RESEARCH PAPER | Chemistry | Volume : 5 | Issue : 11 | November 2015 | ISSN - 2249-555X

Healing Property of the Poly Phenolics of Cassia Fistula

KEYWORDS: Cassia fistula Linn, Legumineosae, Quercetagetine– 6 – O - glucoside, Paper chromatography, Nitrofuracin.

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ABSTRACT *Fresh flowers of Cassia fistula is found to contain Quertagetine – 6 – O - glucoside. The structures of the above compound have been ascertained by UV, chemical reactions, PC and hydrolytic studies. The glycoside isolated from the flowers has sample healing property.*

Introduction
Cassia fistula Linn, popularly known as konnei in Tamil belongs to *Caesalpiniaceae*. Which is one of three orders of *Legumineosae* family, its root, bark , seeds, and leaves are laxavative nature, its fruits are usually applied in rheumatism and snakebites. The bark is given to Ladies for smooth delivery. The yellow flowers from cassia fistula were chosen for healing property and their results are given below.

Experimental
Extraction and Fractionation
Fresh flowers (800 g) of cassia fistula collected from the bank of Cauvery at kumbakonam taluk in Thanjavur District, during october, were extracted with 90% MeOH (4x500 ml) under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate successively fractionated with benzene (3x250 ml), peroxide free ether (4x250 ml) and ethyl acetate. The ethyl acetate fraction only taken for further studies.

Ether fraction (Quercetagetine)
The Et_2O fraction was concentrated in vacuo and kept in ice – chest for a week. The yellow colour solid that separated was filtered and examined. It came out as yellow crystals (M.P. 324 - 325^{0}C) (yield 0.02%) , on recrystallization from MeOH. It was soluble in MeOH , Et_2O, EtOAC, Me_2CO and $CHCl_3$, but not soluble in water. It developed a yellow colour with NaOH and a reddish orange colour with Mg – HCl. It appeared dull black under UV which on exposer to NH_3 fluorescent yellow – green. It respond to Horhammer – Hansel, Gibbs, Wilson's boric acid test but did not answer Molisch's test.

Ethyl Acetate fraction (Quercetagetine – 6 – O - glucoside)
The residue from EtOAC fraction was taken up in Me_2CO and left an ice – chest for two days. When pale yellow solid was separated. It came out as pale yellow plates a recrystallisation from MeOH. It is freely soluble in aq. NaOH, hot water, EtOH, and EtOAC, but insoluble in Et_2O and $CHCl_3$. It gave a greenish-brown colour with alc.Fe^{3+}, an intence yellow colour with NaOH, red colour with Mg-HCl. It appeared as fluorescent yellow under UV with and without NH_3. It answered Wilson's boric acid, Gibbs , Horhammer Hansal and Molisch's test.

Hydrolysis of the glycoside
The glycoside (0.05g) was dissolved in hot aq. MeOH (2ml, 50%) and an equal volume of H_2SO_4 (7%) was added to it. The reaction mixture was refluxed at 100° for 2 hrs. The excess of alcohol was distilled off from hydrolysate and the resulting aq. solution was diluted with more water and left under chilled conditions for 2 hrs. A yellow solid that separated was filtered washing and dried. The aqueous filtrate and the washings were extracted with Et_2O. The dry yellow residue on the filter paper was combined with the residue from the dried Et_2O extract and studied for the aglycone.

Identification of Aglycone (Quertagetine)
The residue from the Et_2O fraction was taken up in Me_2CO and left under chilled conditions for a few days. An yellow solid (M.P. 346 - 348^{0}C) is formed. It

developed a red colour with Mg – HCl ; brown colour with alc. Fe^{3+}; turned yellow with NH_3 and deep purple under UV changing to yellowish green on exposure to NH_3; gave +Ve Shinoda test. The Rf values are indicated in the table.

Table – 1 Rf (x100) values of the constituents of the flowers of Cassia fistula.
(Whatman No.1, Ascending, 30± 2^{0}C)

Compound	Developing solvents					
	a	b	c	d	e	f
Aglycone (1)	10	30	57	31	35	42
Aglycone (authentic)	10	30	57	31	35	42
Glycoside (2)	10	40	30	25	70	60
Glycoside (authentic)	10	40	30	25	70	60

1.Aglycone - Quercetagetine
2.Glycoside – 6 – O - glucoside
a . 15% aq. ACOH
b . 60% aq. ACOH
c . n - BuOH : ACOH : H_2O = 4: 1 : 5 (BAW)
d . Water saturated phenol
e . ACOH : Con. HCl : H_2O = 30 : 10 : 3 (Forestal)
f . n – BuOH : ACOH : H_2O = 3 : 1 : 1 (TBA)

Identification of sugar (glucose)
The aqueous hydrolysate after the removal of the agly-

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cone was neutralised with $BaCO_3$ and filtered. The concentrated filtrate on the PC gave Rf values

corresponding to those of glucose. The identity of the sugar was confirmed by direct comparison with an authentic sample of glucose. A quantitative hydrolysis of the same by the Folin-Wu's micro method revealed it to be a monoside.

Table – 2 Rf (x100) values of the sugar from the glycoside from cassia fistula .
(Whatman No. 1, Ascending, 30 ± 2 ^{0}C)

Compound	Developing solvents			
	a	b	c	d
Sugar from glycoside	77	09	39	90
Glucose (authentic)	77	09	39	90

*Solvents

a. 60 % ACOH
b. n - BuOH : ACOH : H2O = 4 : 1 : 5 (BAW)
c . Water saturated phenol
d .ACOH : CON.HCL : H2O = 30 : 10 : 3

Result and Discussion
The flowers of cassia fistula has been found to contain aglycone quertagetine. The UV spectrum to contain aglycone exhibited two major peaks at 365 nm (band I) and 260 nm (band II) to reveal a flavonol skeleton. It had λ_{max} nm 260, 290sh, 365 ; + NaOMe 268,325,345sh,420 ; + $AlCl_3$ 270, 300sh, 360sh, 425 ; + $AlCl_3$ + HCl 270, 300sh, 360sh, 370sh, 400 ; + NaOAC 270, 300sh, 335sh, 400 ; + (NaOAC + H_3BO_3) 265, 300sh, 370. Absorption peaks in NaOMe indicating free –OH group at C–3, C–3' C-4' in the compound. A shift of +35nm on the addition of $AlCl_3$ –HCl showed the presence of a free 5-OH in the A-ring. The presence of free-OH at C-7 was as certained by a shift of +10nm (band II), on the addition of NaOAC. The catechol type of di hydroxyl groups in B-ring as well A-ring was further evidenced by the smaller bathochromic shift of +5 nm on the addition of H_3BO_3. From the previous data compound was identified as Quercetagetine.

UV spectral data with MeOH indicated that it was a flavonol glycoside, while the addition of H_3BO_3 indicating the occupation of 6th position. It had λ_{max} nm 260, 285sh, 360; +NaOMe 290, 325, 405; + AlCl3 260, 275sh ,300sh, 325sh, 400; +(NaOAC + H_3BO_3) 274, 330sh, 395. The UV spectrum of the glucoside showed peaks at 260 nm and 360 nm and a shoulder at 285nm was indicating of flavonol skeleton. The presence of 5-OH group was evidenced from a shift +40 nm on the addition of $AlCl_3$ – HCl. The presence of free 7-OH was revealed by the shift of +15 nm on the addition of NaOAC. The NaOAC data indicating the occupation of 6th position. From the previous data compound was identified as Quercetagetin – 6 – O - glucoside.

Wound Healing activity
The aqueous extract of the flowers of cassia fistula was prepared and concentrated to dryness. The residue was used for the preparation of the drug. Healthy rabbits (wt 1.5kg) served as experimental models. The rear portion of the rabbit was chosen as the area where the wound was to be created and all the hair on the relevant area completely removed by shaving. It has been mentioned in the literature that wounds less than 10 mm diameter could heal by epithelisation only. As such, wound of about 1.5 aq.cm was created. Due care was taken to maintain the experimental animals in an animal house in order to have a control over factors like temperature, humidity and oxygen tension which will have a direct bearing on the healing process. The animals were given food and water to avoid changes due to nutrition and metabolism.

Shaving the operative area with a clean disposable razor is frequently recommended. This may be particularly useful in dense air bearing areas. Shaving may facilitate wound management, it may invite bacterial proliferation and wound infection if the infundibulum of the hair follicle is injured. This can be avoid by using depilatory agents. Care should be taken to remove all shaved hair in the wound will act as a foreign body, inviting infection and compromising the wound healing process.

The weighed Cassia fistula residue from the flowers extract was triturated with required quantity of yellow soft paraffin so as to make 5% ointment. The ingredients were uniformly and thoroughly mixed in a porcelain tile using a flexible spatula as described earlier. The ointment was collected in a glazed paper for use. Health rabbits (wt.1.5 kg) were taken as experimental models . A wound of about 1.5 sq.cm was created. The prepared ointment was applied on the wound continuously for 15 days. The area of the wound was traced every day morning and then the ointment was applied . The percentage of healing were calculated.

Healthy rabbits (wt . 1.5 kg) were taken as experimental models. A wound of about 1.5 sq. cm was created. The area of the wound was traced and then the base paraffin was applied continuously for 15 days. The percentage of healing were calculated. Healthy rabbits (wt.1.5kg) were taken as experimental models. A wound of about 1.5 sq.cm was created. The area of the wound was traced and then nitro furacin was applied continuously for 15 days. The percentage of healing were calculated. Healthy rabbits(wt.1.5kg) were taken as experimental models without treatment . A wound of about 1.5 sq.cm was created. The area of the wound was traced continuously for 15 days. The percentage of healing were calculated.

Table – I
Percentage of wound Healing Activity

Days	Group - I	Group - II	Group – III	Group – Iv
0	0%	0%	0%	0%
3	38.7%	42.69%	46.25%	63.40%
6	50%	68.31%	54.98%	78.09%
9	62.5%	70%	86.65%	95.1%
12	65%	75%	93.03%	100%

Group – I Without Treatment
Group – II Yellow soft paraffin
Group – III Nitro furacin
Group – IV Cassia fistula

Summary and Conclusion
Cassia fistula belongs to Caesalpiniaceae family. The caesalpiniaceae family have been observed to be having many bioactive flavonoids. The flowers of cassia fistula was found to contain quercetagetin, its 6-0- glucoside. This yellow pigment have been characterized by means of

Volume : 5 | Issue : 11 | November 2015 | ISSN - 2249-555X

chemical reactions, PC and UV spectra.

The healing property of the drug keeping rabbit as animal model. The aqueous extract of cassia fistula was used as drug. It is found to be potent drug as the wound was healed totally on the twelfth day. Thus the drug is proved to be effective with respect to wound healing activity.

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