Isolation and characterization of some flavonoids from the leaf of *Tapinanthus globiferus* growing on *Vitex doniana*

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**Abstract.** *Tapinanthus globiferus* (A. Rich) Tiegh (Loranthacae) is a semi-parasitic plant growing on several plant species such as *Vitex doniana*. It is used in ethno-medicine for the treatment of fungal infection, itching, hypertension, ulcers, epilepsy, diabetes and cancer. The aim of this study was to isolate bioactive compound(s) from the leaf of *T. globiferus*. The powdered plant material was extracted with 90% methanol using maceration method 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 and sephadex LH-20 column which led to the isolation of two flavonoids, and the identity of the compounds was determined on the basis of chemical test and NMR analysis. Based on the 1D and 2D NMR data, the compounds were 2-(3’4’-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromane-4-one (quercetin) and 2-(2,4-dihydroxyphenyl)-5,7-dihydroxy-3-(3-methylhexyl)-4H-chromen-4-one. This is the first report of isolation of these compounds from *T. globiferus* growing on *Vitex doniana*.

**Keywords:** *Tapinanthus globiferus*; Leaf; Isolation; NMR.
Introduction

*Tapinanthus globiferus* (A. Rich) Tiegh belonging to the Loranthaceae Family is a semi-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 *Vitellaria paradoxa*, *Kola*, *Citrus*, *Combretum*, *Acacia*, *Aloe* and *Terminalia* as host trees (Waterberg et al., 1989; Polhill and Wiens, 1998). It is commonly known as mistletoe (English), *Kauchi* (Hausa), *afomo* (Yoruba), and *Osisi/Okwuma osa* (Igbo) in Nigeria. *T. globiferus* is used in ethno-medicine to treat itching (Burkill, 2000), tumor (Yineger and Yewhalaw, D., 2007) and removal of placenta after parturition (Sher and Alyemeni, 2011). The plant is also used to treat diseases such as hypertension, ulcers, epilepsy, diabetes, weakness of vision and promoting muscular relaxation before delivery (Bassey, 2012). Ogunbolude et al. (2014) reported the identification and quantification of quercetin and some phenolic acids from *T. globiferus* growing on other host. Biological studies of *T. globiferus* growing on other hosts have been documented. Antitrypanosomal (Abedo et al., 2013) and anticonvulsant (Abubakar et al., 2016) activities of the plant were also reported. 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 *Vitex doniana*.

We report herein, the isolation and characterization of two flavonoids, quercetin and 2-(2,4-dihydroxyphenyl)-5,7-dihydroxy-3-(3-methylhexyl)-4H-chromen-4-one from the leaf *T. globiferus* growing on *Vitex doniana*.

Materials and methods

**General procedures**

NMR data were recorded on a Bruker AVANCE spectrometer (600 MHz) with residual solvent as internal standard. Melting point was determined on an Electro thermal melting point apparatus. Thin layer chromatography (TLC) was carried out using silica gel 60 GF<sub>254</sub> pre-coated aluminium sheets by Sigma Aldrich, Germany. Low pressure column and Vacuum liquid chromatography were conducted using LOBA Cheme silica gel (60-200) mesh in a sintered glass funnel while gel filtration chromatography was performed using sephadex LH-20 (Sima, Spruce Street, St. Louis, MO, USA). Spots on TLC plates were visualized by spraying with 10% H<sub>2</sub>SO<sub>4</sub> followed by heating at 105 °C for 10 min.

**Plant sample**

Plant sample of *T. globiferus* growing on *Vitex doniana* was collected from Dang Shuni Local Government Area of Sokoto State, Nigeria in December 2016. It was identified and authenticated by Namadi Sanusi of the Herbarium Section, Department of Biological Sciences, Ahmadu Bello University Zaria, with a voucher (No. 900107). The plant material was air dried, pulverized, labelled and stored in a polythene bag for further use.

**Isolation of compounds**

The powdered leaf of *T. globiferus* (2.0 kg) was exhaustively extracted with 3 L of 90% methanol for 6 days. The extract was filtered using Whatman No. 1 filter paper and the filtrate was evaporated to dryness using rotary evaporator at 40 °C to afford crude methanol leaf extract (140 g). Some part of the extract (120 g) was partitioned into *n*-hexane, chloroform, ethylacetate and *n*-butanol fractions. The ethylacetate fraction (3 g) was subjected to purification using VLC with *n*-hexane: ethylacetate mixtures as solvent systems. Twenty (20 mL) of a total of 60 collections were made and combined based on their TLC profile to afford four major fractions coded A-D. Fraction D was further purified using a combination of low pressure column and sephadex LH-20 column to afford a
yellow amorphous substance, 10 mg (Compound 1). A portion of the ethylacetate fraction (4 g) was chromatographed on a silica gel column with n-hexane: ethylacetate mixtures as solvent systems and a total of seventy six collections were made and merged based on their TLC profile to afford seven major fractions coded E1-E7. Repeated gel filtration of fraction E3 led to the isolation of a yellow amorphous substance, 8 mg (Compound 2).

Results

Spectral data
Quercetin (1). Yellow amorphous substance; m.p. 315 °C-316 °C. ¹H-NMR (CD3OD, 600 MHz): δH 7.76 (1H, d, J=1.38 Hz, H-2'), 6.92 (1H, d, J=8.4 Hz, H-5'), 6.42 (1H, d, J=1.56 Hz, H-8), 6.22 (1H, d, J=1.50 Hz, H-6). ¹³C-NMR (CD3OD, 600 MHz): δC 146.7 (C-2), 135.8 (C-3), 176.0 (C-4), 161.1 (C-5), 97.9 (C-6), 164.2 (C-7), 93.1 (C-8), 156.9 (C-9), 103.1 (C-10), 122.8 (C-1'), 114.6 (C-2'), 144.8 (C-3'), 147.7 (C-4'), 114.9 (C-5'), 120.3 (C-6').

2-(2,4-dihydroxyphenyl)-5,7-dihydroxy-3-(3-methylhexyl)-4H-chromen-4-one (2). Yellow amorphous substance; m.p 245 °C-246 °C. ¹H-NMR (CD3OD, 600 MHz): δH 7.76 (1H, s, H-3'), 6.92 (1H, d, J=8.5 Hz, H-6'), 7.67 (1H, d, J=8.1 Hz, H-5'), 6.42 (1H, d, J=1.5 Hz, H-8), 6.21 (1H, d, J=1.8 Hz, H-6). ¹³C-NMR (CD3OD, 600 MHz): δC 146.7 (C-2), 100.0 (C-3), 176.0 (C-4), 161.1 (C-5), 97.9 (C-6), 164.2 (C-7), 93.1 (C-8), 156.9 (C-9), 103.1 (C-10), 122.8 (C-1'), 147.3 (C-2'), 114.4 (C-3'), 144.8 (C-4'), 114.6 (C-5'), 114.8 (C-6'), 35.1 (C-1''), 31.6 (C-2''), 28.9 (C-3''), 25.5 (C-4''), 29.3 (C-5''), 13.0 (C-6''), 22.3 (C-1'''').

Discussion
Compound 1 was obtained as a yellow amorphous substance and it tested positive to shinoda and ferric chloride reagent suggesting the compound to be a flavonoid (Silva et al., 1998). The ¹H-NMR spectrum of compound 1 indicated the presence of 1, 2, 3, 5-tetrasubstituted benzene ring A via the meta-coupled protons at δ 6.42 (1H, d, J=1.50 Hz) and δ 6.21 (1H, d, J=1.56 Hz) which were assigned to H-6 and H-8 respectively and 1, 3, 4-trisubstituted benzene ring B was observed via protons at δ 7.76 (1H, d, J=1.38 Hz, H-2'), δ 7.67 (1H, dd, J=1.7 Hz, 8.4 Hz, H-6') and δ 6.92 (1H, d, J= 8.4 Hz, H-5'). The ¹H-¹H-COSY established the correlation between protons that lie adjacent to each other; the observed correlation between proton at δH 6.21 and δH 6.42 ppm and the protons at δH 6.92 and δH 7.76 ppm further confirmed the assignment of ring A and B above (Sani et al., 2015).

The ¹³C-NMR and DEPT experiments of compound 1 revealed the presence of 15-carbon atoms (Table 1), seven aromatic carbon signals, seven quaternary oxygenated carbon atoms and a down field signal due to carbonyl carbon resonating at δ 176.0 (C-4). These signals suggests a flavonoid-quercetin nucleus (Mabry et al., 1970). The HSQC spectrum was used to attach various protons to their respective carbon atoms and the connectivity between various fragments was established through HMBC. It established the correlation between proton at δH 6.21 (H-6) and the carbons at δC 93.1 (C-8), δ 103.1 (C-10), δ 161.1 (C-5), δ 164.2 (H-8) correlated with the carbons at δ 97.9 (C-6), δ 103.1 (C-10), δ 156.9 (C-9), δ 164.2 (C-7) further confirming the presence of 1,2,3,5-tetrasubstituted benzene ring A. Long range correlation at observed at δH 6.92 (H-5') and carbons at δ 122.8 (C-1'), δ 144.8 (C-3'), δ 147.4 (C-4') and δ 7.67 and carbons δ 114.6 (C-2'), δ 144.7 (C-3') and δ 6.21 (H-6') and δ 120.3 (C-6'), δ 144.8 (C-4'), δ 146.7 (C-2) confirmed the presence of 1,3,4'-trisubstituted benzene ring B. Based on the 1D and 2D NMR data of compound 1 and comparison with the reported literature (Mabry et al., 1970; Sani et al., 2015), the structure of compound 1 was confirmed to be a quercetin (Figure 1).
Compound 2 was obtained as a yellow amorphous substance and it gave positive result in the test for flavonoid using shinoda test (Silva et al., 1998). The NMR data of compound 2 exhibited similarities with those of compound 1. $^1$H-NMR spectrum revealed signals for meta-coupled protons at $\delta_H$ 6.42 (1H, d, $J=1.5$ Hz, H-8) and $\delta_H$ 6.21 (1H, d, $J=1.8$ Hz, H-6) typical of a 1, 2, 3, 5-tetrasubstituted benzene ring A and a pair of ortho-coupled protons were observed at $\delta_H$ 6.92 (1H, d, $J=8.5$ Hz, H-6’) and $\delta_H$ 7.67 (1H, d, $J=8.1$ Hz, H-5’) alongside a singlet at $\delta_H$ 7.76 (1H, s, H-3’) which were assigned to 1, 4, 6-trisubstituted benzene ring B, typical of flavonoids. Additional aliphatic signals were observed at $\delta_H$ 0.89 (H-6”), 0.93 (H-1”), 1.36 (H-3”), 1.62 (H-2”), 2.05 (H-4”) 1.34 (H-1”) and 2.21 (H-1”) typical of an aliphatic side chain.

$^1$H-$^1$H-COSY experiment was used to further confirmed the assignment of meta-coupled protons at H-6 and $\delta$ H-8 and ortho-coupled protons at H-6’ and H-5’. Cross peaks correlations observed between protons at H-1” # H-2”, H-3” # H-2”, H-3” # H-4”, H-3” # H-1” further substantiate the presence of aliphatic side chain as part of the structure.

The $^{13}$C-NMR (600 MHz, CD$_3$OD) and DEPT experiment of compound 2 revealed the presence of seven aromatic carbon signals, six quaternary oxygenated aromatic carbon atoms and a down field signal due to carbonyl carbon resonating at $\delta$ 176.0; the absence of a resonance at $\delta$ 135.8 (C-3) as observed in flavonols and the presence of a signal at $\delta$ 120.3 further suggests a flavone type nucleus (Mabry et al., 1970). Aliphatic resonances at $\delta_c$ 35.1 (C-1”), 31.6 (C-2”), 29.3 (C-5”), 28.9 (C-3”), 25.5 (C-4”), 22.3 (C-1””), 13.0 (C-6”) further confirmed the presence of aliphatic side chain of flavone type which is consistent with the proton NMR.

The HSQC spectrum established direct correlation between protons and their respective carbons. Aliphatic side chain was further substantiated by the correlations observed between the proton at $\delta_H$ 1.36 and $\delta_c$ 28.9 # 1.36, and $\delta_H$ 1.34 and 29.3 among others.

The connectivity between various fragments was established through HMBC. It established a long range correlation between the proton at $\delta_H$ 6.21 (H-6) with the carbons at $\delta_c$ 93.1 (C-8), 103.1 (C-10), 161.1 (C-5), 164.2 (C-7) and the proton at $\delta_H$ 6.42 (C-8) correlated with the carbons at $\delta_c$ 97.9 (C-6), 103.1 (C-10), 156.9 (C-9), 164.2 (C-7) which confirmed the presence of ring A. Correlation of proton at $\delta$ 6.92 (C-6’) with carbons at $\delta$ 122.8 (C-1”), $\delta$ 144.8 (C-4”), $\delta$ 146.7 (C-2) and $\delta$ 147.3 (C-2”) further confirmed the position of hydroxyl groups at position 2’ and 4’ on ring B in the flavone nucleus (Pratkit, 2010). Proton at $\delta$ 7.76 (C-5’) correlate with the carbon at $\delta$ 122.3 (C-3”). The absence of a downfield signal at $\delta_c$ 135 and a proton signal at C-3 suggests that, the prenyl chain (aliphatic chain) might be attached to the C-3 of flavone nucleus. Based on the 1D and 2D-NMR data of compound 2, a tentative structure was proposed as 2-(2,4-dihydroxyphenyl)-5,7-dihydroxy-3-(3-methylhexyl)-4H-chromen-4-one (Figure 2).

![Figure 1](image1.png)

**Figure 1.** Structure of compounds 1 and 2.
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Table 1. Summary of 1D and 2D spectral data of Compound 1 (CD3OD, 600 MHz).

| Position | δ 13C | δ 1H, J (Hz) | DEPT | COSY | HMBC |
|----------|-------|--------------|------|------|------|
| 2        | 146.7 |              | C    |      |      |
| 3        | 135.8 |              | C    |      |      |
| 4        | 176.0 |              | C    |      |      |
| 5        | 161.1 |              | C    |      |      |
| 6        | 97.9  | 6.21 (1H, d, J=1.56) | CH  | H-8  | C-8, 5, 7, 10 |
| 7        | 164.2 |              | C    |      |      |
| 8        | 93.1  | 6.42 (1H, d, J=1.50) | CH  | H-6  | C-6, 7, 9, 10 |
| 9        | 156.9 |              | C    |      |      |
| 10       | 103.1 |              | C    |      |      |
| 2'       | 114.6 | 7.76 (1H, d, J=1.38) | CH  |      | C-2, 3', 6' |
| 3'       | 144.8 |              | C    |      |      |
| 4'       | 147.7 |              | C    |      |      |
| 5'       | 114.9 | 6.92 (1H, d, J=8.4) | CH  | H-6' | C-1', 3', 4', |
| 6'       | 120.3 | 7.67 (1H, dd, J=8.4, 1.7) | CH  | H-5' | C-2', 2', 4' |

Table 2. Summary of 1D and 2D spectral data for Compound 2 (CD3OD, 600 MHz).

| Position | δ 13C | δ 1H, J (Hz) | DEPT | COSY | HMBC |
|----------|-------|--------------|------|------|------|
| 2        | 146.7 |              | C    |      |      |
| 3        | 100.0 |              | C    |      |      |
| 4        | 176.0 |              | C    |      |      |
| 5        | 161.1 |              | C    |      |      |
| 6        | 97.9  | 6.21 (1H, d, J=1.56) | CH  | H-8  | C-8, 5, 7, 10 |
| 7        | 164.2 |              | C    |      |      |
| 8        | 93.1  | 6.42 (1H, d, J=1.50) | CH  | H-6  | C-6, 7, 9, 10 |
| 9        | 156.9 |              | C    |      |      |
| 10       | 103.1 |              | C    |      |      |
| 1'       | 122.8 |              | C    |      |      |
| 2'       | 147.3 |              | C    | C-1' |      |
| 3'       | 114.4 | 7.76 (br s, 1H) | CH  | H-6' |      |
| 4'       | 144.8 |              | C    |      |      |
| 5'       | 114.6 | 7.67 (1H, d, J=8.1) | CH  | H-6' | C-3' |
| 6'       | 114.8 | 6.92 (1H, d, J=8.5) | CH  | H-5' | C-1', 2, 4', |
| 1''      | 35.1  | 2.21         | CH2  | H-2''|      |
| 2''      | 31.6  | 1.62         | CH2  |      |      |
| 3''      | 28.9  | 1.36         | CH   | H-2'', H-4'' | C-5'' |
| 4''      | 25.5  | 2.05         | CH2  | H-3'' |      |
| 5''      | 29.3  | 1.34         | CH2  | C-3'' |      |
| 6''      | 13.0  | 0.89         | CH3  |      |      |
| 1'''     | 22.3  | 0.93         | CH3  | H-1''| C-2'', 4'' |

Study limitation

Two compounds were isolated from the ethylacetate fraction of *Tapinanthus globiferus*, however, testing the efficacy of the isolated compounds on different ailments is recommended for possible drug development.
Conclusion

Chromatographic studies of ethylacetate fraction of _Tapinanthus globiferus_ afforded two flavonoids 2-(3', 4'-dihydroxyphenyl)-3, 5, 7- trihydroxy-4H-chromane-4-one (quercetin) and 2-(2, 4-dihydroxyphenyl)-5, 7-dihydroxy-3-(3-methylhexyl)-4H-chromen-4-one. This is the first report of isolation of these compounds from _Tapinanthus globiferus_ growing on _Vitex doniana_.

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Conflict of interests

The authors declare that they do not have any conflict of interests.

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