS AND METHODS

2-1- Generalities

Aluminium silica gel 60 F254 (Merck) was used for Thin Layer Chromatography (thickness: 0.2 mm). We used a UV lamp and vanillin sulfuric acid as reagent. The isolation of the compounds was done on column chromatography performed on silica gel 60 (6.3-20 µm) (Merck). The recording of NMR spectra was done with Brüker AV-400 with a cryoprobe for ^{1}H and ^{13}C.

The values are in $\square_{ppm}$. The solvent used is $CDCl_3$ which peaks are at $\square_{ppm}$ = 7.28 for ^{1}H and 70.00 for ^{13}C. Coupling constants are reported in Hertz (Hz).

2-2- Plant material

The plant was collected in 2021 in Andasibe forest (Alaotra Mangoro region). It is in East of Madagascar, a part of the Island where climate is favorable for vegetation growing. The plant was dried and reduced into fine powder by using mechanical blender.

2-3- Extraction and isolation

400 gr of powder were put into 2 liters of ethanol 95% for ten days, at room temperature. The solution was then filtered, so that we get the raw solution. The solvent was evaporated in order to obtain the raw extract. The residue was then suspended in water and partitioned by using hexane, ethyl acetate and methanol. The ethyl acetate extract was applied to a silica gel column with Hexane and Ethyl Acetate binary mixtures of increasing polarity afforded 11 fractions (Fractions Ac1 – Ac 11). The chromatography was continued on fraction Ac1 by using Hexane – Ethyl Acetate step gradients (95:5 to 75:25) to give 4 subfractions (Fractions Ac1a – Ac1d). Further purification of Ac1a was applied to column chromatography on silica gel with Hexane – Methanol (95:5 to 70:30) to obtain Compound 1 (7 mg) and Compound 2 (9 mg). The fraction Ac1c (Hexane – Methanol 90:10 to 70:30) was eluted with Hexane – Ethyl Acetate (95:5 to 80:20) to obtain Compound 3 (10 mg) and Compound 4 (8 mg). The fraction Ac4 (Hexane – Ethyl Acetate 85:15 to 65:35) was eluted with Hexane – Ethyl Acetate (95:5 to 40:60) to obtain Compound 5 (8 mg). The fraction Ac8 was eluted with Hexane-Methanol (98 :2 to 80 :20) to give 3 subfractions (Fractions Ac8a – Ac8c). Further purification of Ac8a applied to column chromatography with Hexane-Ethyl Acetate (98:2 to 85:15) gave Compound 6 (7 mg) and Compound 7 (8 mg).

3- RESULTS AND DISCUSSIONS

The spectroscopic data and the comparison from the data literature allowed us to determine the structure of the isolated compounds.

Compound 1 is recognized as Stigmasterol, compared to the literature.[5]
Compound 2 is identified as Sitosterol-β-D-glucoside, compared to the literature.[6]
Compound 3 is identified as Kaempferol, compared to the literature.[7]
Compound 4 is recognized as Afzelin, compared to the literature.[8]
Compound 5 is recognized as Astragalin, compared to the literature.[9]
Compound 6 is identified as Quercetin, compared to the literature.[10]
Compound 7 is identified as Quercitrin, compared to the literature.[11]

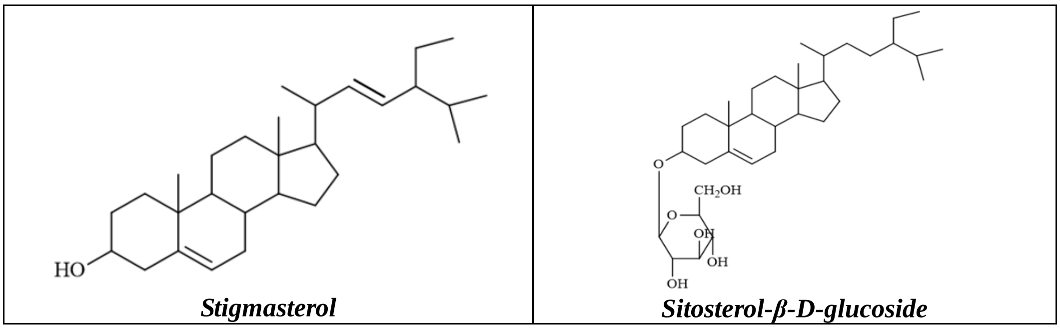
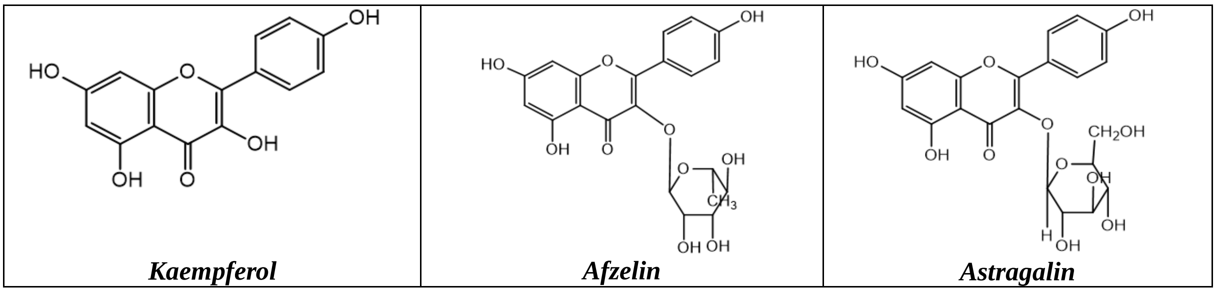


Fig. 1: Steroids isolated from Smilax anceps.



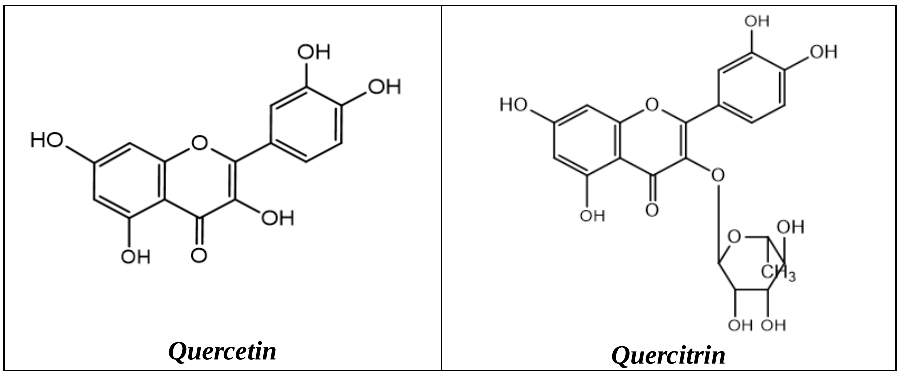


Fig. 2: Flavonoids isolated from Smilax.

Spectral data of isolated compounds

(1) Stigmasterol: white powder

^{1}H NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 0.71 (3H, s, H-29), 0.84 (3H, s, H-19), 0.87 (3H, s, H-18), 0.94 (1H, m, H-24), 0.94 (3H, s, H-27), 1.02 (3H, s, H-26), 1.03 (1H, m, H-14), 1.10 (1H, m, H-9), 1.18 (2H, m, H-28), 1.27 (4H, m, H-16, 17), 1.29 (1H, m, H-21), 1.37 (1H, m, H-20), 1.41 (2H, m, H-11), 1,42 (2H, m, H-15), 1.51 (2H, m, H-8), 1.67 (2H, m, H-7), 1.85 (2H, m, H-2), 1.86 (2H, m, H-1), 2.03 (2H, m, H-12), 2.29 (2H, m, H-4), 3.54 (1H, m, H-3), 5.35 (1H, m, H-6)

^{13}C NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 11.87 (C-29), 11.99 (C-18), 19.04 (C-27), 19.41 (C-26), 19.83 (C-19), 21.09 (C-11), 23.07 (C-21), 24.31 (C-15), 26.06 (C-28), 28.26 (C-16), 29,15 (C-7), 31.69 (C-2), 31.91 (C-8), 33.95 (C-25), 36.15 (C-20), 36.51 (C-10), 37.26 (C-1), 39.78 (C-12), 42.31 (C-4), 50.16 (C-24), 51.24 (C-9), 56.05 (C-17), 56.77 (C-14), 71.81 (C-3), 121.7 (C-6), 129.27 (C-23), 138.33 (C-22), 140.77 (C-5);

(2) Sitosterol-β-D-glucoside: white powder

^{1}H NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 0.65 (3H, s, H-18), 0.86 (3H, s, H-29), 0.87 (3H, s, H-19), 0.88 (3H, s, H-26), 0.90 (5H, m, H-9,14, 27), 0.94 (3H, m, H-17, 24), 0.98 (3H, d, H-21), 1.02 (2H, m, H-28), 1.06 (2H, m, H-22), 1.24 (1H, 3, H-23), 1.37 (1H, m, H-8), 1.38 (1H, m, H-11), 1.43 (2H, m, H-2), 1.53 (2H, m, H-15), 1.81 (2H, m, H-16), 1.88 (2H, m, H-7), 1.97 (1H, m, H-12), 1.99 (1H, m, H-20), 2.12 (1H, d, H-25), 2.42 (2H, t, H-4), 2.74 (2H, d, H-1), 3.96 (1H, m, H-3’), 4.09 (2H, m, H-

4’), 4.26 (2H, m, H-2’, 5’), 4.29 (1H, m, H-3), 4.54 (2H, m, H-6’), 5.04 (1H, m, H-1’), 5.34 (1H, s, H-6)
^{13}C NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 12.89 (C-18), 13.80 (C-29), 19.90 (C-21), 20.10 (C-21), 20.10 (C-26), 20.30 (C-27), 20.90 (C-19), 22.20 (C-11), 23.00 (C-28), 24.30 (C-15), 25.40 (C-23), 27.27 (C-16), 30.30 (C-25), 31.16 (C-2), 32.96 (C-8), 33.09 (C-7), 35.10 (C-22), 37.30 (C-20), 37.80 (C-10), 38.30 (C-1), 40.24 (C-12), 40.86 (C-4), 43.39 (C-13), 46.94 (C-24), 51.25 (C-9), 57.14 (C-17), 57.15 (C-14), 63.70 (C-6’), 72.05 (C-2’), 76.20 (C-4’), 79.39 (C-3, 3’), 79.59 (C-5’), 103.08 (C-1’), 122.80 (C-6), 141.80 (C-5)

(3) Kaempferol: yellow crystals
^{1}H NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 6,137 (1H, d, 2,2 Hz, H-6), 6.285 (1H, d, 2,2 Hz, H-8), 7.739 (2H, d, 8.0 Hz, H-2’, 6’), 7.759 (2H, d, 8.0 Hz, H-3’, 5’)
^{13}C NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 95.781 (C-8), 103.655 (C-6), 105.046 (C-10), 116.720 (C-3’, 5’), 122.888 (C-1’), 131,965 (C-2’, 6’), 136.121 (C-3), 158.968 (C-2), 159.019 (C-9), 161,840 (C-7), 163.167 (C-5), 168,620 (C-4’), 179,362 (C-4)

(4) Afzelin: yellow crystals
^{1}H NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 0.920 (d, H-6”), 3.302 (m, H-5”), 5.362 (d, H-2”, 3”, 4”), 6.137 (sd, 2.2 Hz, H-8), 6.285 (sd, 2.2 Hz, H-6), 6.935 (d, 8.0 Hz), H-3’, 5’), 7.739 (d, 8.0 Hz, H-2’, 6’)
^{13}C NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 17.793 (C-6”), 72.087 (C-2”), 72.136 (C-3”), 72.313 (C-4”), 72.406 (C-5”), 95.781 (C-8), 103.655 (C-6, C-1”), 105.046 (C-10), 116.720 (C-3’, 5’), 122.888 (C-1’), 131,121 (C-2’, 6’), 136.121 (C-3), 158.968 (C-2), 159.019 (C-9), 161.840 (C-7), 163.167 (C-5), 168.620 (C-4’), 179.362 (C-4)

(5) Astragalin: yellow crystals
^{1}H NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 3.318 (H-4”), 3.333 (H-3”), 3.598 (H-5”), 3.708 (H-6”), 3.711 (H-2”), 5.362 (H-1”), 6.137 (sd, 2.2 Hz), 6.290 (sd, 2.2 Hz), 6.935 (d, 8.0 Hz), 7.739 (d, 8.0 Hz)
^{13}C NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 62.301 (C-6”), 71.500 (C-4”), 75.100 (C-2”), 76.900 (C-3”), 81.600 (C-5”), 95.781 (C-8), 101.22 (C-6), 103.655 (C-1”), 105.048 (C-10), 116.720 (C-3’, 5’), 122.866 (C-1’), 131.121 (C-3), 158.968 (C-2), 158.999 (C-9), 161.620 (C-4’), 163.167 (C-5), 163.167 (C-5), 165.000 (C-7), 179.362 (C-4)

(6) Quercetin: yellow crystals
^{1}H NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 6.20 (s, H-6), 6.39 (s, H-8), 6.91 (s, H-5’), 7.31 (d, H-6’), 7.32 (d, H-2’)
^{13}C NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 94.700 (C-8), 99.800 (C-6), 105.800 (C-10), 116.400 (C-5’), 117.000 (C-2’), 122.900 (C-1’), 129.000 (C-6’), 136.300 (C-3), 146.500 (C-3’), 149.800 (C4’), 158.600 (C-9), 159.400 (C-2), 163.100 (C-5), 165.900 (C-7), 179.700 (C-4)

(7) Quercitrin: yellow crystals
^{1}H NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 0.95 (d, H-6”’), 3.37 (t, H-4”’), 3.44 (m, H-5”’), 3.75 (d, H-3”’), 4.23 (s, H-2”’), 5.35 (d, H-1”’), 6.20 (s, H-6), 6.39 (s, H-8), 6.91 (d, H-5’), 7.31 (d, H-6’), 7.32 (s, H-2’)
^{13}C NMR (400 Mhz in $CDCl_3$): □$_{ppm}$ = 17.70 (C-6”’), 71.90 (C-2”’), 72.10 (C-4”’, 3”’), 73.30 (C-5”’), 94.70 (C-8), 99.90 (C-6), 103.60 (C-1”’), 105.90 (C-10), 116.50 (C-5’), 117.00 (C-2’), 122.90 (C-1’), 129.00 (C6’), 136.30 (C-3), 146.50 (C3’), 149.80 (C-4’), 158.60 (C-9), 159.40 (C-2), 163.10 (C-5), 165.90 (C-7), 179.70 (C-4).

The molecules isolated during this study are biologically active. This would partly explain the traditional use as an anti-inflammatory, an antioxidant and an antibacterial.

Stigmasterol is known for its antioxidant and anti-inflammatory properties.[12]

Sitosterol-β-D-glucoside has analgesic properties and is an excellent compound to protect from cancer.[13]

Kaempferol has an anti-inflammatory and anticarcinogenic effect.[14]

Afzelin is an anti-inflammatory and antibacterial agent.[15]

Astragalin has anti-inflammatory and antioxidant properties.[16]

Quercetin, a natural flavonoid, has neuroprotective properties and is an excellent antioxidant.[17]

Quercitrin is an antioxidant and antileishmanial agent.[18]

3- CONCLUSION

The results obtained from our research contribute to highlight the richness of Malagasy biodiversity which includes many species. This paper constitutes (Element de prevue scientifique) a scientific proof of the empirical use of the plant as anti-inflammatory, antioxidant, neuroprotective, analgesic. It is the first time that these secondary metabolites are reported for this species. We contribute to the chemotaxonomy of the genus *Smilax*.

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