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ISOLATION AND PHYTOCHEMICAL STUDIES OF QUERCETIN AND QUERCETIN 3-O-RHAMNOSIDE

K.HEMA*[1] AND D. SUKUMAR[2]

[1]Department of Chemistry, Saveetha School of Engineering, Saveetha University, Chennai-602 105.
[2]Bharathiyar college of Engineering and Technology, Karaikal-609 609

ABSTRACT

In this present work the fresh flowers of Allamanda Cathartica Linn. were collected and the solution of Allamanda Cathartica was extracted with 90% methanol under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate, successively fractionated with benzene, peroxide free diethylether and ethylacetate. The benzene fraction was not yielding any isolable material. The diethyl ether fraction yielded quercetin and ethylacetate fraction yielded quercetin 3-O-rhamnoside (quercitrin). The structure of the isolated compounds was characterized by UV, ^{1}H-NMR and ^{13}C-NMR spectra and identified by paper chromatography (PC).

KEYWORDS: AllamandaCathartica Linn.; quercetin; quercitrin.

K.HEMA
Department of Chemistry, Saveetha School of Engineering,
Saveetha University, Chennai-602105.

1. INTRODUCTION

Allamanda Cathartica (Golden Trumpet) is an ornamental plant of Allamanda genus in the Apocynaceae family. The plant extract was found to be active against gram-positive bacteria. The leaves contain hydrocarbons, long chain esters, triterpene esters, β-amyrin, β-sitosteroland terpenoid. The extract of Allamanda Cathartica Linn. leaves with sodium -bicarbonate (5%) showed high inhibition of ehrlich ascents tumour cells. The aqueousethanol extracts of shoots, leaves and fruits contain an alkaloid [1].The aqueous and alcoholic extract of rootsand leaves exhibit hypotension of male cats. The ethanolic extract of the root is active against P-388 leukemia in vivo in mouse, andinvitro against human carcinoma of the nasopharynx. An alkaloid has also been isolated from the roots. The biologically lactones show anti-fungal and anti-bacteria properties [2]. In the present work, the fresh flowers of Allamanda Cathartica Linn. were collected and fractionated with solvents and the compounds were identified and confirmed by various phytochemical studies.

2. EXTRACTION AND FRACTIONATION

Fresh flowers of Allamanda Cathartica Linn.(1 kg) were collected and extracted with 90% methanol(4 x500ml) under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate, successively fractionated with benzene(3x250ml), peroxide free diethylether (3x250ml) and ethylacetate (4x250ml).The benzene fraction did not yield any isolable material.

2.1 PEROXIDE FREE DIETHYL ETHER FRACTION (FLAVONOL: QUERCETIN)

The diethylether fraction was concentrated in vacuo and left in ice-chest for a week.A yellow solid that separated, was filtered and studied. On crystallization from methanolpale yellow needles were obtained [m.p. 313-15°C, yield – 0.02%]. It was readily soluble in organic solvent and sparingly soluble in hot water. It gave red color with Mg-HCl, olive-green color with alcoholic Fe^{3+}, golden-yellow colour with NH_3 and NaOH, yellow solution with pale green fluorescence with Conc. H_2SO_4 and appeared yellow under UV and UV/NH_3. It reduced ammoniacal $AgNO_3$ in the cold and Fehling's solution on heating. It answered the Horhammer – Hansel, Wilson's boric acid and Gibb's tests [3, 4, 5]. It did not respond to Molisch's test. It had $\lambda_{max}^{methanol}$ nm 255, 269sh, 301sh,370sh, 370;+NaOMe 247sh, 322(dec.); +$AlCl_3$ 267, 328, 390; +$AlCl_3$-HCl265, 301sh,359, 428; +NaOAc 257sh,274, 329, 390(dec.) and +(NaOAc – H_3BO_3) 262, 304sh, 388nm and it had R_f values as depicted in Table-1. It was identified as quercetin and the identity was confirmed by co-and mixed-pc and m.p with an authentic sample of quercetin from physalis minima [6].

Table 1

R_f(X100) value of the constituents of the flowers of AllamandaCathartica Linn.

Compound	Developing solvents							
	H_2O	5% aq. AcOH	15% aq. AcOH	30% aq. AcOH	60% aq. AcOH	n-BuOH:AcOH H_2O = 4:1:5(upper phase)(BAW)	Water saturated with phenol	AcOH : Conc. HCl : H_2O = 30:3:10(forestal)
Aglycone (From ether fraction)	-	01	04	16	41	86	46	47
Quercetin (authentic)	-	01	04	17	40	86	46	47

(Whatman No. 1, Ascending, 30± 2 ^{0}C)

2.3 ETHYL ACETATE FRACTION (FLAVONOL-3-O-RHAMNOSIDE)

The ethylacetatefraction was concentrated in vacuo and left in ice-chest for few days. A yellow solid that separated, was filtered and studied. It came out as pale yellow leaflets,m.p. 182-85 °C (yield 0.05%) on recrystallization from methanol. It was soluble in ethanol and ethyl acetate but insoluble in cold water. It gave green color with alcoholic Fe^{3+}, pink colour with Mg-HCl and ayellow precipitate with aqueous (basic) lead acetate. It reduced ammoniacal$AgNO_3$solution but not Fehling's solution. It appeared deep blue purple under UV that turned yellowish green on exposure to NH_3. It responded to Wilson's boric acid, Molisch's and Gibb's test. But itdid not answer to the Horhammer Hansel test. It had $\lambda_{max}^{methanol}$ nm 256 ,266sh, 300sh, 350; +NaOMe270, 326, 393; +$AlCl_3$276, 303sh, 332, 430; +$AlCl_3$-HCl 272, 303sh, 353, 400; +NaOAc272,320sh and+(NaOAc-H_3BO_3)259, 301sh,368. It had $R_f$values depicted in Table-2.

Table 2

R_f(X100) value of the constituents of the flowers of AllamandaCathartica Linn.

Compound	Developing solvents							
	H_2O	5% aq. AcOH	15% aq. AcOH	30% aq. AcOH	60% aq. AcOH	n-BuOH:AcOH:H_2O = 4:1:5(upper phase) (BAW)	Water saturated with phenol	AcOH : Conc. HCl : H_2O = 30:3:10 (forestal)
Glycoside (From ethyl acetate fraction)	42	34	53	74	80	82	65	81
Quercitrin (authentic)	42	35	53	74	80	83	65	81

Whatman No. 1, Ascending, 30 ± 2 °C)

2.4 HYDROLYSIS OF THE GLYCOSIDE

The glycoside 0.05gm was dissolved in hot aqueousmethanol and an equal volume of H_2SO_4(7%) was added to it. The reaction mixture was refluxed at 100 °C for 2 hours and the hydrolytic products identified as described below.

2.5 IDENTIFICATION OF AGLYCONE (QUERCETIN)

The aglyconewas crystallized with dimethyl ketonegave yellow needles at melting point 316 – 318 °C. Theaglycone that resulted was characterized as quercetin, as mentioned under diethyl ether fraction.

2.6 IDENTIFICATION OF SUGAR (RHAMNOSE)

The concentrated filtrate from the neutralized aqueoushydrolysate when examined byPC gave $R_f$values depicted in Table-3 corresponding to those of rhamnose. The identity of the sugar was also confirmed by direct comparison with an authentic sample of rhamnose. A quantitative hydrolysis revealed the aglycone: sugar ratio to 1:1 confirming the presence of a monoside. The glycoside was therefore identified as quercetin-3-O-rhamnoside and confirmed by co-and mixed – PC with an authentic sample of quercetin-3-O-rhamnoside obtained from the flower of Leucaenaleucocephala [7].

Table 3
R_f(X100) value of the constituents of the sugar from the glycoside of AllamandaCathartica Linn.

Compound	Developing solvent			
	60% aq. AcOH	n-BuOH:AcOH:H_2O = 4:1:5 (upper phase)(BAW)	Water saturated with phenol	AcOH : Conc. HCl : H_2O = 30:3:10(forestal)
Sugar from the glycoside of ethyl acetate fraction	75	31	60	91
Rhamnose (Authentic)	75	31	60	92

(Whatman No. 1, Ascending , 30± 2 °C)

3. RESULTS AND DISCUSSION

The flower of Allamanda Cathartica Linn**.** has been found to contain quercetin and its 3-O-rhamnoside-quercitrin.The UV spectrum of the flavonolaglycone obtained from the ether fraction exhibited λmax at 370nm (band I) and 255nm(band II) indicating a flavonol skeleton [8]. Its NaOMe spectrum degenerated with time.Flavonols possessing free-OH group at the C-3, C-3′ and C-4′ –positions are known to be unstable in NaOMe[9]. It could be inferred that there are free-OH group at C-3, C-3′ and C-4′ in the compound. A shift of +58nm on the addition of $AlCl_3$-HCl is indicative of the presence of a free-OH at C-5 in the A-ring[10]. A Comparison of $AlCl_3$ and $AlCl_3$-HCl spectra revealed an additional bathochromic shift of 30nm in the case of $AlCl_3$spectrum (without acid), which again point to the presence of catechol type of B-ring [11]. The presence of a free-OH at C-7 is an evident from the bathochromic shift of 20nm in band II on the addition of sodium acetate [12].The presence of a catechol type of B-ring is also an evident from the bathochromic shift of 16nm noticed in band I on the addition of H_3BO_3. Based on these observations; the aglycone has been unambiguously characterized as quercetin [13, 14]. The UV spectrum of the glycoside exhibited 2 major absorption peaks at 350nm (band I) and 256nm (band II). The band I absorption of the glycoside is reminiscent of a flavonol skeleton. A comparison of band I absorption of the glycoside and that of the aglycone revealed that there may be 3-glycosylation in the flavonol.A bathochromic shift of 43nm (band I)in NaOMe confirmed the presence of a free-OH at C-4′. The $AlCl_3$ spectra(with and without HCl) showed four absorption peaks to reveal the presence of a free 5- OH group. It was confirmed by bathochromic shift of 50nmon the addition of ($AlCl_3$ –HCl) in the glycoside .The presence of a free –OH at C-7 was an evident from the +16nm (band II) shift on the addition of sodium acetate. The band I absorption in $AlCl_3$spectrum is 30nm more than that noticed on addition of $AlCl_3$-HCl. This is indicative of the existence of an o-dihydroxyl group in the B-ring.In the ^{1}H- NMR spectrum (400 MHz, DMSO-d6, TMS) the A ring protons at C-6 andC-8 appear at δ 6.21 and δ 6.39 ppm respectively. The 5-OH proton resonates at δ 12.65ppm.The proton at C-5′ appears at δ 6.80 ppm. The protons at C-2′ and C-6′ appear at δ 7.51 and δ 7.59 ppm respectively. The methyl protons of rhamnose moiety observed as a double at δ 0.99 ppm. The H-1″ of the rhamnoside resonates at δ 5.08 ppm. The remaining sugar protons appear in the range δ 3.38 ppm- 3.70 ppm. Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C-NMR (100MHz, DMSO-d6, and TMS) data and a complete assignment is given in Table-4. Based on this the aglycone and the glycoside have been characterized as quercetin and quercitrin. The structure of quercetin and quercetin 3-O-rhamnoside is shown in the Fig. 1 & 2.

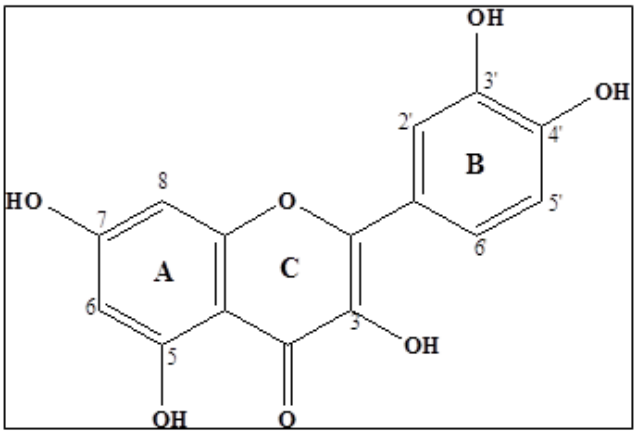

Figure 1
Structure of quercetin

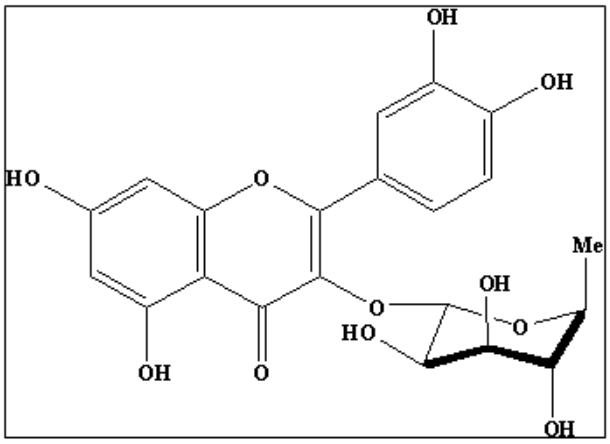

Figure 2
Structure of quercetin 3-O-rhamnoside

Table 4
^{13}C – NMR data and their assignments for the glycoside from the flowers of AllamandaCathartica Linn.

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
Quercitrin (authentic)	156.4	134.4	172.7	161.2	98.6	164	93.5	157.0	104.2	121.0	115.4	145.1	148.3	115.8	121.0
Glycoside from ethyl acetate fraction	156.3	132.2	172.1	161.2	98.3	164	93.6	159.2	108.5	121.6	115.4	145.5	148.3	115.8	121.6

Compound	C-1"	C-2"	C-3"	C-4"	C-5"	C-6"
Quercitrin (authentic)	101.9	70.4	70.6	71.5	70.1	17.3
Glycoside from ethyl acetate fraction	100.00	70.0	70.0	73.0	70.0	18.6

CONCLUSION

In the present work, the fresh flowers of Allamanda Cathartica Linn. were subjected to phytochemical studies. The results of the study showed that the flower containsQuercetin and Quercitrin. The structure of the isolated compound was characterized by UV, ^{1}H-NMR and ^{13}C-NMR spectral studies.

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