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A New Flavonoid Glycoside from Trichilia Emetica

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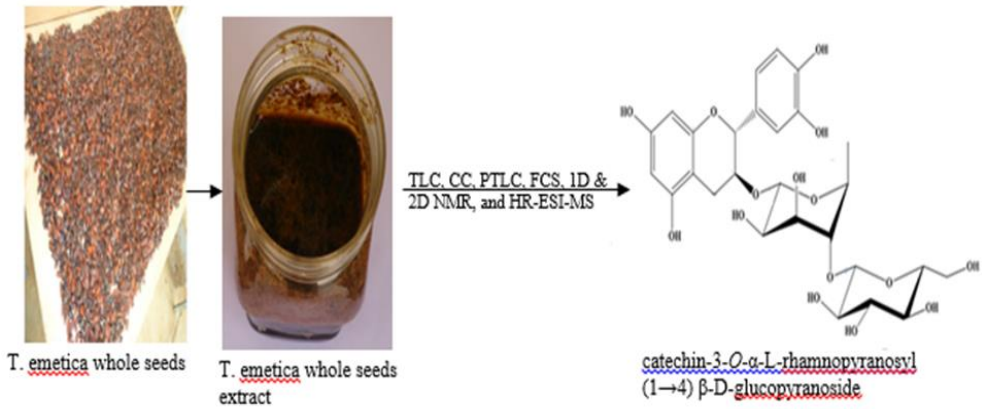
ABSTRACT

A new compound, catechin-3-*O*-α-L-rhamnopyranosyl (1→4) β-D-glucopyranoside, was isolated from the ethylacetate soluble fraction of the aqueous extract of the whole seeds of Trichiliaemetica(Family: Meliaceae). The structure of this compound was established on the basis of nuclear magnetic resonance (NMR), Infrared (IR) spectroscopy and ultraviolet-visible (UV-vis) spectrophotometry. The compound was alsosubjected to antimicrobial activity and brine shrimp lethality bioassay. The pure compound showed either no or reduced activity against the tested microorganisms, it alsoshowed no cytotoxicity.

GRAPHICAL ABSTRACT



Introduction

Flavonoids are large group of biologically active ingredients (above 5000) which have been identified in different plantspecies.[1] They are known to perform several important functions in plants. It is the most important pigment for producing colours needed to attract pollinating animals. It is also required for UV filtration, nitrogen fixation, cell cycle inhibition, and as chemical messengers.[2] Studies have shown that flavonoids (specifically catechins) are "the most common group of polyphenolic compounds in the human diet, and because of their possession of a wide spectrum of biochemical activities such as antimicrobial, antioxidant, anti-inflammatory, antichistosomal, antiplasmodial, and antitrypanosomalanticarcinogenic, and in the prevention of cardiovascular diseases have been a subject of comprehensive studies in the recent years.[3-9] Many groups have isolated and identified the structures of flavonoids with anticancer, antifungal, antiviral and antibacterial activity.[10]

Trichiliaemetica belongs to Mahogany family that is comprised of over 50 genera and about 600 plant species of trees, native to tropical and subtropical regions. *T. emetica*is is widely used in traditional Africa medicine for diverse therapeutic applications, such as the treatment of hernia, gastric ulcer, haemorrhoids and teniasis.[11-12]Previous qualitative phytochemical studies on *T. emetica* have showed a substantive amount of limonoids,sesquiterpenoid and flavonoids.[12-17] This study reports, for the first time, the isolation and structural elucidation of Catechin-3-*O*-α-L-rhamnopyranosyl (1→4) β-D-glucopyranoside from this plant species.

Materials and Methods

The ^{1}H and ^{13}C NMR spectra were recorded on a BrukerAvance (400 MHz) spectrometer withTMS (Tetramethylsilane) as an internal standard. A Thermo Instruments HPLC system mass spectrometer with an electrospray ionization (ESI) source was used for recording of the mass and UV spectra. Column chromatography (CC) was

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performed on Fluorochem silica gel (60 Å). Thin layer chromatography (TLC) and Preparative thin layer chromatography (PTLC) was conducted on precoated E. Merck TLC silica gel 60 F254 glass plates, and visualization of the compound was done using UV lamp UVL-14 EL hand held 220 V 50 Hz 4W 254 nm white light by UVP. The optical rotations were measured using ADP 440+ polarimeter. A Thermo Scientific 1300 gas chromatograph was equipped with a TRIPLUS RSH autosampler and an ITQ 900 MS detector was used for the analysis of the silylated sugars. The conditions of the GC were as follows: column, Rxi®-ms (length: 30 m, ID: 0.25 mm, df: 0.25 µm). Inlet temperature 250 ºC, split flow 30 ml/min, split ratio 20 and carrier flow rate 1.5 ml/min. The temperature program starts at 172 ºC, held for 1 min, then increased at 10 ºC/min up to 210 ºC, held for 1 min then 20 ºC/min up to 220 ºC, held for 1 min, then a final ramp of 10 ºC/min to 280 ºC and held for 1.5 min. Total run time is about 15 min.

Extraction and isolation

Air-dried *T. emetica* whole seeds (1.0 kg) were boiled in water under reflux for 20 minutes at 100 °C. The extract was concentrated and suspended in water, acidified with 2M HCl to PH 2 and successively partitioned with CHCl3 and EtOAc, respectively. The solvents were removed to give the respective crude fraction 31.02 and 26.50 g. The residual water yielded 11.93 g on concentration. The $CHCl_3$ and H_2O fractions gave poor TLC profiles (Hexane-EtOAc-MeOH 3:2:1 and EtOAc-MeOH-H_2O 5:3:1) which were not further investigated. The EtOAc fraction was subjected to silica gel column chromatography using 100% hexane and followed by $CHCl_3$ with increasing amount of MeOH to yield fractions D1 (1.32 g), D2 (1.04 g), D3 (4.74 g), D4 (4.22 g), D5 (5.90 g) and D6 (3.20 g), respectively. Repeated column chromatography of Fraction D5, followed by Prep-TLC (Hexane-EtOAc-MeOH 5: 3: 1), was finally purified with Flash chromatographic system (FC) with linear gradient of methanol/water (containing 0.1% formic acid) at a flow rate of 18 mL/min afforded the new compound, Catechin-3-*O*-α-L-rhamnopyranosyl (1→4) β-D-glucopyranoside.

Acid hydrolysis

Sample (2.5 mg) was dissolved in methanol (10 ml) and 10 ml of 5% H_2SO_4 was added and refluxed for 6 hours. On cooling, the methanol was evaporated under vacuum and the H_2O washed with EtOAc (2 × 5 ml). The aqueous portion was concentrated and compared to standard sugars using the TLC systems (CH_2Cl_2/MeOH/H_2O, 17:6:1) while the EtOAc portion contained the aglycon.[18-19] The sugar was identified as D-glucose andrhamnose.

For further confirmation of the sugar, the method described by Metuteaet al.[20] with modification was adopted. The H_2O portion was evaporated to dryness under the reduced pressure at 40 ^{O}C. The dried H_2O extract was silylated by reacting 0.5 mg of the extract with $NH_2OH.HCl$ (0.1 ml) in dry pyridine (0.1 ml) and heated at 60 ^{O}C for 40 minutes. The mixture was allowed to cool and BSTFA: TMCS (99:1, 0.1 ml) was added and allowed to react at room temperature for 30 minutes. Hexane (0.5 ml) and H_2O (0.3 ml) was added and vortexed for 1 minute. The silylated product was then analysed by GC. The silylated derivatives of sugars standards (glucose, galactose, mannose and rhamnose) prepared in the same manner were used as references.

Bioassays

The antimicrobial activity of the pure compound was determined by the disc diffusion method using the method described earlier.[21]Pure compound 1 mg was dissolved in 1 ml of chloroform and applied to sterile filter paper disc at a concentration of 500 µg/disc. Kanamycin disc (50 µg/disc) was used as a reference standard.

This compound was also screened for cytotoxic activity using brine shrimp lethality assay.[22, 23] In this experiment, pure compound (500 µg) was dissolved in DMSO 0.5 ml and the solution was applied against Artemiasalima for 24 hours in a simplified in vivo assay. Vincristine sulphate was used as a positive control.

Result and Discussion

The compound(23 mg)was obtained as brown needles which was decomposed before melting, with optical rotation $[\alpha]_D^{24.4}$ -9.34 (MeOH: c 0.33). The HR-ESI-MS gave a molecular ion at *m/z* 599.1967 $[M + H]^+$(Calcd for 599.1977) consistent with a molecular formula $C_{27}H_{34}O_{15}$.

The positive ESI-MS gave a fragment at m/z 437 ([M+H-162]$^+$ and 291 [M+H-162-146]$^+$ indicating the presence of a pentose unit and a terminal hexose (**Fig. 1**).The IR spectrum showed a band at 3368, 2933, 1520, 1141, 1102 and 818 cm^{-1}. UV spectrum (MeOH, _max, nm): 241, 273.

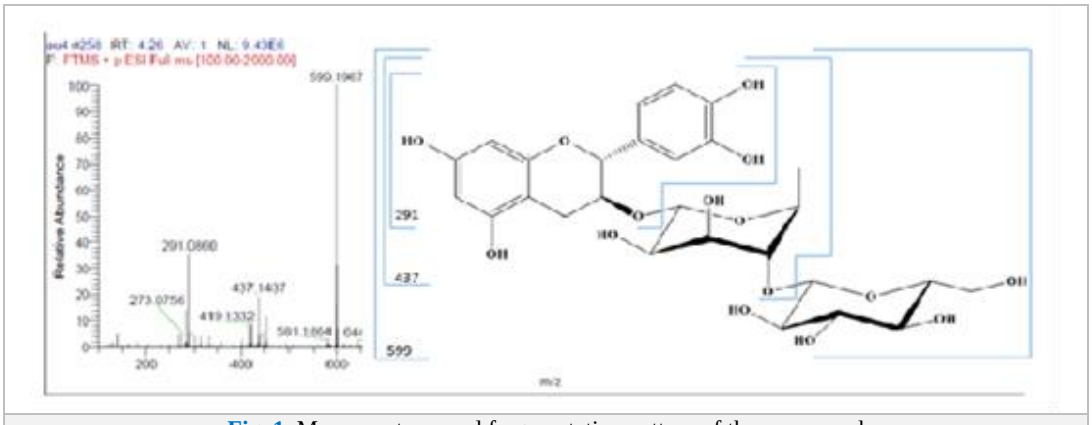

Fig. 1. Mass spectrum and fragmentation pattern of the compound

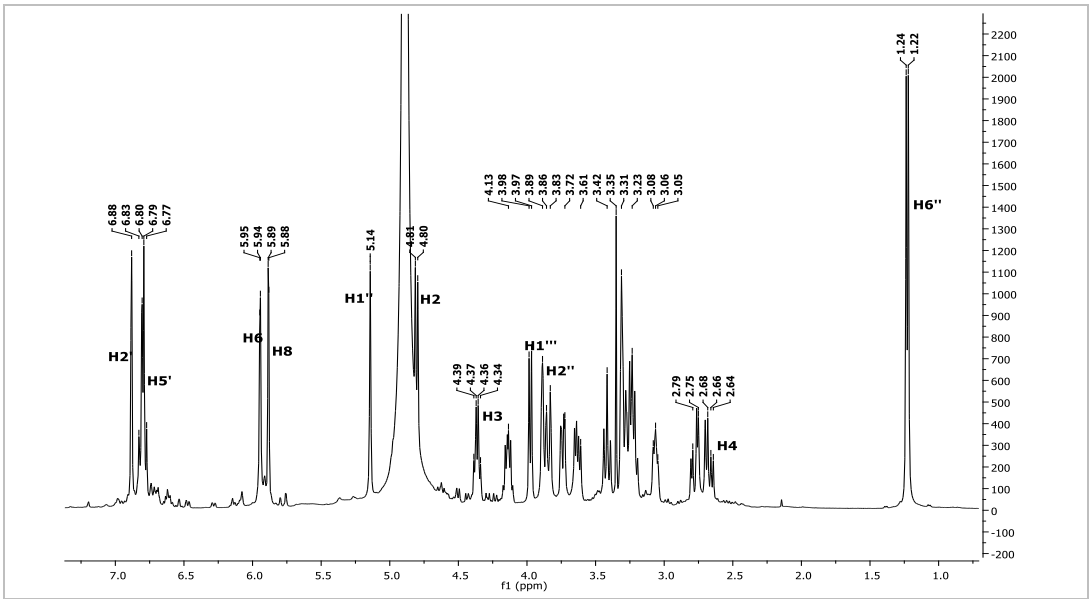

Fig. 2. ^{1}H NMR spectrum of the compound

The ^{1}H NMR spectrum (**Fig. 2**) shows two aromatic doublets at δ_H5.88, 5.94 (each, d, 2.0 Hz) assignable to the A-ring and three aromatic proton signals at δ_H6.88 (d, 1.7 Hz), 6.77 (d, 8.1 Hz), and 6.80 (dd, 1.7, 8.1 Hz) attributable to a 1,3,4-trisubstitued B-ring. Additionally, the spectrum showed a singlet at δ_H 5.14 and methyl protons at δ_H 1.22 (d, 6.2 Hz, H-6'') indicative of theanomeric proton (H-1'') and the methyl group of *α-L*-rhamnose; and another anomeric proton at δ_H 3.97 (d, 7.5 Hz, H-1''') assignable to *β-D*-glucopyranose as judged by the coupling constant. Both are linked via an *O*-glycosidic bond. On acid hydrolysis, the compound gave D-glucose and L-rhamnose. The COSY spectrum (**Fig. 3**) displayed correlations between δ_H 4.80 (H-2) and 4.34 (H-3), and also between δ_H 4.34 (H-3) and δ_H2.64 and δ_H2.75 as assigned to position H-4.

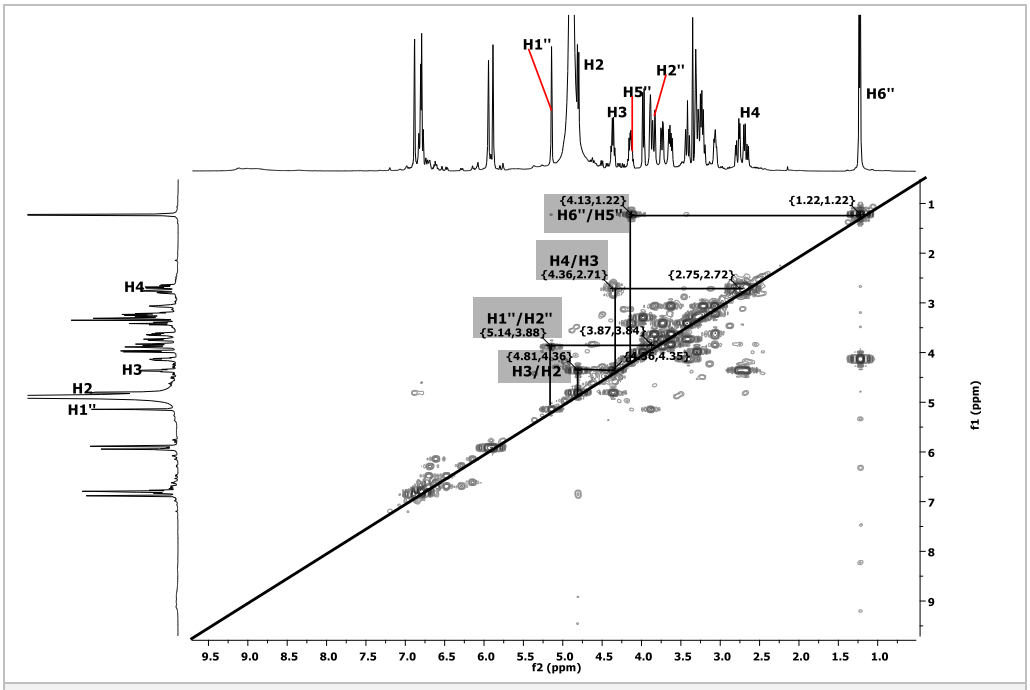

Fig. 3. COSY spectrum of the compound

The DEPT spectrum (**Fig. 4**) resolved twenty seven carbon signals, corresponding to one methyl, two methylene, seventeen methane and seven quaternary.

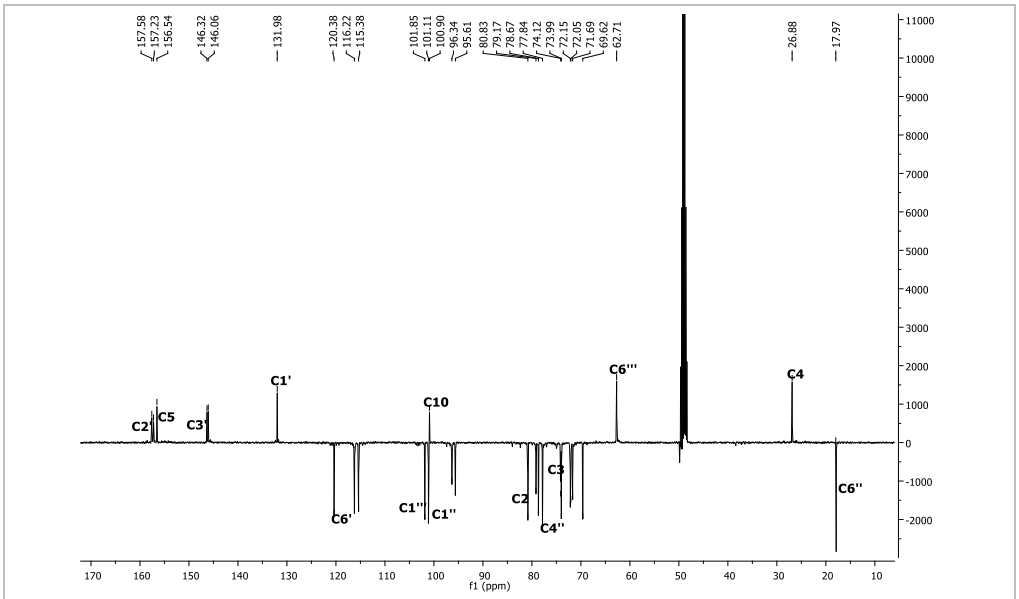

Fig. 4. DEPT spectrum of the compound

The anomeric carbons of glucose and rhamnose appeared at δ_C 101.11 (C-1'') and 101.85 (C-1''') respectively. Other signals for sugars appeared at δ_C 72.05 (C-2''), 72.15 (C-3''), 79.17 (C-4''), 69.62 (C-5''), 17.97 (C-6'') for rhamnose and 73.99 (C-2'''), 77.64 (C-3'''), 71.69 (C-4'''), 78.67 (5''') and 62.71 (C-6''') for glucose moiety. The NMR data of this compound is shown in Table 1.

Table 1. ^{13}C and ^{1}H NMR data of the compound

Atom No.	AU 8 CD30D δc (m)	AU 8 CD3OD δ_H (*J* in Hz)	HMBC [H→C]
2	80.8	4.80 (d, 6.7)	C-3, C-4, C-9, C-1', C-2', C-6'
3	74.1	4.34 (m)	C-2, C-4, C-10, C-1', C-1''
4	26.8	2.64 (dd, 5.2, 16.3)	C-2, C-3, C-5, C-10, C-1''
		2.75 (dd, 7.2, 16.3)	
5	156.5		
6	96.3	5.94 (d, 2.0)	C-5, C-7, C-8, C-10
7	157.2		
8	95.6	5.88 (d, 2.0)	C-5, C-7, C-8, C-9, C-10
9	157.6		
10	100.9		
1'	132.0		
2'	115.4	6.88 (d, 1.7)	C-2, C-1', H-3', C-6'
3'	146.3		
4'	146.1		
5'	116.2	6.77 (d, 8.1)	C-2, C-1', C-2', C-4', C-6'
6'	120.4	6.80 (dd, 1.7, 8.1)	C-2, C-1', C-2', C-3'
Rhamnose 1''			
	101.1	5.14 (br s)	C-3, C-3'', C-4'', C-5''
2''	72.1	3.89 (m)	
3''	72.2	3.72 (m)	
4''	79.2	3.42 (m)	
5''	69.6	4.13 (m)	
6''	17.9	1.22 d (6.21)	
Glucose 1'''			
	101.9	3.97 (d, 7.4)	C-3'', C-4'', 5'', 5'''
2'''	73.9	3.35 (m)	
3'''	77.6	3.31 (m)	
4'''	71.7	3.23 (m)	
5'''	78.7	3.05 (m)	
6'''	62.7	3.61 (dd, 6.1, 11.9)	
		3.83 (dd, 1.7, 11.9)	

The GC analysis of the TMS derivatives of this compound hydrolysates showed that this compound contains D-glucose and L-rhamnose. The obtained values for sugars were comparable to their reported values except for a 6.1 ppm downfield shift of C-4'' due to interglycosidic bond between C-4'' and C-1''' that established a 1→4 linkage between rhamnose and glucose. The ^{13}C spectral data were assigned through the assistance of the HSQC spectrum (**Fig. 5**), where the carbon resonances of the A-ring at δ_C 95.81 (C-6) and δ_C 96.54 (C-8) correlated with meta-coupled (1.98 Hz) protons at δ_H 5.94 (H-6) and δ_H 5.88 (H-8), thus showing that the C-rhamnosylation did not occur in the A-ring. In the C-ring, the HSQC assignment was more important in the C-2, C-3 and the two sugar units. The C-2 signal of the C-ring appeared at δ_C 81.03 and it coupled with the proton doublet at δ_H 4.80 (6.7 Hz) assigned to position H-2. The down field position of the C-2 chemical shifts (δ_C 80.80) observed in the DEPT spectrum and the appearance of a doublet at 4.80 (d, 6.7 Hz) attributed to position 2, indicating that the flavan moieties possessed the 2,3-trans configuration.[24-27] The attachment of the sugar units at C-3 is evident from the downfield shift 5.8 ppm of the C-3 signal to δ_C 74.1 that was coupled with the proton multiplet at (δ_H4.34) assigned to the proton at C-3 of the C-ring.

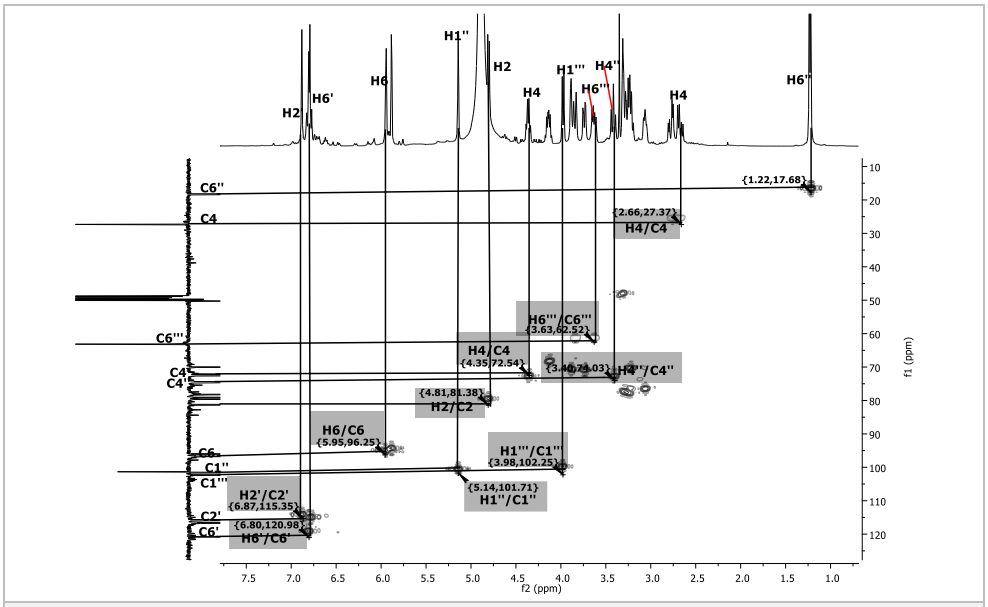

Fig. 5. HSQC spectrum of the compound

This attachment was confirmed by HMBC spectrum (**Fig. 6 and 7**), as correlations from H-1''' (5.14) to C-4'' (δ_C 79.17) and H-1'' (3.99) to C-3 (δ_C 76.22) were observed confirming the above mentioned link. Based on this evidences, the structure of the compound was elucidated as catechin-3-*O*-α-L-rhamnopyranosyl (1→4) *β-D*-glucopyranoside. The isolation and characterization of this compound has been reported for the first time.

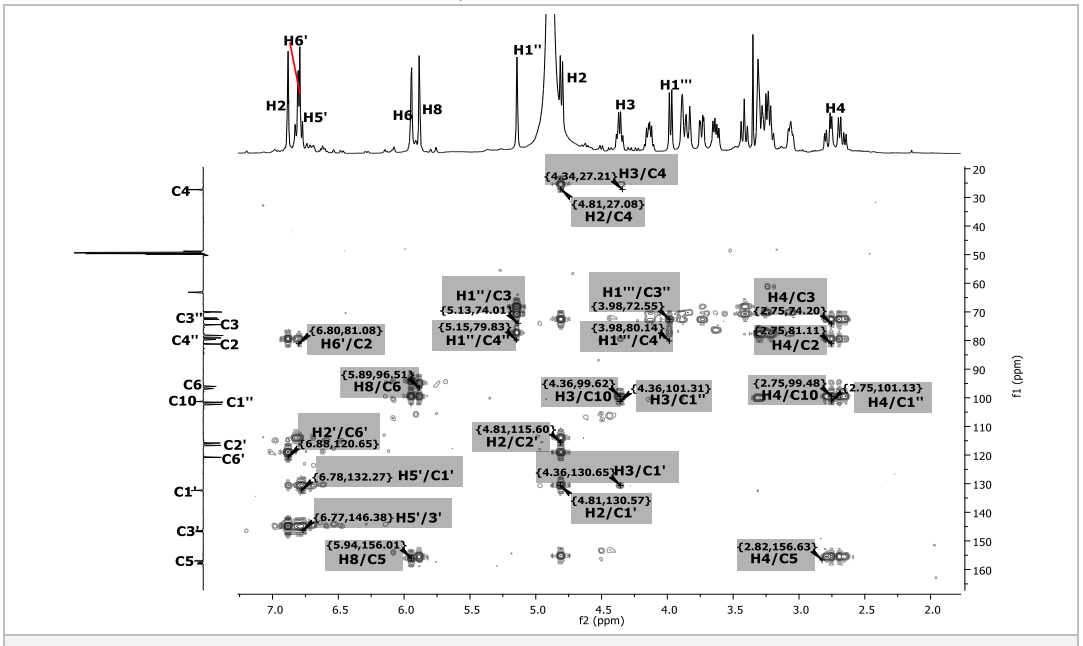

Fig. 6. HMBC spectrum of the compound

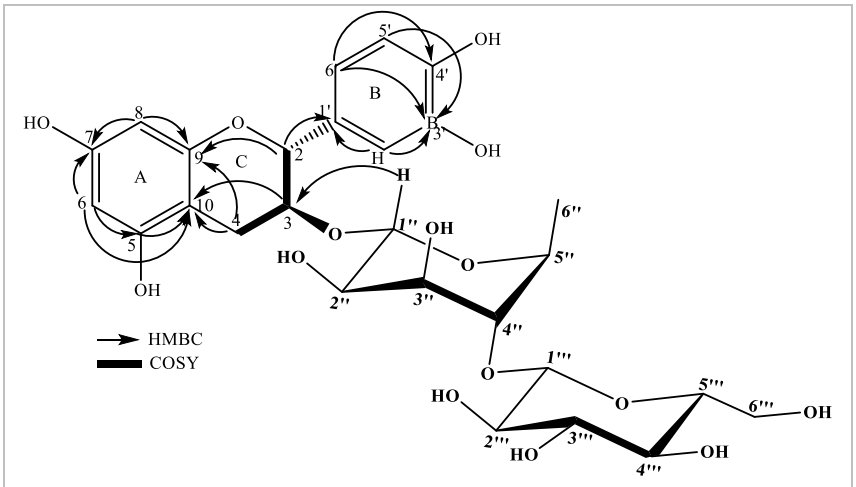

Fig. 7. HMBC and COSY correlations of the compound

Acid hydrolysis

The retention times of standards were as follows: rhamnose; 3.99 min and D-glucose; 5.49 min which is comparable to the result obtained.

Bioassays

The pure compound showed either no or reduced activity against the tested microorganisms. Similarly, the reference substance exhibited cytotoxic activity and the LC_{50} obtained from the bestfit line slope was 0.3 µg/ml while thecompoundshowed no cytotoxicity.

Conclusion

Anew catechin glycoside was isolated from T. emetica for the first time. The antimicrobial and cytotoxic activities showed that this compound have reduced or no activity against these species. The present result may provide more information about flavonoids profiles of this plant species.

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Disclosure statement

No conflict of interest in this report.

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