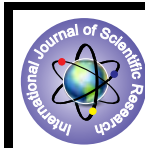


Antibacterial Activity of Kaempferol-3-O-Glucoside



Chemistry

KEYWORDS : Antibacterial - Prosopis juliflora - Kaempferol-3-O-glucoside- Staphylococcus aureus- Escherichia coli.

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ABSTRACT

The fresh flowers of prosopis juliflora belongs to fabaceae family have been found to contain Kaempferol and its glycoside Kaempferol-3-O-glucoside. The structure of the compounds have been ascertained by paper chromatography and UV spectral values. The glycoside isolated from the flowers of prosopis juliflora showed highest antibacterial activity against gram positive organism Staphylococcus aureus and gram negative organism 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 (I.L.Finar ; 1975). Many plants and their isolates have been constantly screened for their possible antimicrobial activity.(M.M.Iwu and C.O.Chiori ; 1984),(M.A.Rao and E.V.Rao ; 1985). Extracts of various medicinal plants containing flavonoids have been reported to possess antimicrobial activity (PKhanna et al ,1980). The mechanism of antimicrobial activity action of the flavonoids by inhibition of respiration and reproduction of microbes has been proved by Pawers (J.J.Pawers ; 1964). The antibacterial activity of isoflavonoids and flavonoids (S.S.Gnanamanikam and J.W.Mansfiels ; 1981) and glycosides of Luteolin and Apigenin has been reported (M.Miski et al ;1983).Quercetin has been to inhibit vireses and bacteria (K.Masayuki et al ; 1984) The root and rhizome oils of Kaempferia galangal showed activity against S.aureus and E.coli (L.S.R.Arambewela et al 1999).During the present investigation the antibacterial activity of the flavonoid glycoside isolated from prosopis juliflora has been evaluated against Styphylococcus aureus and E.coli.

2. Experimental:

2.1 Extraction and fractionation

The fresh flower petals (2 kg) of prosopis juliflora collected at Jayankondam of Ariyalur 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 kaempferol and EtOAc fraction yielded Kaempferol-3-O-glucoside.

2.2 Characterization:

Kaempferol: Yellow solid, M.p 276-278 °C. In UV spectroscopy it had λ_{max} MeOH 265, 294sh, 322sh, 367; +NaOMe 279, 315, 420;+AlCl₃ 260, 303, 350, 424; AlCl₃-HCl 255, 303sh, 348, 424;+NaOAc 263, 303, 368 and NaOAc-H₃BO₃ 257, 297sh, 320sh, 365. It's R_f values are listed in T-2. It was identified as Quercetin and the identity was confirmed by mixed PC.

Kaempferol-3-O-glucoside; Yellow solid M.p 224-226°C. It had λ_{max} MeOH 264,301sh350;+NaOMe 273,324,398sh,410;+AlCl₃ 3262,303sh350,426sh.; AlCl₃-HCl 260,303sh ,350,426;+NaOAc 267,301,352 ; NaOAc-H₃BO₃ 276,301,320,352nm. It's R_f values has represented in T-1&T-2.

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

3.1.Disc diffusion method (B.D.Paul;1974)

Yellow solid namely Kaempferol-3-O-glucoside 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 Di-

methyl 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 inhibition (J.C. Maruzzella and A.H. Practical ; 1958). 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 (T.G. Mariyan et al ; 1993).

4. Results

The isolated compound namely Kaempferol-3-O-glucoside was screened for their antibacterial activity at two different concentrations (100,200 µg) against the gram positive organism S.aureus and gram negative organism E.coli. Tab 1.3 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.aureus and Norfloxacin against E.coli. The % of inhibition was calculated by considering 100% inhibition of the standard drugs.

The drug Kaempferol-3-O-glucoside effectively inhibits the S.aureus by 89.3% at 100µg and a minimum inhibition of about 38.80% at 200µg concentration. The same the drug shows a maximum inhibition in the growth of the E.coli by 71.5% at its lower concentration (100µg) and less inhibition (32.4%) at higher concentration. Results observed implies the bacteriostatic effect of Kaempferol-3-O-glucoside against both the gram positive organism at its lower concentration itself.

5. Discussion

The flowers of prosopis juliflora were found to contain Kaempferol and its glycoside Kaempferol-3-O-glucoside . The structures of the compounds have been ascertained by chemical reactions, paper chromatographic and UV spectroscopic values. The result observed in the present study indicates the bacteriostatic activity of Kaempferol-3-O-glucoside 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.

Table -1 R_f (x100)values of the G1 from the yellow flowers of prosopis juliflora (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
Kaempferol-3-O-glucoside	33	43	53	62	66	52	50	75

Aglycone from hydrolysis	02	01	03	22	31	98	98	76
Kaempferol (authentic)	02	01	02	22	31	97	98	76

***Solvent Keys**

A= H_{20} 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= Kaempferol-3-O-glucoside

Table -2 R_f (X100) values of the sugar from the G1 from the flower of prosopis juliflora (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

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

Spray reagent : Aniline hydrogen phthalate.

G1- Kaempferol-3-O-glucoside

Table-3 Antibacterial activity of Kaempferol-3-O-glucoside from the flowers of *prosopis juliflora*

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 8mm	89.3 38.80	16mm 6mm	71.5 30.34

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