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A Study on Active Constituent of *Loranthus acaciae.*

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Short Communication

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ABSTRACT

It was studied that the fresh flowers of *Loranthus acaciae* has a active ingredient quercetin 3-0 glucoside by U.V. NMR & Paper chromatography, such quercetin 3-0 glucoside showed antimicrobial activity against *Sollmonella typhi, Klebsiella pneumoniae* and *Escherichia coli.*

INTRODUCTION

The plant *Loranthus acaciae* belongs to the family Loranthaceae, occur at agricultural area, natural forests, riparian Zones, Ruderal, Urban areas and wet lands. The flowers of this plant are hermaphroditic with five petals connate in the lower part farming a tubular and curved corolla about 40mm long and with a short calyx 2-3mm long [1].

From the literature survey it was observed that *Loranthus acaciae* is a very good medicinal plant [2] because it is used in complementary and alternative cancer therapy in Europe and it has highly potent in curing circulatory problems, laxative and convulsant [3].

In many of the medicinal plants the flowers also has more effect to cure the diseases due to the presence of phyto chemical constituent flavonoid, so that the present work was aimed to isolation, identification and study of antimicrobial activity of flavonoid present in the flowers of *Loranthus acaciae*.

The flavonoids are polyphenolic compounds possessing 15C atoms two benzene rings joined by a linear three carbon chain. The various flavonoids differ from one another only the state of oxidation of the central ring. Most frequently flavonoid occur combined with sugar as glycosides and any one flavonoid aglycone may occur in a single plant in several glyloside combination [4].

MATERIALS AND METHODS

Extraction and Fractionation

Fresh flowers 2kg of *Loranthus acaciae* collected from the forest area of Villupuram, Tamil Nadu during December were extracted with 85% ethanol (4X500ml) under refuse. The alcoholic extract was concentrated in vacuo and the aqueous concentrate was successively fractionated with C_6H_6 (2 X 500ml) peroride free ether (2 X250ml) and ethyl acetate (5 X 250ml).

Ethyl acetate fraction

There were two layers obtained, aqueous and organic layer. The organic layer was concentrated in vacuo and left in an ice chest for a few days. When a yellow solid separated which was filtered and studied when crystalised from methanol, it came out as yellow crystals called flavonol. It was subjected to PC, UV and NMR.

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Identification of Sugar

The aqueous layer (Solution of glycoside) obtained in the Ethyl acetate fractionation was dissolved in hot MeOH (10ml) an equal volume of H_2SO_4 7% was added and the mixture was gently refluxed at 100°C for 2 hours. The excess of alcohol was distilled off in vacuo and the resulting solution was extracted with Ether there were residue and a queous layers obtained.

The aqueous layer was neutralized with $BaCO_3$ and filtered. The concentrated filtrate on PC gave Rf values corresponding to glucose. The identify of the glucose was confirmed by PC with authentic sample of glucose.

Identification of aglycone

The residue from the above Ether fraction was taken up in acetone and left in an ice-chest for a few days an yellow solid that separated was filtered and studied by PC and UV.

Antimicrobial activity

The isolates of *Salmonella typhi, Pasteurella multocida, Streptococcus pyogenes, Klebsiella pneumonia* and *E. Coli* were cultured at over night at 37°C on nutrient agar medium 5 plates per micro organism.

The suspensions of each bacterial isolate were prepared in isotonic sodium chloride solution. Dried per tri dish 5 per each micro organism were flooded with the appropriate suspension of the bacterial isolates then the sterile 6mm diameter filter papers were impregnated with the appropriate concentrations 50, 100, 150, 200, 250mg of glycoside sample and placed on the corresponding 25 plates 5 each of *Salmonella typhi, Pasteurella multocida, Streptococcus pyogenes, Klebsiella pneumonia* and *E. Coli.* After the incubation at 37°C for 24 hrs. All the plates were observed for Zones of growth inhibition and the diameters of the Zones measured in mm using calibrated ruler, results shown in table 3.

RESULTS AND DISCUSSION

Organic layer of Ethyl acetate fraction had the following UV values MeOH Max nm 255, 269, 370. +NaOMe 247, 306, 420 + $Alcl_3$ 272, 304, 333, 460, $Alcl_3$ – Hcl 264, 303, 358, 426. +NaOAC 276, 329, 390 and NaOAC – H_3O_3 261, 303, 388. The Rf value of PC depicted in Table 1 and C^{13} NMR values are represented in Table 2.

The UV results of aglycon yielded the Max MeOH nm 257, 269sh, 301sh, 370. +NaOMe 247sh, 321, +$Alcl_3$ 327, 303sh, 433 $Alcl_3$ – Hcl – 268, 303sh, 399, +NaOAC 277, 334, +NaOAC– H_3BO_3 267, 298sh, 387, nm. It's Rf value depicted in table 1.

Table 1: Paper chromatography

S. No	Compound	Developing Solvents								
		A	B	C	D	E	F	G	H	I
1.	Glycoside (Yellow crystals) from E to AC fraction	02	15	27	52	68	49	42	68	76
2.	Quercetin 3-0 glucoside (Authentic)	02	15	28	53	69	50	42	68	75
3.	Hydrolysed Aglycon	01	04	17	40	85	38	48	80	72
4.	Quercetin (Authentic)	01	04	17	39	83	39	47	80	70
5.	Sugar from the hydrolysate of glycoside	–	–	–	–	77	09	38	–	91
6.	Glucose Authentic	–	–	–	–	77	09	39	–	90

Rf (X100) values of the components of *Loranthus acaciae* flowers.
What man No: 1 Ascending 30+2°C

Solvent Key

A – H_2O
B – 5% aq. HOAC
C – 15% aq. HOAC
D – 30% aq. HOAC
E – 60% aq. HOAC
F – nBuoH, HOAC, H_2O 4: 1: 5
G – Water Saturated Phenol
H – HOAC, ConHcl, H_2O (Furestal) 30: 3:10

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I – t- BuoH 27%, HOAC: H_2O 3: 1: 1 [TBA]

Spraying agent: Aniline hydrogen Pthalate.

Table 2: C^{13} NMR Spectral data

Compound	C-1	C-2	C-3	C-4	C-5	C-6
Quercetin Authentic δPPM	122.22	115.11	144.81	147.37	115.45	120.32
Quercetin from sample	122.15	115.53	144.41	148.41	116.15	121.57
Glycoside Authentic (Glucose)	101.17	74.05	75.88	76.4	70.35	66.98
Quercetin 3-0 glucoside sample	101.5	74.2	76.1	76.8	70.4	67.1

From all the above results. It was conclude that the flower of Loranthus acacae consisted of a ingredient Quercetin 3-0-glucoside.

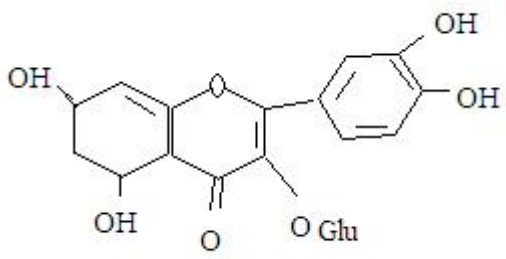

Table 3: Antimicrobial Activity – Zone of Intimation

Concentration of Sample (Mg)	Micro- Organisms				
	Sollmonella typhi,	*Pasteurella multocida*	*Streptococcus pyogenes*	*Klebsiella pneumoniae*	*E. coli*
50	–	–	–	–	–
100	–	–	–	–	–
150	–	–	–	–	–
200	17 mm	–	–	18 mm	16 mm
250	5 mm	–	–	10 mm	7 mm

Antimicrobial activity exhibited by *Loranthus acaciae* flowers due to the presence of active ingredient Quercetin 3-O glucoside only at 200 – 250 mg concentration for *Sollmonella typhi, Klebsiella pneumoniae* and *Escherichia coli.* It was concluded that the *Loranthus acaciae* flower may be used to cure the diseases which are caused by above 3 micro organisms.

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