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 *A. 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 antioxidative (Ammar et al., 2010; Ghasemzadeh et al., 2010), anticarcinogenic (Seelinger et al., 2008), antimicrobial (Zhou et al., 2007; Pereira et al., 2007), antimitagenic (Liverio et al., 1994), anti-inflammatory (Ueda et al., 2002), antiallergic (Mastuda et al., 2002) and anti-obesity (Kamisoyama 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). Ogunbolode 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 *A. 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 Wamakko 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 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 ratio 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 eleven (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 sufficient 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 $^1$H-NMR spectrum (in CD$_3$OD, 500 MHz) of the compound L1 showed signals at $\delta_H$ 5.89 (1H,d, $J$ = 2.0 Hz, H – 8), $\delta_H$ 5.71 (1H, d, $J$ = 2.0 Hz, H – 6), $\delta_H$ 2.66 (1H, dd, $J$ = 4.0, 16.3 Hz, H – 4b), $\delta_H$ 2.69 (1H, dd, $J$ = 4.5, 16.3 Hz, H – 4a), $\delta_H$ 4.73 (1H, brs, H – 2), $\delta_H$ 4.65 (1H, d, H – 3), $\delta_H$ 6.65 (1H, d, 1.0 Hz, H – 2′), $\delta_H$ 6.66 (1H, d, $J$ = 2.5 Hz, H – 6′) and $\delta_H$ 6.89 (1H, s, H – 5′). The $^{13}$C – NMR (500 MHz, CD$_3$OD) and DEPT experiments of L1 showed the presence of 15 carbon atoms; signals at $\delta_C$ 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 (CH$_2$), seven methine (CH) 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 $^1$H-NMR (CD$_3$OD, 500 MHz) of the compound L2 revealed chemical shift values/integration as follows: $\delta_H$ 6.34 (1H, d, $J$ = 2.0 Hz, H-8), $\delta_H$ 6.18 (1H, d, $J$ = 1.5 Hz, H-6), $\delta_H$ 7.34 (1H, d, $J$ = 2.0 Hz, H-2′), $\delta_H$ 6.89 (1H, d, $J$ = 8.5, H-5′), $\delta_H$ 7.30 (1H, dd, $J$ = 2.0, 8.3 Hz, H-6′), $\delta_H$ 5.35 (1H, d, $J$ = 1.5 Hz, H-1′) and $\delta_H$ 3.66 - 3.76 (m, sugar protons). The $^{13}$C-NMR and $^{13}$C-DEPT experiments (500 MHz, CD$_3$OD) of L2 revealed the presence of 21 carbon atoms. Seven aromatic carbon peaks were observed for L2 at $\delta_C$ 132.0 (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 (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 – tetrasubstituted benzene ring A) was assigned from the protons at $\delta_H$ 5.89 (1H, d, $J$ = 2.0 Hz, H – 8) and $\delta_H$ 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 $\delta_H$ 6.65 (1H,
The presence of an aliphatic ring was clearly discerned from the proton chemical shift values observed at $\delta_c 4.73$ (1H, s, H-2) and $\delta_c 6.89$ (1H, s, H-5). The presence of an aromatic ring was confirmed by the absorption at $\delta_c 7.30$ (1H, d, J = 2.0 Hz, H-6) and $\delta_c 6.84$ (1H, d, J = 16.3 Hz, H-3). The presence of an aliphatic ring was confirmed by the absorption at $\delta_c 6.18$ (1H, d, J = 2.0 Hz, H-6) and $\delta_c 6.89$ (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 $\delta_c 3.66$ ($J = 5.0$ Hz) of the -CH$_2$ group suggests the sugar to be a glucuronoside (Boots et al., 2008). The $^1$H-^1H COSY established the correlation between protons that are adjacent to each other, proton at $\delta_c 6.18$ correlates with the proton at $\delta_c 6.34$ ppm, while proton at $\delta_c 6.89$ was found to be correlating with the proton at $\delta_c 7.34$ ppm. These correlations further confirmed the position of the protons in the $^1$H-NMR of L2 (Sani et al., 2015). The $^{13}$C-NMR (500 MHz, CD$_3$OD) and DEPT experiments of compound L2 revealed the presence of 21 carbon atoms, seven aromatic carbon atoms signals at $\delta_c 105.3$ and $\delta_c 105.5$ (C-10), 100.3 (C-6), 95.0 (C-8), 122.8 (C-1), 123.0 (C-6), 116.9 (C-5), 116.4 (C-2). Eight quaternary oxygenated carbon atoms at $\delta_c 167.4$ (C-7), 163.1 (C-5), 159.1 (C-9), 149.9 (C-4'), 146.5 (C-3'), 136.1 (C-3), and 159.1 (C-2) and a down field signal due to carbonyl resonating at $\delta_c 179.5$ which further suggesting the compound to be Quercetin (Tang et al., 2000). The chemical shift value $\delta_c 103.5$ 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 -CH$_2$ absorption at $\delta_c 64.4$ (C-6'). Suggest the sugar to be a glucuronoside which is consistent with $^1$H-NMR data of quercetin glucuronoside (Boots et al., 2008). The HMQC spectrum of L2 was used to attach each proton to their respective carbon atoms. Proton at $\delta_H 6.18$ correlates with the carbon at $\delta_c 100.3$, proton at $\delta_H 6.34$ correlates with the carbon at $\delta_c 95.0$, proton at $\delta_c 6.89$ correlates with the carbon at $\delta_c 123.0$, proton at $\delta_c 7.34$ correlates with carbon at $\delta_c 116.4$, proton at $\delta_c 5.35$ correlates with anomic carbon at $\delta_c 103.5$, $\delta_c 3.76$ correlated with $\delta_c 72$, $\delta_c 3.74$ correlated with $\delta_c 73.7$ and $\delta_c 3.66$ correlated with $\delta_c 64.4$ among others. The connectivity between various fragments was established through Heteronuclear Multiple Bond Correlation spectroscopy (HMBC). It established the correlation between proton at $\delta_H 6.18$ (H-6) with the carbons at $\delta_c 167.4$ (C-7) and $\delta_c 105.5$ (C-10) proton at $\delta_H 6.34$ (H-8) showed a long range correlation with the carbons at $\delta_c 163.1$ (C-5) and $\delta_c 105.5$ (C-10) which further confirmed the 1,2,3,5- tetra-substituted benzene ring A. Correlation between proton at $\delta_H 6.84$ (H-5') with the carbons at $\delta_c 122.8$ (C-1'), $\delta_c 146.5$ (C-3') and $\delta_c 149.9$ (C-4'); proton at $\delta_H 7.30$ (H-6') is correlating with carbons at $\delta_c 116.4$ (C-2') and $\delta_c 146.5$ (C-3') and proton at $\delta_H 7.34$ (H-2') is correlating with carbons at 123.0 (C-6'), $\delta_c 149.9$.
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(C-4') and δ_c 159.1 (C-2) further confirmed the 1,3',4'-trisubstituted benzene ring B. Correlation between proton at δ_H 3.74 (H-3') with carbon at δ_c 136.1 (C-3) and proton at δ_H 3.66 (H-4') with carbon at δ_c 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 – ß – D – glupyranoside (Figure 2).

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Table 1: 1D and 2D spectral data summary for Compound L1 (CD_3OD, 500 MHz)

| Position | δ^1H (Hz) | δ^13C | DEPT | COSY |
|----------|-----------|-------|------|------|
| 1        | -         | -     | -    | -    |
| 2        | 4.73 (1H, brs) | 78.0 | CH   | -    |
| 3        | 4.65 (1H, d, J = 4.5) | 64.9 | CH   | -    |
| 4        | 2.69 (1H, dd, J = 4.5, 16.3) | 28.2 | CH2  | H-4a |
|          | 2.66 (1H, dd, J = 4.0, 16.3) |     |      | H-4b |
| 5        | -         | 155.7 | C    | -    |
| 6        | 5.71 (1H, d, J = 2.0) | 95.0 | CH   | H-8  |
| 7        | -         | 156.6 | C    | -    |
| 8        | 5.89 (1H, d, J = 2.0) | 94.1 | CH   | H-6  |
| 9        | -         | 156.2 | C    | -    |
| 10       | -         | 98.4  | C    | -    |
| 1'       | -         | 130.6 | C    | -    |
| 2'       | 6.65 (1H, d, J = 1.0) | 114.7 | CH   | -    |
| 3'       | -         | 144.5 | C    | -    |
| 4'       | -         | 144.4 | C    | -    |
| 5'       | 6.89 (1H, s) | 114.9 | CH   | H-6' |
| 6'       | 6.66 (1H, d, J = 2.5) | 117.9 | CH   | H-5' |
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.

Acknowledgements

Our special gratitude to Mr. Mubarak B. Dambatta of Cardiff University, School of Chemistry, United Kingdom for running the 1D and 2D-NMR spectroscopy.

Conflict of interest

The authors declare no conflict of interest.

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Table 2: 1D and 2D-spectral data summary for Compound L2 (CD$_3$OD, 500 MHz)

| Position | $\delta^1$H (Hz) | $\delta^{13}$C | DEPT | COSY | HMBC |
|----------|-----------------|--------------|------|------|------|
| 1        | -               | -            | -    | -    | -    |
| 2        | -               | 159.1        | C    | -    | -    |
| 3        | -               | 136.1        | C    | -    | -    |
| 4        | -               | 179.5        | C    | -    | -    |
| 5        | -               | 163.1        | C    | -    | -    |
| 6        | 6.18 (1H, d, $J$= 1.5, H-6) | 100.3      | CH   | $H - 8$ | C-7; C-10 |
| 7        | -               | 167.4        | C    | -    | -    |
| 8        | 6.34 (1H, d, $J$= 2.0, H-8) | 95.0      | CH   | $H - 6$ | C-5; C-10 |
| 9        | -               | 159.1        | C    | -    | -    |
| 10       | -               | 105.5        | C    | -    | -    |
| 1'       | -               | 122.8        | C    | -    | C-3'; C-4' |
| 2'       | 7.34 (1H, d, $J$= 2.0, H-2') | 116.4      | CH   | H-5' | -    |
| 3'       | -               | 146.5        | C    | -    | -    |
| 4'       | -               | 149.9        | C    | -    | -    |
| 5'       | 6.89 (1H, d, $J$= 8.5, H-5') | 116.9      | CH   | H-2' | C-1'; C-3'; C-4' |
| 6'       | 7.30 (1H, dd, $J$= 2.0, 8.3) | 123.0      | CH   | -    | -    |
| 1''      | 5.35 (1H, d, $J$= 1.5, H-1'') | 103.5      | CH   | -    | -    |
| 2''      | 3.78 (1H, d, $J$= 3.0, H-2'') | 72.0       | CH   | -    | -    |
| 3''      | 3.74(1H, dd, $J$=3.0, 9.5 H-3'') | 73.3       | CH   | -    | C-3 |
| 4''      | 3.68 (1H, H-4'') | 71.9       | CH   | -    | C-4 |
| 5''      | 3.67 (1H, d, $J$= 3.5) | 72.1       | CH   | -    | -    |
| 6''      | 3.66 (2H, d, $J$= 5.0) | 64.4      | -CH$_2$ | -    | -    |
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