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Research Paper

Antibacterial Studies on the Isolates of Radermachera Xylocarpa

CHEMISTRY

KEYWORDS : Radermachera xylocarpa (Roxb.) K.Schum., isoquercitrin, Bignoneaceae, , Escherichia coli, and Bacillus subtilis

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ABSTRACT

Radermachera xylocarpa (Roxb.) K.Schum. syn. Bignonia xylocarpa Roxb, Stereopermum xylocarpum (Roxb.), popularly is known as padri tree in English. The fresh flowers of Radermachera xylocarpa has been found to contain the glycoside isoquercitrin. The flavonoid glycoside has been characterized by means of modern physical methods like UV, 1H-NMR, 13C.NMR, hydrolytic studies, chemical reactions and chromatographic techniques. The isolated pigment is found to be having substantial antimicrobial property.

INTRODUCTION

Radermachera xylocarpa belonging to family Bignoneaceae is screened for its biochemical contents. The plant is known for its antiseptic property. Its resin is used for the treatment of skin diseases. Its root bark is bitter in taste and astringent in nature. It is also known as yellow snake tree. The leaves gave flavonoids, dinatin and its glycoside. The roots of the plant yielded O-acetyl oleanic acid and stigmasterol. It is a tree with compound leaves and long dehiscent pods. The pod appears "Snake"y with irregularly placed tubercles on the surface and slightly curved apex. Seeds many, winged, light, creamish – yellow, attached to hard cord like replum. In case of snake bite, seeds are made in to paste and applied on bitten part as well as taken orally[1]. The extracts from R. xylocarpa are used as antivenom[2].

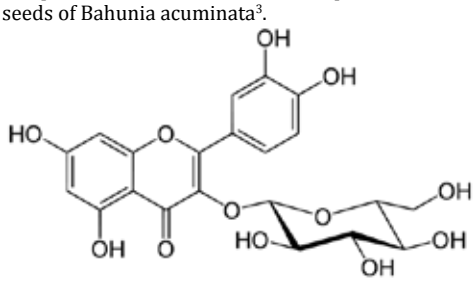
EXPERIMENTAL

Extraction and fractionation:

Fresh creamy white flowers (1 kg) of R. xylocarpa collected from Kodaikanal hills during June were extracted with 80% MeOH (4 X 500 ml) under reflux. The alc. extract was concentrated in-vacuo and the aq. concentrate successively fractionated with benzene (3 X 250 ml), peroxide-free Et_2O (3 X 250 ml) and EtOAc (4 X 250 ml). The benzene and Et_2O fractions did not yield any isolable material.

EtOAc fraction : (flavonol glycoside : isoquercitrin)

The EtOAc fraction was concentrated in vacuo and left in an ice chest for a 2 days. A yellow solid that separated was filtered and studied. It was recrystallised from MeOH when it afforded yellow crystals, m. p. 229-30 °C (yield 0.1%). It was freely soluble in EtOAc and MeOH and sparingly in water. It gave an olive-green colour with alc. $FeCl_3$, 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 did not answer the Horhammer- Hansel[3] test but responded to the Wilson's boric acid[4], Gibbs'[5] and Molisch's tests. The pigment had R_f as indicated in Table I-11 and had λnm 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; (+NaOAc/H_3BO_3) 265, 300 sh, 372. The ^{1}H- and ^{13}C-NMR of the glycoside are appended. The identity of the glycoside was confirmed by direct comparison with an authentic sample of the same from the seeds of Bahunia acuminata[3].



Isoquercitrin

Hydrolysis of the glycoside :

To a solution of the glycoside (0.1 g, 0.2 m mole) in hot aq. MeOH (10 ml, 50%) an equal volume of H_2SO_4 (10%) was added and the mixture refluxed at 100 °C for 2 h. The aq. hydrolysate was worked up in the usual way.

Identification of the aglycone : (flavonol: quercetin)

The aglycone on recrystallisation from methanol gave yellow leaflets, m.p. 316 - 18 °C (yield 0.02%) which was identified as quercetin by colour reactions, behaviour under UV and R_f. The Et_2O fraction was concentrated in vacuo and left in an ice chest for about a week. A yellow solid that separated was filtered and studied. It came out as pale yellow needles m.p. 316-18 °C on recrystallization from MeOH. It was soluble in organic solvents and sparingly in hot water. It gave a red colour with Mg-HCl, olive-green colour 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 answered Wilson's boric acid, Horhammer – Hansel and Gibbs' tests but did not respond to Molisch's test. It had λnm 255, 269 sh, 301 sh, 370; (+NaOMe) 247 sh, 321 (dec.); (+$AlCl_3$) 272, 304 sh, 333, 458; (+$AlCl_3$/HCl) 265, 301 sh, 359, 428; (+NaOAc) 257 sh, 274, 329, 390; (+NaOAc/H_3BO_3) 262, 304 sh, 388 and had R_f values of quercetin. The ^{1}H- and ^{13}C-NMR of the flavonol are appended. It was identified as quercetin and the same was confirmed by co- and mixed-PC and m.m.p with and authentic sample of quercetin from Physalis minima[4].

Identification of the sugar : (glucose)

The aq. solution from the above hydrolysate was neutralized 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 were in favour of a monoside. The identity of the sugar was also confirmed by direct comparison with an authentic sample of glucose.

Antibacterial property of the glycoside

In the course of this investigation the antimicrobial activity of the isolated quercetin 3-O-rutinoside, has been analysed, using *E. coli* a Gram negative microorganism and *B. subtilis* a Gram positive organism. The nephloturbidity meter has been employed for the determination of the antimicrobial activity. Streptomycin has been used as a reference.

RESULTS AND DISCUSSION

The fresh flowers of ***R. xylocarpa*** 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 257 nm (band II) showing a flavonol skeleton. A bathochromic shift of 47 nm in band I observed in its NaOMe spectrum indicated the presence of a free 4′-OH group. The $AlCl_3$-HCl spectra of the glycoside as well as its aglycone showed 3 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. A 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 group is 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 a o-dihydroxyl group in the B-ring could be inferred from a shift of + 10 nm noticed in the glycoside and + 18 nm noticed in case of the aglycone on the addition of H_3BO_3. In the AlCl3 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 a catechol type di-OH group in the B-ring.

In the ^{1}H-NMR spectrum (400 MHz, DMSO-d_6, TMS) of the glycoside, the protons at C-6 and C-8 appear at δ 6.18 and 6.42 ppm respectively. The C- 5′ proton appears as a doublet at δ 6.81 ppm. The 5-OH proton resonates at δ 12.64 ppm as distinct singlet. The –OH protons at C-7, C-3′ and C-4′ show up to δ 9.7, 9.45 and 9.22 ppm respectively. The H-1′′ signal of the flavonol-3-O glucoside is found at δ 5.45 ppm. The remaining glycosyl protons appear in the range δ 3.4 to 3.8 ppm.

Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C-NMR (100 MHz, DMSO-d_6, TMS) data and a complete assignment is given (Table I-12). Due to glycosylation at 3-position, C-2 and C-4 carbons absorb at δ 156.3 and 177.2 ppm respectively. C-1′′, absorbs at δ100.9 ppm. The rest of the carbons of the sugar unit appear between δ 69.9 ppm and 77.6 ppm.

Based on this the glycoside has been characterized as isoquercitrin (quercetin-3-O-glucoside). The isolated yellow pigment is more active against E. subtilis than E. coli. The selective inhibition of growth of Gram-positive bacteria has been observed among most of the antibiotics[5].

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