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Isolation of Taxifolin from Trichilia Emetica Whole Seeds

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Abstract

Taxifolin was isolated from the boiled water extract of *Trichilia emetica* whole seeds. The structure was determined by IR, ^{1}H and ^{13}C NMR and mass spectroscopic methods, as well as comparison of the data obtained with that reported in the literature.

Keywords: Trichilia emetica; Taxifolin; Meliaceae; Spectroscopy; Limonoids.

1. Introduction

Trichilia emetica Vahl (Also known as Cape, Natal or Woodland Mahogany) belongs to the Meliaceae (Mahogany family). They are evergreen tree reaching 20-35 m in height and are widely distributed in the tropics and the subtropical regions of Africa [1]. Phytochemical studies of the root and stem bark of this plant have led to the isolation of compounds with complex and interesting structures called limonoids (a tetranortriterpenoids) with common name *trichilin*, others are tannin and kurubasch aldehyde [2, 3, 4, 5, 6, 8]. The leaves, stem and root of *T. emetica* have been used in African folk medicine for the treatment of chronic wounds, fever, pneumonia, jaundice, gonorrhea, intestinal disorder such as hepatitis, and also to induce labour in women. Additionally, the seed oil is used in combination with *Cyathula natalensis* Sond to treat leprosy [2, 7, 8]. To the best of our knowledge, the isolation of Taxifolin from the whole seeds of *T. emetica* is been reported for the first time.

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2. *Materials and Methods*

Flash chromatography (Reveleris^{TM} Flash Chromatographic System) fitted with Reveleris® C18 column with silica flash cartridges of 18 g (Reveleris^{TM} SRC Cartridges); detection, ELSD (Evaporation Light Scattering Detection) and photodiode array detector (254-280 nm); mobile phase, linear gradient of methanol/water (containing 0.1% formic acid) at a flow rate of 18 mL/min was used for the isolation of the compound. Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) was performed on (silica gel 60 F_{254}, 25 Glass plates 20 × 20 cm, E. Merck, Germany). Thermo Instruments HPLC system mass spectrometer with electron spray ionization (ESI) source was used for recording of the mass spectra. Visualization of the compound was done using UV lamp UVL-14 EL hand held 220V 50Hz 4W 254nm white light by UVP. The IR spectrum (KBr) was recorded on a Perkin Elmer 100 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 400.13 and 100.62 MHz using tetramethylsilane (TMS) as an internal standard.

2.1. Preparation of the material

2.1.1. Collection of the material

*T. emetic*a seeds were collected from Kumasi, Ghana, in February 2013 and identified by a botanist Mr Martin A. Akoh of Kwame Nkruma University of Science and Technology, Kumasi, Ghana. A voucher specimen TBG-2014-1 was deposited at the herbarium of Treborth Botanical Garden Bangor, UK.

2.1.2. Extraction

T. emetica whole seeds 1.0 kg was reflux in water at 100^{o}C for 30 minute, filtered under gravity using Whatman No. 1 filter paper. The solvent was removed using rotary evaporator at 40^{o}C to give 72.14 g dark viscous residue. The residue was used as the crude extract for the experiment.

2.1.3. Purification

The crude extract 72 g was subjected to (450 × 95 mm) column containing 200g of silica gel (20-120 mesh) using the wet packing method in hexane. Subsequently, the column was eluted with a mixture of hexane/chloroform (100:0 and 50:50 V/V) and chloroform/methanol (100:0 to 0:100 V/V). Ninety six (96) fractions were collected, based on their TLC profiles were combined into five primary fractions A-E. All the combine fractions were dried down under vacuum at 40 ^{o}C. Fraction B was further purified by column chromatography and prep-TLC. Final purification was carried out by Flash system using 50% methanol in water to give taxifolin (5 mg).

3. Result and discussion

Taxifolin was obtained as yellow needles. It has a molecular formula of $C_{15}H_{12}O_{7}$ which was established on the basis of ESI-HRMS at *m/z* 305.0660$[M + H]^{+}$(Calcd for 305.0662) (Fig. 1). The IR spectrum of this compound has a broad band at 3369 cm^{-1} region corresponding to phenolic and alcoholic O-H stretching. Another band at

1638 cm^{-1} is due to carbonyl and aromatic C=C stretching. The DEPT spectrum (Fig. 2) showed fifteen carbon signals, which consist of seven methine and eight quaternary carbon. Also, the resonance for the keto group appeared at C-4 (δc 198.4).

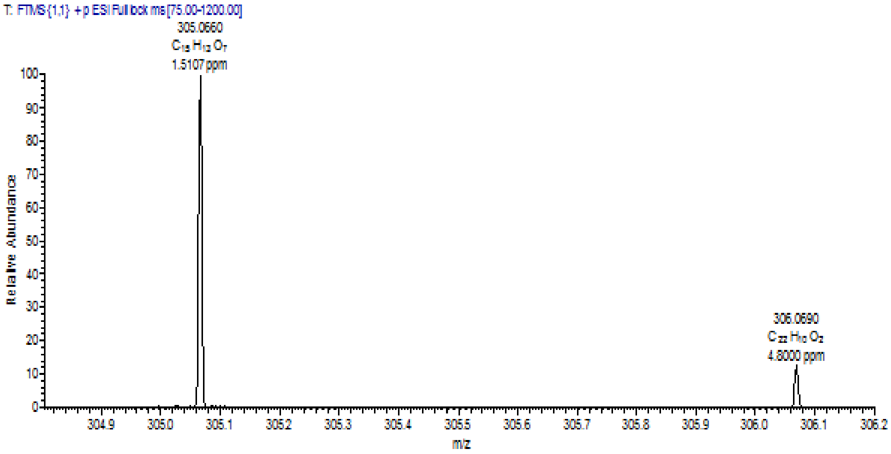


Figure 1: Mass spectrum of taxifolin

The ^{1}H NMR spectrum (Fig. 3) showed that the resonance of proton H-2 had been split by proton H-3 resulting in a doublet at δ_H 4.91 (d, 11.5 Hz), while proton H-3 was split by proton H-2 yielding a doublet at δ_H 4.51 (d, 11.5 Hz). These protons signals H-2 and H-3 were seen to couple to each other in COSY correlation (Fig. 4). The down field position of the C-2 chemical shifts (δ_C 85.1) observed in the DEPT spectrum and the appearance of a doublet at 4.91 (d, 11.1 Hz) attributed to position 2, indicate that the flavan moieties possessed the 2,3-trans configuration [9, 10, 11].

The proton spectrum also showed that H-2' was split by H-6' in a meta position resulting in a doublet at δ_H 6.98 (d, 1.3 Hz), proton H-5' was split by proton H-6' yielding a doublet at δ_H 6.81(d, 8.2 Hz) while proton H-6' was split by proton H-5' and H-2' resulting in a doublet of doublet at δ_H 6.87 (dd, 1.8, 8.2 Hz) attributed to ring B. The proton at H-6 and H-8 split each other in a Meta position and each yielding a doublet at δ_H 5.90 and 5.94 (d, 1.3 Hz, each) assigned to ring B.

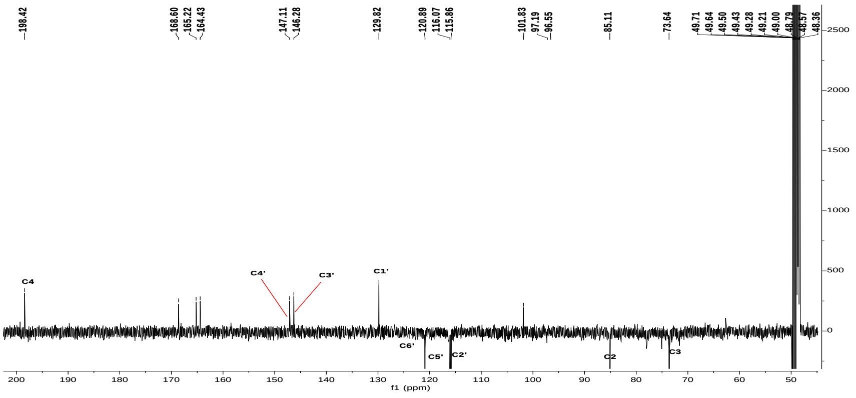


Figure 2: DEPT spectrum of Taxifolin in CD$_3$OD

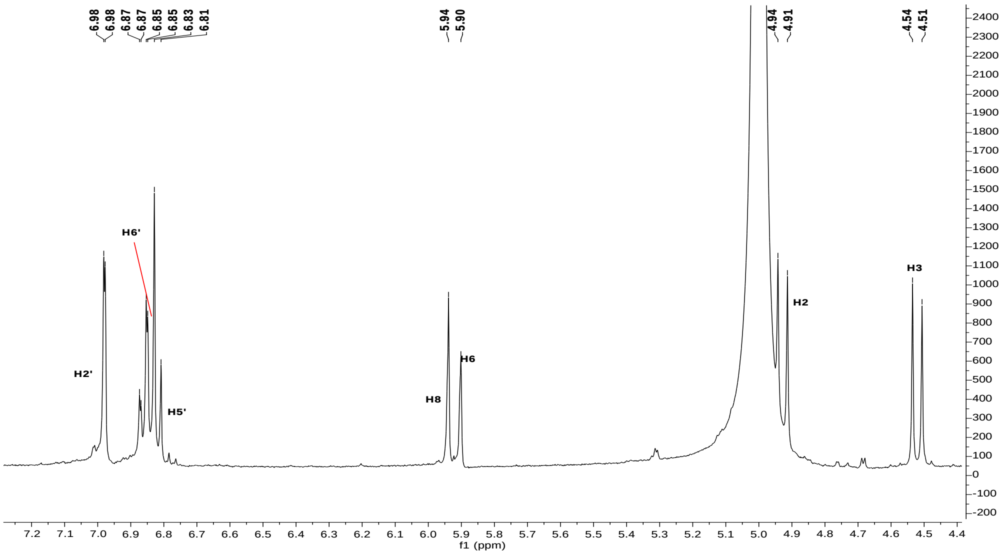


Figure 3: ^{1}H NMR spectrum of Taxifolin in CD_3OD

The structure was further confirmed by long-range heteronuclear multiple bond correlation (HMBC) as observed in (Fig. 4), where the ring C methine protons H-2 (δ_H 4.91) correlated with C-3, C-4, C-1', C-2'and C-6' (δc 116.1); and H-3 (δ_H 4.51) correlated with C-2, C-4, C-1' (δc 129.8). Ring B also showed correlations of H-2' to C-2, C-4', and C-6' (δc 116.1); H-5' to C-2, C-1' and C-3' (δc 144.9); and H-6' to C-2, C-1' and C-3' (δc 146.2). The ^{1}H and ^{13}C NMR value obtained in this study is comparable to literature value [14] (Table 1).

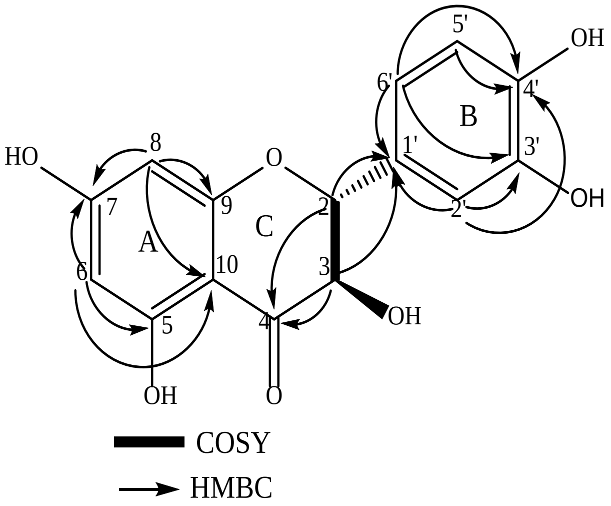
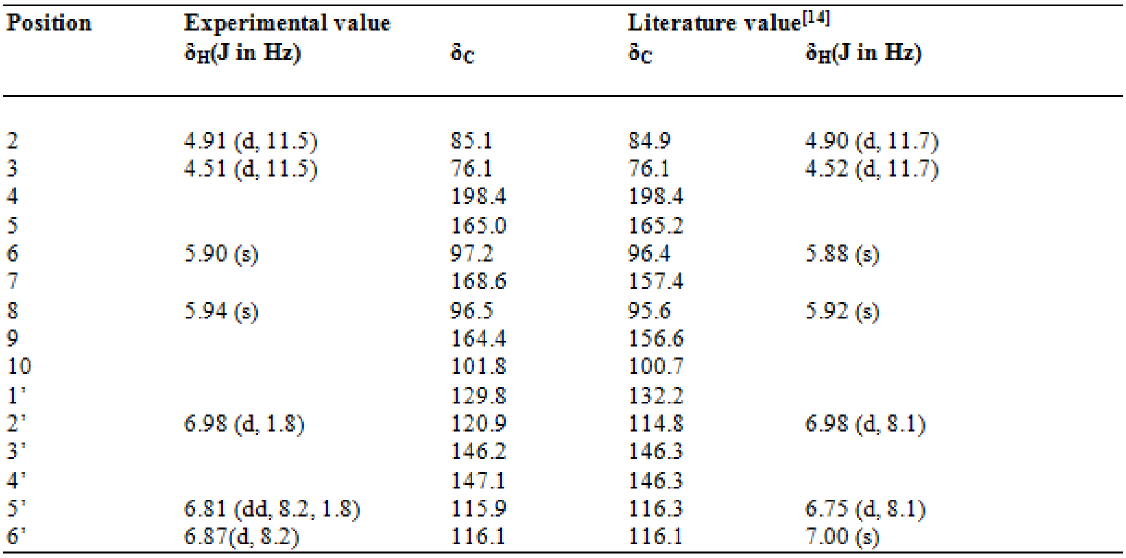


Figure 4: HMBC and COSY correlations of Taxifolin

This compound has been isolated from the stem of *Quercus acuta* Thunberg [12], whole plant and fruit of *Cayratia japonica* [13].

Table 1: ^{1}H and ^{13}C NMR data of Taxifolin in deuterated methanol (CD_3OD)



Position	Experimental value		Literature value[14]	
	δ_H(J in Hz)	δ_C	δ_C	δ_H(J in Hz)
2	4.91 (d, 11.5)	85.1	84.9	4.90 (d, 11.7)
3	4.51 (d, 11.5)	76.1	76.1	4.52 (d, 11.7)
4		198.4	198.4	
5		165.0	165.2	
6	5.90 (s)	97.2	96.4	5.88 (s)
7		168.6	157.4	
8	5.94 (s)	96.5	95.6	5.92 (s)
9		164.4	156.6	
10		101.8	100.7	
1'		129.8	132.2	
2'	6.98 (d, 1.8)	120.9	114.8	6.98 (d, 8.1)
3'		146.2	146.3	
4'		147.1	146.3	
5'	6.81 (dd, 8.2, 1.8)	115.9	116.3	6.75 (d, 8.1)
6'	6.87(d, 8.2)	116.1	116.1	7.00 (s)

Source: Wang et al., 2012 [14]

4. Conclusion

This is the first report of the isolation and identification of taxifolin from *T. emetica*. The work was carried out by using different chromatographic separation and spectroscopic techniques.

Acknowledgement

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