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FLAVONOIDS AND GALLIC ACID FROM LEAVES OF SANTALOIDES AFZELII (CONNARACEAE)

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ABSTRACT

Fractionation of the ethyl acetate fraction of the ethanolic extract of the dried powdered leaves of *Santaloides afzelii* (Connaraceae) on silica gel column chromatography afforded gallic acid and two flavonoids glycosides identified as quercetin-3-*O*-rhamnoside and myricetin-3-*O*-rhamnoside. Their structures were elucidated by ^{1}H and ^{13}C-NMR data and UV data. It is the first time that these compounds are reported in the plant.

Keywords: *Santaloides afzelii*, Connaraceae, Polyphenol, Flavonoids glycosides, Gallic acid

INTRODUCTION

Santaloides Afzelii (R.Br. ex Planch) G. Schellenb belongs to the plant family Connaraceae. It is a scandent to lianous shrub or small tree, widely dispersed in tropical Africa and used in ethnomedicine for the treatment of diverse ailments[1]. The Connaraceae family consists of about 20 genera and 350 species of tanniferous tropical trees and shrubs[2]. The leaves in this family are alternate, without stipules and pinnate compounds. The plants of this family possess analgesic and anti-inflammatory activities[3]. The aqueous leaves extract of *Byrsocarpus coccineus* (Connaraceae) contains flavonoids glycosides and may be a potential remedy for the treatment of certain central nervous system disorders in human[4-5].

Polyphenols compounds, including anthocyanins, flavonols, and phenolic acids, are among the most bioactive natural molecules found in the plants because of their antioxidant activities[6]. The risk of prostate cancer and pancreatic cancer may decrease at higher dietary flavonoids intakes[7-8]. Gallic acid can be regarded as a promising candidate for development as a topical anti-HSV-2 agent and inhibited the growth of lung cancer cells[9-10].

In tropical countries, particularly in Côte d'Ivoire, *Santaloides Afzelii* is often used in traditional medicine by villagers[11]. On the other hand macerate of the leaves is used as a wash to stabilize household[12].

In the present paper, we report the isolation and identification of major phenolic compounds from leaves of *Santaloides Afzelii.* To our best knowledge, there are no previous reports on the chemical constituents of *Santaloides Afzelii* (Connaraceae) in the literature.

EXPERIMENTAL

General

Using liquid chromatography with UV photodiodearray detection (LC-UV) and post-column derivatization it is possible to get sure data on polyphenols. Further structural information is provided by the combination of HPLC (LC) with mass spectrometry (MS). The structures were established on the basis of one and two-dimensional NMR spectral experiments and ultraviolet (UV) spectrometry.

A Brucker Avance 400 spectrometer was used for ^{1}H and ^{13}C- NMR spectra recorded at 400 and 100 MHz, respectively. The spectra were recorded at 23°C using an external reference (TMS) in MeOH-d_4 or DMSO-d_6 in a sealed capillary tube placed inside the NMR cell. Chemical shifts are reported relative to Me_4Si for ^{1}H and ^{13}C. The reproducibility of ^{13}C NMR shift was about ±0.05 ppm. Chemical assignments were made using either DEPT 135, or HMBC or HSQC techniques or common chemical shift assignments rules. Flash column chromatography was performed on Macherey-Nagel Silica gel 60 (15-40µm). TLC plates (Macherey-Nagel, ALUGRAM® SILG/UV_{254}, 0,2mm silica gel 60Å) were visualized under UV light at 254nm and/or by dipping the TLC plates in a solution of phosphomolibdic acid (3g) in EtOH (100mL) followed by heating with a heat gum. ESI-MS was recorded on a Shimadzu GC MS-QP 2010 with electron-impact ionization (70 eV). HRMS in the positive ion mode was performed using a Q-TOF Ultima Global hybrid quadrupole time-of-flight instrument (Waters-Micromass).

High-Performance Liquid Chromatography (HPLC) Analysis

Analytic HPLC was performed using a RP-18 (5µm) Lichro CART® 150-4,6mm at 25°C. The binary elution system was composed with acetonitrile (solvent A) and 0.2% TFA/water (solvent B). Separations were performed at room temperature by solvent gradient elution: 10-20% B during 40 min, 20-30% B during 5 min, 30-40% B during 5 min, 40-45% B during 5 min and then return to the initial conditions (10% B) in 5 min to re-equilibrate the column. The flow rate for both analysis and washing cycles was 0.8 mL/min. The concentration of each sample was 0.1 mg/mL in methanol and detection wavelengths were 254, 280, 325 and 530 nm.

Plant material

The leaves of *Santaloides Afzelii* were collected in November 2009 at the beginning of the dry season from Korhogo in north of Côte d'Ivoire. A voucher sample was identified by Prof. Aké-Assi Laurent, Faculty of Science and Technology, Cocody-Abidjan University where a specimen was deposited. The collected plant materials were washed under running and shed dried.

Phytochemical screening

Phytochemical screening was performed to establish the type of secondary metabolites present in the plant. Air-dried leaves of *Santaloides Afzelii* were tested for the presence of flavonoids, anthraquinones, alkaloids, terpenoids and steroids, tannins and saponins using Harborne method[13]. It shows the presence of flavonoids, triterpenes, steroids, tannins and alkaloids.

Extraction procedure

The air-dried powdered sample (450 g) was exhaustively extracted with hexane at room temperature by constant stirring. The residue was extracted with 70% EtOH (3 x 500mL) at room temperature by constant stirring during 24 hours. After filtration on cotton then watmann paper, the extract was concentrated under reduced pressure at 40°C to afford a brown residue. The residue (20g) was suspended in water and partitioned successively with CH_2Cl_2 (3x 200mL) and AcOEt (3x 200mL). The obtained extracts were separately dehydrated with anhydrous sodium sulfate and evaporated under vacuum after filtration to give dichloromethane extract (1.82g) and ethyl acetate extract (5.10g).

Isolation and purification

The ethyl acetate fraction (5g) of *Santaloides Afzelii* leaves was subjected to column chromatography on silica gel 60 with solvents gradients CH_2Cl_2-AcOEt and AcOEt-MeOH to give 12 fractions (F_1-F_{12}). Fractions F_5 and F_8 were purified by flash column chromatography on silica gel 60, eluting with CH_2Cl_2-MeOH (10-1) to afford compound **1** (342mg) and compound **2** (219mg) respectively. The different fractions were checked by TLC and HPLC. Another aliquot of each compound was dissolved in CD_3OD or DMSO and analyzed by NMR for chemical structure determination.

Compound 1: Quercetin-3-*O*-rhamnoside ; Yellow powder; HPLC R_t 48.63 min ; UV vis λ_{max} 256.348 nm (methanol) ; HREI-MS (m/z) 448.0905; **$C_{21}H_{20}O_{11}$** (calcd 448.0903). ^{1}H NMR (400 MHz, DMSO-d6): 12.00 (1H, s, OH-5); 7.34 (1H, d, J = 2Hz, H-2'); 7.32 (1H, dd, J = 8.3Hz/2Hz, H-6'); 6.93 (1H, d, J = 8.3Hz, H-5'); 6.22 (1H, d, J = 2Hz, H-6); 6.39 (1H, d, J = 2Hz, H-8); 5.25 (1H, d, J = 1.2Hz, H-1''); 4.24 (1H, dd, J = 3.3Hz/1.2Hz, H-2''); 3.78 (1H, dd, J = 9.3Hz/3.3Hz, H-3''); 3.40 (1H, m, H-4''); 3.17 (1H, m, H-5''); 0.82 (3H, d, J = 6.1Hz, H-6''). ^{13}C NMR (100 MHz, DMSO-d6): 179.7 (C-4); 166.0 (C-7); 163.2 (C-5); 159.3 (C-2), 158.6 (C-9); 149.8 (C-4'); 146.4 (C-3'); 136.3 (C-3); 123.0 (C-6'); 122.9 (C-1'); 116.9 (C-2'); 116.4 (C-5'); 105.9 (C-10); 103.6 (C-1''); 99.8 (C-6); 94.7 (C-8). 73.3 (C-5''); 72.1 (C-3'' and C-4''); 71.9 (C-2''); 17.7 (C-6'').

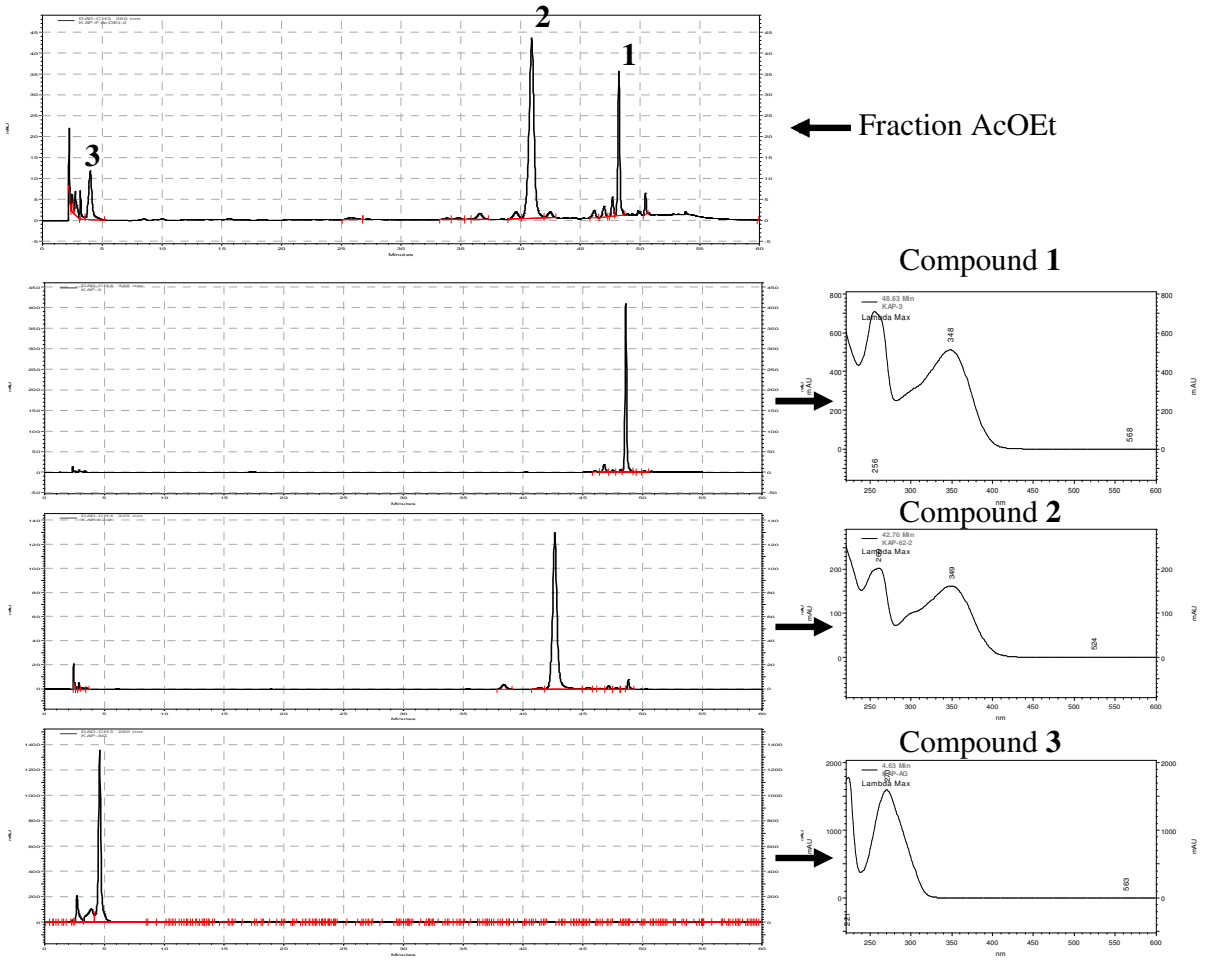

Fig.-1: HPLC and UV analysis of sample. HPLC column:Lichro CART® RP-18 (5µm)150x4.6mm; gradient elution: acetonitrile (solvent A) and 0.2% TFA/water (solvent B), detection wavelength: 280 nm. Flow-rate: 0.8mL/min.The ethyl acetate fraction of *Santaloides Afzelii* leaves was purified by flash chromatography on silica to afford 1 and 2 as crystalline compounds.

Compound 2: Myricetin-3-*O*-rhamnoside; Yellow powder; HPLC R_t 42.70 min ; UV vis λ_{max} 260.349 nm (methanol) ; The HREI-MS (m/z) 464.0851, **$C_{21}H_{20}O_{12}$** (calcd 464.0853). ^{1}H NMR (400 MHz, DMSO-d6): 12.68 (1H, s, OH-5); 6.38 (1H, d, J = 2Hz, H-6); 6.20 (1H, d, J = 2Hz, H-8); 6.90 (2H, d, J = 2Hz, H-2'/H-6'); 5.20 (1H, d, J = 1.8Hz, H-1''); 3.89 (1H, dd, J = 3.2Hz/1.8Hz, H-2''); 3.55 (1H, dd, J = 10.6Hz/3.2Hz, H-3''); 3.17 (1H, m, H-4''); 3.34 (1H, m, H-5''); 0.84 (3H, d, J = 6.1Hz, H-6''). ^{13}C NMR

(100 MHz, DMSO-d6): 177.7 (C-4); 164.0 (C-7); 161.2 (C-5); 157.4 (C-9); 156.4 (C-2); 145.7 (C-5' and C-3'); 136.4 (C-4'); 134.2 (C-3); 119.5 (C-1'); 107.8 (C-2'); 107.8 (C-6'); 103.9 (C-10); 101.9 (C-1''); 98.7 (C-8); 93.5 (CH-6); 71.7 (C-4''); 70.9 (C-5''); 70.7 (C-3''); 70.4 (C-2''); 17.9 (C-6'').

Compound 3: Gallic acid; HPLC R_t 4.52 min; UV vis λ_{max} 270 nm (methanol); The ESI-MS *m/z* 169 [M - H].

RESULTS AND DISCUSSION

The HPLC analysis (Figure 1) of the ethyl acetate fraction of the ethanolic extract of the dried powdered leaves of *Santaloides afzelii* indicated the presence of three (3) majors compounds **1**, **2** and **3**. A preliminary study of the UV spectra (Figure 1) of compounds **1**, **2** and **3** showed absorbance bands at 256/260, 348/349 271 nm respectively, characteristics of phenolic compounds[14].

Compound 1

The HREI-MS spectrum of compound **1** revealed a molecular ion peaks M^+ at m/z 448.0905 corresponding to the molecular formula $\mathbf{C_{21}H_{20}O_{11}}$ (calcd 448.09033)**.**

The ^{1}H-NMR spectrum of compound **1** showed an ABX spin coupling system at δ_{ppm} 7.34 (d, J = 2Hz), 7.32 (dd, J = 8.3Hz/2Hz) and 6.93 (d, J = 8Hz) assigned to H-2' , H-6' and H-5'. It also showed an AB spin coupling system of two protons at δ_{ppm} 6.39 (d, J = 2Hz) and 6.22 (d, J = 2Hz) assigned to H-8 and H-6[14]. The signal at δ 12.00 showed the presence of the proton of OH group only on carbon C-5. The signal of the anomeric proton of rhamnose at δ_{ppm} 5.25 showed a doublet with coupling constant J = 1.2 Hz, indicating α configuration. The ^{13}C NMR spectrum of compound **1** showed 21 resonances. The DEPT NMR experiment revealed 10 quaternary carbons and 11 primary or tertiary carbons. It showed six carbon signals of a sugar moiety at δ_{ppm} 103.56, 71.92, 72.12, 72.05, 73.27 and 17.67 assigned to C-1", C-2", C-3", C-4", C-5" and C-6" respectively. The position of rhamnose was also confirmed in HMBC spectrum by observation of a peak between δ_H 5.25 (H-1") and δ_C 136.25 (C-3).

Compound 2

The HREI-MS spectrum of compound **2** revealed a molecular ion peaks M^+ at m/z 464.0851 corresponding to the molecular formula $\mathbf{C_{21}H_{20}O_{12}}$ (calcd 464.08525).

The ^{1}H-NMR spectrum of compound **2** showed a system of two proton at δ_{ppm} 6.90 (d, J = 2Hz) corresponding to H-2' and H-6'**.** It also showed an AB spin coupling system of two protons at δ_{ppm} 6.38 (d, J = 2Hz) and 6.20 (d, J = 2Hz) attributed to H-8 and H-6. The signal at δ 12.68 showed the presence of the proton of OH group only on carbon C-5. The signal of the anomeric proton of rhamnose at δ_{ppm} 5.20 showed a doublet with coupling constant J = 1.8Hz, indicating α configuration. The ^{13}C NMR spectrum of compounds **2** showed 21 resonances. The DEPT NMR experiment revealed 11 quaternary carbons and 10 primary or tertiary carbons. It also showed six carbon signals of a sugar moiety which appeared at δ_{ppm} 101.86, 70.42, 70.73, 71.72, 70.91 and 17.88 assigned to C-1", C-2", C-3", C-4", C-5" and C-6" respectively. The position of rhamnose was also confirmed in HMBC spectrum by observation of a peak between δ_H 5.20 (H-1") and δ_C 134.20 (C-3).

The flavones and flavonols present two major absorption bands in the UV analysis in the ranges of 320-385 nm (Band I) and 250-285 nm (Band II)[15]. The substitution of the proton of hydroxyl (in position 3) of a flavonol leads to a hypsochrom effect on band I which shifted to 345 and 365 nm[16]. The UV spectra of compounds **1** and **2** showed low absorption bands which confirmed the position of rhamnose.

The comparison of the UV, H-NMR and EI-MS spectra data with reported values leads to the identification of compound **1** and **2** as quercetin 3-*O*-α-rhamnoside and myricetin 3-*O*-α-rhamnoside respectively[17-20].

Compound 3

The HPLC-MS-ESI analytical technique showed UV vis λ_{max} absorbance band at 270 nm (methanol) (Figure 1) and ESI-MS (negative mode) *m/z* 169 [M - H]. Compound **3** was identified as gallic acid by comparing its retention time, ESI-MS and UV data with standard or reported literature values[21-22].

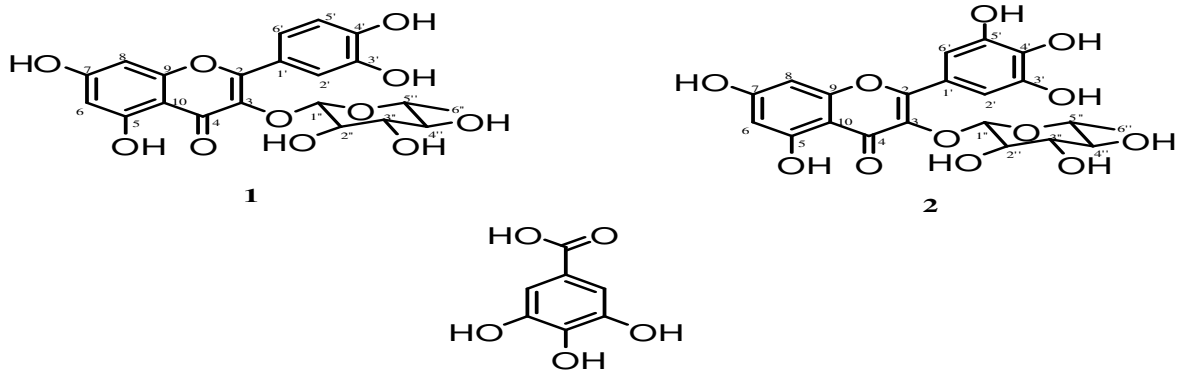

Fig.-2: Structure of compounds 1, 2 and 3 isolated from *Santaloides Afzelii* leaves

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