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Phytochemical Studies On the Flowers of Ipomoea Aquatica

Chemistry

KEYWORDS : Ipomoea aquatica , Convolvulaceae

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ABSTRACT

The fresh flowers of Ipomoea aquatic of Convolvulaceae family has been found to contain kaempferol and astragalin. The structures of the flavonoids has been characterized by means of modern physical methods like UV, 1H – NMR, 13 C – NMR, PC, chemical reactions and hydrolytic studies.

EXPERIMENTAL

Extraction and Fractionation:-

Fresh flowers (500g) of Ipomoea aquatica collected from the banks of Cauvery in Kumbakonam during December were extracted with 85% ethanol (4x500ml) under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrated successively fractionated with benzene (b.p-60-80°C) (3x250ml), peroxide – free Et2O (3x250ml) and EtOAc (4x250ml)

The benzene fraction did not yield any isolable material.

Et2O Fraction: (flavonol - Kaempferol)

The residue from the Et2O fraction was taken up in Me2CO and left in an ice – chest for a few days, when yellow needles (MeOH) m.p. 278-80oC (yield 0.02%) were separated. It has λ MeOH max nm, 253sh, 266sh, 294sh, 322sh, 367, 370, + NaOMe 278, 316, 416, + AlCl3 with and without HCl, 256sh, 269, 303sh, 348, 424; + NaOAC, 274, 303, 387, and + (NaOAc+H3BO3) 267, 297sh, 320sh, 372., it was soluble in organic solvents but insoluble in water.

It developed a reddish orange color with Mg-HCl and yellow colour with NaOH. It appeared pale yellow under UV as well as on exposure to NH3. It responded to Wilson's boric acid4. Harhammer – Hansel5 Gibb's tests6 and Molishc's test. It had Rf values as depicted in Table I-1.

TABLE I-1
Rf (X 100) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF I – AQUATICA
(Whatman No.1, Ascending, 30± 2°C)

COMPOUND	Developing Solvents*									
	A	b	c	d	e	f	g	H	i	j
Aglycone from Et_2O fraction	-	-	05	15	49	93	67	62	87	94
Kaempferol (Authentic)	-	-	05	15	50	93	67	62	87	95
Glycoside fcom EtoAc fraction	13	40	42	68	77	70	71	70	53	-
Kaempferol 3-glucoside (Authentic)	13	40	43	68	77	71	71	71	53	-

*Solvent Key :-a. H2O;b. 5%aq. HOAc; c. 15%aq. HOAc: d. 30%aq. HOAc;e. 60%aq. HOAc;f. nBuOH:HOAc: H2O=4:1:5(Upper phase); g. Phenol Saturated with water; h. HOAc: con. HCl: H2O=30:3:10 (forestal); i. HOAc: con. HCl: H2O=30:3:10
j. EtOAc; HCOOH: H2O=10:2:3

EtOAc Fraction : - (Flovonol – 3 – O – glucoside : astragalin)
The EtOAc fraction was concentrated in vacuo and left in an ice – chest for a day when yellow solid separate which was filtered and studied.

When crystallized from MeOH, it came out as yellow long needless, m.p.176-78°C (yield 0.1%). It was freely soluble in aq. NaOH, hot water, EtOH and EtOAc but insoluble in Et2O, Me2CO and CHCl3. It gave a greenish – brown colour with alc. Fecl3, and intense yellow colur with NaOH, red colour with Mg-HCl and yellow precipitate with aq. Lead acetate. It appeared as a dark purple spot in UV light which turned yellow in burning with NH3. It answered Wilson's boric acid, Gibb's, and Molisch's tests but did not respond to the Horthammer – Hansel test. It had λ MeOH max nm 264, 301sh., 350., + NaOMe 273, 324, 398.,+AlCl3 with and without HCl 275, 304, 353, 397 sh; + NaOAc 269, 301sh, 351. It had Rf values as depicted in Table I-1.

Hydrolysis of the glycoside:-

The glycoside (0.05g, 0.2m mole) dissolved in hot aq. MeOH (2ml, 50%) was hydrolyzed with H2SO4 (5%) at 100°C for about 2 hrs and the hydrolytic products identified as described below.

IDENTIFICATIONOF AGLYCONE: - (FLAVONOL –Kaempferol)

The (yellow) aglycone on recrystallisation from MeOH afforded a yellow crystalline solid m.p. 278-80°C which was identified as Kaempferol, by colour reactions behavior under UV and Rf values (Table I-1). It had the same UV spectral values, mentioned under Et2O fraction.

IDENTIFICATION OF SUGAR: (glucose)

The aqueous hydrolysate after the removal of the aglycone was neutralized with BaCO3 and filtered. The concentrated filtrate on PC gave Rf values corresponding to those of glucose. The glycoside was thus identified as astragalin and this was confirmed by co-PC with an authentic sample of astragalin, isolated from Hydrangea macrophylla. The 1H and 13C – NMR of the glycoside are appended [Fig1-1, and Fig 1-2].

TABLE I-2
Rf (X 100) VALUES OF THE SUGAR FROM THE GLUCOSIDE FROM I.AQUATICA
(Whatman No.1, Ascending, 30±2°C)

COMPOUND	Developing Solvents			
	f	g	h	i
Sugar from the hydrosate of EtoAc fraction	77	09	39	90
Glucose (authentic)	77	09	39	90

*Solvent Key:-e.60%aq. HOAc; f. nBuOH: HOAc: H2O=4:1:5(U); g. Phonal Saturated with Water; h. HOAc: con. HCl; H2O=30:3:10 (forestal); i. HOAc: con. HCl : H2O =30:3:10

FIG I-1 H – NMR SPECTRUM (DMSO FROM EtoAC FRACTION OF IOPOMEA AQUTICA

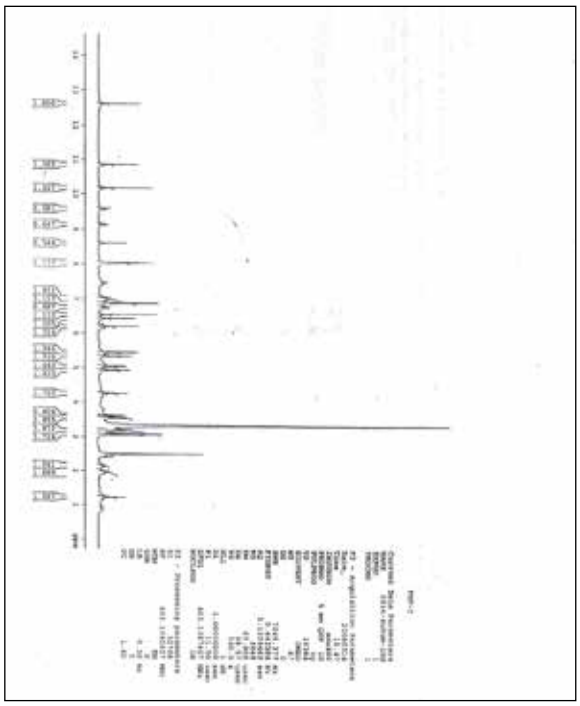
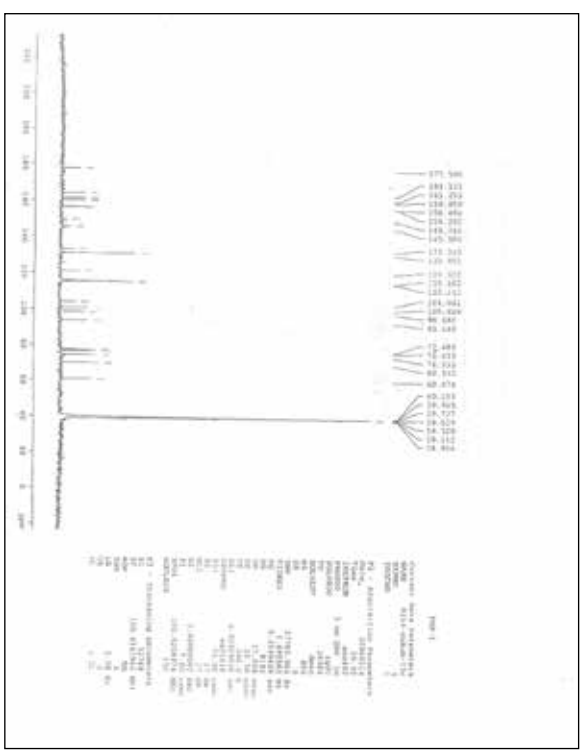


FIG I -2 13 C – NMR SPECTRUM (DMSO – d6) OF THE GLOCOSIDE FROM EtoAC FRACTION OF IPOMEA AQUTICA



RESULTS AND DISCUSSION

The fresh flowers of I.Aquatica have been found to contain Kaempferol and its 3-0-β glucoside. The UV spectrum of the flavonol aglycone obtained from the Et2O fraction exhibited two major peaks at 367 nm (band I) and 266 nm (band-II) which showed a flavonol skeleton. A bathochromic shift of 49 nm on the addition NaOMe revealed the presence of the free 4'-OH group in the B-ring. A shift of +57 nm on the addition of the AlCl3 – HCl showed the presence of a free 5-OH in the A-ring. The presence of a free OH at C-7 was ascertained by a shift of +8 nm (band II) on the addition of NaOAc. The AlCl3 spectrum was exactly same as that of the (AlCl3-HCl) revealing the absence of catechol type of substitution there was only +5 nm shift on the addition of (NaOAc-H3BO3).

The band I UV absorption of the glycoside is at 350 nm which is again indicative of a flavonal skeleton. A comparison of band I absorption of the glycoside and the aglycone reveals that there may be 3-glycosylation in the flavonol. A bathochromic shift of 48 nm (band I) ascertained the presence of free – OH at C-4'. The AlCl3 spectra (with and without HCl) showed four absorption peaks to reveal the presence of a 5-OH group. It was confirmed by the bathochromic shift of 47 nm on the addition of (AlCl3-HCl). The presence of a free –OH at C-7 was evident from the +5 nm (band II) shift on the addition of NaOAc. The H3BO3 spectrum is exactly same as that of NaOH indicating the absence of catechol type of substitution in B-ring.

In the 1H-NMR spectrum (400 MHz, DMSO-d6 TMS), the A-ring protons at C-6 and C-8 appear separately as doublets at δ 6.249 ppm and δ 6.420 ppm respectively. The 5-OH proton resonates at δ 12.609 ppm. In the B-ring, the protons at C-2', 3',5' and 6' due to the free rotation of the phenyl ring appear as two pairs of Ortho coupled doublets at δ 6.944 and δ 8.017 ppm. The H-3',5', doublet occurs up field from the H-2',6' doublet due to the shielding effect of oxygenation at C-4'as also due to deshielding influence of C-ring operating on H-2' and H-6'. The H-1'' signal of the glucose moiety appears at δ 5.442 ppm found down-field from the bulk of the sugar protons. The remaining glucosyl portons appear at δ 3.319 ppm. The β- linkage of the glucose to 3-OH is evident from the large coupling constant 7.28 Hz of H-1".

Comparing 13C-NMR (100 MHz, DMSO-d6, TMS) spectrum of the glycoside (recorded with a AMX 400 spectrometer) with that of the aglycone, the carbonyl carbon at C-4 of the glycoside appears at 1.6 ppm downfield to that of the aglycone. Due to the glycosylation at C-3, its ortho carbon of the glycoside C-4 appears at 2.4 ppm downfield to that of the aglycone. All the other carbons of the glucoside at A and C-rings appear at downfield. In the B-ring the 1', 3' and 5' carbons appear up field to that of the aglycone and the 2', 4' and 6' carbons down field. A complete assignment of the 13C-NMR spectrum of glycoside is available in Table I-3.

On this basis the identity of the pigments obtained from Et2O and Et2OAc soluble can be confirmed as kaempferol and its 3-glucoside respectively.

TABLE I.3
C-NMR DATA AND THEIR ASSISGNMENT FOR THE GLYCOSIDE FROM I.AQUATICA

COMPOUNDS	C2	C3	C4	C5	C6	C7	C8	C9	C10
Astragalin from the literature	156.4	133.4	177.5	161.1	98.8	164.2	93.8	156.4	104.0
Glycoside from I.Aquatica δppm	156.404	133.245	177.500	161.253	98.686	164.133	93.648	156.404	104.041

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COMPOUNDS	C1'	C2'	C3'	C4'	C5'	C6'			
Astragalin from the literature	120.9	131.0	115.1	159.9	115.1	131.0			
Glycoside from I.Aquatica δppm	120.928	130.882	115.112	159.959	115.112	130.882			

COMPOUNDS	C1"	C2"	C3"	C4"	C5"	C6"			
Sugar from the literature	101.4	74.2	76.5	70.1	77.2	61.0			
Glycoside from I.Aquatica δppm	100.928	74.232	76.453	69.932	77.483	60.874			

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