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Isolation and Characterization of Quercetin 3,7-O-β-d-diglucoside from *Cascabela thevetia* (Flowers)

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ABSTRACT

The present work deals with the isolation, identification of the compound from the flowers of *Cascabela thevetia.* The structure of the isolated compound was elucidated by physical and chemical methods. The isolated compound was characterized using various spectroscopic data such as UV, ^{1}H NMR, ^{13}C NMR, MS.

Keywords: *Cascabela thevetia*, UV, NMR (^{1}H, ^{13}C) and MS, Quercetin 3,7-O-β-d-diglucoside

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INTRODUCTION

Cascabela thevetia (L.) belongs to the family *Apocynaceae* and commonly known as the Mexican Oleander is a native plant of Mexico and Central America and a close relative to *Nerium oleander* [1]. It is an evergreen tropical shrub or small tree that bears yellow, trumpet like flowers and its fruit are deep green or black in colour encasing a large seed that bears resemblance to a Chinese plant "be-still tree" [2,3]. The leaves of *Cascabela thevetia* are used to control toothache; the barks of the plant have shown decongestant activity [4]. In plants, flavonoid 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 [5]. We have isolated a new flavonoid glycoside namely Quercetin 3,7-O-β-d-diglucoside from the flowers of the above plant.

MATERIALS AND METHOD

Collection of Flowers

Fresh flowers of *Cascabela thevetia* were collected from S. Pudur, Sivagangai (Dt), Tamil Nadu, India, during the month of August and identified by Dr. S. John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. SS005 dated: 03/06/2016). St. Joseph's College (Campus), Trichirappalli, Tamil Nadu, India.

Extraction and fractionation

Fresh flowers (3 kg) of *Cascabela thevetia* 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) (4x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Ethyl acetate fraction was taken for further study.

Ethyl acetate fraction (Flavonol-di-glycoside-quercetin 3, 7-O-β-d-diglucoside)

The ethyl acetate fraction was concentrated in vacuo and the residue obtained was taken up in acetone and left in an ice chest for 2 hours. A yellow solid (m.p. 187 - 188^{0}C) separated was recrystallized from methanol. It gave deep pink colour with Mg-HCl, olive green colour with alc.Fe^{3+} and yellow colour with NaOH. It responded to Wilson's boric acid test, Gibb's test and Molisch's test [6]. But did not respond to Horhammer-Hansel test [7]. It had R_f values as depicted in table 1

It had $\boldsymbol{\lambda}_{max}$ MeOH 256, 268sh, 355; + NaOMe 268, 301sh, 405; + $AlCl_3$ 276, 303sh, 335, 432; + $AlCl_3$ – HCl 268, 297, 303sh, 354, 393; + NaOAc 255, 320sh, 354 and + NaOAc - H_3BO_3 258, 300sh, 376 nm.

The ^{1}H, ^{13}C and mass spectra of the glycoside are appended in Figure I.

Hydrolysis of the glycoside

To a solution of the glycoside (50 mg) in hot aqueous methanol (5 ml, 50%), and equal volume of H_2SO_4 (7%) was added and the mixture was refluxed at 100^0C for 2 hrs. The aqueous hydrolysate was extracted with Et_2O. The residue from Et_2O was studied further [8].

Identification of the aglycone (Flavonol-Quercetin)

The residue from the ether fraction of the hydrolysate was taken up in acetone and left under chilled conditions for a few days. A yellow solid was obtained. It came out as yellow needless on crystallization from methanol (m.p 317-319^{0}C). It was sparingly soluble in hot water and soluble in organic solvents. It answered Horhammer-Hansel test, Wilson's boric acid test and Gibb's test [7]. But did not answer Molisch's test [6]. It gave yellow colour with NH_3 and NaOH. It gave olive green colour with alc.Fe^{3+} and red colour with Mg-HCl. It had R_f values as depicted in table 1.

It had It had λ_{max} MeOH 255, 270, 300sh, 373; + NaOMe 247, 327sh, 420; + $AlCl_3$ 271, 304sh, 332, 459; + $AlCl_3$ – HCl 265, 301sh, 360, 428; + NaOAc 272, 329, 393 and + NaOAc - H_3BO_3 262, 304sh, 389 nm. It was identified as quercetin.

Table 1: R_f X 100 values of the glycoside and aglycone from the flowers of *Cascabela thevetia*

(Whatman No.1, Ascending, 30$\pm$2^{0}C)

Compound	Developing solvents								
	a	b	C	d	e	f	g	h	i
Glycoside	43	47	64	71	88	59	31	89	69
Aglycone (Complete hydrolysis)	-	-	03	17	40	85	44	48	72

*** 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

Identification of the sugar: (Glucose)

The filtrate after the removal of the aglycone was neutralized with $BaCO_3$. The concentrated filtrate, when examined by paper chromatography gave R_f values as depicted in table 2. These values are identical with those of glucose. The identity was confirmed by comparison with an authentic sample of glucose [8].

Table 2: R_f x 100 values of the sugar from the glycoside from the flowers of *Cascabela thevetia*(Whatman No.1, Ascending, 30±2^{0}C)

Compound	Developing solvents			
	f	g	h	j
Sugar from the glycoside	17	37	37	24
Glucose(authentic)	17	38	37	24

j = n BuOH : Benzene : Pyridine: H_2O = 5:1:3:3

Spray reagent : Aniline hydrogen phthalate

RESULTS AND DISCUSSION

The fresh flowers of *Cascabela thevetia* have been found to contain Quercetin 3,7-O-β-d-diglucoside.

The UV spectrum of the glycoside showed two major peaks at 355 nm (band I) and at 256 nm (band II), indicating a flavonoids skeleton. A comparison of band I absorption of the glycoside (MeOH) spectrum and that of the aglycone reveals that there may be 3-glycosylation in the flavonol. This is supported by Horhammer-Hansel test [8]. The glycoside responded to this test but the aglycone did not respond. The $AlCl_3$ spectrum of the glycoside and the aglycone revealed the presence of O-dihydroxyl group in the B-ring by the bathochromic shift of 39 nm (band I) and 30 nm (band I) over and above the $AlCl_3$-HCl spectrum respectively. A free 5-OH group in the glucose was confirmed by a bathochromic shift of 38 nm relative to that of MeOH spectrum. This is also supported by the fact that the glycoside and the aglycone answered Wilson's boric acid test [9].

A bathochromic shift of 50 nm in the glycoside and 47 nm in the aglycone was seen in band I of NaOMe spectrum as compared with their respective MeOH spectrum, showed the presence of free 4'-OH in both [10].

The absence of a free –OH at C-7 of the glycoside was observed by the NaOAc spectrum (band II) in which no bathochromic shift was noticed. The corresponding aglycone however showed a bathochromic shift of 17 nm, supporting the presence of free 7-OH and thereby revealing the glycosylation at C-7. The presence of O-dihydroxy group can further be confirmed by NaOAc-H_3BO_3 spectrum, which showed a bathochromic shift of 21 nm. On this basis it can be concluded that there are 3- and 7- glycosylation [9,10].

In ^{1}H NMR spectrum (DMSO-d_6, TMS) of the glycoside, the 5-OH proton resonates at δ 12.65 ppm, as a distinct singlet. The A-ring protons at C-6 and C-8 appear as doublets at δ 6.205 ppm and at δ 6.416 ppm respectively. The C-5' proton appears at δ 6.9 ppm(d). The proton at C-2'

appears at δ 7.65 ppm(d) and the proton at C-6'appears at δ 7.562 ppm(dd). The H-1''and H-1''' of the glycose moieties appear at δ 5.35 ppm and at δ 4.9 ppm respectively.

Supporting evidence is given by ^{13}C-NMR (DMSO-d_6) (TMS) (Figure I.2) spectrum. The signal position and their complete assignments to different carbons are given in Table 3. Due to glycosylation at C-3 and at C-7 these two signals shifted upfield and the two ortho carbon atoms showed downfield shift. C-1" of the glucose moiety appears at δ 101.4 ppm. C-1''' of the glucose moiety appears at δ 100.2 ppm. The remaining sugar carbons resonate between δ 61.0 ppm to δ 77.4 ppm [8,10].

The structure of the glycoside is further evidenced by mass spectrum (Figure I. 3) of the glycoside. It had a peak at m/z 626 and at m/z 625 for M^+ ion and M^+-1 ion respectively. The fragmentation pattern following RDA and other common fragmentation pattern are shown in Figure I.4. 7-O-glycosylation is evidenced by the peak present at m/z 339. The presence of O-di-hydroxy group in B ring is evidenced by the peak present at m/z 302. Other peaks are also in favour of the structure of the compound [8,10].

Based on the above evidences, the glycoside has been characterized as Quercetin 3,7-O-β-d-diglucoside.

Table 3: ^{13}NMR spectral data and their assignments for the glycoside from the flowers of *Cascabela thevetia*

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Quercetin from literature (δ ppm)	146.9	135.5	175.8	160.7	98.2	163.9	93.3	156.2	104.0
Glycoside G_1(δ ppm)	155.4	133.7	177.1	160.9	99.4	162.8	94.5	156.8	104.1

Compound	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
Quercetin from literature (δ ppm)	122.1	115.3	145.0	148.1	116.5	122
Glycoside G_1(δ ppm)	122.0	115.6	144.5	148.2	116.3	121.5

Compound	C-1''	C-2''	C-3''	C-4''	C-5''	C-6''
3-O-glucoside from literature(δ ppm)	101.3	74.4	76.6	70.2	77.4	61.0
Glycoside G_1(δ ppm)	101.4	74.3	76.5	70.1	77.4	61.0

Compound	C-1'''	C-2'''	C-3'''	C-4'''	C-5'''	C-6'''
7-O-glucoside from literature (δ ppm)	100.3	73.3	76.6	70.0	77.3	61.0
Glycoside G_1 (δ ppm)	100.2	73.4	76.5	69.8	77.4	61.0

Mass fragmentation pattern

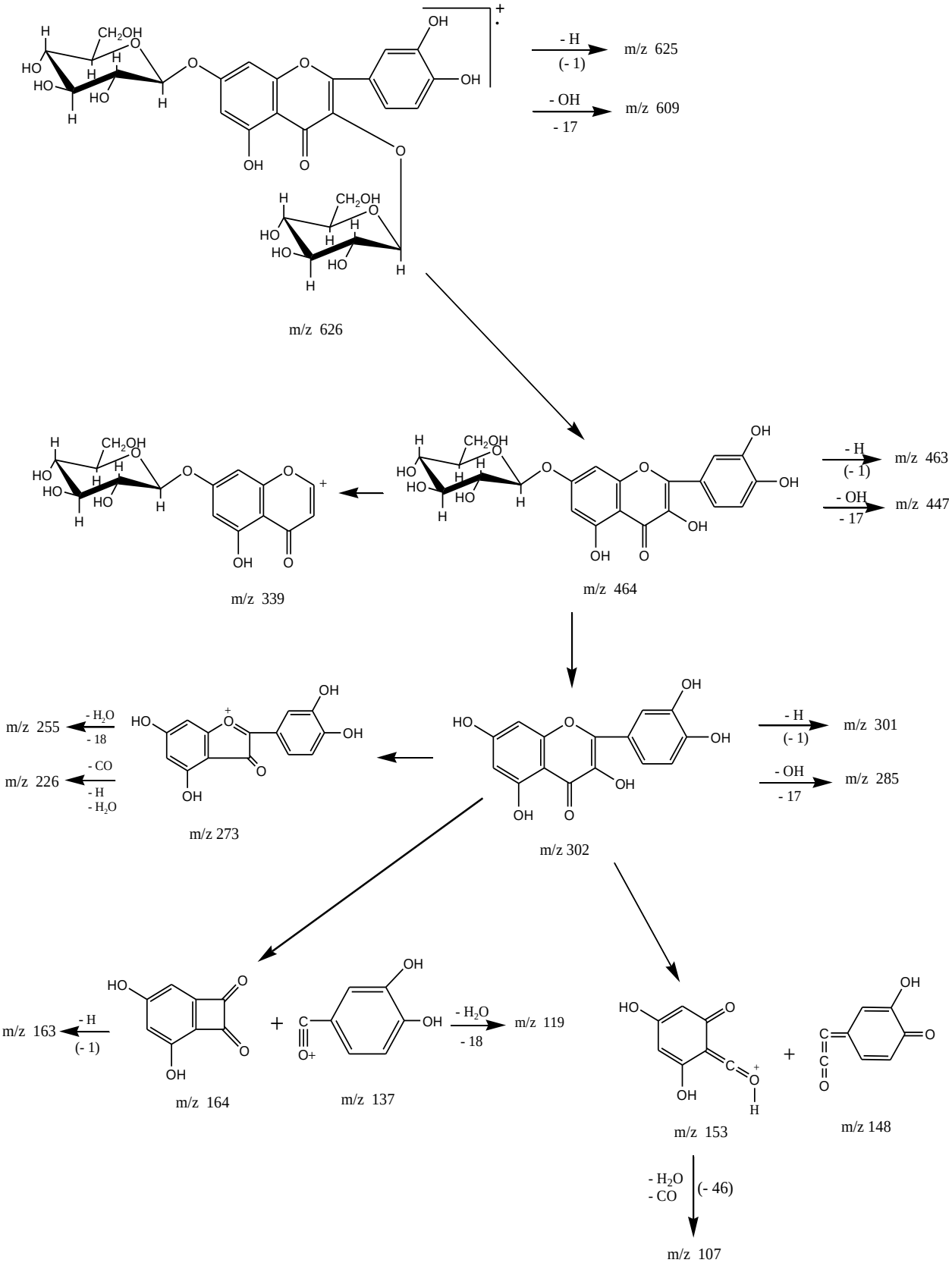
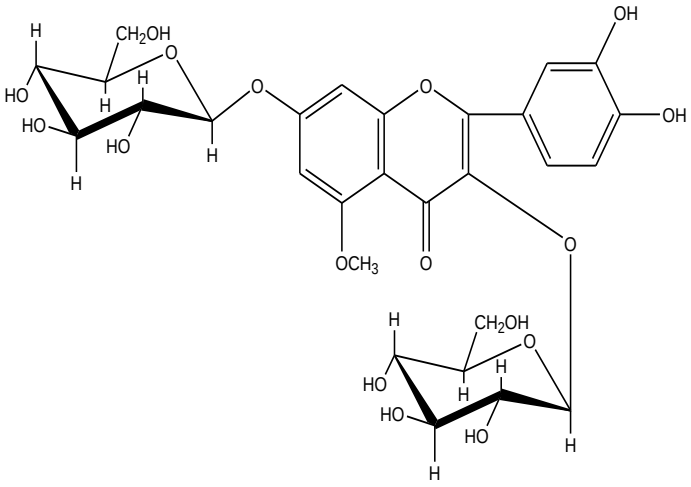


Figure: 1



Quercetin 3,7-O-β-d-diglucoside

CONCLUSION

A new compound Quercetin 3,7-O-β-d-diglucoside has been isolated from the ethyl acetate fraction of flowers of *Cascabela thevetia.* However, subjecting it to biological testing will definitely give fruitful results.

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