International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr

Research Article Open Access

Isolation and Characterization of 7, 4' di-O-methyl kaempferol-3-O-rhamnoside from *Bauhinia tomentosa* flowers

S. Solomon*[1], N. Muruganantham[2], M.M. Senthamilselvi[3]

[1]*Department of Chemistry, Periyar E.V.R. College (Autonomous), Trichy, Tamil Nadu, India.*
[2]*Assistant Professor, Department of Chemistry, Roever Engineering College, Perambalur, Tamil Nadu, India.*
[3]*Principal, Government Arts College, Ariyalur, Tamil Nadu, India.*

A B S T R A C T

Flavonol glycoside 7,4'di-O-methyl kaempferol-3-O-rhamnoside was isolated from the flowers of Bauhinia tomentosa. The structures of the isolated compound was elucidated through their physical and chemical methods. The isolated compound was characterized using various spectroscopic data such as UV, ^{1}H NMR, ^{13}C NMR, MS.

Keywords: Bauhinia tomentosa, UV, NMR (^{1}H, ^{13}C) and MS, 7,4'di-O-methyl kaempferol-3-O-rhamnoside.

A R T I C L E I N F O

CONTENTS

Article History: Received 21 January 2016, **Accepted** 19 March 2016, **Available Online** 10 April 2016

***Corresponding Author**
S. Solomon
Research Scholar,
Department of Chemistry,
Periyar E.V.R.College,
Tiruchirappalli, Tamil Nadu, India.
Manuscript ID: IJMPR2887

PAPER-QR CODE

Citation: S. Solomon, *et al.* Isolation and Characterization of 7, 4' di-O-methyl kaempferol-3-O-rhamnoside from *Bauhinia tomentosa* flowers. *Int. J. Med. Pharm. Res*., 2016, 4(2): 85-89.

1. Introduction

Plants and plant based drugs have been used to treat various diseases from time immemorial which is proved to be less toxic and free from side effects. The genus Bauhunia belongs to Leguminosae family, subfamily Caesalpiniaceae which comprises about 300 species distributed in tropical and sub tropical regions. Several therapeutic properties are attributed to Bauhinia species, which includes anti-ameboic, anti-diabetic, analgesic, anti-rheumatic, hypocholesteromic activities[1]. Bauhinia tomentosa is a shrub prevalent among south India and is widely used in ayurvedic preparations. This plant has been reported to possess antioxidant [2], gastroprotective [3], hepatoprotective [4] and antimicrobial

effect [5]. Aderogba and his colleagues [6] reported that the ethanolic extract of B. tomentosa leafs contain kaempferol-7-O-rhamnoside, kaempferol-3-O-glucoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside. It was previously reported that B. tomentosa could stimulate the immune system and could act as a potential anti-inflammatory agent [7]. In plants, flavonoids aglycones (i.e., flavonoids without attached sugar) occur in a variety of structural forms. All contain fifteen carbon atoms in their basic nucleus two six-membered rings linked with a three carbon unit which may or may not be a part of a third ring [8].

2. Materials and Methods

Collection of Flowers

Fresh flowers of Bauhinia tomentosa were collected from Jail Corner, Trichy district, Tamil Nadu, India, during the month of January and identified by Dr. S. John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. SS003 dated: 08/01/2016). St.Joseph's College (Campus), Trichy, Tamil Nadu, India.

Extraction and fractionation

Fresh flowers (1kg) of Bauhinia tomentosa collected at Jail corner, Trichy district, Tamil Nadu, India were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (3x250ml), peroxide free diethyl ether (3x250ml) and ethyl acetate (8x250ml). Ethyl acetate fraction alone was taken for further study.

Ethyl acetate fraction: (Flavonol glycoside -7, 4'di-O-methyl kaempferol-3-O-rhamnoside): The EtOAc fraction was concentrated in vacuo. The residue from EtOAC fraction was taken up in acetone and left in an ice chest for two hours. A yellow solid (m.p. 184-186^{o}C) (yield 0.2%) separated was recrystallized from methanol. It gave greenish brown colour with alc.$FeCl_3$, an intense yellow colour with NaOH, deep pink colour with Mg-HCl. It responded to Wilson's boric acid test (Flavones and flavonols having free 5-OH give yellow colour with borocitric acid), Gibb's test (Flavones and flavonols having no substitution at C-8 will give blue or green colour with 2,6-dichlorobenzoquinonechlorimide) and Molish's test [9] (2 drops of 20% alcoholic solution of α-naphthol is added to about 2ml of dilute aqueous solution of glycoside followed by the addition of 2ml of Conc.H_2SO_4 through the sides of the test tube. Deep violet colour is produced). But did not respond to Horhammer-Hansel test [10] (when Zirconium oxychloride dissolved in acetic acid is sprayed in paper flavonol with free 3-OH group will give yellow colour) [11]. It had R_f values as depicted in table (1.1).

Hydrolysis of the glycoside

50 mg of the glycoside was dissolved in hot aqueous methanol (5 ml, 50%). To this, an equal volume of H_2SO_4 (7%) was added and the mixture was refluxed at 100^{0}C for 2 hrs. The aqueous hydrolysate was extracted with Et_2O. The residue from Et_2O was studied further.

Identification of the aglycone

(7, 4'-di-O-methyl Kaempferol)

The aglycone that separated from the Et_2O layer, on crystallization gave a yellow solid (m.p 278- 280^{o}C) yield (0.04%). It was soluble in organic solvents and sparingly soluble in hot water. It developed a reddish orange colour with Mg-HCl and intense yellow colour with NaOH. It responded to Horhammer-hansel test [10], Gibb's test and Wilson-boric test [9]. But did not answer Molisch's test. It had R_f values as depicted in table 1.1. It had λmaxMeOH 266, 382; + NaOMe 275, 393; + $AlCl_3$ 269, 303, 348, 422; +$AlCl_3$-HCl 268, 303, 347, 422; + NaOAc 268, 305, 317, 352; + NaOAc-H_3BO_3 265, 302 sh, 351 nm [12].

Identification of sugar: (Rhamnose)

The aqueous solution from the above hydrolysate was neutralized with $BaCO_3$ and filtered. The concentrated filtrate on chromatographic examination (PC) gave a single spot and its R_f values are depicted in table 1-2 and the values are agreed with those of rhamnose. The identity of the sugar was confirmed by Co-chromatography with an authentic sample of rhamnose [13].

3. Results and Discussion

The fresh flowers of Bauhinia tomentosa have been found to contain 7,4'di-O-methyl Kaempferol-3-O-rhamnoside. The UV spectrum of the glycoside showed two major peaks at 358 nm (band I) and 258 nm (band II), showing the presence of a flavonoid [14]. A bathochromic shift of 24 nm found in the MeOH spectrum of aglycone compared to the glycoside, suggests that the site of glycosylation could be at C-3. This is supported by the fact that the glycoside is not responded to Horhammer – Hansel test but the aglycone responded[15]. The presence of free –OH at C-5 in the glycoside and in the aglycone is evident from their positive response to Wilson boric acid test. This is also supported by the fact that a bathochromic shift of 41 and 40 nm observed in the glycoside and in the aglycone respectively in the $AlCl_3$-HCl spectra[16].

A comparison of NaOAc spectrum[18] (band II) of the glycoside and its aglycone with their respective MeOH spectrum suggests the absence of 7-OH. The absence of any characteristic shift in (band I) NaOAc-H_3BO_3, suggests the absence of catechol type of substitution in B ring[18].

In the ^{1}H NMR spectrum (DMSO-d_6; TMS) (fig 1.1) of the glycoside, the signal appearing at δ 12.59 ppm corresponds to the –OH at C-5. The C-6 proton appears as a doublet at 6.2 ppm due to meta coupling with C-8 protons and the C-8 protons appears as a doublet at 6.4 ppm due to meta coupling with C-6 proton. Protons at C-2', C-3', 5'and 6'appear as two pairs of ortho coupled doublets. The proton at C-3'and C-5'occur at δ 6.8 ppm as a doublet.

The protons at C-2' and C-6'appear at 7.56 ppm as a doublet, due to deshielding effect of oxygen substituent at C-4'. The presence of $-OCH_3$ group is evidenced by the presence of a signal at δ 3.8 ppm [17].The H-1'' signal of rhamnose appears at 5.33 ppm. The rhamnosyl $-CH_3$ protons appear at δ 1 ppm as a doublet. Other rhamnosyl protons appear between 3 to 4 ppm [18]. Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C-NMR (DMSO-d_6-TMS) (Fig 1-2) data. The signal positions and their complete assignments to

different carbons are given in table 1-3. Due to glycosylation, the C-3 carbon shows an up field shift and the two ortho carbons C-2 and C-4 show downfield shift [13]. Presence of $-OCH_3$ group is evidenced by the downfield shift found at C-4' and at C-7. Methyl carbon of this $-OCH_3$ group resonates at δ 59.5 ppm. Methyl carbon of rhamnose resonated at δ 17.6 ppm [19]. The structure of the glycoside is further evidenced by the mass spectrum of the aglycone obtained from the glycoside. The mass spectrum is shown in the fig 1-3. The spectrum of the aglycone had a peak at m/z 314 for M^+ ion. The fragmentation pattern (fig 1-4) following RDA (Retro Diels Alder) and other common fragmentation pattern is, in favour of the structure of the compound [11].

Table 1.1: R_f X 100 Values of G1from the flowers of Bauhinia tomentosa
(Whatman No.1, Ascending, 30 ± 2^0C)

Compound	*Developing solvents								
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**
Glycoside G_1	12	39	42	68	78	70	70	52	71
Aglycone from G_1	-	-	5	-	49	92	66	86	63

*** Solvent Key**
a = H_2O
b = 5% aq. HOAc
c = 15% aq. HOAc
d = 30 % aq. HOAc
e = 60 % aq. HOAc
f = n. BuOH: HOAc: H_2O = 4:1:5 (Upper phase)
g = Phenol saturated with water
h = HOAc: Conc. HCl: H_2O = 30:3:10
i = t BuOH : HOAc : H_2O = 3:1:1

It had λmax MeOH 258, 358 ; + NaOMe 273, 396; + $AlCl_3$ 275, 304, 353, 399 ; + $AlCl_3$ –HCl 274, 304, 352, 399; + NaOAc 259, 305, 317 sh, 353; + NaOAc-H_3BO_3 265, 301 sh, 351 nm. The ^{1}H and ^{13}C- NMR spectra of the glycoside are appended in Fig. 1.1 and 1.2

Table 1.2: R_f (X100) Values of the sugar form the glycoside G_1 from the flowers of Bauhinia tomentosa
(Whatman No.1 Ascending, 30 ± 2^0C)

Compound	* Developing solvents				
	e	**f**	**g**	**h**	**i**
Sugar from G_1	76	30	61	92	78
Rhamnose (authentic)	75	30	60	92	79

J = n BuOH: Benzene: Pyridine: H_2O = 5:1:3:3 Spray reagent: Aniline hydrogen phthalate

Table1-3: ^{13}C-NMR spectral data and their assignments for the aglycone and glycoside G_1 from the flowers of Bauhinia tomentosa

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Kaemepferol from literature	146.8	135.6	175.9	160.7	98.2	163.9	93.5	156.2	103.1
Glycoside G_1	156.3	133.1	177.2	161.07	97.7	164.3	92.6	156.3	103.7

Compound	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
Kaemepferol from literature	121.7	129.5	115.4	159.2	115.4	129.5
Glycoside G_1	121.04	130.7	115.1	168	115.1	130.7

Compound	C-1''	C-2''	C-3''	C-4''	C-5''	C-6''
Kaemepferol from literature	101.9	70.4	70.6	71.5	70.1	17.3
Glycoside G_1	101.88	70.45	70.8	71.7	69.97	17.6

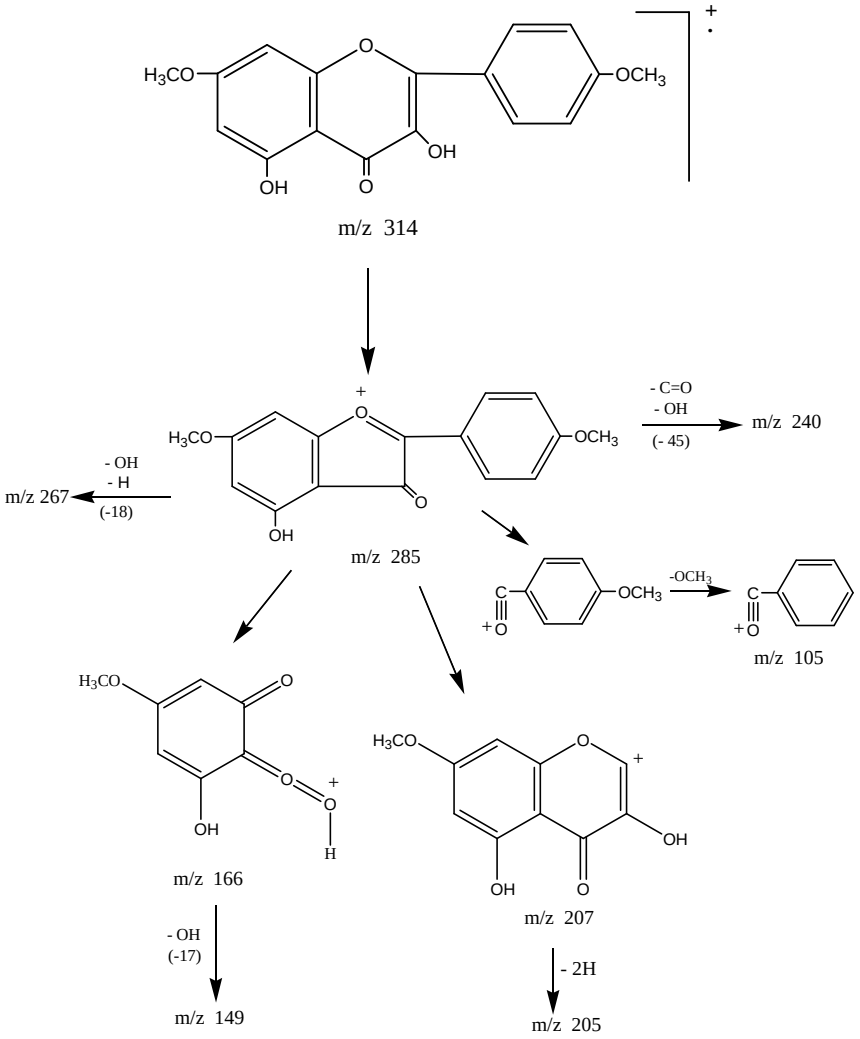

Figure 1: Mass fragmentation Pattern

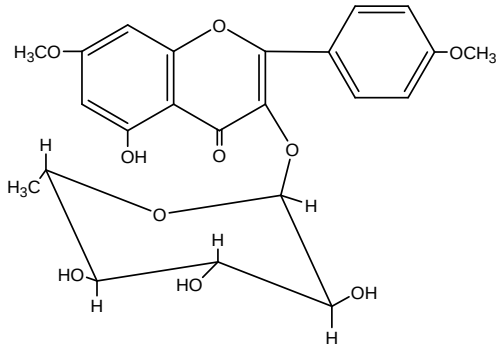

Figure 2: 7,4'-di-O-methyl Kaempferol-3-O-rhamnoside

Based on the above evidences the glycoside has been characterized as 7,4'-di-O-methyl Kaempferol-3-O-rhamnoside[18].

4. Conclusion

The plant Bauhinia tomentosa is a known for its traditional medicine. Isolation of the new compound 7, 4'di-O-methyl kaempferol-3-O-rhamnoside Bauhinia tomentosa will definitely be useful in medicinal field.

5. References

[1] Khalil NM, Pepato MT, Brunetti IL. Free radical scavenging profile and myeloperoxidase inhibition of extracts from antidiabetic plants: Bauhinia for ficata and Cissussicyoides. Biol Res 2008; 41:165–71.

[2] Aderogba MA, McGaw LJ, Ogundaini AO, Eloff JN. Cytotoxicity study of antioxidant flavonoids from Bauhinia tomentosa leaf extract. Niger J Nat Prod Med 2008:12:50–4.

[3] Gonzalez-Mujica F, Motta N, Márquez AH, Capote-Zulueta J. Effects of Bauhinia megalandra aqueous leaf extract on intestinal glucose

absorption and uptake by enterocyte brush border membrane vesicles. Fitoterapia 2003, 74: 84–90.
[4] Viana EP, Santa-Rosa RS, Almeida SSMS, Santos LS. Constituents of the stem bark of Bauhinia guianensis. Fitoterapia 1999; 70(1):111–2.
[5] Bodakhe SH, Ram A. Hepatoprotective properties of Bauhinia tomentosa tabark extract. Yakugaku Zasshi 2007:127:1503–7.
[6] Gopalakrishnan S, Vadivel E. Antibacterial and antifungal activity of the bark of Bauhinia tomentosalinn. Int J Pharm Sci 2011:2:104–9.
[7] Kannan N, Renitta RE, Guruvayoorappan C. Bauhinia tomentosa stimulates the immune system and scavenges free radical generation in vitro. J Basic ClinPhysiolPharmacol 2010;21(2):157–68.
[8] Middleton E. The flavonoids. Trends PharmacolSci 1984: 5:335-338.
[9] F.E. King, T.J.King and L.C.Manning, JChem.Soc., 1957, 563.
[10] L.Horhammer and R.Hanse, Arch.Pharm.Berl., 1955, 288, 315.
[11] K.R.Markham,“ Techniques of Flavonoid Idendifications” Acadamic Press,London,1982.
[12] V. Ragunathan, Ph.D. Thesis, Bharathidasan Univ., Tiruchirappalli, 1992, p. 62.
[13] K.R.Markham,“ Techniques of Flavonoid Idendifi cations”Acadamic Press,London,1982.P.88.
[14] K.R.Markham,“ Techniques of Flavonoid Idendifi cations”Acadamic Press,London,1982.P.82.
[15] O.Barbara, J.F.Sanz and J.A.Marco, J.Nat.Prod., 1986, 49, 702.
[16] T.A.Geissman,Ed."The Chemistry of flavonoid Co mpound”, Pergamon Press, London, 196.
[17] T.J. Mabru, K.R. Markham and M.B. Thomas. The systematic identification of flavonoids”Spring er-Verlog, Newyork, 1970, P.271.
[18] K.R.Markham and T.J.Mabry, Phytochem., 1968, 7, 1197.
[19] M.R.Vignon and J.A.Vettero, Tetrahedr,lett.,1976, 28, 2445.