



# Isolation and Structural Elucidation of Novel Polyphenol from the Flowers of Hibiscus Viti Folios

## KEYWORDS

Kaempferol-3-O-galactoside, Bathochromic shift, Hibiscus vitifolios Glycoside, polyphenol.

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## ABSTRACT

The naturally occurring Poly phenol Kaempferol – 3-0 galactoside has been isolated from the flowers of Hibiscus vitifolios and the structure was confirmed by spectroscopic and paper chromatography techniques.

## Introduction

Many research work have been reported that the bio active component flavonoid present in plants play vital role in cure various diseases.<sup>1,8,9</sup> Hibiscus vitifolios belongs to the family Malvaceae widely occur in India, Africa, Australia and Egypt. Traditionally its leaves and bark are used for the treatment of Jaundice and diabetes. Mucilage from its root is applied to hair and skin to kill parasites. Hence the present work was aimed to isolate and elucidate the structure of flavonoid from the flowers of Hibiscus vitifolios with the help of modern techniques like UV, paper chromatography <sup>13</sup>C NMR, HSQC and <sup>1</sup>H NMR.<sup>2,3,4</sup> Flavonoids are naturally occurring polyphenols, It is comprised of C, O and H.

## Materials and Methods:

## Extraction and fractionation:

Fresh flowers of 2kg of Hibiscus Vitifolios collected from in and around Kumbakonam during December were extracted with 85% EtOH. The alcoholic extract was concentrated in Vacuo and the aqueous concentrate successively fractionated with benzene, Ether and Ethyl acetate<sup>5,6</sup>.

## EtOAc Fraction:

The residue from EtOAc fraction yield yellow needles on recrystallisation from MeOH, Melting Point (259-261°C) It developed red colour with Mg-HCl, Brown colour with a/c. Fe<sup>3+</sup>, yellow colour when viewed under UV light with and without NH<sub>3</sub> responded to willson boric acid, Gibbs tests but did not answers the Hor hammer – Hansel test<sup>7</sup>.

## UV Studies:

It had λ max MeOH nm 262, 301 sh, 347 (+NaOMe) 270 324,398 (+ AlCl<sub>3</sub> with and without HCl) 275, 304, 352, 396, Sh (+NaOAc) 269, 306, 318 Sh, 352 (+NaOAc/H<sub>3</sub> BO<sub>3</sub>) 260, 300 Sh, 308.

<sup>13</sup>C NMR Values and Rf Values from chromatography are tabulated in table 1 and 2 respectively.

## Hydrolysis of the glycoside:

The glycoside 0.05g dissolved in not aq MeOH was hydrolyzed with H<sub>2</sub>SO<sub>4</sub> (10%) at 100°C for about 2hr and the hydrolytic products identified as described below.

## Identification of Sugar:

The aqueous hydrolysate after the removal of the aglycone was neutralized with BaCO<sub>3</sub> and filtered. The concentrated

filtrate or paper chromatography gave Rf values corresponding to those of galactose. The identity of the sugar was also confirmed by direct comparison with an authentic sample of galactose

**Table: 1**  
**C13 NMR Spectral data of Flavonoid of H. Vitifolios**

	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C <sub>7</sub>	C <sub>8</sub>	C <sub>9</sub>	C <sub>10</sub>	C <sub>1'</sub>
Kaempferol 3-0- galactoside (Authentic)	156.4	133.4	177.5	161.1	98.8	164.2	93.8	156.4	104.0	120.9
Flavonoid from H.Vitifolios	156.4	133.3	177.5	161.1	98.8	164.2	93.7	156.4	104.2	120.9

C <sub>2</sub> '	C <sub>3</sub> '	C <sub>4</sub> '	C <sub>5</sub> '	C <sub>6</sub> '	C <sub>1</sub> ''	C <sub>2</sub> ''	C <sub>3</sub> ''	C <sub>4</sub> ''	C <sub>5</sub> ''	C <sub>6</sub> ''
131.0	115.1	159.9	115.1	131.0	101.9	71.3	73.1	68.0	75.7	60.3
130.9	115.1	160.0	115.1	131.2	101.9	71.3	73.3	68.1	75.8	60.3

**Table : 2**

**R<sub>f</sub> values of Flavonoid of the flowers of H. Vitifolios. (Whatman No.1, Ascending 30 ± 2°C )**

Compound	Developing Solvents							
	a	b	c	d	e	f	g	h
Flavonoid from EtOAc	10	15	33	63	88	89	85	85
Kaempferol – 3 -0 galactoside (Authentic)	10	15	34	62	86	89	85	85

## Solvent Key

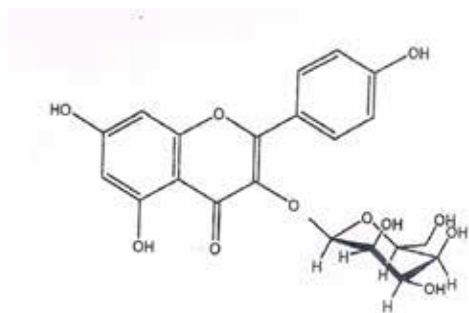
a – H<sub>2</sub>O, b- 5%, aq. HOAc, C – 15% aq HOAc, d – 30% aq HOAc, e – 60% aq – HOAc, f – BAW [n- Butanol, Acetic Acid, Water].

g – Phenol saturated with water

h – Forestol.

### Results and Discussions:

The UV spectrum of the flavonoid exhibited 2 major peaks at 262 (band II) 347nm (band I) indicated the glycosylation at 3 – position in the flavonoid. A bathochromic shift of 47nm (band I) noticed in its NaOMe spectrum indicated the presence of free OH at C<sub>4</sub>'. The AlCl<sub>3</sub> spectra showed four absorption peaks revealed the free 5-OH group. The shift in NaOAc showed that the presence of free OH at C<sub>7</sub>. In the <sup>1</sup>H – NMR of the flavonoid, the OH protons at C<sub>5</sub>, C<sub>7</sub>, C-4' resonates at 12.68, 10.23 and 9.38 respectively. The C<sub>3</sub>' and C<sub>5</sub>' protons appears at 6.78. C<sub>6</sub> and C<sub>8</sub> protons at 6.41 and 6.52. the H -1" signal of the glucose moiety appears at 5.5. The remaining galactosyl protons appear in the range 3 to 3.9 ppm. The complete assignment of C<sup>13</sup> NMR signals revealed that the structure flavonoid in Kaempferol – 3-O galactoside. Further it was confirmed by UV and PC values.



Kaempferol – 3-O – galactoside

### Conclusion:

The bio active component present in the flowers of medicinally important plant *Hibiscus vitifolios* is Kaempferol – 3-O – galactoside.

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