Research Paper Chemistry

Phytochemical Studies And Antibacterial Investigations on Gliricidia Sepium

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ABSTRACT

The fresh pale pinkish flowers of Gliricidia sepium were found to contain the flavonol glycoside, isoquercitrin. The structure of the isolated polyphenolic has been ascertained by means of modern physical methods like UV, H-1 NMR, C-13 NMR, chemical reactions, chromatographic examinations and hydrolytic studies. The isolated yellow pigment is observed to be antibacterial. This property has been compared with standard drugs.

Keywords : Gliricidia sepium, isoquercitrin, Bacillus subtilis and Escherichia coli

INTRODUCTION

Gliricidia sepium, often simply referred to as Gliricidia (common names: MatazRatón; Cacao de nance, Cachanance, it is commonly known as "Madreado" Honduras; Kakawate in the Philippines; Madre Cacao or Madre de Cacao in the Philippines and Guatemala; and Madero negro in Nicaragua), is a medium size leguminous tree belonging to the family Fabaceae. It is considered as the second most important multi-purpose legume tree, surpassed only by Leucaena leucocephala. Gliricidia sepium (Jacq.) Kunth.ex.walp. popularly known as Vivasayathagarai in Tamil belongs to the family Papilionoideae of Fabaceae. It is an introduced plant, native of South America. It is a medium sized tree and can grow from 10 – 12 m high, fairly free from pests and diseases. It is valued as a green manure for paddy in Tamil Nadu. Its leaves and bark are reported to possess insecticidal activity[1]. This tree is used in Mexico as shade for cocoa and coffee plantations and for this reason it is called 'Madrecacao' (mother of cocoa).

It is also used as a poison for rodents and in fact the Latin name Gliricidia means rodent poison. It is used as a hedge plant and the flowers are utilized as food in some places in Mexico[2]. In Panama, decoction of leaves used in utricaria, rash and also in burns and erysepalas[3]. In Guatemala and Costa Rica, bark decoction is used against bacterial and protozoal infections[4]. Branches of Gliricidia sepium is used to reduce fever in children and adults. It has also been used to treat infections produced by *Microsporumcanis*, *Trychophytonmentagrophytes* and *Neisseriagonorrohae*[5].

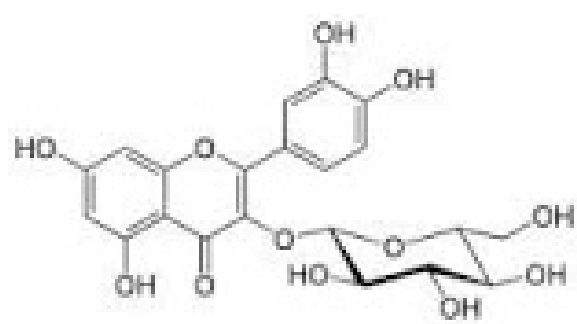
EXPERIMENTAL

Extraction and fractionation:

Fresh flowers of (1kg) of G. sepium were collected from Cholakad of Kolli hills in Namakkal District during November. The flowers have been extracted with 85 % MeOH (4X500 ml) under reflux. The alc. extract was concentrated *in vacuo* and subsequently fractionated with petroleum ether (b.p. 60 - 80° C) (3X250 ml), peroxide free Et_2O (3X250 ml) and EtOAc (4X250 ml).

Crystalline solids could not be isolated from petroleum ether and Et_2O fractions

EtOAc fraction: (flavonol glycoside – Isoquercitrin)



The EtOAc fraction was concentrated *in vacuo* and left in an ice-chest for 2 days. A yellow solid that separated was filtered and studied. It was recrystallised from MeOH when it afforded yellow crystals, m.p. 229-230° C (yield 0.1 %). It was freely soluble in MeOH and EtOAc and sparingly soluble in water. It gave an olive green colour with alc. Fe^{3+}, deep pink colour with Mg-HCl, yellow colour with NaOH and appeared deep purple under UV that turned yellow on exposure to NH_3. It responded to the Wilson's boric acid[6], Molisch's and Gibb's tests[7] but did not answer the Horhammer – Hansel test[8]. It had nm 257, 269 sh, 299 sh, 362; +NaOMe 272, 327, 409; +$AlCl_3$ 275, 303 sh, 333, 430; +($AlCl_3$ – HCl) 274, 303 sh, 353, 401; +NaOAc 271, 320 sh, 372 and +(NaOAc – H_3BO_3) 265, 300 sh, 372.

Hydrolysis of the glycoside:

To a solution of glycoside (0.1g, 0.2m mole) in hot aq. MeOH (10 ml, 50 %) and an equal volume of H_2SO_4 (7 %) was added to it. The reaction mixture was refluxed at 100^0 C for 2 h. The excess of alcohol was distilled off from hydrolysate and the resulting aq. solution was diluted with more water and left under chilled conditions for 2 h. A yellow solid that separated was filtered, washed and dried. The aq. filtrate and the washings were extracted with Et_2O. The dry yellow residue on the filter paper was combined with the residue from the dried Et_2O extract and studied for the aglycone.

Identification of aglycone: (quercetin)
The yellow aglycone that separated was taken up in Me_2CO and left in an ice-chest for a day when a pale yellow solid (m.p. 316 - 318° C) (yield 0.01 %) was obtained. It was soluble in organic solvents and sparingly in hot water. It gave red colour with Mg-HCl, olive green colour with alc. Fe^{3+}, golden yellow with NH_3 and NaOH. Yellow solution with a pale green fluorescence with conc. H_2SO_4 and appeared yellow under UV and UV/NH_3. It reduced ammonical $AgNO_3$ in the cold and Fehling's solution and heating. It answered Horhammer – Hansel and the Wilson's boric acid tests It gave a pentaacetate, m.p. 200 - 201° C, a petabenzoate m.p. 188 - 190° C and a pentamethyl ether m.p. 152 - 153° C. It had nm 255, 269sh, 301sh, 370; +NaOMe 247sh, 321; +$AlCl_3$ 272, 304sh, 333, 458; +($AlCl_3$ – HCl) 265, 301sh, 359, 428; +NaOAc 257sh, 274, 329, 390 and +(NaOAc – H_3BO_3) 262, 304sh, 388.

It was identified as quercetin and the same was confirmed by Co-PC, mixed – PC and m.m.p. with an authentic sample of quercetin isolated from Guazuma ulmifolia[9].

Identification of sugar: (glucose)
The aq. solution from the above hydrolysate was neutralised with $BaCO_3$ and filtered. The concentrated filtrate on chromatographic examination (PC) gave R_f values corresponding to those of glucose. The running properties of the glycoside was in favour of monoside. The identity of the sugar was also confirmed by direct comparison with an authentic sample of glucose. The glycoside was therefore identified as quercetin 3 – O – glucoside (isoquercitrin) and confirmed by Co- PC, mixed PC and m.m.p with an authentic sample of quercetin 3 – O – glucoside isolated from Guazuma ulmifolia[10].

RESULTS AND DISCUSSION
The fresh flowers of G.sepium have been found to contain quercetin - 3 O – β -glucoside. The UV spectrum of the glycoside showed two major absorption peaks at 362 nm (band I) and 257nm (band II) showing a flavonol skeleton. A bathochromic shift of 47 nm (band I) in observed in its NaOMe spectrum indicated in the presence of a free 4' – OH group. The $AlCl_3$-HCl spectra of the glycoside as well as its aglycone showed three absorption peaks and a shoulder indicating a free 5 – OH group in both. The glycoside as well as its aglycone did not exhibit any intense UV fluorescence ascertaining the presence of a free hydroxyl group at C-5 in both. The bathochromic shift of 39 nm and 58 nm respectively in $AlCl_3$ – HCl spectra was yet another evidence for the same. The presence of a C-7 -OH in evident from a shift of +14 nm in the case of the glycoside and +19 nm in the case of the aglycone on the addition of NaOAc. The presence of an o-dihydroxyl group in the B-ring could be inferred from a shift of +10 nm noticed in the case of the aglycone on the addition of H_3BO_3. In the $AlCl_3$ spectrum an absorption peak was noticed at 430 nm (band I) which on addition of HCl reduced by 29 nm. This is another evidence for the presence of di –OH group in the B-ring.

In the ^1H – NMR spectrum (400 MHz, DMSO-d_6, TMS) of the glycoside the protons at C-6 and C-8 appear as-doublets at δ 6.2 and δ 6.4 ppm respectively. The 5-OH proton resonates at δ 12.7 ppm as a distinct singlet. The C-5' proton appears as a doublet at δ 6.8 ppm. The H-1" signal of the flavonol – 3 – O – glucoside is found at δ 5.9 ppm. The remaining glucosyl protons appear in the range δ 3.4 – 3.5 ppm. Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C – NMR (67.89 MHz, DMSO-d_6, TMS) data. Due to glycosylation at 3-position, C-2 and C-4 carbons absorb at δ 156.3 and 177.4 ppm respectively. C-1" absorbs at δ 101.0 ppm. The rest of the carbons of the sugar unit appear between δ 60.9 and δ 77.3 ppm C-6" appear at δ 17.4 ppm.

Based on this the glycoside has been characterised as isoquercitrin (quercetin 3 – O – glucoside).

TABLE - 1
BACTERIOSTATIC EFFECT OF ISOLATED FLAVONOID GLYCOSIDE FROM G. SEPIUM

Compound	Dose µg/ml	Percentage protection	
		B.subtilis	E.coli
Isolated glycoside from G. sepium	50 100 200	78 82 82	40 51 59
Streptomycin	50 100 200	65 68 73	54 56 60
Benzylpenicillin	50 100 200	76 89 92	60 65 71

RESULTS AND DISCUSSION
The antibacterial activity of a flavonoid glycoside, has been investigated by measuring and comparing the turbidity of the control with that of the flavonoid drug. The observed percentage of protection depicted in Table - 1 indicates that the bacteriostatic effect is a dose dependent one. The Gram-negative bacteria E. coli has been inhibited to a lesser extent as compared to B. subtilis, a Gram-positive organism. This suggests that there exists a pattern of selective toxicity among the flavonoid glycosides towards the Gram-positive group. These results are in conformity with the observations of earlier researchers that the Gram-positive bacteria are selectively inhibited by flavonoids and isoflavonoids of plant origin [11]-[15].

This pattern of 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 antibiotics[16]. It has been suggested that the cell wall thickness of these bacteria is the basis for their sensitivity. Gram-positive bacteria in general, have thin cell walls whereas the Gram-negative organisms have thicker cell walls.

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REFERENCES

[1] The Wlth. of India, Raw Materials, C.S.I.R. , New Delhi, 1956, 4, 137. | [2] R.L. Delizo, V.F. Del Fierro. Vegetative propagation of Madre Cacao, Gliricidia sepium (Jack) Steud, by cuttings using Alpha Naphthalene Acetic Acid (ANAA). Araneta Res J., 1974, 21, 20. | [3] L.V. Asolkar, K.K. Kakkar, J. Chakre editors. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles, Part I, New Delhi: CSIR, 1992, 177. | [4] I. Berger,A.C. Barrientos, A.Caceres, M. Hernandez, L. Rastrelli, C.M. Passreiter Plants used in Guatemala for the treatment of protozoal infections. II: Activity of extracts and fractions of five Guatemalan plants against Trypanosoma cruzi. J Ethnopharmacol, 1998, 62(2), 107. | [5] M.P Gupta, Plantas Medicinales Iberoamericanas. I ed. Bogota: Presencia Ltda; 1995, 378.| [6] C. W. Wilson, J. Amer. Chem. Soc., 1928, 48.| [7] J. Shinoda, J. Chem. Pharm. Soc., 1928, 48.| [8] L. Horhammer and R. Hansel, Arch. Pharm. Berl., 1955, 288, 315.| [9] D. Sukumar, Ph.D Thesis, Bharathidasan University, Tiruchirappalli, 1989, 97.| [10] idem, ibid., 1989, 97.| [11] J.G. Wyman and H.D. van-Etten, Phytopath., 1978, 68, 583| [12] S.S. Gnanamanickam and D.A. Smith, Phytopath., 1980, 70, 894| [13] M.G. Sethuraman, Ph.D. Thesis, Bharathidasan Univ., 1988, 152| [14] C.G.G. Barnabas and S. Nagarajan, J. Madras Univ., 1979, 166, 51| [15] N.B. Pappano, S.E. Blanco, N. B. Debattista, R.F. Segovia and F.H. Ferretti, Commun. Biol., 1985, 4, 23.| [16] J.S. Glasby, Éncylcopedia of Antibiotics', John Wiley, London, 1976, 372.