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Isolation and Characterization of Kaempferol from *Tapinanthus globiferus* Growing on *Balanites aegyptiaca*

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Abstract

Objective: *Tapinanthus globiferus* is a hemi-parasitic plant used in ethonomedicine to treat different ailment including fungal infections. The aim of the study was to isolate the bioactive compound from leaf of *T.globiferus* growing on *Balanites aegyptiaca*.

Material and Methods: The plant material was collected, identified pulverized to powder using mortar and pestle. The powdered plant material was subjected to maceration with 90% methanol to obtained crude methanol leaf extract which was further partitioned with solvent of increasing polarity to obtained *n*-hexane, chloroform, ethylacetate and *n*-butanol fractions.

Results: Chromatographic separation of ethylacetate fraction using a combination of silica gel column and sephadex LH-20 column led to the isolation of flavonoid (3,5,7- trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one). The structure of the compound obtained was established by Spectroscopic analysis (FTIR, NMR).

Conclusion: Chromatographic studies of ethyl acetate fraction of *T. globiferus* growing on *Balanites aegyptiaca*, afforded a 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one.To the best of our knowledge, this is the first report of isolation of this compound from *Tapinanthus globiferus* growing on *Balanites aegyptiaca*.

Introduction

Tapinanthus globiferus belonging to the family: Loranthaceae is a hemi-parasitic plant grows on the branches of large trees species including Balanite aegyptiaca, kola, Citrus, Combretum, taminalia, Acacia, Ficus glumbosa, and Tamarindus indica as host plant [1,2]. *T. globiferus* is a woody, spreading shrub with blackish, smooth stems made rough by the presence of lenticels. The leaves are opposite but sometimes alternate. The leaf length varies from 7-15(20) cm while the leaf width could be 3-10(15) cm. The leaves are thick, ovate, obtuse, rounded to cuneate at base. Petiole length up to 2 cm long and grooved axially, nerves pinnate with barely prominent and irregular lateral nerves. The inflorescence is a sub-sessile fascicle with up to 6 flowers. The

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flower is bisexual with a red corolla tube up to 2 cm long and a swollen base that is greenish in color. Calyx forming a short tube enclosing the corolla tube. The stamens are five alternating with the petals and partly fused to the petals. The unattached portion of the filament curls up as soon as the petal lobes open. The fruits are one-seeded, globose and green when immature [3].

T. globiferus is commonly known as mistletoe (English), *Kauchi* (Hausa), *Eme-emi afomo* (Yoruba), and *Osisi/Okwuma osa* (Igbo) [4]. In Nigeria *T. globiferus* has been used in ethonomedicine to treat different ailments including itching[5], tumour, syphillis, fever and removal of placenta after parturition[5].The plant is also used to treat diseases such as hypertension, ulcers, epilepsy, diabetes, weakness of vision and promoting muscular relaxation. *T. globiferus* growing on *Loranthaceae* and *Viscaceae* are *hemiparasitic* plants and their preparations in the form of extracts, infusions, tinctures or tea bags are widely used in various cultures in almost every continent to treat or manage various health problems including hypertension, *diabetes mellitus*, inflammatory conditions, irregular menstruations, menopause, epilepsy, arthritis, cancer, etc [6,7] reported the identification and quantification of two flavonoids and three phenolic acids from *T. globiferus* growing on *Zanthoxylum zonthoxyloide* which include; Quercetin, Rutin, Chlorogenic acid, Galic acids and Caffeic acid. [8] reported the isolation of (-) Epicatechin and Quercetin-3-β-D-glupyranoside from *T. globiferus* growing on *Acacia nilotica.* Bioactive assay of *T. globiferus* growing on other host have been reported. Antifungal activities [9], antimalarial activity [10], anti-inflammatory and anti-oxidant properties [11] and cardiovascular effects [12] activities of the plant were reported. To the best of our knowledge, no work has been reported on the isolation and characterization and of *T. globiferus* growing on *Balanites aegyptiaca.*

We report herein isolation and chracterization of flavonoid (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) from the leaf of *T. globiferus* growing on *Balanite aegyptiaca.*

Materials and Method

General procedures

NMR data were recorded on a Bruker AVANCE spectrometer (400 MHz) with residual solvent as internal standard. A pre coated TLC plates was used to carry out thin layer chromatography by one way ascending technique. Capillary tubes were used to manually apply the sample on the TLC plate and the chromatogram was developed in an air tight chromatographic tank at room temperature with appropriate solvent systems. Low pressure column and vacuum liquid chromatography were conducted using LOBA chem silica gel (60-120) mesh size in a sintered glass funnel while gel filtration chromatography was performed using LH-20. The spots were visualized under UV spectrophotometer (254-366 nm) and (10 % Sulphuric acid) as spray reagent followed by heating in an oven at 105 ºC for about 10 minutes.

Plant Material

The plant material (leaf of *T. globiferus*) was collected from Gunburawa village village in wamakko local government area of Sokoto State Nigeria. The sample was identified by Abdulazeez Salihu at the Herbarium Unit of Biological Sciences, by comparing with specimen number (UDUH/ANS/0135). The plant material was shed dried, pulverized to powder and stored in a polythene bag prior to extraction.

Extraction

One thousand two hundred grams (1200 g) of the powdered sample was macerated with 5 L of methanol with occasional agitation for 3 days, the extract was filtered and the solvent was evaporated with aid of rotary evaporator at 40 ºC to obtain crude methanol leaf extract of *T. globiferus.* Some part of the methanol leaf extract (210 g) was suspended in 700 mL of distilled water which was then filtered and partitioned with solvent of increasing polarity to obtain n-hexane (HF), chloroform (CF), ethylacetate (EF) and n-butanol (BF) fractions.

Isolation

Ethylacetate fraction (5 g) was subjected to column chromatography using gradient elution technique starting with mixture of chloroform: ethyl acetate 50:50, 40:60, 20:80 and 10:90 followed by 100 % ethyl acetate, ethylacetate:

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methanol 95:5 and 90:10 and the column was finally washed with 100 % methanol. A total of 54 collections of 20 mL each were made and combined based on their TLC profile to afford (9) major fractions coded E1 to E9. Fraction E3 was subjected to further purification using low pressure column chromatography.

Fraction E3 (0.5 g) was subjected to silica column chromatography using 100 % chloroform and mixture of chloroform: ethyl acetate (95:5, 93:7, 90:10, 80: 20 and 70:30) and total of 18 collections (1 mL each) were made and combined based on their TLC profile to afford major fractions coded E3A- E3H. Fraction E3D was further subjected to purification using Sephadex LH-20 and methanol as eluting solvent. A total of 12 collections were made and combined based their TLC profile to give 2 major fractions coded E3D1-E3D2. Repeated gel filtration of fractions E3D1-E3D2 led to the isolation of yellow amorphous compound coded as M1.

Characterization of compound

Compound M1 was subjected to spectroscopic analysis (FTIR and NMR) in order to elucidate its structure.

Results

Kaempferol yellow amorphous, m.p 176-178 ^{0}C. FTIR

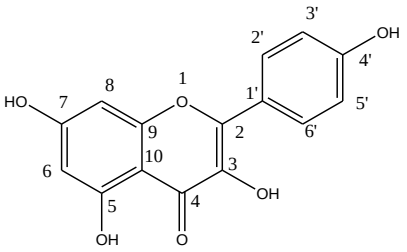

Figure 3.1 ;3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one

OH=3250 cm^{-1}, C-H =2930 cm^{-1}, C=O =1680 cm^{-1}, C=C = 1480 cm^{-1}, C-O=1050 cm^{-1}. ^{1}H-NMR (MeOD, 400 MHz) δ_H 6.29 (1H, d, *J*=2.2 Hz, H-6), δ_H 6.52 (1H, d, *J*=2.2 Hz, H-8), δ_H 8.15 (2H, d, *J*=8.0 Hz, H-2', 6'), δ_H 7.12 (2H, d, *J*= 8.0 Hz, H-3',5'). ^{13}C-NMR (δ ppm, 400 MHz): 175.16 (C-4), 167.23 (C-7), 161.13 (C-5), 156.93 (C-9), 147.38 (C-2), 160.08 (C-4'), 130.5 (C-6'), 137.49 (C-3), 122.73 (C-1'), 117.09 (C-3') 130.60 (C-2'), 117.09 (C-5'), 104.06 (C-10), 100.0 (C-6), 94.06 (C-8). 3,5,7- trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one.

Table1 *NMR spectra data of M1 (MeOD, 400MHz) and comparison with literature*

Position	^{1}H NMR (M1)	^{1}HNMR [16]	^{13}C NMR (M1)	^{13}CNMR [16]
2			147.4	147.1
3			137.5	136.7
4			175.2	176.6
5			161.1	162.4
6	6.29, d, J= 2.2Hz	6.27, d J= 2.2Hz	100.6	99.2
7			164.2	165.0
8	6.25, d, J= 2.2Hz	6.54, d, J= 2.2Hz	94.1	94.5
9			156.9	157.8
10			104.1	104.2
1'			122.7	123.4
2'	8.15, d, J= 8.0Hz	8.14, d, J= 8.0Hz	130.6	130.5
3'	7.12, d, J = 8.0Hz	7.02, d, J = 8.0Hz	117.1	116.4
4'			160.1	160.2
5'	7.12, d, J= 8.0Hz	7.02, d, J =8.0Hz	117.1	116.4
6'	8.15, d, J= 8.0 Hz	8.14, d, J =8.0Hz	130.5	130.5

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Discussion

Chromatographic separation of ethylacetate fraction of *T. globiferus* leaf growing on *Balanites aegyptiaca* being the most active fraction led to the isolation of a yellow amorphous solid coded M1. FTIR spectrum of M1 in (KBr) exhibited the absorption at 3250 cm^{-I} due to O-H stretching, 2930 cm^{-I} is due to C-H stretching which is in good agreement with [13]. A carbonyl C=O stretching around 1680 cm^{-I} C=C bending around 1480 cm^{-1} also in good agreement with what was reported by [13] and a sharp C-O stretching around 1050 cm^{1} due to ether linkage in the chromen nucleus typical of flavonoids [14]. The compound M1 was obtained as yellow amorphous solid, ^{1}H-NMR and ^{13}CNMR show chemical shift values typical of flavonoids. The ^{1}H-NMR spectrum of compound M1 indicated the presence of a 1,2,3,5-tetrasubtituted benzene ring A via the presence of meta-coupled protons at δ_H 6.25 (1H, d, J = 2.2 Hz, H-8) and δ_H 6.29 (1H, d, J = 2.2 Hz, H-8); and a 1',4'-disubtituted benzene ring B was clearly discerned via ortho-coupled protons at 7.12 (2H, d, J=8.0 Hz, H-3', 5') and δ_H 8.15 (2H, d, J= 8.0 Hz, H-2', 6') typical of a flavonol nucleus kaempferol [15]. Protons at position 2'and 6' are in the same chemical environment and likewise protons at position 3' and 5' are also in the same chemical environments, these resonances are in good agreements with what was reported for kaempferol by [15].

^{13}C NMR spectral analysis revealed the presence of 15 carbon signals, typical of flavonoidal skeleton which comprises of Seven (7) quaternary oxygenated carbons signals at δ_C 137.5 (C-3), 147.4 (C-2), 156.9 (C-9), 161.1 (C-5), 164.2 (C-7), 160.1 (C-4') and a downfield signal due to a carbonyl carbon at δ_C 175.2 (C-4); eight (8) aromatic carbon signals at δ_C 100.6 (C-6), 94.1 (C-8), 104.1 (C-10), 122.7 (C-1'), 130.6 (C-2'), 117.1 (C-3'), 117.1 (C-5') and 130.5 (C-6'). These chemical shift values are in good agreement to the values reported for kaemferol by [15]. Based on the FTIR and 1D-NMR data of M1 and comparison with existing data in the literature [16], the structure of M1 was confirmed to be a flavonol (Kaempferol).

Conclusion

Chromatographic studies of ethyl acetate fraction of *T. globiferus* growing on *Balanites aegyptiaca,* afforded a 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one.To the best of our knowledge, this is the first report of isolation of this compound from *Tapinanthus globiferus* growing on *Balanites aegyptiaca.*

Conflict of interest

There is no conflict of interest among the authors

Consent for publications

The authors approved the manuscript for publication

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