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Phytochemical And Antibacterial Effect Of *Delonix Elata*

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ABSTRACT

The fresh creamy white flowers of Delonix elata were found to contain the flavonol glycoside, tiliroside. 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.

INTRODUCTION

Delonix elata is a hermaphroditic, deciduous tree. Flowers in the hot season or during the early rains, in east Africa this is normally around December and August-March in India. Fruit ripening is between May and July***. Delonix elata*** (L.) Gamble. Syn. ***Poinciana elata*** Linn. called Gul mohar or Gold mohur, popularly known as vadha mudakki in Tamil belongs to Caesalpinioideae[1],[2]. A strikingly ornamental medium – sized tree, planted in avenues and gardens in all warmer and damper parts of India. It has a spreading crown of feathery foliage and bears flowers early in the hot season as the foliage falls and the branches are nearly bare. The leaves and barks of the plant are widely used by Siddha and Ayurveda practitioners for treating several conditions. ***D.elata*** used as anti – inflammatory, rheumatism[3], anti-microbial[4],[5] and possess antioxidant[6] activities.

The bark of this plant reported to contain β-sitosterol, saponins, alkaloids, carotene, hydrocarbons, phytotoxins and flavonoids. Flowers of this plant reported consist of tannins, saponins, flavonoids, steroids, alkaloids, carotenoides[7], [8]. Several experimental studies have revealed biological and pharmacological properties of phenolics compounds, especially their antimicrobial activity, antiviral, anti-inflammatory and cytotoxic activity[9], [10].

The leaves of the plant were reported to have cytotoxic, hepatoprotective and antioxidant properties. Seven flavonoid glycosides were isolated and identified from the leaves of ***D. elata***[11]. With a view to locating additional flavonoids, the flowers of ***D. elata*** have been investigated and the results are presented hereunder.

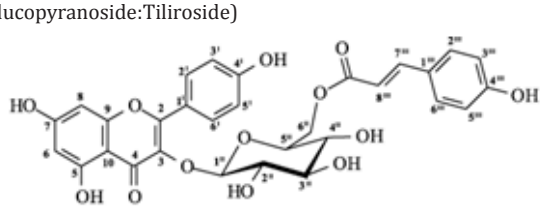
EXPERIMENTAL

Extraction and fractionation:

The fresh flowers of (1 kg) of ***D.elata*** collected from Sivagiri hills in Erode District during February were extracted with 90 % MeOH (4X500 ml) under reflux. The alc. extract was concentrated *in vacuo* and the aq. extract successively fractionated with petroleum ether (b.p. 60 - 80° C) (3X250 ml), peroxide free Et_2O (3X250 ml) and EtOAc (4X250 ml).

The petrol and ether fractions did not yield any isolable solid and were discarded.

EtOAc fraction: (Kaempferol 3-O – β -D-(6"-p-coumaroyl) glucopyranoside:Tiliroside)



The residue from EtOAc fraction was taken up in Me_2CO 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 came out as yellow needles, m.p. 262-264° C (yield 0.1 %). It was freely soluble in EtOAc, MeOH and sparingly soluble in Et_2O and $CHCl_3$. It developed pink colour with Mg-HC1, green colour with Fe^{3+}. It appeared purple under UV that turned yellow on exposure to NH_3. It responded to Wilson's boric acid, Molisch's and Gibb's tests but did not answer the Horhammer - Hansel test. It had 267, 298sh, 314, 358sh nm. +NaOMe 275, 305sh, 369; +$AlCl_3$ 275, 307, 320 sh, 358 sh, 395 sh; +($AlCl_3$ – HCl) 275, 307, 320 sh, 356 sh, 395 sh; +NaOAc 275, 298 sh, 310, 372 and +(NaOAc – H_3BO_3) 267, 298 sh, 314, 358 sh.

Hydrolysis of the glycoside:

The glycoside (50 mg) was dissolved in 5 ml of hot aq. MeOH and subjected to an equal volume of H_2SO_4 (7 %) in a boiling water bath for 2 h. The excess of methanol was distilled off *in vacuo* and the resulting aq. solution was extracted with Et_2O. The residue from the Et_2O fraction was studied as described below.

Identification of aglycone: (flavonol: kaempferol)

The aglycone on recrystallisation from MeOH gave yellow leaflets, m.p.277 - 279° C (yield 0.02 %). It had 250 sh, 266, 365; +NaOMe 271, 291 sh, 316, 413 (dec); +$AlCl_3$ 260 sh, 268, 303 sh, 350, 424; +($AlCl_3$ – HCl) 258 sh, 269, 303 sh, 348, 419; +NaOAc 275, 301, 385 (dec) and +(NaOAc – H_3BO_3) 267, 296 sh, 320 sh, 370. It was soluble in organic solvents but insoluble in water. It developed a reddish orange colour with Mg-HCl and yellow colour with NaOH. It appeared pale yellow under UV and UV/NH_3. It responded to Wilson's boric acid, Gibb's and Horhammer - Hansel tests. It was identified as kaempferol by Co-PC, mixed – PC and m.m.p. with an authentic sample isolated from ***Ligustrum perrottetii***[12].

Identification of Phenolic acid: (p-coumaric acid)

The acid crystallised from MeOH as colourless needles, mp-219-220° C. It produced pale yellow colour with alkalis, greenish brown with Fe^{3+} and decolourised Br_2-water. It gave effervescence with HCO_3^-. Colourless under UV changing to blue under UV/NH_3. It had 255, 305. It was identified as p-hydroxycinnamic acid (p-coumaric acid) and the identity was further confirmed by direct comparison with an authentic sample.

Identification of sugar: (glucose)

A portion of aq. filtrate was neutralised with $BaCO_3$ and filtered. The concentrated filtrate on chromatographic examination (PC) gave R_f values corresponding to those of glucose. The identity of the compound was also confirmed by Co-PC, mixed – PC and m.m.p with an authentic sample.

RESULTS AND DISCUSSION

Tiliroside has been isolated from the fresh flowers of ***D.elata***.

The flavonol glycoside had 250sh 266, 365 nm. A bathochromic shift of 9 nm in band II of NaOAc with decomposition of band I suggested free 3,7,4'-OH groups. NaOMe spectrum showing de-

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composition indicated the presence of free 3 and 4'-OH groups. A bathochromic shift of 54 nm in band I of $A1C1_3$/HC1 spectrum suggested the presence of 3 and/or 5-OH groups. A hypsochromic shift of only 5 nm in band I of $AlCl_3$/HCl spectrum compared to $A1C1_3$ spectrum and no characteristic shift in band I of NaOAc/H_3BO_3 revealed the absence of o-dihydroxyl in B-ring. Based on these data the flavonol was identified as 3,5,7,4'-tetrahydroxyflavone (kaempferol) and the identity confirmed by direct comparison including co, mixed-PC and m.m.p with an authentic compound[13].

The phenolic acid had 255, 305 nm. From these data the phenolic acid was identified as p-hydroxycinnamic acid (p-coumaric acid) and the identity was further confirmed by direct comparison with an authentic sample[14]. Sugar was identified as D-glucose by Rf in different solvents and confirmed by co-PC with an authentic sample.

The fact that kaempferol 3-O-glucoside (astragalin) and p-coumaric acid were obtained from this compound indicated that it was an acylated derivative of kaempferol 3-O-glucoside. The of this compound compared with that of kaempferol 3**-** O-glucoside revealed that the p-coumaroyl group is attached to one of the sugar OH groups and not to the phenolic OH groups of the aglycone. The ^{1}H NMR spectrum of the compound gave evidence for a monosubstituted kaempferol and a p-D-glucopyrsnosyl moiety esterified with E-p-coumaric acid. Doublets at **δ** 6.18 and 6.41 ppm (5-2 Hz) could be assigned to H-6 and H-8 respectively. The doublets at δ8.02 (J- 9Hz, 2H**,** H-2',6') and 7.40 ppm (J=9 Hz, 2H**,** H-3l.5') suggested 4'-oxygenated B-ring. The signal for the anomeric proton of glucose (**δ** 5.48 ppm, J-7Hz**)** indicated a β-configuration[15]. The stereochemistry of p-coumaric acid was concluded[16] from the doublets at **δ** 7.37 and 6.13 ppm exhibiting an expected coupling constant of 16 Hz**.**

The ^{13}C NMR spectrum besides revealing the nature of the aglycone, sugar moiety and acyl group indicated the position of glycosylation and acylation. The signal at ***δ*** 99.00 and 93.60 are due to C-6 and C-8. ^{13}C NMR spectra provided evidence 4' for oxygenation at C-3, C-5, C-7 and C-4' as well as for p-substituted benzene (B-ring). The chemical shift of 101.18 ppm for glucose C-1 resembling the anomeric carbon resonance of the sugar of kaempferol 3-O**-** glucoside[17] (δ 101.4) was 1ndicative of 3-O-glycosylation. Careful comparison of the chemical shifts of C-2, C-3, C-5 and C-7 of the flavonoid part with reported values for kaempferol and its 3-O-glucoside also led to the conclusion that this compound is a 3-O-glucoside. The ^{13}C signals of C-1 to C-4 of glucose compared well with those reported for 3-O-β-D-glucopyranoside. The site of esterification of glucose at C-6 was decided by comparison of the δ values of C-5 and C-6 of glucose moiety of the glycoside with C-5 and C-6 of glucose in kaempferol 3-O-β-D-glucopyranoside**.** The shift of carbon resonances (Δ***S)*** of glucose carbons for this compound were observed as C-4 (+ 0.08 ppm**),** C-5 (-2.03 ppm) and C-6 (+2.23 ppm) as compared to those in kaenpferol 3-O-glucoside suggested that the p-coumaroyl groups was attached at C-6 of glucose. The ^{13}C chemical shifts observed for this compound were in good agreement with those published[18] for E-p-coumaric apid. Based on these findings, the compound was characterized as kaempferol 3 – O – β – D - (6" – E – p – coumaroyl glucopyranoside**)** (tiliroside) earlier isolated from ***Tilia argentea***[19].

TABLE I-1
BACTERIOSTATIC EFFECT OF ISO LATED FLAVONOID GLYCOSIDE FROM *D. ELATA*

Compound	**Dose** *μg/ml*	Percentage protection	
		B.subtilis	E.coli
Isolated glycoside from ***D. elata***	50 100 200	70 80 90	55 69 79
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 I - 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[20]-[24].

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[25]. 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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