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Spectral Characterization And Evaluation of Antimicrobial Activity of Flavonoid From The Flowers of Glyceridia Sepium

KEYWORDS: Glyceridia sepium, human pathogens, kaempferol, pyronoside, glycoside.

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ABSTRACT *The flavonoid was isolated by extraction and fractionation with different solvents from the fresh flowers of Glyceridia sepium belongs to the family fabaceae and it was identified as Kaempferol 3,7-O-β-D-diglucopyronoside with the help of UV ,1H NMR&13C NMR spectroscopic techniques ,then it's antimicrobial activities against some human pathogens were studied by disk diffusion method . It has been proved that, the flavonoid possess anti microbial activity against all the tested strains and it's zone of inhibition nearly equal to the drug Ciproflaxacin which was used as a standard drug. Thus the compound kaempferol 3, 7 - O - β - D - diglucopyronoside obtained from the flowers of Glyceridia sepium is one of the potential element for therapeutic use*

INTRODUCTION

It is estimated that 2% of all carbon photosynthesized by plant is converted in to flavonoids or closely related compounds[1,2].Flavonoid is one of the largest groups of naturally occurring phenols. Flavonoids commonly occur as flavonoid -O-glycoside or flavonoid -C-glycoside[1,2].These compound show a broad spectrum of biological activities[4,5].In this investigation it was aimed to elucidate the structure and antimicrobial activities of flavonoid from the flowers of Glyceridia sepium belongs to the family fabaceae,It is a medium size leguminous tree and can grow 10 to 12 meters height. The flowers are located on the end of branches, these flowers have a bright pink to lilac colour that is tinged with white. A pale yellow spot is usually at the flower's base

EXPERIMENTAL

Extraction and Fractionation:

Fresh flowers of 2kg of Glyceridia sepium collected from forest area of Muthandi Kuppam Village, Panruti ,Tamilnadu, India were extracted with 85% methanol (5x500ml) under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate successively fractionated with benzene (3x250ml) ether (3x250ml) and ethyl acetate (4x250ml).The residue from ethyl acetate fraction called flavonoid came out as yellow solid .It developed red colour with Mg-Hcl, brown colour with alc. Ferric soln , developed yellow colour when viewed under uv light with and without NH_3 , it responded willson boric acid , molisch's test and Gibb's test. The flavonoid which was subjected to UV ,[1] H NMR and[13] C NMR spectroscopic studies[6,7]

Antimicrobial Activity

The flavonoid was tested for its antimicrobial activity[8]against some human pathogens like Escherichia coli, klebsilla pneumonia, pseudomonous aeruginosa, proteus vulgaris, streptococcus mutan and streptococcus pyogenes by disk diffusion method[9,10]. Disks measuring 6mm in diameter were punched from whatman No.1filter paper sterilized by dry heat at 140°C for an hour. They were immensed in solution which was prepared with different concentrations by using dimethyl formamide. Disks of each concentration were placed in nutrient agar medium inoculated with fresh bacteria strains separately. The incubation was carried out at 37°C for 24 hr. Ciprofloxacin was used as a standard at a concentration of 10µg/ ml. After the incubation period is over the zone of inhibition produced by the flavonoid with different organisms in different plates were measured and recorded immediately by using zone reader[11].

RESULTS AND DISCUSSION

The UV Spectral values of the flavonoid showed two absorption maxima at 350 nm (band I) at 255nm (bandII). Indicating the presence of glycosylation at 3-OH position. A stable bathochromic shift of 47 nm (Band I) noticed in NaOMe spectrum indicated the presence of free OH at . Abathochromic shift of 48nm in band I of flavonoid in the $AlCl_3$-HCl spectra confirmed the presence of free-OH group at C_5. In the ^{1}H-NMR data the signal at 6.2 ppm and 6.4 ppm corresponds to the proton at C_6 and C_8respectively. The doublet appearing in the region of 8.6 ppm corresponds to the proton at C_2' &C_6'. While the proton of C_5 appears at 6.8 ppm. H- 1" proton resonates at 5.4 ppm which indicates glucose moiety. The remaining sugar protons appear in the range of 3.0 to 3.7 ppm . The signal at 1.6 ppm indicates glycosylation at C_3The above details were assigned based on literature survey [1,7]. ^{13}C NMR spectral values of flavonoid were compared with the standard values[7] is shown at Table 1

Table : 1 Comparison of ^{13}C NMR Spectral values of flavonoid from the flowers of Glyceridia Sepium with standard values

	C_4	C_2	C_3	C_5	C_6	C_7	C_8	C_9	C_{10}	C_1'	C_2'	C_3'	C_4'	C_5'	C_6'
Kaempferol 3,7-O-β-D-digluoPyranoside from literature	177.5	156.4	133.4	161.1	98.8	164.2	93.8	156.4	104.0	120.9	131.0	115.1	159.9	115.1	131.0
Flavonoid	177.9	156.7	133.3	161.7	98.3	164.4	94.0	156.8	104.4	121.3	131.3	115.6	160.4	115.5	131.4

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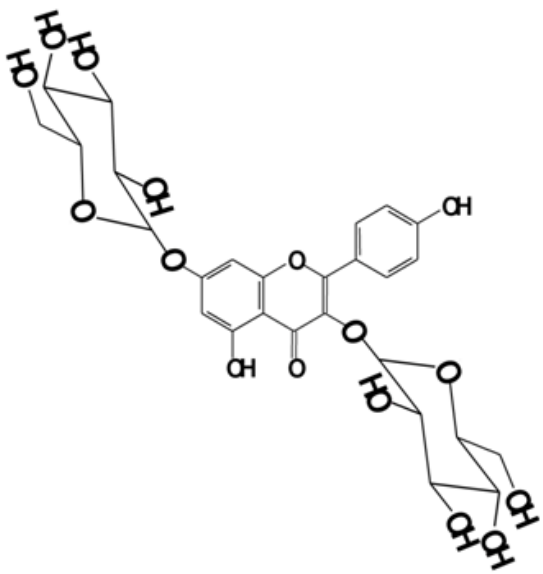
	C1″	C2″	C3″	C4″	C5″	C6″	C1‴	C2‴	C3‴	C4‴	C5‴	C6‴
Kaempferol 3,7-O-β-D-digluoPyranoside from literature	101.3	74.4	76.6	70.2	77.4	61.0	100.3	73.3	76.6	70.0	77.3	61.0
Flavonoid	101.3	74.3	76.9	70.3	77.2	61.6	99.1	73.5	76.6	70.0	77.2	61.3

From UV, ^{1}H NMR & ^{13}C NMR spectroscopic studies it was confirmed that the structure of flavonoid present in the flowers of Glyceridiasepium is kaempferol 3,7-O-β-diglucopyronoside,is as follows.



Kaempferol 3,7-O-β-D-diglucopyronoside

Table : 2 Antibacterial Activity of Flavonoid Zone of Inhibition in (Millimeters)

Micro Organism Used						
Drug	EC	KP	PA	PV	SM	SP
Ciproflaxacin [Standard] 10µg/ml	19	23	20	22	20	22
Flavonoid at 50µg/ml	18	22	19	20	20	21
Flavonoid 100µg/ml	18	23	20	21	20	21

EC	-	Escherichia Coli
KP	-	Klebsiella Pneumoniae
PA	-	Pseudomonas Aeruginosa
PV	-	Proteus Vulgaris
SM	-	Streptococcus Mutans
SP	-	Streptococcus Pyogens

Antibacterial activity of flavonoid isolate is nearly equal to ciprofloxacin which was used as a standard drug against the micro organisms shown in the table 2. From this, it was concluded that, the flavonoid isolate from flowers of glyceridia sepium has therapeutic effects.

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