Research Paper

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Investigations On Caryota Urens

Chemistry

KEYWORDS : Caryota urens, Streptococcus aureus, Escherichia coli

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INTRODUCTION

Caryota urens Linn. (Tamil:Koondal panai) belonging to the Palmae is a lofty handsome plain, distributed in forests of India, Srilanka and Burma1 .The sap of this palm is vary, high in simple sugars. In India and other Asian countries, the palm is tapped for its syrup which is often fermented into an alcoholic beverage called toddy. The syrup is also processed into a granular suger called jaggery.2 Fresh flowers of C.Urens (1 Kg) have been collected from Kolli hills in Namakkal district during May and June. The fresh flowers have been collected and extracted with 85% MeOH. It was concentrated in vacuo. The aqueous extract was successively fractionated with benzene (2x500ml), peroxide free Et20 (4x500ml) and EtOAc (4x500ml). The benzene did not yield any isolable material. Et20 Fraction: (Flavonol - quercetin)

The Et20 fraction was concentrated in vacuo and kept in and ice-chest for a week. An yellow solid that separated was filtered and examined. On crystallization from MeOH, yellow needles were obtained (m.p. 313-15°C, yield 0.02%). It was readily soluble in organic solvents and sparingly in hot water. It gave a red colour with Mg-HCl, olive green colour with alc.Fe3+, golden yellow colour with NH3 and NaOH yellow solution with a pale green fluorescence with cone. H2SO4 and appeared yellow under UV/ NH3. It reduced ammonical AgN03 in the cold and Fehling's solution on heating. It answered the Horhammer- Hansel3, Wilson's boric acid 4 and Gibb's tests5. It did not answer molisch's test. It had

255nm, 269sh, 370; +(NaOMe) 262sh, 321, 420 (dec);+(AlCl3) 267, 303, 458; + (A1C13HC1) 267,: 303, 351, 428; + (NaOAc) 275, 328, 390 and +(NaOAc- H3BO3) 262,303 sh, 386nm and had Rf values and depticted in Table I-A. The !H-NMR and 13C-NMR spectra of flavonol are appended. It was identified as quercetin and the identity was confirmed by co and mixed PC and m.m.p with and authentic sample or quercetin from Physalis minima. EtOAc fraction:(Flavonol glycoside : Kaempferol - 3 - O - glucoside)

The EtOAc fraction was concentrated in vacuo and left in an ice-chest for a few days.The yellow sold that separated was filtered and recrystallised from MeOH when yellow needles (m.p 176-78°C, yield 0.01%) were obtained. It had nm 265,352; +(NaOMe)275, 388;+(Alcl3) 275, 306,348, 406; +(AICI3-HCI) 274, 306, 350, 406; + (NaOAc) 275,366 and + (NaOAc-H3BO3) 265, 360. It developed a pink colour with (Mg-HCl), greenish brown colour with FeCl3 and formed and yellow precipitate on tereatment with lead acetate. It appeared purple when viewed under UV which turned yellow on exposure to NH3. It responded to Molisch's test but not to Horhammer-Hansel test. It had Rf values as depicted in table I-A. The H and 13C nmr spectra of the glycoside are appended.

HYDROLYSIS OF THE GLYCOSIDE

To a solution of the glycoside in hot MeOH, an equal volume of H2S04 (7%) was added. The mixture was refluxed at 100°C for 2h. The excess of alcohol was distilled off in vacuo and the resulting solution was extracted with Et20. The residue from Et20 from fraction was studied as described below. IDENTIFICATION OF AGLYCONE(Kaempferol)

The residue from Et20 fraction was taken in Me2CO and left under chilled conditions for a few days when an yellow solid (m.p 276-78C, yield 0.01%) was obtained. It had nm 266, 320, 370; + (NaOMe) 278, 316, 420; + (AlCl3) 268, 303, 350, 424; + (A1C13-HC1) 269, 302, 352 420; + (NaOAc) 274, 386 and + (NaOAc- H3BO3)267,320, 372. It developed a red colour with Mg-HCl and yellow colour with NH3. It appeared yellow when viewed under UV light, with and without NH3. Its Rf Values are given in Table I-A.

IDENTIFICATION OF THE SUGAR : (Glucose)

A portion of the aq.filtrate was neutralized with BaCO3 and the concentrated filtrate was subjected to PC. The Rf values corresponded to those of glucose. The identity of the sugar was also confirmed by direct comparison with the authentic sample.

TABLE -IA
Rf(xlOO) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF C.URENS
(WHATMAN NO. I ASCENDING 30 ± 2°C)

	DEVELOPING SOLVENTS								
Compound	a	b	C	D	e	f	g	H	i
Aglycone from Et_20 fraction	-	01	04	17	38	85	39	48	.72
Quercetin (Authentic)	- .	01	04	17	38	85	39	48	72
Aglycone from Hydrolysafe of.	-	5	-	49 .	93	67	62	87	94
Kaempferol (Autheritiac)	-	5	-	50	93	67	62	87	94
Glycoside from EtyOAc	13	40	43	68	77	71	71	83	71.
Astragalin (Authentic)	13	40	43	68	77	71	71	83	71

SOLVENT KEY

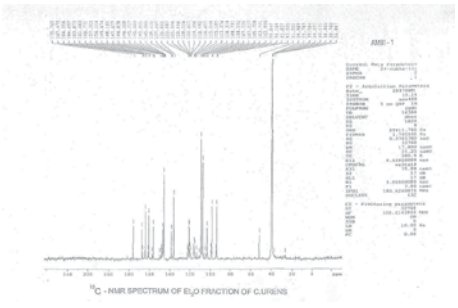
a = H20, b=5%aq. AcOH, c=15% aq.AcOH, d j 30% aq. AcOH

e = 60%aq. AcOH, f = BAW [n-BuOH;AcOH;H20 = 4:1:5 (upper phase)]

g = Phenol saturated with water

h = Forestal (AcOH:a Conac:HCl:H20 = 30:3:10)

i = TBA (t-BuOH:AcOH:H20 = 3:1:1)



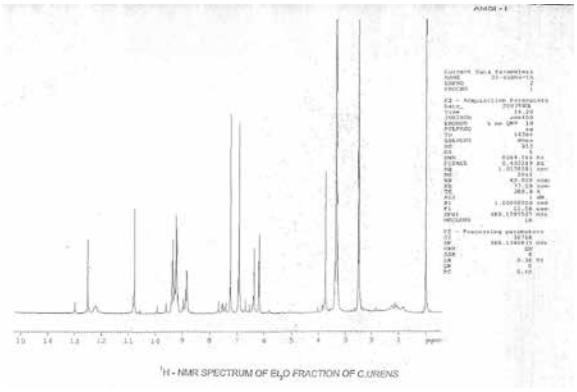
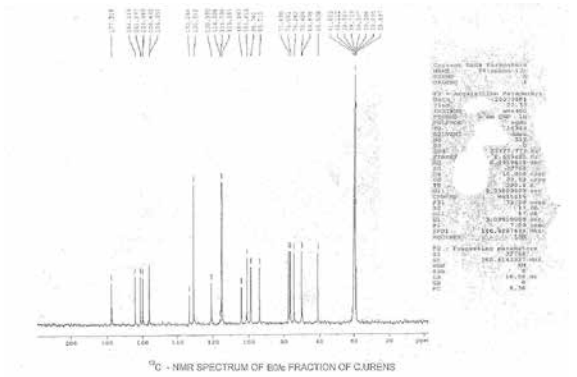


TABLE -II
13C NMR SPECTRAL DATA OF THE AGLYCONE FROM C. URENS AND ASSIGNMENT OF SIGNALS TO VARIOUS CARBONS

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Aglycone (6ppm)	147.677	135.865	175.765	161..483	98.849	166.356	93.837	156.114	103.741
From Literature	147.5	136.5	176.4	161.0	99.6	166.0	94.5	156.7	104.0

Compound	C-l'	C-2'	C-3'	C-4'	C-5'	C-6'
Aglycone (6ppm)	122.007	116.057	145.405	148.101	116.057	121.594
From Literature	123.0	116.0	145.7	148.1	116.5	121.0



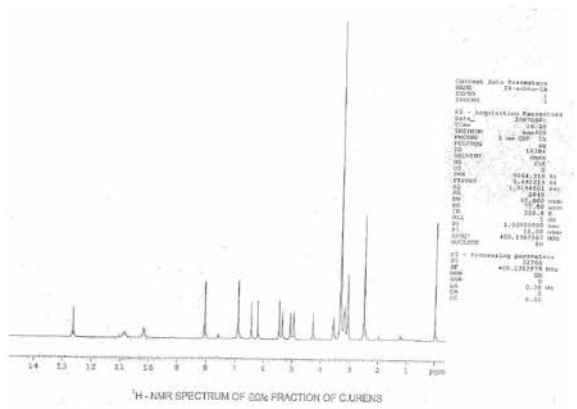


TABLE -III
13C NMR SPECTRAL DATA OF THE GLYCOSIDE FROM C. URENS AND ASSIGNMENT OF SIGNALS TO VARIOUS CARBONS

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	c-10
Glycoside (£ppm)	156.351	133.288	177.518	161.277	98.761	164.219	93.713	156.435	104.067
From literature	156.3	133.0	177.4	161.1	98.7	164.1	93.6	156.3	104.1

Compound	c-r	C-2'	C-3'	C-4'	C-5'	C-6'
Glycoside *(Sppm)*	120.970	130.918	115.161	159.989	115.161	130.918
From Literature	121.0	130.7	115.0	159.8	115.0	130.7

Compound	C-l"	C-2"	C-3"	C-4"	C-5"	C-6"
Glycoside (?ppm)	101.016	74.282	77.496	70.404	76.501	60.928
From Literature	101.4	74.2	77.2	70.1	76.5	61.0

RESULTS AND DISCUSSION

The fresh flowers of C. Urens have been found to contain quercetin, astragalin.

The UVspectrum of the Et20 soluble exhibited A^^ at 70 nin (band I) and 255 nm (band II) indicating a flayonol skeleton. A shift of+58 nm on the addition of AICl3 -HC1 indicates the presence of free -OH at C-5 in a ring. A comprison of A1C13 and AICI3-HCI spectra revealed an additional bathochromioc shift of 30 nm in the case of AICI3 spectrum (with out acid) which again points to the presence of catechol type of B-ring. It is further substantiated by the bathochromic shift of 16nm noticed in band I on the addition of H3 BO3. The presence of a free -OH at C-7 is evident from the bathochromic shift of 20nm in band II on the addition of NaOAc. It has already been evidenced from the appearance of a new band at 330 nm in the NaOMe spectrum.

In the H-NMR spectrum (400MHz, DMSO -d6, TMS) (VII) of the aglycone, the hydroxyl proton at C-5 shows up at £12.506 ppm, as a distinct singlet. The sharp singlets at 510.786 and £9.603 ppm correspond to - OH protons at C-7 and C-3. The doublet at £9.316 ppm (J=9Hz) accounts for the hydroxyl protons at C-3' and C-4'. The C-5' proton appears as a doublet at 5 6.680 ppm (J=9Hz). The signals due to the protons at C-2' and C-6' overlap at £7.5.26 ppm. A-ring protons at C-6 and C-8 could be located at £6.183 (d,J=2.5Hz) and £6.407 ppm (d,J=2.5Hz) respectively.

Supporting evidence for the structure of the flavonol is provided by the 13C-NMR (100MHz, DMSO-d6, TMS) (VIII) spectral data. A complete assignment of the various signals is provided in table II. I

The UV spectrum of the flavOnol glycoside showed A^mx at 352nm (band I) and 265nm (band II). A bathochromic shift of 54nm is observed in band I of its NaOMe spectrum indicates the presence of a free OH group at C-4'. The A1C13 spectrum of the glycoside showed four absorption maxima indicating a free - OH group at C-5 which is further supported by a bathochromic shift of 53nm in its A1C13-HC1 spectrum and positive, response of the glycoside to Wilson's boric acid test. The presence of a -OH group at C-7 could be inferred from a bathochromic shift of 12nm (in band I) noticed in its NaOAc-H3B03. Further, a bathochromic shift of 18nm (band I) observed in the MeOH spectrum of the aglycone obtained after hydrolysis of the glycoside as compared to that of the glycoside suggests that the site of glycosylation could be at C-3 which is also supported by the fact that the glycoside did not respond to the Horhammer-Hansel test whereas the aglycone did.

Based on these observation, the glycoside has been characterized as Kaempferol 3-O-glucoside.

The 400 ,MHz 1H-NMR (DMSO -d6) spectrum, of the flavonol glycoside shows a signal at 812.623 ppm revealing the presence of a free 5-OH proton. The A-rimg protons of C-6 and C-8 appear at 5 6.207 and 5 5.354 ppm respectively. In the B-ring, the protons at C-2',3',5' and 6' appear as two pairs at 8 6.882 and

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8 8.040 ppm The H-3' and 5' appear at upfield from the H-2',6' due to the shielding effect of oxygenation at c-4' as also due to the deshielding influence of C-ring operating on H-2' and H-6'. The H-l" signal of the glucose moiety appears at 8 5.464 ppm, found down field from the bulk of the sugar protons. The 7-OH proton appears at 8 10.865 ppm. The OH proton at C-4' appears at 8 10.118 ppm. The remaining glucoses protons appears in the range 8 3.191 to 8 3.334 ppm supporting evidence for the structure of the glycoside was provided by the analysis of 1 C-NMR (100MHz, DMSO-d6,TMS) data and the complete assignment of signal to various carbons is as given in table III

2. DETERMINATION OF ANTIBACTERIAL ACTIVITY:
The flavonoids isolated from C. urens have been investigated for their antibacterial activity.

A standard volume -|2.5ml) of Mueller-Hinton agar medium that would support the growth of the test organisms was added to several, labelLed, sterile, Stoppered and identical Petri-dishes. Solutions of the test compound at six different concentrations viz 25, 50, 50, 100, 200, 300, 400jj.g/ml in sterile water were prepared. Standards containing streptomycin at concentration of 50; 100, 200j.ig/ml and a control containing no drug were prepared. A standard inoculum of a suspensions of turbidity equal to a McFarland standards 0.5 of the test organism was added to all the Petri-dishes. The small amount of the drug that is carried over with this inoculum is easily removed by diffusion into the agar and the effect is negated by spreading the inoculum over a large area. All these manipulation were carried out with utmost care under aseptic conditions. After inoculation, the plates were immediately incubated incubated at 37°C and Minimum Inhibitory Concentration (MIC) is found out after 48h.of incubation.

The number of colonies that grown on this subculture after incubation is then counted and compared to the number of CFU/ml (CFU-Colony Forming Units) in the original inoculum. % resistance at that drug concentration =[(Nc-Nd)/Nc] x 100 Where Nc = number of colonies on the control Petri-dish. Nd = number of colonies on a Petri-dish with-drug. The % of resistance in each is as depicted in Table V

TABLE-III
EFFECT OF EtOAc ISOLATE OF CARYOTA URENS AGAINST BACTERIAL GROWTH

Compound	Dose In µg	Nc CFU/ ml	S.aureus		E.coli	
			Nd x10^5 CFU/ml	% Inhibition	Nd x 10^5 CFU/ml	% inhibition
I.EtOAc	25	10^6	5	50	5.5	45
Fraction	50		2.5	75	4.2	58
	100		1.5	85	4	60
	200		0.3	97	2.5	75
	300		0.2	98	2	80
	400		0.1	99	1	90
2.Streptomycin	50		2	80	4	60
	100		1	90	2.5	75
	200		0.4	96	1.4	86

RESULTS AND DISCUSSION
The antibacterial activity of the isolated flavonoid glycoside has been investigated by counting the number of colonies on each Petri-dish. A standard inoculum of the test- organism of Mcfarland standard 0.5 corresponds to 106 CFU/ml. it can be inferred from the Table III, that antibacterial effect is a dose dependent one. For S.aureus the MIC is found to be 200 fig while, for E.coli, MIC is 400f.ig. The Gram-negative bacteria, E.coil has been inhibited to lesser extent when compared to S.aureus the Gram-positive cocci. This reveals that there exists a pattern of selective toxicity among the flavonoid glycosides towards the Gram-positive organism. It confirms the observation of earlier researchers that the Gram-positive bacteria are selectively inhibited by flavonoids and is flavonoids of plant origin .6 Such selectivity of chemicals towards Gram positive bacteria is not . restricted to compounds of plant origin. It is the general phenomenon observed among most of the antibiotics7 . It has been suggested that the cell will thickness for their sensitivity. The Gram-negative organisms have a thick coating of lipopolysaccharides while the Gram-positive bacteria have thin cell walls. Acknowledgements The authors thank SIF of I.I.Sc. Bangalore, for their help in recording the nmr spectra.

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