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Antimicrobial Activity of *Ipomoea Pes-Caparae* (L) R. Br

Chemistry

KEYWORDS : Ipomoea pes-caprae, Convolvulaceae, melanoxetin, 3',4' , 6 ,8 – tetrahydroxflavonol-5'- methylether-7-O-neohesperidoside

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ABSTRACT

Ipomoea pes-caprae, also known as Beach Morning Glory or Goat's Foot, is a common pantropical creeping vine belonging to the family Convolvulaceae. It grows on the upper parts of beaches and endures salted air. The rare flavonol melanoxetin and 3',4' , 6 ,8 – tetrahydroxflavonol-5'- methylether-7-O-neohesperidoside have been identified by means of modern physical methods like UV, H-1 nmr, C-13 nmr , chemical reactions, chromatographic techniques and hydrolytic studies.

INTRODUCTION

Ipomoea pes-caprae (L)R.Br., (Syn.) *Convolvulus pes-caprae.L., Ipomoea biloba* Forssk., popularly known as (railroad wine in English) adapukodi in Tamil, belongs to Convolvulaeceae. It is distributed throughout Pantropical, Somaliland, tropical Asia, Sumatra, Malaysia and Singapore. It is cultivated along wind-swept inland sites. The juice squeezed from the plant is used in Malaysia to treat fish stings. The leaves are used in Indonesia to hasten the bursting of boils; sap from the young leaves are boiled in coconut oil and used to treat sores and ulcers, and the seeds, chewed with areca nut, sooth abdominal pains and cramps. In Philippines, the boiled leaves are used to treat rheumatism [(1)]. It showed anti-inflammatory[(2-3)] antimicrobial, homolytic, analgesic [(4)] antinociceptive, antipyretic[(5)] activities.

Isoquercitrin has been recorded from leaves of *I. pes-caprae* [(6)]. With a view to locating additional flavanoids, the leaves of *I. pes-caprae* have been investigated and the results are presented here under.

EXPERIMENTAL

Fresh flowers (1kg) of *I. pes-caprae* collected from the beach of Karaikal, during November were extracted with 85% MeOH(5 x 500ml) under reflux. The alc. extract was concentrated *in vacuo* and the aq. concentrate was successively fractionated with petroleum ether (60 - 80^0 C) (4 x 250 ml), peroxide – free Et_2O (3 x 250 ml) and EtOAc (4x 250ml). The petrol fraction did not yield any crystalline solid .

Et_2O fraction: (flavonol : Melanoxetin)

The Et_2O fraction was concentrated *in vacuo* and left in an ice-chest for a week. An yellow solid that separated was filtered and studied. On crystallisation from MeOH, pale yellow needles were obtained. (m.p. 226 - 28^0 C, yield - .01%). 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. Fe^{3+}, golden – yellow colour with NH_3 and NaOH, yellow solution with a pale green fluorescence with conc. H_2SO_4 and appeared yellow under UV with or without ammonia. It reduced ammonical $AgNO_3$ in cold and Fehling's solution on heating. It answered the Horhammer – Hansel, Wilson's boric acid and Gibb's tests. It did not respond to Molisch's test. It had nm 247, 260sh, 308sh, 318, 362; +NaOMe 250, 290, 335(dec.); +$AlCl_3$ 268sh 280, 316sh, 387; +($AlCl_3$/HCl) 248, 359sh, 308sh, 362; +NaOAc 254, 260sh, 308sh, 318, 361; +(NaOAc/H_3BO_3) 254, 260sh, 309sh, 319, 372nm and had R_f values as depicted in table(I - 14). The ^{1}H-NMR and ^{13}C-NMR of the flavonol are appended (fig.I – 11, 12). It was identified as melanoxetin and the identity was confirmed by co-and mixed –PC and m.m.p. with an authentic sample of melanoxetin from Acacia karroo, A.montana [(7)].

EtOAC fraction: (flavonol glycoside – 3',4' , 6 ,8 – tetrahydroxflavonol-5'- methylether-7-O-neohesperidoside):

The residue from EtOAc fraction was taken up in Me_2CO and left in an ice- chest for two days when a pale yellow flakes separated, m.p. 222 - 24^0 C (yield 0.05%). It was freely soluble in EtOAc and MeOH, sparingly in water and insoluble in Et_2O and $CHCl_3$. It gave a magenta colour with Mg-HCl; greenish- brown colour with NaOH; appeared yellow under UV which turned bright fluorescent yellow when fumed with NH_3. It answered the Horhammer – Hansel, Molisch's and Gibb's tests but did not respond to the wilson's boric acid test. It had nm 260, 297sh, 371; +NaOMe 327, 433(dec.); +$AlCl_3$ 274, 477 ; +($AlCl_3$/HCl) 267, 364, 441; +NaOAc 260, 324, 396; +(NaOAc/H_3BO_3) 263, 394 nm .

Hydrolysis of the glycoside:

To a solution of the glycoside (0.05 g) in hot MeOH (20 ml) , an equal volume of H_2SO_4 (10%) was added and the mixture gently refluxed at 100^0 C for 2 h. The excess of alcohol was distilled off and the resulting aq. solution extracted with Et_2O.

Identification of the aglycone: (3',4',6,7,8 – pentahydroxy flavonol- 5' – Methylether):

The residue from the Et_2O fraction when dissolved in a small quantity of Me2CO and left in an ice-chest for a few days yielded a yellow solid. It was soluble in organic solvents but insoluble in water. It appeared fluorescent green when viewed under UV with and without NH_3. It gave a pink colour with Mg-HCl and yellowish green colour with NaOH, $AlCl_3$ and NaOAc. It responded to the Horhammer – Hansel and Gibbb's tests but did not respond to the wilson's boric acid test and Molishch's test. It had nm 262, 345sh, 374 ; + NaOMe 276, 321, 435sh(dec.); +$AlCl_3$ 267, 366, 460; +($AlCl_3$/HCl) 270, 365, 435; + NaOAc 270, 377(dec.); and + (NaOAc/H_3BO_3) 257, 387 nm.

Identification of the sugar: (glucose and rhamnose)

The aq. filtrate after the removal of the aglycone was neutralised with $BaCO_3$ and the concentrated filtrate on PC indicated the presence of two sugars viz. glucose and rhamnose. The running properties of the glycoside were also in favour of a bioside. The identity of the sugars was confirmed by co – PC with authentic samples of glucose and rhamnose.

Enzymatic hydrolysis with pectinase:

The glycoside was resistant to hydrolysis by the enzyme – pectinase indicating that it is a neohesperidoside and not a rutinoside.

RESULTS AND DISCUSSION

The fresh flowers of *I. pes-caprae* is found to contain the rare flavonol melanoxetin and 3',4',6,8- tetrahydroxyflavonol – 5'-methylether – 7 – O- neohesperidoside.

The UV spectrum of the aglycone showed two bands at 362 nm (band I) and 247 nm (band II) which shows a flavonol skeleton. No bathochromic shift is observed, instead a decomposition is noted to find a continual decrease in intensity. From this it was inferred that there may be hydroxylation in 3,3' and 4' [(8)]. A comparison of $AlCl_3$ and $AlCl_3$/ HCl spectra revealed an additional bathochromic shift of 25nm in the case of $AlCl_3$ spectrum which points out the presence of a catechol type of B and A – rings. The presence of a free –OH at C-7 is evident from the bathochromic shift of 7nm in band II, on the addition of NaOAc

[9]. The presence of the catechol type of B and A- rings is also evident from the bathochromic shift 10 nm noticed in band I, on the addition of H_3BO_3 [10-11].

In the ^1H – NMR spectrum (400MHz, DMS-d_6, TMS) of the aglycone , the sharp singlets at δ10.8 ppm and δ9.69 ppm correspond to – OH proton at C-7 and C – 3 respectively. The doublet at δ9.40 ppm (J = 2.2 Hz) and δ9.36 ppm (J = 2.0Hz) accounts for the hydroxyl protons at C – 3' and 4'. The C-5' proton appears as a doublet at δ6.89ppm , (J = 2.5 Hz). The signals due to the protons at C – 2' and C – 6'appear at δ7.87 and δ7.79 ppm respectively. A ring phenolic proton at C – 8 could be located at δ9.5 ppm.

Supporting evidence for the structure of the flavonol is provided by the ^{13}C-NMR (100 MHz, DMSO – d_6, TMS) spectral data.

The principal absorption maxima of the glycoside and its aglycone are respectively 371nm (band I), 260nm (band II) and 374nm (band I), 262nm (band II) indicating a flavonol skeleton in both [12]. A bathochromic shift of 62nm in the glycoside and 61nm in the aglycone observed in their NaOMe spectra is suggestive of free – OH at C – 4' [13] in both. The decomposition of NaOMe spectra with passage of time indicates the presence of free –OH groups at C- 3, C-3' and C-4' [14]. The absence of a free –OH at C – 5 is evident from the enormous bathochromic shift of 70nm noticed in the glycoside and 61nm in the algycone in the $AlCl_3$ –HCl spectra [15]. It is further confirmed by the negative reaction of both the glycoside and the aglycone in the Wilson's boric acid test. No change was observed in band II of the glycoside on the addition of NaOAc which reveals the absence of a free – OH at C-7. The corresponding aglycone however shows a bathochromic shift of 8 nm supporting the presence of a free 7 –OH, arising due to hydrolysis. The lesser extent of this shift is in favour of the C – 6 and C-8 oxygenation in the glycoside and the aglycone. Vicinal trihydroxylated A – ring (hydroxyls at C-6, C – 7 and C -8) in the aglycone is evidenced by its colour reactions and the decrease in intensity of its NaOAc spectrum with time [16]. A bathochromic shift of 23 nm in glycoside and 13 nm in the aglycone on the addition of H_3BO_3 further supported the presence of catechol type of substitution in B –r ing [17].

In the ^1H – NMR spectrum (400MHz, DMSO – d_6, TMS) of the glycoside (fig I - 13), the C – 5 proton is strongly deshielded by 4 – keto group and appears as a signlet at δ 7.2 ppm [18]. The high – field signals of C – 2' and C – 6' protons resonating at δ 6.2 ppm (d, J = 2 Hz) and 6.6 ppm (d, J = 2 Hz) indicate the presence of 3' , 4', 5' – trioxygenation pattern in the B – ring[19] . Again the non- equivalence of the C – 2' C – 6' protons and C – 2' proton appearing at a higher field than the C – 6' proton indicate the presence of –OCH_3 group at C – 5' which is evidenced by a 3 proton signal at δ 3.9 ppm [20]. The H – 1" of the glucose and H – 1"' of rhamnose resonate at δ5.0 and 4.7ppm respectively. The broad signal appearing in the range δ 1.0 – 1.3 ppm representing the C -6"' protons (methyl protons of rhamnose) clearly reveals the presence of neohesperidoside. Had it been a rutinoside there should have been a dsignal at δ 0.8 – 1.1 ppm[21] . The rest of the sugar protons appear in the range of δ3.1 – 3.8 ppm.

Additional evidence for the structure of the glycoside was provided by the ^{13}C – NMR (100 MHz, DMSO-d_6, TMS) spectral data . Due to the glycosylation at C – 7, the signals of C – 6 and C -8 appear at δ 130.8 and δ 124.8 ppm respectively. The deoxy C – 5 appears at δ121.91 ppm. The carbonyl carbon (C -4) appears at δ 176.00 ppm. The methoxyl carbon resonates at δ 52.00 ppm. The appearance of C – 6"' signal at δ20.90 ppm and the signal of C -6" at δ 60.40 ppm (not at 66.00 ppm) clearly shows that the glycoside is a 7 – O – neohesperidoside [22-23].

ANTIMICROBIAL ACTION OF ISOLATED *IPOMOEA PES-CAPRAE* **(L)R.Br.,**

The isolated flavonoid glycosides from the fresh flowers were chosen for the investigation of their bacteriostatic effect on Gram positive (Bacillus subtilis) and Gram negative (Escherichia coli) strains.

A nutrient broth medium of pH 7 containing peptone 1% (w/v), yeast 0.5% (w/v) and sodium chloride 0.5% (w/v) was prepared in distilled water and sterilised 15lb/cm^2 pressure. 8 ml of this medium was taken in each of the identica', sterlile, labelled and plugged with cotton assay - tubes. Standard aq.solutions of the test compounds in sterile water containing 50, 100 and 200 µg/ml were injected into no test compound and standards containing streptomycin and benzyl penicillin at concentrations of 50, 100 and 200 µg / ml were also arranged. One loopful of the inoculums of a suitable dilution of overnight broth culture of the test organism was added. All these manipulations were carried out with care under aseptic conditions. The assay – tubes were incubated at 37 $\pm 0.5^0$ C for 36 hr and the resultant turbidities were measured with a nepheloturbiditymeter. The percentage of bacteriostatic effect was computed by employing the relationship, % inhibition = where Tc is the turbidity of the control and Tt is that of the specific treatment. Moreover subculturing from the treatment showing no visible turbidity (no visible growth) into a broth free of the test compound was also carried out to make sure that all the bacterial cells had been destroyed by the given concentration of the test compound. The percentage inhibitions are depicted in Table .

TABLE

BATERIOSTATIC EFFECT OF ISOLATED FLAVONOID GLYCOSIDES

Compound	Dose µg / ml	Percentage protoection	
		B. subtilis	E. coli
1	50	60	50
	100	77	65
	200	96	80
Streptomycin	50	65	54
	100	68	56
	200	73	60
Benzylpencilin	50	76	60
	100	89	65
	200	92	71

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