International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

Research Article Open Access

Isolation and characterization of *6-C-β-D xylosyl, 7-O- β-D-glucosyl quercetin* from *Cucurbita maxima* (Pumpkin) flowers

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

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

A B S T R A C T

In Indian system of medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of diseases, India has been one of the pioneers in the development and practice of well-documented original systems of medicine, particularly Ayurveda, Siddha and Unani. A compound has been isolated from the flowers of Cucurbita maxima. The isolated flavonol glycoside was identified as 6-C-β-D xylosyl, 7-O-β-D glucosyl quercetin. The chemical structure of this compound was elucidated based on spectroscopic data like UV, NMR (^{1}H, ^{13}C) and MS. This is the first report of isolation of this compound from Cucurbita maxima flowers.

Keywords: Cucurbita maxima, UV, NMR (^{1}H, ^{13}C) and MS, 6-C-β-D xylosyl, 7-O-β-D-glucosyl quercetin.

A R T I C L E I N F O

CONTENTS

Article History: Received 25 March 2016, **Accepted** 30 April 2016, **Available Online** 27 May 2016

***Corresponding Author**
N. Muruganantham
Assistant Professor, Department of Chemistry, Roever Engineering College, Perambalur, Tamilnadu, India.
Manuscript ID: IJCPS2951

PAPER-QR CODE

Citation: N. Muruganantham, *et al.* Isolation and characterization of 6-*C-β-D xylosyl*, 7-O-β-D-glucosyl quercetin from *Cucurbita maxima* (Pumpkin) flowers. *Int. J. Chem, Pharm, Sci.*, 2016, 4(5): 267-271.

1. Introduction

Pumpkin is the common name for the genus Cucurbit of the family Cucurbitaceae (gourd family), a group that includes the pumpkins and squashes. The pumpkin varies much in form, being sometimes nearly globular, but more generally

oblong or ovoid in shape. It is an annual creeper with stems up to 30 feet (9 m) long, furnished with large claspers. The leaves are large and rough like Melons. The flowers are large like Lilies and yellow in colour. The fruit is very large and contains white flattish seeds.

Herbal medicine is the study and use of medicinal properties of plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Many of these phytochemicals have beneficial effectson long-term health when consumed by humans and can be used to effectively treat human disease [1].

Pumpkin contains naturally active components that comprise of polysaccharides, fixed oils, para-aminobenzoic acid, peptides, sterol, and proteins (*Buchbauer G et. al., 1998)* [2]; (*Kuhlmann H et. al., 1999* [3]; *Matsui T et. al., 1999* [4]; *Appendino G et. al., 1999* [5]). The fruits are a noble source of carotenoid and γ -aminobutyric acid (*Murkovicet. al., 2002* [6]; *Gonzalez E et. al., 2001* [7]; *Rodriguez-Amaya DBet. al.,* 1999 [8]; *Arima HK et. al.,* 1990 [9]). Three new multiflorane-type triterpene esters, i.e. 7α-hydroxymultiflor-8-ene- 3α, 29-diol 3acetate-29-benzoate, 7α-methoxy multiflor-8-ene-3α,29-diol 3,29-dibenzoate,and 7β-methoxymultiflor-8-ene-3α,29-diol 3,29-dibenzoate, were isolated from seeds of Cucurbit maxima, along with the known compound, multiflora-7,9(11)-diene-3α,29-diol 3,29-dibenzoate [10].The flowers of Cucurbita maxima Duch. Afforded a 4:1 mixture of spinasterol and 24-ethyl-5α-cholesta-7, 22, 25-trien-3β-ol. Their structures were elucidated by extensive 1D and 2D NMR analyses [11]. The present work has been aimed at isolation and structure elucidation of bio active compound from ethyl acetate soluble fraction of Cucurbita maxima flowers.

2. Experimental

Extraction and fractionation

Fresh flowers (3 kg) of Cucurbita maxima collected from O. Koothur village, Ariyalur district, Tamilnadu, India. The flowers 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 (8x250ml) and ethyl acetate (8x250ml). Ethyl acetate fraction alone was taken for further study.

Ethyl acetate fraction

(6-C-β-D xylosyl, 7-O-β-D-glucosyl quercetin) (G)

The ethyl acetate fraction was concentrated in vacuo. The solid obtained from EtOAc fraction was taken up in acetone and left in an ice chest for three hours. A yellow solid (m.p 228-230^{0}C) (yield 0.3%) separated was recrystallised 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 (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), Horhammer-Hansel test (*L.Horhammer et al.,1955*)[12]. (when Zirconium oxychloride dissolved in acetic acid is sprayed in paper flavonol with free 3-OH group will give yellow colour) and Molish's test (*F.E.King et al., 1957)* [13](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). It had R_f values as depicted in table (1.1) (*K.R. Markham et al., 1982)*[14].

Hydrolysis of the glycoside (G)

Acid hydrolysis

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

Identification of the above residue

(Flavonol-C-glycoside: quercetin-6-C-β-D-xyloside) (G')

The residue obtained from Et_2O layer, on crystallization gave a yellow solid (m.p 218- 220^{o}C) yield (2%). It gave an olive green colour with alc. Fe^{3+}, deep pink colour with Mg-HCl and yellow colour with NaOH. It responded to Horhammer-hanseltest L. *Horhammer et al., 1955*[12], Gibb's test, Wilson-boric and Molisch's test (*F.E.King et al., 1957)* [16]. It had λmax MeOH 252, 302, 382; + NaOMe 253, 324, 434; + $AlCl_3$ 252, 290, 303, 452; +$AlCl_3$-HCl 203, 292, 303, 420; + NaOAc 270, 382; + NaOAc-H_3BO_3 252, 405 nm.

Identification of sugar (glucose)

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 agreed with those of glucose. The identity of the sugar was confirmed by Co-chromatography with an authentic sample of glucose. (*K.R. Markham et al., 1982)* [14].

3. Results and Discussion

The fresh flowers of Cucurbita maxima have been found to contain 6-C-β-D xylosyl, 7-O-β-D-glucosyl quercetin. The UV spectrum of the glycoside G showed two major adsorption peaks at 363 nm (bank I) and 250 nm (band II) showing the presence of flavonoid. (*K.R.Markham et al., 1982)* [17]. No bathochromic shift (band I) of the glycoside G' as compared to the glycoside G, suggests the C-3 hydroxyl group is free in both. This is also supported by the fact that the glycoside G and the G' responded to Horhammer-Hansel test. O-di hydroxyl groups in B ring is evidenced by the bathochromic shift seen in $AlCl_3$-HCl spectrum and NaOAc-H_3BO_3 spectrum as compared with the MeOH spectrum in G and G'. (*O.Barbara et al.,1986)* [15]. Appearance of additional peak at 324 nm in G'in the NaOMe spectrum indicates the presence of free 7-OH and also suggests that the site of glycosylation could be at C-7. The presence of free –OH at C-5 in the glycoside G and in the glycosideG' is evident from their positive response to Wilson boric acid test. This is also supported by the fact that the bathochromic shift of 40 and 38 nm could be observed in the glycoside G, G' respectively in the $AlCl_3$-HCl spectra with respect to their MeOH spectra..(*T.A. Geissman et al., 1961)* [20].

Comparison of NaOAc spectra (band II) of the glycoside G with G'suggests 7-glycosylation. Bathochromic shifts of 22 nm and 23 nm (band I) seen in NaOAc-H_3BO_3 spectra of glycoside G andG' respectively as compared with their respective MeOH spectra, suggest the presence of Catechol type of substitution in B-ring. This is also supported by the bathochromic shift noticed in $AlCl_3$ spectrum of G and G' as compared with their respective MeOH spectra. It is evedinced by Molisch's test that the compound G' which was obtained by the acid hydrolysis of G, also possesses a sugar moiety, proving the C-glycosylation. Further the compound G' is not hydrolysable by hot acid treatment and that confirms C-glycosylation. In the ^1H NMR spectrum (DMSO-d_6- TMS) (Fig 2.1) of the glycoside G, the signal appearing at δ 12.65 ppm corresponds to –OH at C-5. C-2' proton is appearing at δ 7.29 ppm. C-6' proton is appearing at δ 7.55 ppm (odd). C-5'proton is seen at δ 6.9 ppm as a doublet. C-8 proton appears as a singlet at δ 6.5 ppm, indicating the substitution at C-7 and also at C-6. The anomeric proton of glucose moiety appears at δ 5.1 ppm and that of xylose appears at δ 5.3 ppm. The rest of the sugar protons resonate in the range of δ 3.0 to δ 4.0 ppm. Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C NMR (DMSO-d_6- TMS) data. The signal positions and their complete assignment to different carbons are given in table 1-3. Presence of glucose and Xylose sugar moieties are clearly evidenced by the ^{13}C spectral data. Due to O-glycosylation, the C-7 carbon shows an upfield shift and the ortho carbon C-8 shows downfield shift. Due to C-glycosylation the C-6 carbon shows downfield shift.The structure of the glycoside G is further evidenced by mass spectrum. The mass spectrum of the glycoside had a peak at m/z 596 for M^+ ion. The fragmentation pattern (fig 1.1) following RDA and other common fragmentation pattern are in favour of the structure of the compound. ((*K.R.Markham et al., 1968)* [17]. Appearance of the peak at m/z 440 is the evidence for the presence of two glycosyl moieties in A ring. Presence of two –OH groups in B ring is evidenced by the peak at m/z 302 and at m/z 110.

Table 1.1: R_f X 100 Values of Glycoside (G) from the flowers of Cucurbit maxima (Ethyl acetate fractions)
(Whatman No.1, Ascending, 30 ± 2^0C)

Compound	* Developing solvents								
	a	b	c	d	e	f	g	h	i
Glycoside G_1	06	11	13	38	52	42	57	62	52
Aglycone from G_1	-	-	04	17	38	84	40	47	71

* Solvent Key
a = H_2O
b = 5% aq. CH_3COOH
c = 15% aq. CH_3COOH
d = 30 % aq. CH_3COOH
e = 60 % aq. CH_3COOH
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 250, 383; + NaOMe 252, 435; + $AlCl_3$ 253, 306, 457; + $AlCl_3$ – HCl 252, 308, 423; + NaOAc 251, 384; + NaOAc-H_3BO_3 252, 405 nm.

Table 1.2: R_f (X100) Values of the sugar obtained from the glycoside $G_2$from the flowers of Cucurbita maxima.
(Whatman No.1 Ascending, 30 ± 2^0C)

Compound	* Developing solvents			
	f	g	h	j
Sugar from G_2	16	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.

Table I -3: ^{13}C – NMR Spectral data and their assignments for the glycoside G from the flowers of Cucurbita maxima

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	103.1
Glycoside G1	147	135.5	177.2	161.3	108	162	95.2	156	104

Compound	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
Quercetin from literature (δ ppm)	122.1	115.3	145.0	147.6	115.6	120.0
Glycoside G1	122.1	115.1	145	149	115.6	121

Compound	C-1''	C-2''	C-3''	C-4''	C-5''	C-6''
Quercetin from literature (δ ppm)	100.2	73.3	76.6	69.8	77.4	60.9
Glycoside G1	100.83	73.37	76.68	68.22	77.11	61.01

Compound	C-1'''	C-2'''	C-3'''	C-4'''	C-5'''
Quercetin from literature (δ ppm)	74.6	70.3	78.5	70.0	70.0
Glycoside G1	74.04	70.52	78.5	70.31	70.03

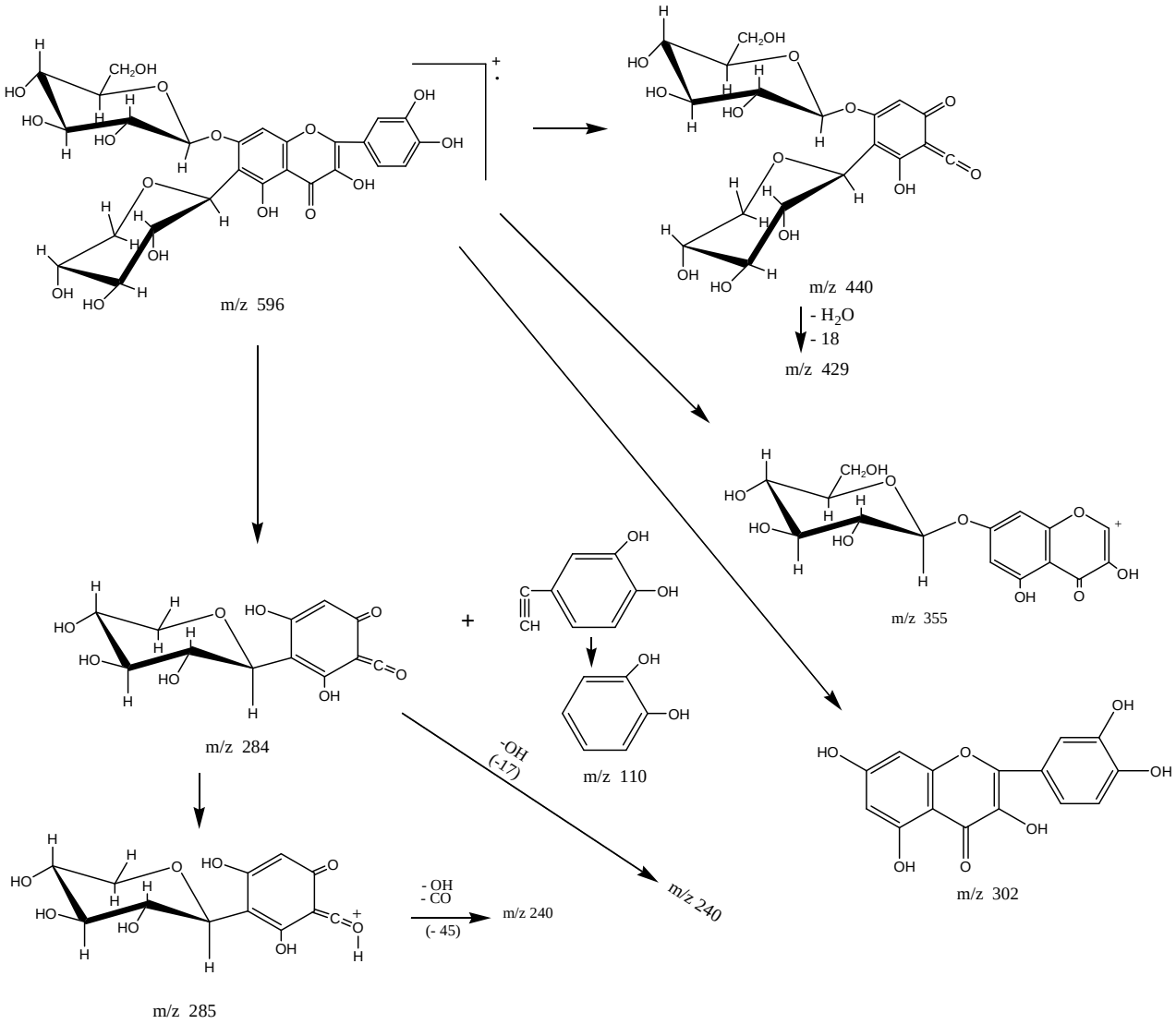

Figure 1.1: Mass fragmention Pattern of the Glycoside

Based on the above evidences, the glycoside G has been characterized as 6-C-β-D xylosyl, 7-O-β-D glucosy lquercetin.

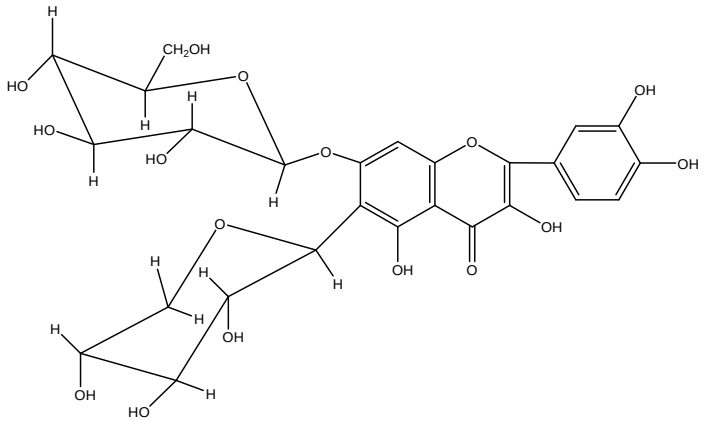

Figure 1.2: 6-C-β-D xylosyl, 7-O-β-D glucosyl quercetin.

4. Conclusion

Our pharmaceutical industry continuously search new lead molecules having better therapeutic action and less side effect, In recent years lead molecules from natural origin had gaining more popularity due to less side effect and better therapeutic action. In recent years, ethno-botanical and traditional used of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. The best classical approaches the isolation of the compound of Flavonol 6-C-β-D xylosyl, 7-O-β-D-glucosyl quercetin, from the flowers of Cucurbita maxima for management of various diseases.

5. References

[1] Miro M. (1995). Cucurbitacine and their pharmacological effects. Phyto Res, 9:159-168.

[2] Buchbauer G, Boucek B, Nikiforov A (1998) on the aroma of Austrian pumpkin seed oil: correlation of analytical data with olfactoric characteristics. Ernahrung/Nutrition 22(6): 246–249.

[3] Kuhlmann H, Koetter U, Theurer C (1999) Sterol contents in medicinal pumpkin (Cucurbita pepoconvar. citrullinina var. styriaca) depending on genotype and location. ActaHorticulturae 492: 175– 178.

[4] Matsui T, Guth H, Grosch W (1998) A comparative study of potent odorants in peanut, hazelnut, and pumpkin seed oils on the basis of aroma extract dilution analysis (AEDA) and gas chromatography olfactometry of headspace samples (GCOH). Lipid–Fett 100(2): 51–56.

[5] Appendino G, Jakupovic J, Belloro E, Marchesini A (1999) Multi- floranetri terpenoid esters from pumpkin. An unexpected extrafolic source of PABA. Phytochemistry 51: 1021–1026.

[6] Murkovic M, Mulleder U, Neunteufl H (2002) Carotenoid Content in Different Varieties of Pumpkins. J Food Composition Anal 15: 633–638.

[7] Gonzalez E, Montenegro MA, Nazareno MA, Lopez de Mishima BA (2001) Carotenoid composition and vitamin A value of an Argentinian squash (Cucurbita moschata). Arch Latinoam Nutr 51(4): 395–399.

[8] Rodriguez-Amaya DB (1999) Latin American food sources of carotenoids. Arch Latinoam Nutr 49(3 Suppl 1): 74S–84S. Rodriguez-Amaya DB (1999) Latin American food sources of carotenoids. Arch Latinoam Nutr 49(3 Suppl 1): 74S–84S.

[9] Arima HK, Rodriguez-Amaya DB (1990) Carotenoid composition and vitamin A value of a squash and a pumpkin from northeastern Brazil. Arch LatinoamNutr 40(2): 284–292.

[10] Takashi Kikuchi, Shinsuke Ueda, Jokaku Kanazawa, Hiroki Naoe, Takeshi Yamada and Reiko Tanaka , Three New Triterpene Esters from Pumpkin (Cucurbitamaxima) Seeds, Molecules 2014, 19, 4802-4813

[11] Consolacion Y. Ragasa and Kathleen Lim, Sterols from Cucurbita maxima, Philippine Journal of Science134 (2): 83-87, December 2005

[12] L.Horhammer and R. Hanse, Arch. Pharm. Berl., 1955, 288, 315

[13] F.E. King, T.J.King and L.C. Manning, J. Chem. Soc., 1957, 563

[14] K.R. Markham, “Techniques of Flavonoid Idendifications” Acadamic Press, London, 1982.

[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,1961

[17] K.R.Markham and T.J. Mabry, Phytochem., 1968, 7, 1197.