Isolation and characterization of some flavonoids from the leaf of *Tapinanthus globiferus* growing on *Acacia nilotica*

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*Tapinanthus globiferus* is used ethnomedicinally for the treatment of bacterial infections, inflammation, stomach pain, ulcers among others. The aim of the study was to isolate bioactive compounds from the leaf of *T. globiferus* growing on *Acacia nilotica*. The powdered plant material was extracted with 90 % methanol using cold maceration and the resulting crude methanol leaf extract was partitioned into *n*-hexane, chloroform, ethylacetate and *n*-butanol fractions. The ethylacetate fraction was chromatographed on a silica gel, sephadex LH-20 column and preparative thin-layer chromatography. (−)-Epicatechin and Quercetin 3-O-β-D-glucopyranoside were isolated and characterized by means of physiochemical and spectroscopic (1D and 2D-NMR) analyses for the first time from *T. globiferus* growing on *A. nilotica*.

**Keywords:** *Tapinanthus globiferus*; Flavonoids; Isolation; NMR.

1. Introduction

The plant kingdom, with its remarkable diversity of natural compounds, has merited special interest (Lewinsohn and Gijzen, 2009). Among these compounds, flavonoids have received much research and attention (Harborne and Williams, 2000; Kesarkar *et al*., 2009; Buer *et al*., 2010). They not only function as stress protectants in plants (Hahlbrock and Scheel, 1989; Cespedes *et al*., 2001), and UV protectants (Goto and Kondo, 1991; Li *et al*., 1995), but also have multi-beneficial biological activities such as antioxidantive (Ammar *et al*., 2010; Ghasemzadeh *et al*., 2010), anticarcinogenic (Seelinger *et al*., 2008), antimicrobial (Zhou *et al*., 2007; Pereira *et al*., 2007), antimitugenic (Liverio *et al*., 1994), anti-inflammatory (Ueda *et al*., 2002), antiallergic (Mastuda *et al*., 2002) and anti-obesity (Kamisoayama *et al*., 2008) properties.

*Tapinanthus globiferus* (A. Rich) belonging to the Loranthaceae family is a hemi-parasite with glabrous pendulous stems up to 1.2 m long with roots that mostly grows on the branches of a large number of tree species including *Acacia, Vitellaria, Kola, Citrus, Combretum, Aloe and Terminalia* as host trees (Waterberg *et al*., 1989; Polhill and Wiens, 1998). Ogunbolude *et al.* (2014) reported the identification and quantification of quercetin and some phenolic acids from *T. globiferus* growing on other host. Extensive literature search revealed that there is no report yet on the isolation and characterization of any compound from the plant *T. globiferus* growing on *Acacia nilotica*. We report herein, the isolation and characterization of (−)-epicatechin and Quercetin-3-O-β-D-glucopyranoside from the leaf of *T. globiferus* growing on *A. nilotica*.

2. Materials and Methods

2.1 General Procedures

The NMR experiments were conducted on a Bruker AVANCE spectrometer (500 MHz) with residual solvent (TMS) as internal standard. The melting points of the isolated compounds were determined on an electrothermal melting point apparatus. Thin layer chromatography (TLC) was carried out using silica gel 60 F254 pre-coated aluminium sheets (Sigma Aldrich, Germany). Column chromatography was conducted using LOBA Cheme silica gel (60–200) mesh in a sintered glass funnel. Gel filtration chromatography was performed using sephadex LH-20 Spots on TLC plates were visualized by spraying with 10 % H2SO4 followed by heating at 105 °C for 10 minutes.
2.2 Collection, Identification and Preparation of Plant Material

*Tapinanthus globiferus* growing on *Acacia nilotica* was collected at Dundaye village of Wammakko Local Government Area of Sokoto State, Nigeria in August 2019. It was identified and authenticated by Malam Abdul-azeez of the Herbarium unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, with a voucher specimen number (UDUH/ANS/0327). The plant material was shade dried, pulverized and stored in a polythene bag for further use.

2.3 Extraction and Isolation of Compounds

The pulverized leaf of *T. globiferus* (1.437 kg) was exhaustively extracted with 4.5 L of 90 % methanol for 9 days with occasional shaking. The extract was filtered and the filtrate was evaporated to dryness using a rotary evaporator at 40 °C to afford crude methanol leaf extract (128.5 g). The crude extract (120 g) was suspended in 500 mL of distilled water, then filtered and partitioned successively with solvents of increasing polarity to afford *n*-hexane, chloroform, ethylacetate and *n*-butanol fractions. The ethylacetate fraction (3.7 g) was subjected to silica gel column chromatography with eluting solvents systems consisting of different ratio of mixtures of *n*-hexane: ethyl acetate, 100 % ethylacetate, different ratios of mixtures of ethyl acetate: methanol, and 100 % methanol. TLC was used to monitor the column. A total of 150 collections were made and combined based on their TLC profile to afford six (11) major fractions coded EA-EK. Fraction EC was purified using sephadex LH-20 column with dichloromethane (100 %), mixtures of dichloromethane: ethylacetate, mixtures of ethylacetate: methanol to methanol (100 %) as solvent systems. Two (2) mL each of a total of 88 collections were made and combined based on their TLC profile to afford six (6) major fractions coded EC1-EC6. Fractions EC3 and EC6 were merged and further purified using preparative TLC with a mixture of ethylacetate: chloroform: methanol: water in the ratio of 15: 4: 4: 1 as solvent system. After development, the plates were dried and bands of interest were scraped using spatula and then dissolved in insufficient quantity of methanol and ethyl acetate. It was filtered, the filtrates were allowed to dry. TLC analysis of the samples obtained using a mixture of ethylacetate: chloroform: methanol: water (15: 4: 4: 1) as solvent system gave a single homogenous spot which led to the isolation of compounds L1 and L2.

3. Results and Discussion

3.1 Results

**Spectral data**

The isolate L1 is a yellow crystalline solid compound which is soluble in methanol and ethylacetate with a melting point of 178 - 179 °C. The 1H–NMR spectrum (in CD3OD, 500 MHz) of the compound L1 showed signals at δH 5.89 (1H, d, J = 2.0 Hz, H – 8), δH 5.71 (1H, d, J = 2.0 Hz, H – 6), δH 2.66 (1H, dd, J = 4.0, 16.3 Hz, H – 4b), δH 2.69 (1H, dd, J = 4.5, 16.3 Hz, H – 4a), δH 4.73 (1H, brs, H – 2), δH 4.65 (1H, d, H – 3), δH 6.65 (1H, d, 1.0 Hz, H – 2′), δH 6.66 (1H, d, J = 2.5 Hz, H – 6′) and δH 6.89 (1H, s, H – 5′). The 13C–NMR (500 MHz, CD3OD) and DEPT experiments of L1 showed the presence of 15 carbon atoms; signals at δC 155.7 (C – 5), 156.6 (C – 7), 156.2 (C – 9), 98.4 (C – 10), 28.2 (C – 4), 144.5 (C – 3), 78.0 (C – 2), 144.4 (C – 4′), 95 (C – 6), 94.1 (C – 8), 114.7 (C – 2′), 117.9 (C – 6′) and 114.9 (C – 5′). The DEPT further revealed the multiplicity of the carbons as one methylene and seven methane (CH3) and seven quaternary (C) carbon atoms.

The isolate L2 is also a yellow crystalline solid compound, soluble in methanol but insoluble in chloroform; m.p 223 - 224 °C. The 1H-NMR (CD3OD, 500 MHz) of the compound L2 revealed chemical shift values/integration as follows: δH 6.34 (1H, d, J = 2.0 Hz, H-8), δH 16.18 (1H, d, J = 1.5 Hz, H-6), δH 7.34 (1H, d, J = 2.0 Hz, H’2), δH 6.89 (1H, d, J = 8.5, H-5), δH 7.30 (1H, dd, J = 2.0, 8.3 Hz, H-6), δH 5.35 (1H, d, J = 1.5 Hz, H-1′) and δH 3.66 - 3.76 (m, sugar protons). The 13C-NMR and 13C-DEPT experiments (500 MHz, CD3OD) of L2 revealed the presence of 21 carbon atoms. Seven aromatic carbon peaks were observed for L2 at δc 123.0 (C-6′), 122.8 (C-1′), 95.0 (C-8), 105.5 (C-10), 122.8 (C-1′), 116.9 (C-5′) and 116.4 (C-2′). Other peaks include 159.1 (d, C-2), 136.1 (C-3), 179.5 (C-4), 73.3 (C-3′), 71.9 (C-4′), 64.4 (C-6′) and 72.1 (C-5′).

3.2 Discussion

Compound L1 was obtained as a yellow solid substance; the sharp melting point observed by the compound indicates its purity (John, 1964) and it tested positive to ferric chloride reagent suggesting the presence of phenolic nucleus (Silva et al., 1998). The 1D- and 2D-NMR data of compound L1 revealed chemical shift values typical of flavonoids (Yusuf et al., 2019). The presence of an AX system (1,2,3,5 – tetra-substituted benzene ring A) was assigned from the protons at δH 5.89 (1H, d, J = 2.0 Hz, H – 8) and δH 5.71 (1H, d, J = 2.0 Hz, H – 6), while an ABX system (1,3,4 – trisubstituted benzene ring B) was depicted via the protons at δH 6.65 (1H,
the resonances around δ 6.89 (1H, d, J = 2.0 Hz, H-2') and δ 6.89 (1H, s, H-5'). The presence of an aliphatic ring was clearly discerned from the proton chemical shift values observed at δ 4.73 (1H, s, H-2) and δ 4.65 (1H, d, J = 4.5 Hz, H-3) representing an oxymethine and a carbinol proton respectively, consistent with a saturated ring C (Yusuf et al., 2019). Two protons with signals at δ 4.65 (1H, dd, J = 4.5, 16.3 Hz, H-4a) and δ 2.66 (1H, dd, J = 4.0, 16.3 Hz, H-4b) assigned to C-4 are characteristic of a flavonoid of 3-flavon basic nucleus (Yusuf et al., 2019). The chemical shift value for H-2 δ 4.73 which appeared as a singlet is an indication that compound L1 is an (−) – epicatechin rather than (+) – catechin (Yusuf et al., 2019; De mello et al., 1996). 1H-1H COSY established the correlations between the protons at δ 4.65 (H-3) δ 2.69 (H-4b), and δ 2.66 (H-4a) which confirmed the assignment of ring C while the cross peaks correlations observed between δ 6.65 (H-2) and δ 6.89 (H-5') further strengthened the assignment of ring B. The 13C – NMR (500MHz, CD3OD) and DEPT experiments indicated the presence of 15 carbon atoms. Compound L1 exhibited 7 aromatic carbon peaks at δc 95.0 (C-6), 94.1 (C-8), 98.4 (C-10), 130.6 (C-1), 114.7 (C-2'), 114.9 (C-5'), and 117.9 (C-6'), five oxygenated carbon atoms at 155.7 (C-5), 156.6 (C-7), 156.2 (C-9), 144.4 (C-4') and 144.5 (C-3') and the three aliphatic carbons at δ 78.0 (C-2), 64.9 (C-3) and the methylene carbon at 28.2 (C-4) suggested that the compound L1 to be an epicatechin (Abdullahi et al., 2017; Yusuf et al., 2019). The absence of a down field signal around δc 82 (C-2) confirms compound L1 to be an (−) – epicatechin rather than (+) – catechin (De mello et al., 1996; Yusuf et al., 2019). HSQC experiments established the attachment of various protons to their respective carbons, the protons at δ 4.73 correlated with δc 78.0 and δ 2.69, 2.66 correlated with δc 28.2 among others. Comparison with a reference NMR data (Yusuf et al., 2019) showed a good match and based on the above, the structure of compound L1 was confirmed to be (2R, 3R) -3,4-dihydro-2-(3, 4-di hydroxy phenyl)-2H-chromene-3, 5, 7 triol or (−) -epicatechin (Figure 1).

Compound L2 was obtained as a yellow solid substance; it gave positive reaction with ferric chloride suggesting the presence of phenolic nucleus and Fehling’s test indicating the presence of sugar moiety (Silva et al., 1998). The sharp melting point demonstrated by the compound indicated its purity (Tang et al., 2000). The basic skeletal structure of L2 was confirmed from the 1H-NMR data. The presence of two meta-coupled protons (ring A) at δ 6.18 (1H, d, J = 1.5 Hz) and δ 6.34 (1H, d, J = 2.0 Hz) assignable to H-6 and H-8 respectively was observed. An ABX system was clearly discerned from the protons signals at δ 6.89 (1H, d, J = 8.5 Hz, H-5'), δ 7.34 (1H, dd, J = 2.0 Hz, H-2') and δ 7.30 (1H, dd, J = 2.0, 8.3 Hz, H-6'). The sugar moiety was defined by the resonances around 3.65 – 3.76, the doublet at δ 3.66 (J = 5.0 Hz) typical of the –CH2 group suggest the sugar to be a glupyranoside (Boots et al., 2008). The 1H-1H COSY established the correlation between protons that are adjacent to each other, proton at δ 6.18 correlates with the proton at δ 6.34 ppm, while proton at δ 6.89 was found to be correlating with the proton at δ 7.34 ppm. These correlations further confirmed the position of the protons in the 1H-NMR of L2 (Sani et al., 2015). The 13C-NMR (500 MHz, CD3OD) and DEPT experiments of compound L2 revealed the presence of 21- carbon atoms, seven aromatic carbon atoms signals at δc 105.5 (C-10), 100.3 (C-6), 95.0 (C-8), 122.8 (C-1'), 123.0 (C-6'), 116.9 (C-5') and δ 116.4 (C-2'). Eight quaternary oxygenated carbon atoms at δ 167.4 (C-7), 163.1 (C-5), 159.1 (C-9), 149.9 (C-4'), 146.5 (C-3'), 136.1 (C-3), 159.1 (C-2) and a down field signal due to carbonyl resonating at δc 179.5 which further suggesting the compound to be Quercetin (Tang et al., 2000). The chemical shift value δc 103.5 was assigned to the anomeric carbon (C-1'). Other resonances such as 72.0 (C-2'), 73.3 (C-3'), 71.9 (C-4') and 72.1 (C-5') were characteristic of sugar absorptions and the -CH2 absorption at δc 64.4 (C-6'). Suggest the sugar to be a glupyranoside which is consistent with 1H-NMR data of quercetin glupyranoside (Boots et al., 2008). The HSQC spectrum of L2 was used to attach each proton to their respective carbon atoms. Proton at δ 6.18 correlates with the carbon at δc 100.3, proton at δ 6.34 correlates with the carbon at δc 95.0, proton at δ 6.89 correlates with the carbon at δc 116.9, proton at δ 7.30 correlates with carbon at δc 123.0, proton at δ 7.34 correlates with carbon at δc 116.4, proton at δ 5.35 correlates with anomeric carbon at 103.5, δ 3.76 correlated with δc 72, δ 3.74 correlated with δc 73.7 and δ 3.66 correlated with δc 64.4 among others. The connectivity between various fragments was established through Heteronuclear Multiple Bond Correlation spectroscopy (HMBC). It established the correlation between proton at δ 6.18 (H-6) with the carbons at δc 167.4 (C-7) and δc 105.5 (C-10) proton at δ 6.34 (H-8) showed a long range correlation with the carbons at δc 163.1 (C-5') and δ 105.5 (C-10) which further confirmed the 1,2,3,5- tetra-substituted benzene ring A. Correlation between proton at δ 6.84 (H-5') with the carbons at δc 122.8 (C-1'), δc 146.5 (C-3') and δc 149.9 (C-4'); proton at δ 7.30 (H-6') is correlating with carbons at δc 116.4 (C-2') and δc 146.5 (C-3') and proton at δ 7.34 (H-2') is correlating with carbons at 123.0 (C-6'), δc 149.9
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(C-4') and δC 159.1 (C-2) further confirmed the 1,3',4'-trisubstituted benzene ring B. Correlation between proton at δH 3.74 (H-3") with carbon at δC 136.1 (C-3) and proton at δH 3.66 (H-4") with carbon at δC 179.5 (C-4) also confirmed the glucose attachment at carbon position 3. Based on 1D and 2D-NMR data of L2 and comparison with the reported literature (Beck and Haberlein, 1999; Tang et al., 2000), the structure of L2 was confirmed to be Quercetin -3 – O – β – D – gluopyranoside (Figure 2).

Figure 1: (-)-Epicatechin

Figure 2: Quercetin -3 - O - ß - D - gluopyranoside

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4. Conclusion

Chromatographic studies of ethylacetate fraction of T. globiferus afforded two flavonoids (2R, 3R)-3, 4-di hydro-2- (3, 4-di hydroxyl phenyl)-2H-chromene-3, 5, 7 triol or (-)-epicatechin and Quercetin 3-O-β-D-glucopyranoside.

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Conflict of interest

The authors declare no conflict of interest.

References


Table 2: 1D and 2D-spectral data summary for Compound L2 (CD3OD, 500 MHz)
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