FLAVONOL AND ITS GLYCOSIDE FROM THE STEM OF *Trichilia emetica*

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Abstract: The powdered stem bark of *Trichilia emetica* (Family: Meliaceae) was extracted with methanol. The resulting methanol extract was dissolved in water and successively partitioned against equal volume of chloroform and ethyl acetate. The ethyl acetate fraction was subjected to silica gel column chromatography and eluted with chloroform and chloroform: methanol in various ratios, with further re-chromatography of some eluates and final purification with flash chromatographic system with linear gradient of methanol/water (containing 0.1% formic acid) at a flow rate of 18 mL/min. Two pure compounds were isolated, and was structurally identified on the basis of 1D- and 2D-NMR experiments, and MS data analyses. Also, the data obtained were directly compared to those reported in the literature. It was concluded that the compounds isolated were quercetin and quercetin-3-*O*-*β*-D-glucopyranoside.

Keywords: Flavonol, quercetin, quercetin-3-*O*-*β*-D-glucopyranoside, *Trichilia emetica*, column chromatography

Introduction

Quercetin is a plant flavonol belonging to a group of natural substances with phenolic structure built around a core flavone structure. The structure consists of two phenyl ring A and B linked through an oxygen-containing pyrone ring C arranged in a C_6-C_3-C_6 configuration (Balasundram *et al.,* 2006; Merken and Beecher, 2000). Quercetin has five hydroxyl groups and it is one of the most abundant dietary flavonoids with an average daily consumption of 25-50 milligrams (Formica and Regelson, 1995). They are found in many fruits, vegetables, leaves and grains; and are in substantial amount in red onions and kale. They are also used as an ingredient in dietary supplements, beverages and foods (Slimestad *et al.,* 2007). However, during red wine making, flavonols play a major role in terms of quality. They influence the colour by forming molecular complexes with anthocyanins, a phenomenon called co-pigmentation (Schwarz *et al.,* 2005; Hilbert *et al.,* 2015). Furthermore, flavonols have been linked to many positive health benefits (Krishnaiah *et al.,* 2011; Qin *et al.,* 2011).

The structure of quercetin is common to all family members of flavonoids, and changes in substitution patterns to ring C result in the major flavonoid classes, such as flavonols, flavones, flavanones, flavanols and anthocyanidins (Hollman and Katan, 1999). Of these flavonoids, flavonols and flavones are the most widely distributed and structurally diverse (Harborne *et al.,* 1999). Similarly, substitutions such as oxygenation, alkylation, glycosylation, acylation, and sulfation to rings A and B may give rise to different compounds within each class of flavonoids. Furthermore, individual differences within each group result from the variation in number and arrangement of the hydroxyl groups as well as from the nature and extent of alkylation and/or glycosylation of these groups (Hollman and Katan, 1999; Pietta, 2000).

Apart from flavanol, other flavonoids groups based on the oxidation state of the central ring are flavones, flavonols, flavanones, isoflavones, and anthocyanins. The structural difference of the compounds of this group is relatively due to the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation (Camargo *et al.,* 2015). Presently, flavonoids are the subject of comprehensive studies because of its possession of wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability to modify the gene expression (Harborne and Turner, 1984).

Trichilia emetica belongs to Mahogany family that is comprised of over 55 genera and over 600 plant species of trees, native to tropical and subtropical regions. This plant has long history of use in Africa's folk lore. In South Africa, the decoction of the root and bark is used as an emetic, purgative and also as a remedy for colds, pneumonia, and intestinal disorders such as hepatitis. In Zimbabwe, the bark is used to induce abortion (van der Vossen and Mkamilo, 2007; Orwal *et al.,* 2009). Infusion mixed with *Pseudocedralakotschii* and *Nauclealatifolia* is used in the treatment of malaria. The powdered bark mixed with root of *Securidacalongependonculata*are used for teniasis (Diallo *et al.,* 2003; Komane *et al.,* 2011).

Several limonoids have been reported from the stem bark of *T. emetica;* trichilin A, trichilin B, trichilin C, trichilin D, trichilin E, trichilinin, rohituka 3, rohituka-7, rohituka-5, nymania 1, drageana 4, seco-A-protolimonoids, Tr-A, Tr-C, Tr-B, sendanin and drageana 4; and nymania 1 and Tr B showed selective inhibitory activity toward DNA repair-deficient yeast mutants (Gunatilaka *et al.,* 1998; Komane *et al.,* 2011). This study reports for the first time, the isolation of flavonol from the stem bark of *T. emetica*.

Materials and Methods

Experimental methods

Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) was performed on (silica gel 60 F254, 25 Glassplates 20 × 20 cm, E. Merck, Germany). Flash column chromatography was performed on Fluorochem silica gel 60A. Visualization of the compound was done using UV lamp UVL-14 EL hand held 220 V 50 Hz 4 W 254 nm white light by UVP.1H and 13C NMR spectra were recorded on a Bruker spectrometer at 400.13 and 100.62 MHz using tetramethylsilane (TMS) as an internal standard. Thermo Instruments HPLC system mass spectrometer with electron spray ionization (ESI) source was used for recording of the mass spectra. Flash chromatography (RevelerisTM Flash Chromatographic System) fitted with Reveleris® C18 column with silica flash cartridges of 18 g (RevelerisTM 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. The optical rotations were measured in methanol solution ona ADP 440+ polarimeter

Collection of plant materials

The sample was collected from Kumasi, Ghana, and identified by a botanist Mr. Martin A. Arkoh 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.

Extraction and isolation

The dried powder of *T. emetica* stem (1.80 kg) was extracted four times with 2.50 L of methanol at 100°C for 1 h. The extracts were combined and concentrated to dryness under reduced pressure yielding 137.97 g dark brown gummy extract. The methanolic extract (138 kg) named **TEM**, was suspended in 1 L of H_2O, and partitioned successively with the same volume of chloroform and ethyl acetate to yield chloroform soluble (**TEMC,** 50 g), ethyl acetate-soluble (**TEME,** 52 g), and water-soluble (**TEMW,** 32 g) fractions. The yield of each partition is illustrated in Fig. 1. The ethyl acetate fraction (30 g) was subjected to silica gel column chromatography (CC) with chloroform and methanol used as the eluents at gradient mixtures from 100% chloroform: 0% methanol to 0% chloroform: 20% methanol. Fractions 29 to 45 (**TEME I**, 4. 70 g) obtained with 93% chloroform in methanol and fractions 70 to 75 (**TEME II**, 0.95 g) obtained with 86% chloroform in methanol were separately combined and each subjected to further column chromatography. The combination was carried out according to the similarity of each collection in thin layer chromatography (TLC). Active fractions **TEME I**-13-16 and **TEME II**-5-7 were further purified with flash chromatographic system with linear gradient of methanol/water (containing 0.1% formic acid) at a flow rate of 18 mL/min. **TEME I**afforded compound X (13.10 mg) while **TEME II** yielded compound Z (6.00 mg), respectively. Their structures were shown in Fig. 2. Each compound was identified by 1D- and 2D-NMR and MS and checked for purity in comparison with authentic samples with purities of 95 – 97% by HPLC (Fig. 3).

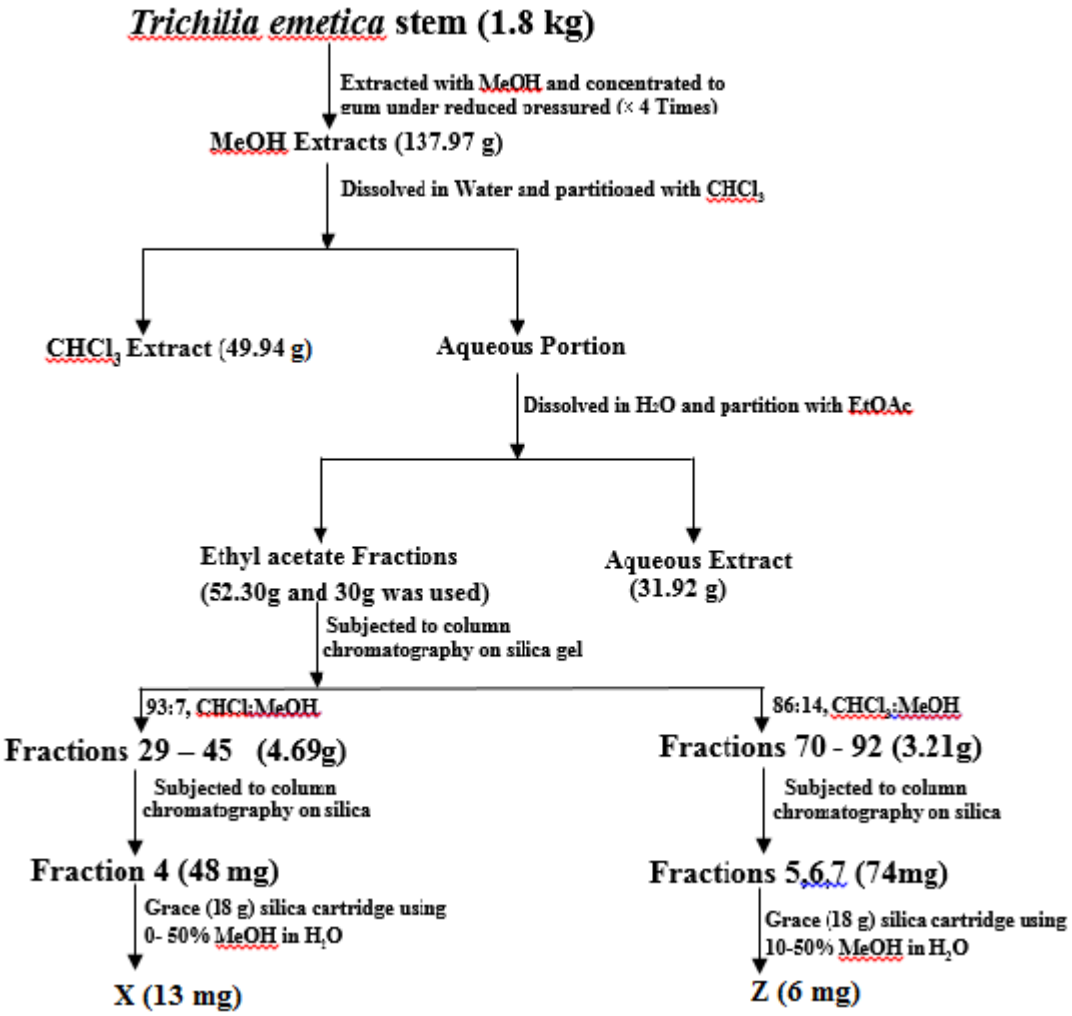


Fig. 1: The sample preparation scheme from *T. emetica* stems

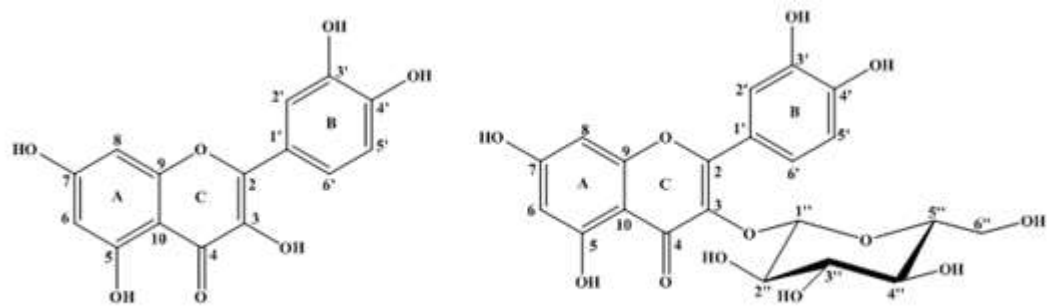
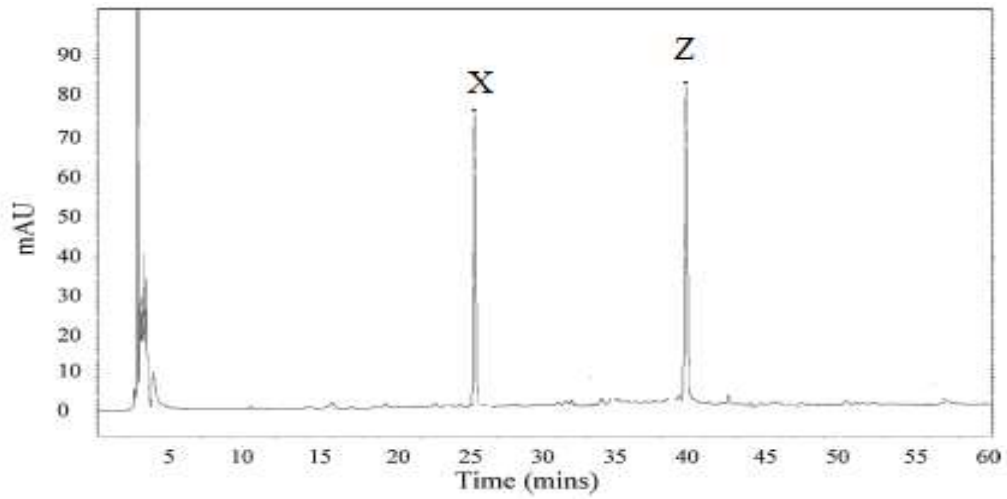


Fig. 2: Structures of compounds X and Z



Peaks: X, quercetin and Z, quercetin-3-*O*-β-D-glucopyranoside

Fig. 3: HPLC chromatogram of the compounds from *T. emetica* stems

Results and Discussion

Compound X (13 mg); Yellow needles, optical rotation $[\alpha]_D^{24.4}$ -8.15 (MeOH: c 0.33). ESI-HRMS (positive ion mode): *m/z* 303.0503 $[M + H]^+$,1H-NMR (CD3OD) *δ*: 6.20 (1H, d, *J* = 2.06 Hz, H-6), 6.40 (1H, d, *J* = 2.06 Hz, H-8), 7.75 (1H, d,*J*= 2.20 Hz, H-2'), 6.89 (1H, d, *J* = 8.6 Hz, H-5') and 7.63 (1H, dd, *J* = 8.5, 2.1 Hz, H-6'); 13C-NMR (CD3OD) (Table 1). By comparison with the literature data (Kim *et al.*, 2011; Wei *et al.*, 2013, Carini*et al.*, 2015) compound **X** was identified as quercetin Fig. 2.

Compound Z (6 mg); Yellow needles, optical rotation $[\alpha]_D^{24.4}$ -11.31 (c 0.33 in MeOH). ESI-HRMS (negative ion mode): m/z 463.0892 $[M - H]^-$, 1H-NMR (CD3OD)δ: 6.22 (1H, d, *J* = 2.1 Hz, H-6), 6.41 (1H, d, *J* = 2.1 Hz, H-8), 7.73 (2H, d,*J* =2.1, H-2'), 6.88 (1H, d,*J* = 8.5 Hz, H-5'), 7.59 (2H, dd,*J* = 8.5, 2.1, H-6'), 5.26 (1H, d, *J* = 7.5 Hz, H-1"), 3.23~3.58 (6H, m, H-2"-5") 13C-NMR (CD3OD) (Table 1). The 1H-NMR and 13C-NMR data (Table 1) were in agreement with that of quercetin-3-*O*-*β*-D-glucopyranoside (Hilbert *et al.*, 2015; Kwom and Bae, 2011; Kang *et al.*, 2012).

Compound X (13 mg) (Figs. 4 – 7) was obtained as yellow needles with optical rotation of (MeOH: c 0.33). It has a molecular formula of $C_{15}H_{10}O_7$ which was established on the basis of ESI-HRMS at *m/z* 303.0503 $[M + H]^+$(Calcd for 303.0506). The DEPT spectrum (Fig. 5) showed fifteen carbon signals, which consisted of five methine and ten quaternary carbons. The ^{1}H NMR spectrum (Fig. 4) for ring B showed three aromatic protons signals at δ_H7.75 (d, 2.2 Hz, H-2'), δ_H 6.89 (d, 8.6 Hz, H-5') and δ_H7.63 (dd, 2.2, 8.5 Hz, H-6') indicating a 1,2,3-trisubstituted aromatic ring in the form of an ABX spin-system. In the COSY spectrum (Fig. 7) these signals δ_H7.63 (H-6') andδ_H 6.89 (d, 8.6 Hz, H-5') were mutually coupled, and similarly, the meta-coupled aromatic proton signals at δ_H 6.20 (d, 2.1 Hz) and δ_H 6.40 (d, 2.1 Hz) were assigned to H-6 and H-8 in the A-ring. The DEPT spectrum also showed resonances for the keto group at C-4 (δc 175.91) for ring C and its nature was further confirmed by long-range coupling observed in HMBC (Fig. 6), where the ring A methine protons H-6 (δ_H 6.20) correlated with C-5, C-7, C-8 and C-10 (δc 103.11); and H-8 (δ_H6.40) correlated with C-4, C-6, C-7, C-9 and C-10 (δc 103.11). Ring B was confirmed by the HMBC correlations of H-2' to C-1', C-3', C-4' and C-6' (δc 120.27); H-5' to C-1', C-2', C-3' and C-4' (δc 147.35); and H-6' to C-2', C-3' and C-4' (δc 147.35). On the basis of NMR data (Table 1), Compound X was identified asquercetin and this was confirmed by comparison of its ^{1}H and ^{13}C NMR data with those reported in the literature (Kim *et al.*, 2011; Wei *et al.*, 2013).

Table 1: ^{1}H and ^{13}C NMR data of compound X and Z

Atom No.	Compound X $CD_3OD\delta_C$(m)	Compound Z $CD_3OD\delta_C$ (m)	Compound X $CD_3OD\delta_H$ (m, *J* in Hz)	Compound Z $CD_3OD\delta_H$ (m, *J* in Hz)
2	146.57 (C)	159.01 (C)		
3	135.81 (C)	135.62 (C)		
4	175.91 (C)	179.50 (C)		
5	161.09 (C)	163.06 (C)		
6	97.82 (CH)	99.60 (CH)	6.20(d, 2.06)	6.22 (d, 2.1)
7	164.14 (C)	166.02 (C)		
8	93.0 (CH)	94.42 (CH)	6.40 (d, 2.06)	6.41 (d, 2.1)
9	156.81 (C)	158.47 (C)		
10	103.11 (C)	105.42 (C)		
1’	122.74 (C)	123.07 (C)		
2’	114.58 (CH)	115.99 (CH)	7.75 (d, 2.2)	7.73 (d, 2.1)
3’	144.80 (C)	145.91 (C)		
4’	147.35 (C)	149.85(C)		
5’	114.81 (CH)	117.55 (CH)	6.89 (d, 8.6)	6.88 (d, 8.5)
6’	120.27 (CH)	123.19 (CH)	7.63 (dd, 2.1, 8.5)	7.59 (dd, 2.1, 8.5)
1’’		104.30 (CH)		5.26 (d, 7.5)
2’’		75.73 (CH)		3.50 (m)
3’’		78.39(CH)		3.34 (m)
4’’		71.21 (CH)		3.23 (m)
5’’		78.12 (CH)		3.58 (m)
6’’		62.55 (CH_2)		3.72 (dd, 2.3, 11.8) 3.57 (dd 5.3, 11.8)

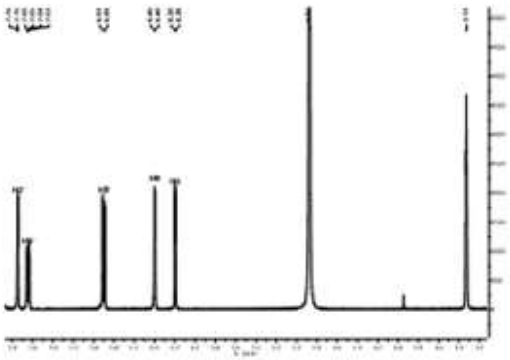


Fig. 4: ^{1}H NMR spectrum of compound X

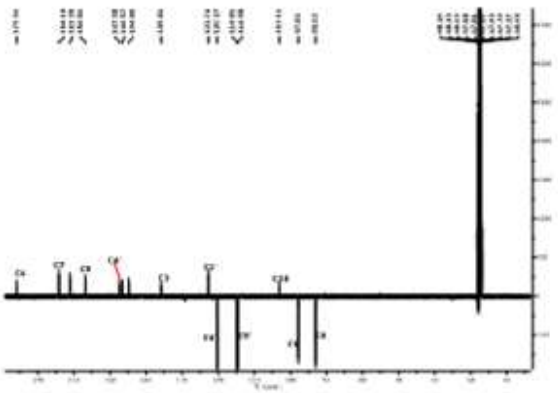


Fig. 5: DEPT spectrum of compound X

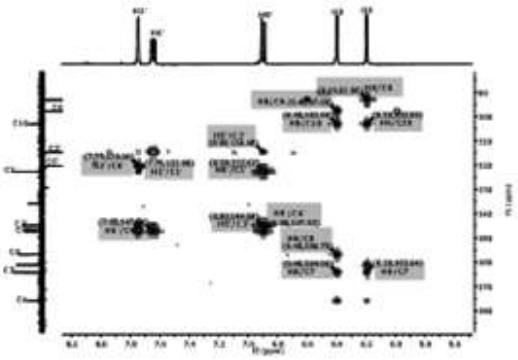


Fig. 6: HMBC spectrum of compound X

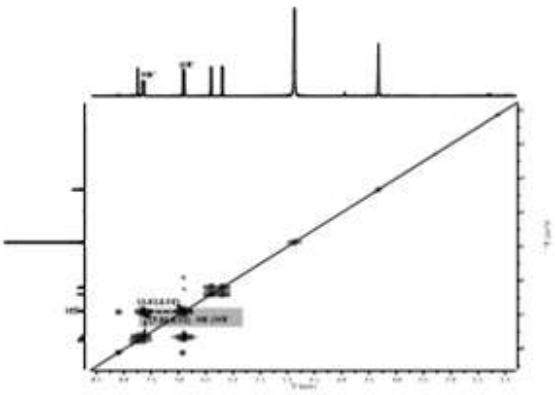


Fig. 7: COSY spectrum of compound X

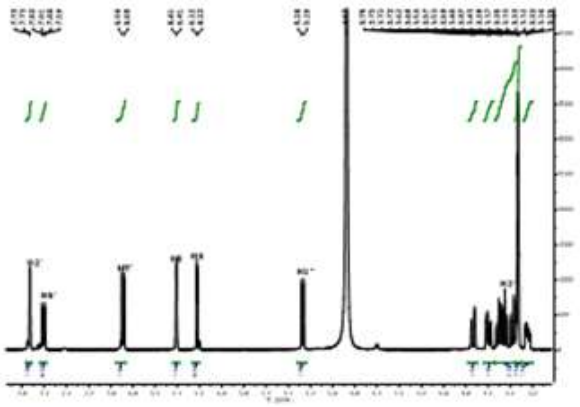


Fig. 8: ^{1}H NMR spectrum of compound Z

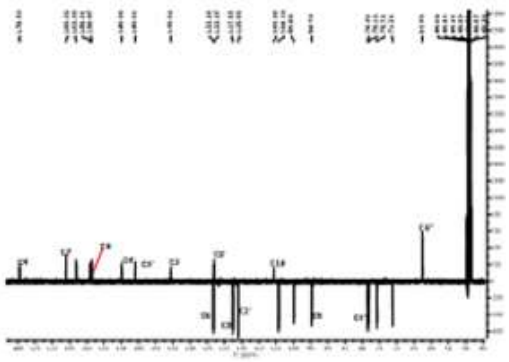


Fig. 9: DEPT spectrum of compound Z

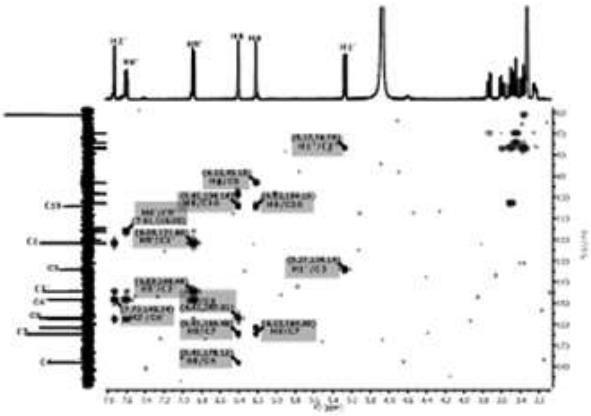


Fig. 10: HMBC spectrum of compound Z

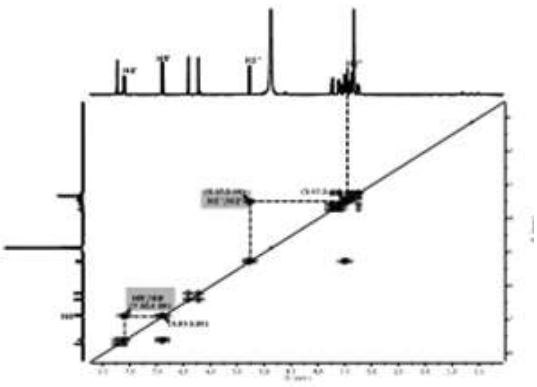


Fig. 11: COSY spectrum of compound Z

Compound Z (6 mg) (Figs. 8 – 11) was also obtained as yellow needles, with optical rotation of -11.31 (c 0.33 in MeOH). It has a molecular formula of $C_{21}H_{20}O_{12}$ which was established on the basis of ESI-HRMS at m/z 463.0892 [M − H]$^{-}$ (Calcd for 463.0876). The DEPT spectrum (Fig. 9) showed twenty one carbon signals, which consist of one methylene, ten methine and ten quaternary carbon atoms. The comparison of the ^{1}H and ^{13}C NMR data (Table 1) with that of compound X revealed close similarity except for the presence of signals for glucose moiety. Hence, the aglycone is suggested to be a quercetin (Fig. 2). The ^{1}H spectrum (Fig. 8) showed one anomeric proton at δ_H5.27 (d, 7.5 Hz, H-1''), indicating a β-glycosyl moiety based on the coupling constant. In the COSY spectrum (Fig. 11), the correlation between the anomeric proton signalδ_H5.27 (d, 7.5 Hz, H-1'') with δ_H3.50 (m) assigned to position 2'' was observed. The remaining ^{13}C NMR data for glucose moiety are consistent with those of glucose. The attachment of the glucose unit at C-3 (δc 135.62) was apparent from the H-1'' to C-3 HMBC connectivity (Fig. 10). The NMR data (Table 1) were thus consistent with quercetin-3-*O*-β-D-glucopyranoside, and this was confirmed by comparison of its ^{1}H and ^{13}C NMR data with those reported in the literature (Hilbert *et al.*, 2015; Kwom and Bae, 2011; Kang *et al.*, 2012).

Conclusion

This is the first report of the isolation of quercetin and its glycoside from the stem bark of *T. emetica*. Several chromatographic separation techniques such as TLC, CC and Flash chromatographic system were utilized. The compounds were structurally identified on the basis of nuclear magnetic resonance (NMR), and MS data analyses as well as the direct comparison of the data reported in the literature.

Acknowledgements

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Conflict of Interest

Authors have declared that there is no conflict of interest reported in this work

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