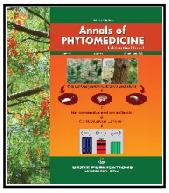
Annals of Phytomedicine 11(1): 673-679, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.1.80

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Review Article : Open Access

Isolation, characterization and structure elucidation of flavonoids from the root bark of *Bauhinia variegata* L.

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Article Info

Article history

Received 1 April 2022

Revised 17 May 2022

Accepted 18 May 2022

Published Online 30 June 2022

Keywords

Bauhinia variegata L.

Quercetin 7-O-methyl ether

Kaempferol-7, 4'-dimethyl ether 3-O-β-D-glucopyranoside

Kaempferol 3-O-β-D-Glucopyranoside

Chemical analysis

1D NMR (^{1}H & ^{13}C NMR) and 2D NMR (HMBC, HSQC & NOESY)

Abstract

Three known flavonoids, quercetin-7-O-methyl ether (1), kaempferol-7, 4'-dimethyl ether 3-O-β-D-glucopyranoside (2) and kaempferol 3-O-β-D-glucopyranoside (3) were isolated from the root bark of *Bauhinia variegata* L., and the structures of the all known compounds were mainly established by extensive spectroscopic analysis, including 1D (^{1}H & ^{13}C NMR), 2D NMR (HMBC, HSQC and NOESY) and chemical studies. In the chemical analysis, compounds 2 and 3 were hydrolysis under acidic conditions to obtain their aglycones and these were identified as kaempferol-7, 4'-dimethyl ether (4) and kaempferol (5), respectively.

1. Introduction

Bauhinia variegata L. (Leguminosae) is a medium sized deciduous tree, found on the rocky hills of Circars, Deccan and Carnatic regions of South India (Gamble *et al.*, 1956). An infusion from its bark is used as an astringent, alterative tonic and useful in scrofula, skin diseases and ulcers. The decoction of the roots is used in dyspepsia and as an antidote to snake bites (Tammanna *et al.*, 2000). Previous phytochemical studies on the stems (Guptal *et al.*,1980, 1984 and 1979), flowers (Wahab *et al.*, 1987; Rahman *et al.*, 1985), root bark (Vijaya Bhaskar Reddy *et al.*, 2003) and seeds (Yadava *et al.*, 2001) of this species have led to the isolation of several flavonoids and their sugar derivatives. Evaluation of the *in vitro* antioxidant potential activity of *B.variegata* plant extract using DPPH (2, 2-diphenyl-1-picrylhydrazyl), and nitric oxide scavenging methods along with phytochemical analysis through thin layer chromatography (Punit *et al.*, 2019). The present work on root bark has resulted in the isolation, characterization and structure elucidation of three known flavonoids, namely; as quercetin-7-O-methyl ether (1), kaempferol-7, 4'-dimethyl ether 3-O-β-D-glucopyranoside (2) and kaempferol 3-O-β-D-glucopyranoside (3). The isolated known compounds were extensively established by various 1D and 2D NMR, mass spectral data and chemical studies. Compounds 1 and 2 were reported first time from the root bark of *B. variegata*.

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2. Materials and Methods

2.1 General experimental procedures

Melting points were measured on a kofler hot-stage apparatus and are uncorrected. Optical rotations were measured in MeOH at 25°C on a Perkin-Elmer241 polarimeter. ^{1}H and ^{13}C NMR spectra were determined on brukeravance 400 and bruker AC 300 spectrometer using DMSO-d_6 and $CDCl_3$ with TMS as internal standard. (^{1}H-^{1}H COSY, HSQC, HMBC and phase sensitive NOESY (with 500 ms mixing time) spectra were recorded using the standard pulse sequences. ESITOFMS and ESI-MS/MS were recorded in positive ion mode on an API Q- STAR PULSA of applied bio-system. Column chromatography was performed on acme silica gel finer than 200 mesh (0.08 mm). TLC was performed using Merck pre-coated silica gel F_{254} plates. Spots were detected on TLC under UV light or by heating after spraying with 10% H_2SO_4 in C_2H_5OH (*v/v*).

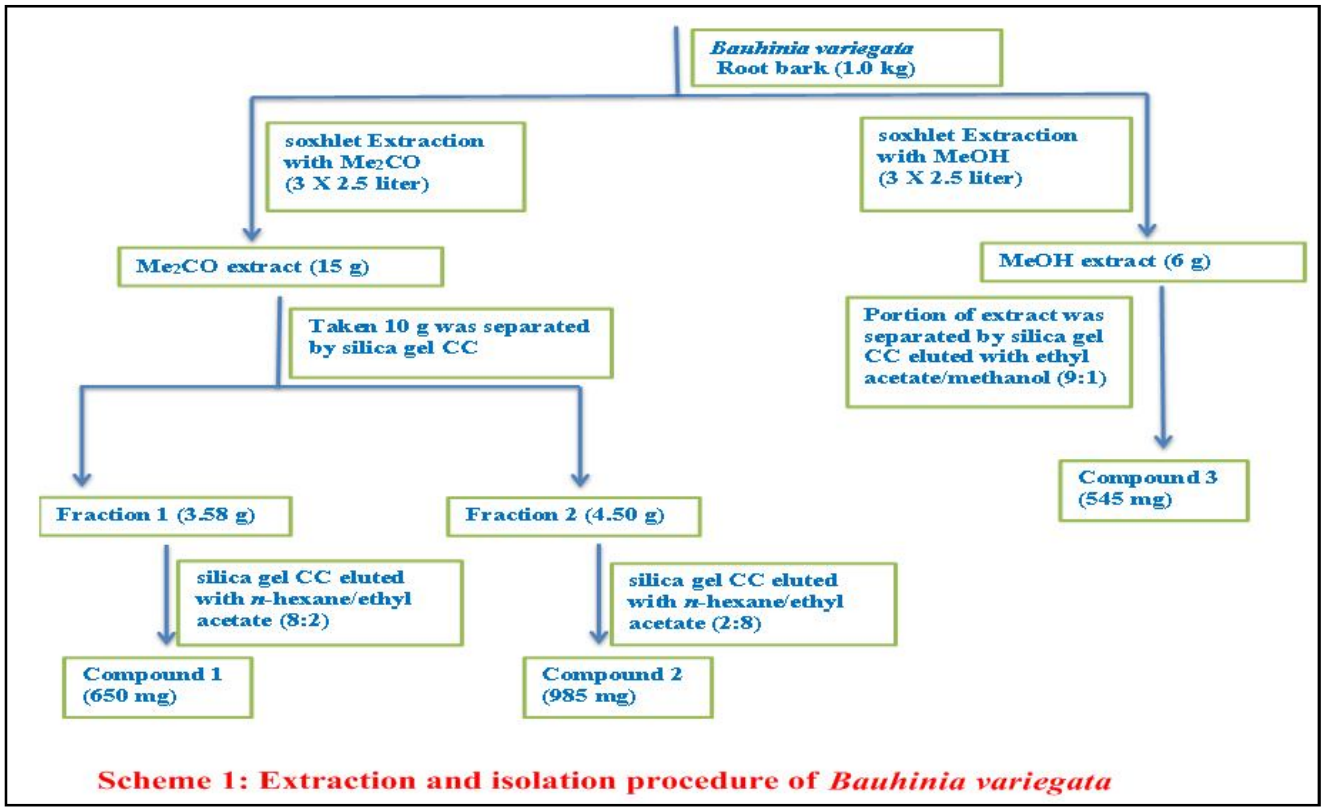
2.2 Plant materials

The root bark of *B. variegata* was collected in December 2019 from Tirumala Hills, Andhra Pradesh, South India. A voucher specimen (BR 005) was deposited in the herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

2.3 Extraction and isolation

Air-dried and powdered root bark of *B.variegata* (1.0 kg) was successively extracted with acetone (Me_2CO) (3 × 2.5 liter) and

MeOH (3 × 2.5 liter) by using Soxhlet apparatus.The filtrate of the solvents were concentrated vacuum distillation under reduced pressure by rotary evaporator at 35ºC to yield 15 g and 6 g, respectively. Portion of the acetone extract (10 g) was dissolved in a minimum amount of methanol (MeOH) and adsorbed over silica gel (20 g, 100-200 mesh, Merck) 60 g of silica gel (100-200 mesh) was taken in silica gel column and on the top of column crude material was loaded. Initially, column was eluted with direct step gradient mixtures as eluents like *n*-hexane: ethyl acetate in the ratios 9:1, 8:2, 7:3, 1:1 and 3:7 collected ten fractions. Based on the TLC pattern, similar fractions are combined it and made into two fractions; Fr.1 (3.58 g) and Fr. 2 (4.50 g). Fraction 1 (2.5 g) was further purified by using silica gel column chromatography eluted with hexane/ethyl acetate (8:2) to give compound 1 as yellow crystalline solid 650 mg). In the TLC analysis Fraction 2 was showing one major compound and some minor pigments appeared. This was further purified by small silica gel column chromatography using the direct solvent mixture like *n*-hexane: ethyl acetate (2:8) yielded a compound 2 as yellow solid (985 mg). The isolated pure compounds were characterized by using various extensive spectral data and established as quercetin-7-O-methyl ether (1) and kaempferol-7, 4'-dimethyl ether 3-O-β-D-glucopyranoside (2). In the TLC analysis of crude methanol extractshowed one major compound. Portion of the crude methanol extract (4 g) was dissolved in minimum amount of methanol (MeOH) and adsorbed over silica gel (10 g, 100-200 mesh, Merck). 40 g of silica gel (100-200 mesh) was taken in silica gel column and on the top of column crude material was loaded. Initially column was eluted with direct solvent like EtOAc and step gradient mixture as EtOAc: MeOH (9:1) to obtain compound 3(545 mg). The isolated pure compound was established as kaempferol 3-O-β-D-glucopyranoside (3) (Scheme 1).



Scheme 1: Extraction and isolation procedure of *Bauhinia variegata*

3. Results and Discussion

Compound 1 was obtained as yellow crystalline solid (650 mg) from acetone extract, mp 212-214ºC. It gave a green color with alcoholic ferric chloride and an orange red color with magnesium-hydrochloric acid. It was yellow under UV and UV/NH_3. It was analyzed for $C_{16}H_{12}O_7$ which is consistent with the presence of $[M+H]^+$ ion at *m/z* 317.0480 and $[M+Na]^+$ ion at *m/z* 339.0309 in the positive ESITOFMS (Electrospray ionization time-of-flight mass spectrometry) spectrum and was corroborated by the presence of sixteen carbon signals in the ^{13}C NMR spectrum. The color reactions together with the UV absorption maxima at 256, 295 (sh), 371 nm suggested compound 1 to be a flavonol derivative (Mabry *et al.*, 1970). UV spectral shifts in the presence of diagnostic reagents indicated free hydroxyl groups at 3, 5, 3' and 4' positions. The IR spectrum showed two strong absorption bands at 3447 and 1654 cm^{-1} due to hydroxyl and chelated carbonyl functions, respectively.

The ^1H NMR spectrum of compound 1 showed a D_2O exchangeable downfield signal at δ 12.79 and was attributed to a chelated hydroxyl at C-5 position. It also exhibited signals for three additional phenolic hydroxyl groups at δ 9.40 (1H) and δ 8.20 (2H), and a methoxyl group at δ 3.85. Two *meta*-coupled doublets (J = 2.0 Hz) at δ 6.23 and 6.47, each integrating for one proton, were assigned to H-6 and H-8, respectively. The methoxyl group at δ 3.85 was placed at C-7 position in compound 1 did not show any bathochromic shift with sodium acetate in the UV spectrum. The three aromatic protons

of ring-B were shown up as typical ABX system at δ 7.69, 7.56 and 7.0 and were assigned to 2', 6' and 5' protons, respectively. Thus, the structure of compound 1 (Figure 1) was established as quercetin-7-O-methyl ether and reported first time from the root bark of *B.variegata* as its spectral data agreed well with literature values (Barbera *et al.*, 1986).

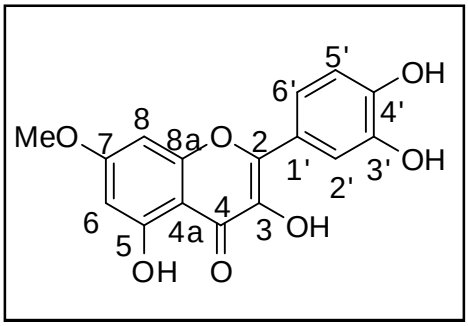


Figure 1: Quercetin-7-O-methyl ether (1).

Compound 2 was crystallized from methanol as yellow solid (985 mg), mp 140-142°C. It had $[\alpha]_D^{25}$-40.7° (*c* 0.1, MeOH). The $[M+H]^+$ and $[M+Na]^+$ peaks at *m/z* 477.1376 and 499.0950 in the positive ESITOFMS spectrum suggested the molecular formula $C_{23}H_{24}O_{11}$ for compound 2. This was corroborated by ^{13}C NMR spectrum which showed signals for all the twenty three carbons of the molecule. Positive Molisch's test, the UV absorption maxima in methanol at 266 and 345 nm, the color reactions and the chromatographic behavior suggested compound 2 to be a flavonol-O-glycoside (Mabry *et al.*, 1970).The addition of sodium methoxide and sodium acetate did not cause any shift in the UV absorption maxima, indicating the absence of free hydroxyl groups at C-4' and C-7 positions, respectively. A bathochromic shift of 55 nm in band I of the aluminium chloride and aluminium chloride-hydrochloric acid spectrum compared to methanol spectrum showed the presence of a free hydroxyl group at 5-position. The IR spectrum showed two strong absorption bands at 3400 and 1650 cm^{-1} due to hydroxyl and chelated carbonyl functions, respectively.

The ^1H NMR spectrum of compound 2 exhibited a D_2O exchan geable downfield signal at δ 12.55 and was ascribed to C-5 hydroxyl as it showed HMBC correlations with C-5 (δ160.9), C-6 (δ97.9) and C-4a (δ105.0). It also showed signals for two methoxyl groups at δ 3.84 and 3.85. Two *meta*-coupled doublets at δ 6.37 and 6.74 which correlated to carbons at δ 97.9 and 92.3, respectively in the HSQC spectrum, were assigned to C-6 and C-8 protons. The methoxyl group at δ 3.85 was placed at C-7, as these protons showed ^3J correlation with this carbon at δ 165.1 in its HMBC spectrum and showed two strong NOE correlations with H-6 (δ 6.37) and H-8 (δ 6.74) in the NOESY spectrum. The ^1H NMR spectrum of compound 2 also showed the presence of typical A_2B_2 doublets of ring-B at δ 8.15 (2H, *J* = 9.0 Hz) and 7.07 (2H, *J* = 9.0 Hz) and were assigned to H-2', 6' and H-3', 5', respectively. The methoxyl group at δ 3.84 was placed at C-4' as these protons showed HMBC correlation with this carbon at δ 161.3, and a strong NOE correlation with H-3' and 5' (δ 7.07). The chemical shift positions of all the protons and carbons were unambiguously assigned by HSQC (Table 1), HMBC (Table 2) and ^1H-^1H COSY (Table 3). An anomeric proton signal at δ 5.50 (d, *J* = 7.3 Hz) and a significant mass fragment at *m/z* 315.1 corresponding to $[M+H-162]^+$ in the ESI-MS/MS spectrum of $[M+H]^+$ ion at *m/z* 477.1 suggested the presence of a hexose sugar residue with β-configuration.

Acid hydrolysis of compound 2 with 2 N HCl afforded D-glucose and an aglycone identified as kaempferol-7, 4'-dimethyl ether (2a) (Figure 3); (Rossi *et al.*, 1997; Nawwar *et al.*, 1985) indicating that the glucosyl moiety in compound 2 was linked to C-3 position. The attachment of glucosyl residue at C-3 was also evidenced by the presence of a cross peak between H-1" (δ 5.50) of glucosyl moiety and C-3 (δ133.7) in the HMBC spectrum.Thus, the structure of compound 2 was characterized as kaempferol-7, 4'-dimethyl ether 3-O-β-glycopyranoside and reported first time from the root bark of *B.variegata*.

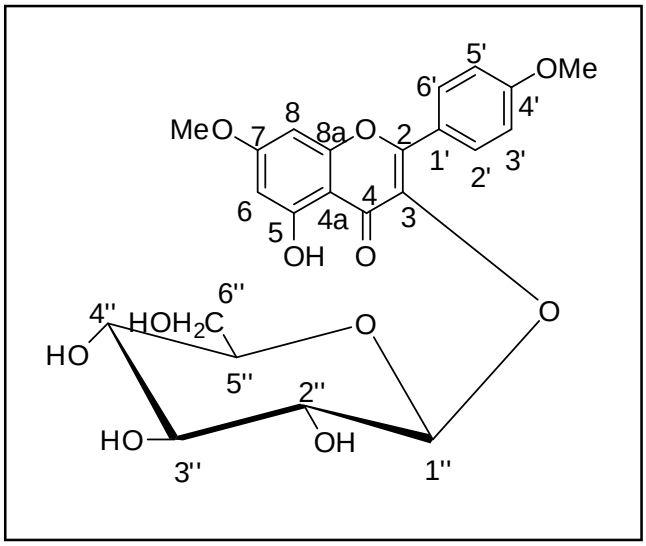


Figure 2(a): kaempferol-7, 4'-dimethyl ether .

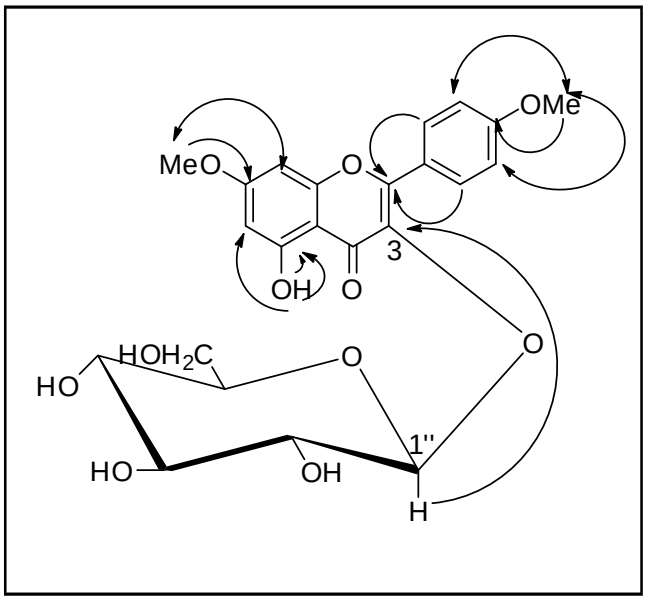


Figure 2(b): Significant HMBC (→) and 3-*O*-β-D-glycopyranoside (2) NOESY (↔) correlations (2).

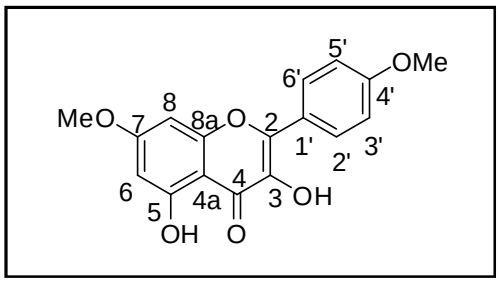


Figure 3: Kaempferol-7, 4'-dimethyl ether (2a).

Table 1: ^{1}H-^{13}C COSY- $^{1}J_{CH}$ (HSQC) data of compound 2

Proton chemical shift (δ)	Correlated carbon chemical shift (δ)	Assignments
8.15 (H-2' and 6')	130.8	C-2' and C-6'
7.07 (H-3' and 5')	113.7	C-3' and C-5'
6.74 (H-8)	92.3	C-8
6.37 (H-6)	97.9	C-6
5.50 (H-1")	100.7	C-1"
3.85 (OMe-7)	56.1	OMe-7
3.84 (OMe-4')	55.4	OMe-4'
3.56 (H-6"a)	60.8	C-6"
3.32 (H-6"b)	60.8	C-6"
3.22 (H-5")	76.4	C-5"
3.18 (H-3")	75.5	C-3"
3.07 (H-4")	69.9	C-4"
3.06 (H-2")	74.2	C-2"

Table 2: HMBC ($^{2-3}J_{CH}$) correlations of compound 2

Proton	Chemical shift (δ)	Correlated carbon (s)
OH-5	12.55	C-4a, C-5, C-6
H-2' and 6'	8.15	C-2, C-1', C-4'
H-3' and 5'	7.07	C-1', C-4'
H-8	6.74	C-4a, C-6, C-7, C-8a
H-6	6.37	C-4a, C-5, C-7, C-8
H-1"	5.50	C-3
OMe-7	3.85	OMe-7
OMe-4'	3.84	OMe-4'

Table 3: ^{1}H-^{1}H COSY data of compound 2

Chemical shift of coupled protons (δ)	Type of coupling	Assignment
8.15 and 7.07	*ortho*	H-2' and H-3'
8.15 and 7.07	*ortho*	H-6' and H-5'
6.74 and 6.37	*meta*	H-8 and H-6
5.50 and 3.06	*vicinal*	H-1" and H-2"
3.18 and 3.06	*vicinal*	H-3" and H-2"
3.07 and 3.18	*vicinal*	H-4" and H-3"
3.22 and 3.07	*vicinal*	H-5" and H-4"
3.56 and 3.22	*vicinal*	H-6"a and H-5"
3.32 and 3.22	*vicinal*	H-6"b and H-5"
3.56 and 3.32	*geminal*	H-6"a and H-6"b

Compound 3 was isolated as yellow needles (545 mg) from methanol, mp 177-178°C. It gave a positive Molisch's test, greenish brown color with alcoholic ferric chloride and an orange color with magnesium-hydrochloric acid. It was purple under UV and yellow under UV/NH_3. The color reactions, positive Molisch's test and UV absorption maxima at 267 and 350 nm suggested the compound to be a flavonol glycoside (Mabry *et al.*, 1970). The IR spectrum showed two strong absorption bands at 3420 and 1657 cm^{-1}due to hydroxyl and carbonyl functions. Acid hydrolysis of compound 3 yielded an aglycone characterized as kaempferol (3a) and a sugar identified as D-glucose. The purple fluorescence of the glycoside compared to yellow of its aglycone under UV suggested that site of glycosylation at 3-OH, which was supported by a hypsochromic shift of 10 nm in band I of the glycoside compared to its free aglycone (Mabry *et al.*, 1970) in methanol. UV spectral data with diagnostic shift reagents revealed the presence of free hydrolysis at 5,7 and 4'-positions, and blocked hydroxyl at 3-position. From the foregoing observations coupled with the identification of the aglycone as kaempferol (3a). Compound 3 could be assigned the structure kaempferol 3-O-β-glucoside (Figure 4). Conformation of the structure kaempferol 3-O-β-glucoside was provided by the ^{1}H NMR spectral data which showed the presence of four aromatic protons of ring B as typical A_2B_2 doublets at δ 8.03 (2H, J = 8.9 Hz, H-2', 6') and 6.87 (2H, J = 8.9 Hz, H-3', 5'). A pair of *meta*-coupled doublets at δ 6.19 and 6.42 was ascribed to H-6 and H-8, respectively. An anomeric proton doublet at δ 5.45 with a coupling constant of 7.3 Hz was ascribed to H-1" of the α-glucosyl residue. A complex multiplet over the range δ 2.90-3.57 integrating for 6 protons were assigned to the remaining ix protons of the glucosyl residue.

Upon comparison of ^{13}C NMR spectrum of Compound 3 with its aglycone, kaempferol (3a) (Figure 4a) (Agarwal *et al.*, 1989) (Table 4), it was observed that the C-3 signal of Compound 3 showed an up fieldshift of δ 2.4, and the C-2 and C-4 signals showed downfield shifts of δ 6.4 and 1.6, respectively from that of kaemperol showing the location of the glucose residue at C-3 position (Agarwal *et al.*, 1989; Markham *et al.*, 1978). Finally compound 3 was characterized as kaempferol 3-O-β-D-glucopyranoside. Final confirmation of compound 3 as kaempferol 3-O-β-D-glucopyranoside was by direct comparison with an authentic sample isolated previously from *Tephrosia calophylla* (Kishore *et al.*, 2003).

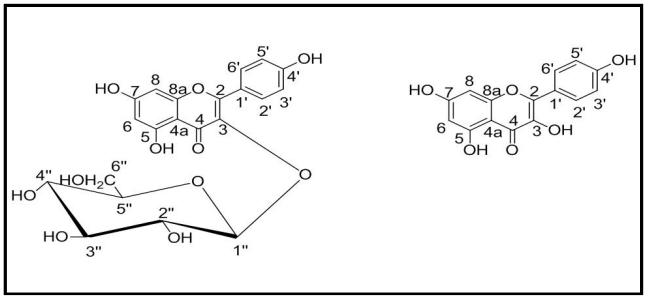


Figure 4: kaempferol-3-*O*-β-D-glucopyranoside (3).

Table 4: Comparison of ^{13}CNMR data in (δ) of compound 3 with its aglycone, kaempferol (3a)

Carbon	Compound 3	Kaempferol (3a)
2	156.2	146.8
3	133.2	135.6
4	177.5	175.9
4a	104.0	103.9
5	161.2	160.7
6	98.7	98.2
7	164.3	163.9
8	93.7	93.5
8a	156.4	156.2
1'	120.9	121.7
2'	130.9	129.5
3'	115.1	115.4
4'	160.0	159.2
5'	115.1	115.4
6'	130.9	129.5
1"	100.9	-
2"	74.2	-
3"	77.5	-
4"	69.9	-
5"	76.4	-
6"	60.9	-

3.1 Quercetin-7-*O*-methyl ether (1)

Isolated as yellow crystals (650 mg) from acetone, mp 212-214°C; $[M + H]^+$ 317.0480 ($C_{16}H_{12}O_7$+H requires 317.0660). UV: λmax (MeOH) (log ε) 256 (4.05), 295 (sh), 371 nm; +NaOMe: 260, 432 nm; +NaOAc: 262, 372 nm; +NaOAc/H_3BO_3:260, 389 nm; +$AlCl_3$: 273, 451 nm; +$AlCl_3$/HCl: 268, 423 nm. IR: ν max (KBr) 3447 (-OH), (s), 2910 (OMe) (s), 1654 (>C=O), 1480, 1400, 1210, 1025, 750, 700 cm^{-1}.^{1}H NMR (400 MHz, Me_2CO-d_6): δ 12.79 (1H, s, OH-5), 9.40 (1H, s, OH-3), 8.20 (2H, s, OH-3', 4'), 7.69 (1H, d, *J* = 2.1 Hz, H-2'), 7.56 (1H, dd, *J* = 8.1, 2.1 Hz, H-6'), 6.47 (1H, s, d, *J* = 2.0 Hz, H-8), 6.23 (1H, s, d, *J* = 2.0 Hz, H-6), 3.85 (3H, s, OMe-7).^{13}C NMR (75 MHz, Me_2CO-d_6): δ 179.4 (C-4), 164.8 (C-7), 163.1 (C-5), 157.7 (C-8a), 156.6 (C-2), 149.0 (C-3'), 145.8 (C-4'), 139.2 (C-3), 122.9 (C-1'), 122.0 (C-6'), 116.2 (C-5'), 115.9 (C-2'), 105.8 (C-4a), 99.3 (C-6), 94.3 (C-8), 56.6 (OMe-7). ESITOFMS: (positive mode) *m/z* (rel. int. %) 655.0787 $[2M+Na]^+$ (43), 633..995 $[2M+H]^+$ (3), 339.0309 $[M+Na]^+$ (96), 317.0480 $[M+H]^+$ (100) ($C_{16}H_{12}O_7$+H requires 317.0660).ESI-MS/MS: (positive mode) *m/z* (rel. int. %) 317.0 $[M+H]^+$ (56), 302.0 $[M+H-CH_3]^+$ (70), 301.0 $[M+H-CH_3-H]^+$ (100), 285.0 $[M+H-CH_3OH]^+$ (11), 274.0 $[M+H-CH_3-CO]^+$ (21), 257.0 $[M+H-CH_3OH-CO]^+$ (9), 256.0 $[M+H-CH_3-CO-H_2O]^+$ (3), 245.0 $[M+H-2CO-H]^+$ (13), 229.0 $[M+H-CH_3OH-2CO]^+$ (10), 167.0 ($^{1,3}A^+$) (1), 137.0 ($^{1,3}A^+$-OCH_3+H)(Ma et al., 1997)or ($^{0,2}B^+$) (2).

3.2 Kaempferol-7, 4'-dimethyl ether 3-*O*-β-D-glycopyranoside (2)

Compound 2was isolated and crystallized from methanol as yellow crystalline solid (985 mg), mp 140-142°C. It had $[\lambda]_D^{25}$-40.7° (*c* 0.1, MeOH); $[M+H]^+$ 477.1019 ($C_{23}H_{24}O_{11}$+H 477.1396).UV: λ max (MeOH) (log ε) 266 (4.28), 345 (4.20) nm; +NaOMe: 267, 354 nm;+NaOAc: 268, 343 nm; +$AlCl_3$: 270, 400 nm. IR: ν max (KBr) 3400 (-OH) (s), 2840 (OMe) (s), 1650 (>C=O) (s), 1600, 1580, 1500, 1450, 1220, 1200, 1150, 1110 cm^{-1}.^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (1H, s, 5-OH), 8.15 (2H, d, *J* = 9.0 Hz, H-2', 6'), 7.07 (2H, d, *J* = 9.0 Hz, H-3', 5'), 6.74 (1H, d, *J* = 2.1 Hz, H-8), 6.37 (1H, d, *J* = 2.1 Hz, H-6), 5.50 (1H, d, *J* = 7.3 Hz, H-1''), 3.85 (3H, s, OMe-7), 3.84 (3H, s, OMe-4'), 3.56 (1H, br d, *J* = 12.0 Hz, H-6''a), 3.32 (1H, br d, *J* = 12.0 Hz, H-6''b), 3.22 (1H, ddd, *J* = 9.0, 9.0, 9.0 Hz, H-5''), 3.18 (1H, dd, *J* = 9.0, 9.0 Hz, H-3''), 3.07 (1H, dd, *J* = 9.0, 9.0 Hz, H-4''), 3.06 (1H, dd, *J* = 9.0, 9.0 Hz, H-2'').^{13}C NMR (75 MHz, DMSO-d_6): δ 177.6 (C-4), 165.1 (C-7), 161.3 (C-4'), 160.9 (C-5), 156.3 (C-2), 156.1 (C-8a), 133.7 (C-3), 130.8 (C-2', 6'), 122.4 (C-1'), 113.7 (C-3', 5'), 105.0 (C-4a), 100.7 (C-1''), 97.9 (C-6), 92.3 (C-8), 76.4 (C-5''), 75.5 (C-3''), 74.2 (C-2''), 69.9 (C-4''), 60.8 (C-6''), 56.1 (OMe-7), 55.4 (OMe-4').ESITOFMS: (positive mode) *m/z* (rel. int. %) 975.1946 $[2M+Na]^+$ (27), 499.0950 $[M+Na]^+$ (33), 477.1376 $[M+H]^+$ (100) ($C_{23}H_{24}O_{11}$+H requires 477.1396). ESI-MS/MS: (positive mode) *m/z* (rel. int. %) 477.1 $[M+H]^+$ (3), 315.1 $[M+H-162]^+$ (98), 300.1 $[M+H-162-Me]^+$ (56), 273.1 $[M+H-162-C_2H_2O]^+$ (18), 272.1 $[M+H-162-Me-CO]^+$ (100), 244.1 $[M+H-162-Me-2CO]^+$ (70), 229.0 $[M+H-162-2Me-2CO]^+$ (33).

3.2.1 Acid hydrolysis of kaempferol-7, 4'-dimethyl ether 3-*O*-β-D-glycopyranoside (2)

A solution of compound **2** (10 mg) in 4 ml of 2N HCl in MeOH (1:1) was heated under reflux for 2h on a water bath, cooled and MeOH distilled off. The yellow precipitate separated was filtered and recrystallized from MeOH to give yellow needles (4 mg) mp 204-206°C. UV: λ max (MeOH) (log) 268 (4.10), 340 (3.80) nm; +NaOMe: 268, 341 nm; +NaOAc: 270, 342 nm; +$AlCl_3$: 270, 395 nm; +$AlCl_3$/HCl: 270, 395 nm. IR: ν max (KBr) 3390 (-OH), 2950 (-OMe), 1654 (>C=O), 1594, 1500, 1245, 1190, 950, 820, 795 cm^{-1}.^{1}H NMR (200 MHz, $CDCl_3$): δ 11.35 (1H, s, OH-5),9.40 (1H, s, OH-3), 8.05 (2H, d, *J* = 8.0 Hz, H-2', 6'), 7.01 (2H, d, *J* = 8.0 Hz, H-3', 5'), 6.42 (1H, d, *J* = 2.0 Hz, H-8), 6.25 (1H, d, *J* = 2.0 Hz, H-6), 3.85 (6H, s, OMe-7, 4').Finally the aglycone was characterized as kaempferol 7,4'-dimethy ether (2a) as its spectral data well agreed with literature data(Rossi *et al.*, 1997 and Nawwar *et al.*, 1985).

3.3 Kaempferol 3-*O*-β-D-glucopyranoside (3)

Isolated and crystallized from MeOH as yellow needles (545 mg), mp 177-178°C (found: C: 56.21, H: 4.50 $C_{21}H_{20}O_{11}$ requires C: 56.25, H: 4.46). UV: λ max (MeOH) (log ε) 267, 350 nm; +NaOMe: 275, 325, 402 nm; +NaOAc: 275, 312 (sh), 284 nm; +NaOAc/H_3BO_3: 265, 357 nm; +$AlCl_3$: 275, 306, 350, 398 nm; +$AlCl_3$/HCl: 278, 304, 358, 398 nm. IR: ν max (KBr) 3420 (br) (-OH), 2924, 1657 (>C=O), 1609, 1507, 1361, 1289, 1205, 1180, 1061, 1013, 968, 732 cm^{-1}.^{1}H NMR (300 MHz, DMSO-d_6): δ 12.60 (1H, s, OH-5), 10.40 (2H, br s, OH-7, 4'), 8.03 (2H, d, *J* = 8.9 Hz, H-2', 6'), 6.87 (2H, d, *J* = 8.9 Hz, H-3', 5'),6.42 (1H, d, *J* = 2.0 Hz, H-8), 6.19 (1H, d, *J* = 2.0 Hz, H-6), 5.45 (1H, d, *J* = 7.3 Hz, H-1''), 2.90-3.57 (6H, m, H-2'', 3'', 4'', 5'', CH_2-6'').^{13}C NMR (75 MHz, DMSO-d_6): δ177.5 (C-4), 164.3 (C-7), 161.2 (C-5), 160.0 (C-4'), 156.4 (C-8a), 156.2 (C-2), 133.2 (C-3), 130.9 (C-2', 6'), 120.9 (C-1'), 115.1 (C-3', 5'), 104.0 (C-4a), 100.9 (C-1''), 98.7 (C-6), 93.7 (C-8), 77.5 (C-3''), 76.4 (C-5''), 74.2 (C-2''), 69.9 (C-4''), 60.9 (C-6''). CIMS (Positive ion mode, CH4) *m/z* (rel. int. %): 449 $[M+H]^+$(16), 287 $[M+H-162]^+$ (100), 180 (54), 162 (9), 145 (4), 121 (2). EIMS (70 eV, DI) *m/z* (rel. int. %): 286 [M-glucosyl]$^+$(100).

3.3.1 Acid hydrolysis of kaempferol 3-*O*-β-D-glucopyranoside (3)

Acid hydrolysis of compound 3(10 mg) with 2N HCl in MeOH (1:1) (13 ml) as described under compound 3gave a solid which on crystallization from MeOH afforded yellow needles (4 mg), mp 274-75°C. It gave a green color with alcoholic Fe^{+3}, pink with Mg-HCl and yellow with Na_2CO_3 and NaOH. It was yellow under UV and UV/NH_3. UV: λ max (MeOH) (log ε) 262, 325 (sh), 364 nm; +NaOMe: 255 (sh), 275, 315 nm; +NaOAc: 274, 301 nm; +$AlCl_3$: 272, 305, 350, 426 nm; +$AlCl_3$/HCl: 272, 305 (sh), 348, 422 nm. IR: ν max (KBr) 3323 (s) (-OH), 1660 (>C=O), 1615, 1520, 1246, 1215, 1093, 1024, 823 cm^{-1}.^{1}H NMR (300 MHz, Me_2CO-d_6): δ 12.15 (2H, s, OH-5, OH-3), 9.70 (1H, br s, OH-7), 9.10 70 (1H, br s, OH-4'), 8.14 (2H, d, *J* = 9.0 Hz, H-2', 6'), 7.0 (2H, d, *J* = 9.0 Hz, H-3', 5'), 6.52 (1H, d, *J* = 2.1 Hz, H-8), 6.26 (1H, d, *J* = 2.1 Hz, H-6).^{13}C NMR (75 MHz, DMSO-d_6): See Table 4:EIMS (70 eV, DI) *m/z* (rel. int. %): 286 $[M]^+$, 285 $[M-1]^+$ (13), 257 $[M-29]^+$ (5), 153 $[A_1+H]^+$ (11), 149 (77), 43 (100). Finally the aglycone**3a**was characterized as kaempferol.

4. Conclusion

Quercetin 7-O-methyl ether (1), kaempferol-7, 4'-dimethyl ether 3-O-β-D-glucopyranoside (2) and kaempferol 3-O-β-glucopyranoside (3) flavonoids were isolated from the root bark of *B.variegata*, and the structures of all known compounds were mainly established by extensive spectroscopic analysis, including 1D (^{1}H &^{13}C NMR), 2D NMR (HMBC, HSQC & NOESY) and chemical studies. In the chemical analysis, compounds 2 and 3 were hydrolysis under acidic conditions to obtain their aglycones and these were identified as kaempferol-7, 4'-dimethyl ether (4) and kaempferol (5), respectively and these compounds were characterized by ESITOFMS spectroscopy.

Acknowledgments

The authors thank Dr. J.P. Brouard, Laboratorie de Chimie des substances Naturelles, MNHN, Paris, France for providing all compounds spectral data for their constant support during this research work for isolation and characterization of secondary metabolites from plant sources.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Mopur Vijaya Bhaskar Reddy (2022). Isolation, characterization and structure elucidation of flavonoids from the root bark of *Bauhinia variegata* Linn. Ann. Phytomed., 11(1):673-679. http://dx.doi.org/10.54085/ap.2022.11.1.80.**