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Research Paper Chemistry

Antibacterial activity of Bauhinia tomentosa Linn

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ABSTRACT

The fresh flowers of Bauhinia tomentosa Linn belongs to Caesalpinioideae family have been found to contain Quercetin -3-O-rutinoside. The structure of the compounds have been ascertained by paper chromatography ,UV,C13 NMR spectral values. The glycoside isolated from the flowers of Bauhinia tomentosa showed highest antibacterial activity against Staphylococcus aureus and Escherichia coli.

Keywords : Antibacterial-Bauhinia tomentosa,Quercetin-3-O-rutinoside-Staphylococcus aureus- Escherichia coli

1. Introduction :

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in India. Chemical substances ,which are capable of inhibiting the growth or causing the death of pathogenic microorganism without affecting the normal tissues of the host are termed antimicrobials.1

Many plants and their isolates have been constantly screened for their possible antimicrobial activity2, 3 . Extracts of various medicinal plants containing flavonoids have been reported to possess antimicrobial activity4. The mechanism of antimicrobial activity action of the flavonoids by inhibition of respiration and reproduction of microbes has been proved by Pawers5. The antibacterial activity of isoflavonoids and flavonoids 6 and glycosides of Luteolin and Apigenin has been reported 7. Quercetin has been to inhibit vireses and bacteria8. The root and rhizome oils of Kaempheria galangal showed activity against S.aureus and E.coli 9.During the present investigation the antibacterial activity of the flavonoid glycoside isolated from Bauhinia tomentosa has been evaluated against Styphylococcus aureus and E.coli.

2. Experimental:

2.1 Extraction and fractionation

The fresh flower petals (2 kg) of Bauhinia tomentosa collected at Kumbakonam of Thanjavur district during the month of December. They were extracted with 85% methanol(5X500 ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with benzene (3X250 ml) peroxide free ether (2X250 ml) and EtOAc (8X250 ml). Only EtOAc fraction was taken up for study. This Et2O fraction yielded Quercetin and EtOAc fraction yielded Quercetin-3-O-rutinoside.

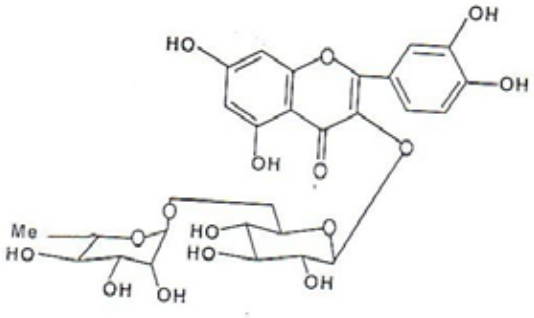
2.2 Characterization:

Quercetin : Yellow solid, M.p 313-350 ˚C. In UV spectroscopy it had λmax MeOH 255,269,370; +NaOMe 247,306,420;+AlCl3 272,304,333,460;AlCl3-HCl 264,303,358,426;+NaOAc 276,329,390 and NaOAc-H3O3 261,303,388. The 100 MHz C13 NMR spectral values are represented in Tab 1.3 .It's Rf values are listed in Tab1.2. It was identified as Quercetin and the identity was confirmed by mixed PC.

Quercetin-3-O-rutinoside (Rutin); Yellow solid M.p 188-189˚C. It had λmax MeOH 254,268 sh,300sh,358;+NaOMe 272,332sh,410;+Alcl3275,303 sh,433; AlCl3-HCl 268,303 sh,364,399;+NaOAc 277,334 sh 93; +NaOAc-H3BO3 267,298sh ,387nm. It's Rf values are represented in Tab 1.1 &1.2. The C13 NMR spectral values are illustrated in Table 1.3.

2.3 Hydrolysis of the glycoside

The glycoside was dissolved in hot aq. MeOH (5ml 50%) and an equal volume of H2SO4 (7%) was added. It was refluxed at 100 ˚C . The excess alcohol was distilled in vacco and extracted with Et2O. The resulting yellow solid was similar to there for free aglygon described earlier. The filtrate after removal of aglycone was neutralized with BaCO3. Their Rf values are illustrated in Table1.2.



Quercetin-3-O-rutinoside

Table 1.1

Rf (x100)values of the G1 from the yellow flowers of Bauhinia tomentosa(whatman No.1 Ascending,30±)

Compound	Developing Solvents							
	A	B	C	D	E	F	G	H
Glycoside	32	43	52	62	67	53	51	76
Aglycone from G1 (complete hydrolysis)	-	-	04	16	39	85	44	47
Quercetin authentic	-	-	04	16	40	85	44	47
Glycoside from (Partial Hydrolysis)	3	7	22	34	59	60	57	67
Quercetin-3-O-rutinoside	3	7	22	34	59	60	57	67

***Solvent Keys**

A= H2O, B= 5%aq.HOAc, C =15%aq.HOAc,D=30%aq. HOac,E=60%aq.HOac,F=n.BuOH;H2O=4:1:5(Upper phase), G=Phenol Saturated with water
H= HOAc:con.HCL:H2O=30:3:10
G1=Quercetin-3-O-rutinoside

Table 1.2
Rf (X100) values of the sugar from the G1 from the flower of Bauhinia tomentosa (Whatman No:1, Ascending, 30+-2)

Compound	Developing solvent				
	F	G	H	I	J
Sugar from G1	18	38	37	-	25
Glucose authentic	17	38	37	-	24
Sugar from G1	34	58	58	55	-
Rhamnose authentic	34	58	59	55	-

J=nBuOH: Benzene: Pyridine: H2O=5:1:3:3

Spray reagent : Aniline hydrogen phthalate.

G1-Quercetin-3-O-rutinoside

Table 1.3
13C- NMR spectral data and their assignment for the aglycone and glycoside G1 from the yellow flowers of Bauhinia tomentosa

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Rhamnetin (from Literature (δ) ppm)	146.35	135.81	175.66	160.98	98.47	164.0	93.55	156.54	103.25
Glycoside G1 (δ ppm)	156.59	133.27	177.34	161.19	99.70	164.16	93.60	156.41	103.95
Rhamnetin 3-O- rutinoside from literature (δ ppm)	156.6	133. 6	177.67	161.53	99.05	164.46	94.13	156.80	104.35

Compound	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
Quercetin (from Literature (δ) ppm)	122.22	115.11	144.81	147.37	115.45	120.32
Glycoside G1 (δ ppm)	122.15	115.53	144.41	148.41	116.25	121.57
Quercetin-3-O- rutinoside from literature (δ ppm)	121.38	115.57	147.22	149.13	116.59	121.61

Compound	C-1"	C-2"	C-3"	C-4"	C-5"	C-6"
Glycoside G1 δ ppm	101.17	74.05	75.88	76.4	70.35	66.98
Glucose from literature δ ppm	101.5	74.2	76.1	76.8	70.4	67.1

Compound	C-1"'	C-2"'	C-3"'	C-4"'	C-5"'	C-6"'
Glycoside G1 δ ppm	100.73	74.05	71.83	70.54	68.2	17.71
Rhamnose from literature δ ppm	100.5	74.2	72.2	70.8	68.2	17.5

3. Antibacterial activity of Quercetin-3-O-rutinoside
3.1.Disc diffusion method 10 .
Yellow solid namely Quercetin-3-O-rutinoside was examined for antibacterial efficiency.

The culture was maintained on slants consisting of nutrient. The test solution was prepared by dissolving 250mg of each extract separately in 5ml of Sterile Dimethyl formamide(DMF).

Nutrient agar medium was prepared and sterilized by autoclave . They poured into sterile petridishes to a uniform depth of 40mm and allowed to solidify at room temperature. After, the test organism were inoculated with in a bacterial culture. Thus provide the uniform surface growth of bacterium . The the sterile filter paper (6mm) containing sample were immersed in test extract and placed over the solidified agar in such a way that there is no overlapping of zone of inhibition11. The organism inoculated petridishes were incubated at 37 ˚C for 48 hours. After incubation the zone of inhibition produced by the sample with different organism were measured and recorded immediately using a zone reader 12.

Table.1.4
Antibacterial activity of Quercetin-3-O-rutinoside

Drug	Concentration	Microorganism used			
		S.aureus	% of inhibition	E.coli	% of inhibition
S1	2mg/ml (Penicillin)	19mm	100	-	-
S2	2mg/ml (Norfloxacin)	-	-	21mm	100
G1	100µg 200µg	16mm 7mm	84.2 36.85	16mm 7mm	76.2 33.34

4 . Results and Discussion :
The isolated compound namely Quercetin -3-O-rutinoside was screened for their antibacterial activity at two different concentrations (100,200 µg) against the gram positive organism S.aureus amd gram negative organism E.coli. Tab 1.4 shows the inhibition along with their % of inhibition of the growth of the organism used by comparing them with the standard antibiotic namely Penicillin against the S.aurus and Norfloxacin against E.coli. The % of inhibition was calculated by considering 100% inhibition of the standard drugs.

The drug Quercetin -3-O-rutinoside effectively inhibits the S.aureus by 84.2% at 100µg and a minimum inhibition of about 36.5% at 200µg concentration. The same the drug shows a maximum inhibition in the growth of the E.coli by 76.2% at its lower concentration (100µg) and less inhibition (33.4%) at higher concentration. Results observed implies the bacteriostatic effect of Quercetin-3-O-rutinoside against both the gram positive organism at its lower concentration itself.

5. Conclusion
The flowers of Bauhinia tomentosa were found to contain Quercetin and its glycoside Quercetin-3-O-rutinoside. The structures of the compounds have been ascertained by chemical reactions, paper chromatographic and UV,C13NMR spectroscopic values. The result observed in the present study indicates the bacteriostatic activity of Quercetin-3-O-rutinoside was a dose dependent one. This conclusion is supported by many of the earlier reports. Which suggests that the flavonoid glycoside exhibit selective toxicity against microorganisms.

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