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Isolation of Quarcetin-3-*O*-β-D-glucopyranoside from the ethanol leaf extract of *Ficus sycomorous* L. (Moracaea)

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ABSTRACT

Ficus sycomorous (Sycamore fig.) Family Moraceae is a plant used in African traditional medicine to treat mental illness, dysentery, cough, diarrhea, tuberculosis and Cancer. In this research work the leaf of the plant was subjected to cold maceration using 75% ethanol. The extract was fractionated using n- hexane, chloroform ethylacetate and n-butanol. The ethylacetate fraction was subjected to column chromatographic separation and purification. Quarcetin-3-O-β-D-glucopyranoside was isolated and the structure of the compound was elucidated by various spectral techniques such as1 and 2D NMR and mass spectrometry.

Keyword: *Ficus sycomorous*, Moraceae, NMR, Quarcetin-3-O-β-D-glucopyranoside

INTRODUCTION

Ficus sycomorus L., a medicinal plant belonging to the family Moraceae comprises about 755 fig tree species worldwide (Van Noort *et al*., 2007). It is a large, semi-deciduous spreading savannah tree, up to 21 (max. 46) m; it is occasionally buttressed. Its leaves are broadly ovate or elliptic, the sub base is cordate, apex is rounded or obtuse and is scabrous above; petiole is 1-5 cm long, with five to seven pairs of yellow lateral veins; lowest pair originates at the leaf base. The plant is widely distributed in tropical Africa stretching from Senegal to South Africa, Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast. In Nigeria the plant is mostly found in semi-arid regions (Williams *et al*., 1980). The plant is referred to by number of local names as Sycamore fig (English), Baure (Hausa, Northern Nigeria), Tarmu (Kanuri), and Kamda (Babur/Bura), among others **(**Hyde *et al.,* 2013).

Ficus sycomorus (moraceae) has many traditional medicinal uses in the treatment of snake bites, jaundice, chest pains, dysentery, cold, coughs and throat infections (Sofowora, 1993). Its leaves are used in African Traditional medicine to treat jaundice, and are antidote for snake bite. In northern Nigeria, the stem bark of *Ficus sycomorus* is used traditionally to treat fungal diseases, jaundice and dysentery (Berg and Corner, 2005). The Hausa and Fulani tribes of northern Nigeria use the stem-bark of *F. sycomorus* to treat diabetes mellitus, fungal diseases, jaundice and dysentery (Hassan *et al*., 2007; Adoum *et al*., 2012). The parts of *Ficus sycomorus* used traditionally for the treatment of tumors and diseases associated or characterized by inflammation include the fruits in different stages of ripening, fresh or dry, tree bark, leaves, twigs and young shoots, and also latex from the bark, fruit and young branches (Lansky *et al*., 2008).

The aim of this research work is to isolate and characterize some bioactive compounds from the leaves of *Ficus sycomorus*.

MATERIALS AND METHODS

Collection and identification

The leaves of *Ficus sycomorus* were collected in the month of April, 2013, from Turunku village, Igabi Local Government Area, Kaduna State. The plant was identified at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, (voucher specimen number 1466). The plant material was air dried under shade until constant weight was obtained and size reduced manually using clean mortar and pestle.

Extraction and Partitioning

The plant material (2500 g) was subjected to cold maceration using 75% ethanol for 72 h. The extract was then filtered using Whatman Filter paper (No 1) and the filtrate was evaporated under reduced pressure and dried to afford 8.20g (%w/w) of a brownish residue referred to as Crude Ethanol Extract. The crude extract was further fractionated successively using n-hexane (2 liters), chloroform (1.3 L), ethyl acetate (1.5 L) and n–butanol (950 mL).

Chromatographic procedure

The ethylacetate fraction (1.5 g) was subjected to silica gel (60-120 mesh size) column chromatography (2 cm x 30 cm length). The column was packed with silica gel of mesh size 60-120 by wet packing method. A padding of cotton was placed at the bottom of the column. A slurry of the silica gel was made using solvent of lowest polarity (Chloroform). The required amount of stationary phase (silica gel) was poured into the column to form a bed of silica and the extract was then poured on to the bed of silica. The column was eluted continuously using chloroform (100%), followed by 9:1, 8:2, 7:3, 6:4 and 1:1 mixture of chloroform and ethylacetae. Finally, the column was washed with 100% methanol. A total of 178 fractions were collected. Collections with similar TLC profile were pulled together to yield eight bulked fractions X_1-X_8 (52.1 mg, 132.3mg, 70.0 mg, 101.6 mg, 88.8 mg, 97,6 mg 69.3 mg and 189.5 mg respectively). Pulled fraction X_4 which showed four distinct spots was subjected to gel filtration using sephadex LH20 eluted with 100 % methanol. Collections 113, 114 and 115 each showed (similar) one major and one minor spots on TLC chromatogram, hence, were pooled together (21.1 mg). The collection was subjected to preparative TLC using ethylacetate: methanol 9:1 as solvent system. The compound was collected by scrapping silica from the TLC plate, dissolved in methanol and filter through filter paper. This yielded one major compound (5.2 mg) which showed single homogenous spot on TLC chromatogram (Plate1) sprayed with 10% sulphuric acid. It was labeled A1.

Structural elicidation of A1

The 1D NMR (^{1}H, ^{13}C, DEPT) and 2D (HSQC, COSY and HMBC) NMR spectra of A1 was obtained on Bruker 500MHz NMR spectrometer and the GC/MS analyses were performed on a Hewlett-Packard 5890 Gas Chromatograph [2 µL of a 1% sample, Ultra 2HP column (25 m x 0.32 mm x 0.52 mm), flow rate: 1 mL/min, injection temperature: 250°C, detector temperature: 290°C] coupled to a Hewlett-Packard 5971A mass selective detector.

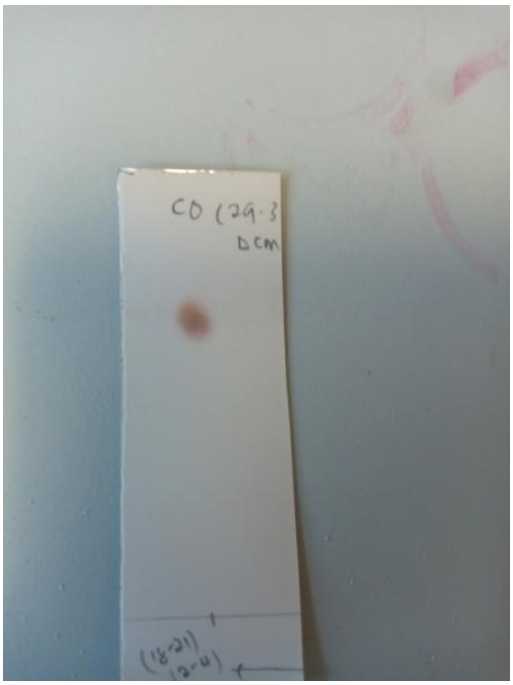

Plate 1: TLC Chromatogram of A1 using ethylacetate: methanol 9:1 as solvent system

The melting point of A1 was determined in open-glass capillaries on Stuart SMP10 melting point apparatus.

RESULTS

The compound was isolated as an amorphous brown powder with TLC Rf value of 0.89, soluble in methanol and had a melting point of 193-195^{0}C. The GCMS spectrum of A1 showed a

molecular ion $[M-H]^+$, peaks at *m/z* 463.3 and 301.0.

The ^{1}H-NMR of A1 (500 MHz, $CDCl_3$): revealed signals at δ_H 7.68 (1H, d, *J* = 2.02 Hz, H-2'), 7.55 (1H, dd, *J* = 2.14, 6.4 Hz, H-6'), 6.83 (1H, d, *J* = 8.4 Hz, H-5'), 6.26 (1H, d, *J* = 1.76 Hz, H-8), 6.09 (1H, d, *J* = 1.89 Hz, H-6), 5.13 (1H, d, *J* = 7.68 Hz, H-1"), 3.75 (1H, dd, *J* = 2.34, 12.0 Hz, H-6"), sugar protons appear in the range δ_H 3.20-3.90.

The ^{13}C- NMR(125 MHz, $CDCl_3$) spectrum of A1 showed 21 carbon signals which include those at δc 179.4 (C-4), 168.8 (C-7), 161.5 (C-5), 157.2 (C-2), 148.0 (C-4'), 144.8 (C-3'), 134.0 (C-3), 121.8 (C-6'), 121.6 (C-1'), 116.1 (C-2'), 114.7 (C-5'), 104.4 (C-1"), 100.3 (C-6), 94.0 (C-8), 103.3 (C-10), 78.3 (C-5"), 78.1 (C-2"), 75.7 (C-3"), 71.2 (C-4") and 62.6 (C-6").

The HMBC spectrum revealed that the Proton signals at δ_H 7.69 showed cross peaks with a carbon at δc 122, 144.7, 148.8 and 157.5. Proton signals at δ_H 7.55 showed cross peaks with a carbon at δc 116.1, 148.8 and 157.2 while anomeric proton signals at δ_H 5.13 showed cross peaks with a carbon at δc 134.1 (Figure 1).

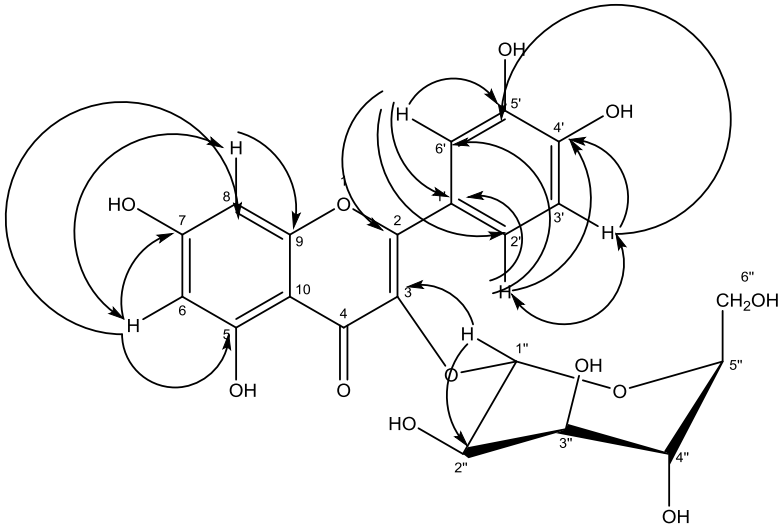

Figure 1: HMBC correlations of compound A1

Table 1: Summary of NMR spectral data of A1

NO.	^{13}C NMR/DEPT-135 (500 MHz) $CDCl_3$	^{13}C NMR (500 MHz) $CDCl_3$**	^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)	^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz) **	HMBC (C → H)	COSY (H → H)
2	159.17 C	158.44				
3	135.80 C	135.64				
4	179.76 C	179.48				
5	163.23 C	162.99				
6	100.04 CH	99.89	6.20 d 1H (2.1)	6.19	5,7,8,10	-
7	166.23 C	165.97				
8	94.85 CH	94.73	6.39 d 1H (2.1)	6.38	6,7,9,10	
9	158.7 CH	158.44				
10	105.8 C	106.68				
1'	123.04 C	123.08				
2'	116.14 CH	117.59	7.58 dd 1H (2.2, 8.5)	7.71	1',3',6'	
3'	146.04 CH	145.87	6.86 d 1H (8.5)	6.91	2',4',5,	
4'	150.00 C	149.33				

5’	117.61	C	116.01				6’
6’	123.4	CH	123.20	7.70 d 1H (8.5)	7.59	1’,2’,4’,5’	5’
1”	104.44	CH	104.39	5.2 d 1H (7.7)	5.23	3,3’	2”
2”	75.87	CH	75.37	3.50 m	3.48	1”,3”	1”,3”
3”	78.54	CH	78.11	3.24 m	3.35	4”	2”,4”
4”	71.37	CH	71.22	3.37 m	3.43	3”,5”,6”	3”,5”
5”	78.27	CH	78.35	3.50 m	3.24	1”,3”,4”	6” α, 6” β
6”a	61.67	CH_2	62.58	4.12 m 1H	3.37	5”	5”
6”b				3.57 m 1H	3.56		5”

** Source: Hadi *et al*., 2017

DISCUSSION

Compound **A1** was obtained as a pink amorphous powder, Melting point 193-195^{0}C. The ^{1}H NMR spectrum showed aromatic protons with signals at δ_H 6.20 (1H, d, *J* = 2.0 Hz), δ_H 6.39 (1H, d, *J* = 2.1 Hz) consistent with the meta-coupled protons H-6 and H-8 of ring-A. Signals at δ_H 7.71 (1H, d, *J* = 2.2 Hz, H-2'), δ_H 7.59 (1H, dd, *J* = 8.5, 2.3 Hz, H-6'), δ_H 6.87 (1H, d, *J* = 8.5 Hz, H-5') corresponding to the catechol protons on ring-B (Ebada, *et al*., 2008). Doublet at δ_H 5.25 d (1H, *J* =7.6 Hz) was assigned to anomeric proton of hexos sugar mainly glucose (Yekta *et al*., 2008). The rest of the protons in the sugar moiety resonated between 3.42 and 3.71. The coupling constant of the anomeric proton H-1” (*J* = 7.6 Hz) define the stereochemistry of glycosidic linkage as β (Guo *et al*., 2012). The ^{13}C NMR spectrum showed the presence of 15 carbon signals in the aglycon including 10 quaternary carbons at δc 1567.2, 134.0, 179.4 161.5, 168.8, 156.4, 103.3, 121.6, 144.8 and 148.06 assigned to C-2, C-3, C-4, C-5, C-7, C-9, C-10, C1’, C-3’ and C-4’ respectively. The five methine carbons appeared at *δ*c 100.3, 94.0, 116.1, 120.9 and 121.8 assigned to C-6, C-8, C-2’, C-5’ and C-6’ respectively (Choudhary *et al.,* 2010). The low field signal at 179.4 was due to the carbonyl group at C-4. The anomeric carbon signals of glucose appeared at δc 104.8. Resonance at δc 61.7 was assigned to the methylene carbon of glucose moiety at C6''. The assignment of these chemical shifts was consistent with HMBC experiment. These spectroscopic data indicated that this compound was a flavonoid glycoside. The HSQC experiment indicated that the proton signals at δ_H 6.26 (H-8) and 6.09 (H-6) correlated with carbon signals at *δ*c 94.5 (C-8) and *δc* 100.3 (C-6), respectively. Additionally, the carbon signals at *δ*c 114.4, (C2’), *δ*c 116.1, (C-5’) and *δ*c 121.8 (C-6’) correlated with the proton signals at δ_H 7.58 (H-2'), 6.69 (H-5'), and 7.58 (H-6'). The Mass spectrum showed the presence of molecular ion peak at *m/z* value of 464.3241 $[M+H]^+$ suggesting the molecular weight of (464) of quercetin-3-*O*-β- D-glucoside. Peak at *m/z* value of 302.5 $[M+H]^+$ was due to loss of glucose moiety from the molecule. Based on these spectral analyses and comparing them with those reported by Hadi *et al.,* (2007), A1 was confirmed to be quercetin-3-*O*-β-D-glucoside (Figure 2). This compound was previously isolated from the leaves of the same plant by Mohamed *et al.,* (2010).

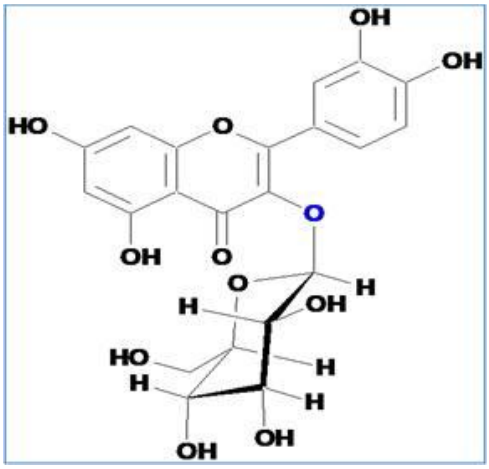

Figure 2: Structure of Quarcetin-3-*O*-β-D-glucopyranoside

CONCLUSION

Based on chromatographic and spectral studies isolated compound was identified as quercetin-3-O-*β*-D-glucoside. Thus, quercetin-3-O-β-D-glucoside was successfully isolated from leaves of *Ficus sycomorus.*

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