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Phytochemical Studies on Flowers of *Plumeria alba*

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Various fractions obtained from *Plumeria alba* contain antiinflammatory activities. The fraction obtained from diethyl ether was found to be a flavonol kaempferol. Aglycone and glucose fractions were also obtained during fractionation. All these fractions were characterized by UV-spectral analysis. The ethyl acetate fraction obtained was analyzed for its antiinflammatory activity which showed a dose-dependent activity against haemolysis.

Key Words: Phytochemical, *Plumeria alba* Linn.

INTRODUCTION

Plumeria alba Linn of apocyanceae is an erect tree with white flowers. It is distributed throught West Indies. In Guyana, the latex is applied to ulcers, herpes and scabies. The root bark is purgative, alternative, detergent and is given for blennorrhagia and herpes. An extract of it is used internally and externally for syphilitic ulcers[1]. It is cultivated in Indian gardens and popularly called 'Peru' in Tamil[2].

EXPERIMENTAL

Extraction and fractionation: About 500 g of the white flowers of *Plumeria alba* collected from the banks of Cauvery in Kumbakonam during December were extracted with 95 % ethanol under reflux. About 2 L of analar grade ethanol is used for extraction. The alcoholic extract was concentrated in vacuo and the aqueous concentrate was fractionated with about 750 mL of benzene, 1 L of peroxide-free diethyl ether and 2 L of ethyl acetate. No crystalline solid could be recovered from benzene fraction.

The residue from the diethyl ether fraction was taken up in ice-chest for a few days, when yellow needles were separated. It was identified as a flavonol Kaempferol. It had λ_{max}^{MeOH} nm 253 sh, 266, 294 sh, 322 sh, 367 sh; + NaOMe 278, 316, 416; + $AlCl_3$ with and without HCl, 256 sh, 269, 303 sh, 348, 424; + NaOAC 274, 303, 387 and (NaOAc + H_3BO_3) 267, 297 sh, 320 sh, 372.

It is soluble in organic solvents but insoluble in water. It developed a reddish orange colour with NaOH. It appeared pale yellow under UV as well as on exposure to NH_3. It responded to Wilson's boric acid[3], Horhammer-Hansel[4] and Gibb's tests[5] but did not respond to the Molisch's test. R_f values as given in Table-1.

It was identified as kaempferol and was confirmed by direct comparison with an authentic sample of kaempferol from *Ipomea aguatica*[6].

TABLE-1
R_f (× 100) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF *Plumeria alba*
[Whatmann filter paper No. 1, Ascending 30 ± 2 ºC]

Compound	Developing solvents							
	A	B	C	D	E	F	G	H
Aglycone form the diethyl ether fraction	3	–	5	18	52	95	97	31
Kaempferol (authentic)	3	–	5	18	52	95	97	61
Glycoside from ethyl acetate fraction	13	40	42	68	77	71	71	53

Solvent key: A = H_2O; B = 5 % acetic acid; C = 15 % acetic acid; D = 30 % acetic acid; E = 60 % acetic acid; F = *n*-BuOH:AcOH:H_2O 4:1:5 (Upper Phase) (BAW), G = Water saturated phenol, H = AcOH:conc. HCl:H_2O = 30:3:10 (Forestal).

The fraction obtained from ethyl acetate was concentrated *in vacuo* and left in an in chest for a few days. The yellow solid that separated was filtered and studied. It comes out as pale yellow needless (m.p. 223-225 ºC) (yield 0.05 %) on crystallization from methanol. It was identified as flavonol glucoside kaempferol-4'-O-glucoside. It was freely soluble in ethyl acetate, methanol, sparingly in water but insoluble in diethyl ether and chloroform. It developed a green colour with alcoholic Fe^{3+}. It appeared dull yellow under UV as well as on exposure to NH_3. It responded to Wilson's boric acid, Gibb's, Horhammer-Hansel and Molisch's tests. It had λ_{max}^{MeOH} nm 260, 293sh, 320, 355; + NaOMe 275, 323sh, 370; + $AlCl_3$ 260, 275, 320, 400; + $AlCl_3$ + HCl 290, 320, 400 and NaOAc 274, 295, 355 and NaOAc/H_3BO_3 270, 290 sh, 355.

Hydrolysis of the glycoside: The glycoside (0.05 g, 0.2 mmol) was dissolved in hot aqueous methanol (2 mL, 50 %) and an equal volume of H_2SO_4 (7 %) was added to it. The reaction mixture was refluxed at 100 ºC for 2 h. The excess alcohol was distilled off from the hydrolyzate and the resulting aqueous solution was diluted with more water and left under chilled conditions for 2 h. The yellow solid that separated was filtered, washed and dried. The aqueous filterate and the washings were extracted with diethyl ether. The dry yellow residue on the filter paper was combined with the residue from the anhydrous diethyl ether extract and studied for the aglycone.

Identification of aglycone: (flavonol; kaempferol): The yellow coloured aglycone on recrystallization from methanol afforded a yellow crystalline solid, m.p. 278-280 ºC, which was identified as kaempferol by colour reactions, behaviour under UV and R_f (Table-1). It had the same UV spectral values mentioned under ethereal fraction and was identified as kaempferol.

Identification of sugar: The aqueous hydrolyzate after removal of the aglycone was neutralized with $BaCO_3$ and filtered. The concentrated filterate on paper chromatography gave R_f values (Table-2) corresponding to those of glucose. The identity of the sugar was further confirmed by direct comparison with an authentic sample of glucose as also by preparation of its osazone and recording its m.p. A quantitative hydrolysis of the same by the Folin-Wu's micro method revealed it to be monoside.

TABLE-2
R_f (× 100) VALUES OF THE SUGAR FROM THE GLYCOSIDE OF *Plumeria alba*
(Whatmann filter paper No. 1, Ascending 30 ± 2 ºC)

Sugar	Developing solvents			
	E	F	G	H
Sugar from the hydrolyzate of EtOAc fraction	77	9	39	90
Glucose (Authentic)	77	9	39	90

Solvent key: E = 60 % acetic and F = *n*-BuOH:AcOH:H_2O = 4:1:5 (Upper phase) BAW, G = Water saturated phenol; H = AcOH:conc. HCl:H_2O = 30:3:10 (Forestal).

Antiinflammatory activity: Fresh blood was collected from healthy sheep and mixed with equal volume of sterilized alsever solution containing 2 % dextrose, 0.8 % sodium citrate, 0.05 % citric acid and 0.42 % NaCl and used within 5 h. Hyposaline (0.36 % 2 mL), phosphate buffer (0.15 m, pH = 7.4 1 mL) and SRBC (1 %, 0.5 mL) were taken in a number of tubes. Solutions of different concentrations of the drug were added in all tubes except one tube. The last tube served as control in which instead of the drug, isosaline (0.85 %, 1 mL) was added. The contents were incubated at 37 ºC for 0.5 h and then centrifuged. The intensity of the supernatant was measured at 560 nm using a photoelectric colorimeter. The control tube that contained no drug was taken as representing 100 % SRBC lysis. The percentage of prevention of drug-treated hyposaline-induced SRBC lysis was calculated from the difference in absorbance readings of the control and drug-treated tubes using the following relation.

$$100 - \frac{\text{Reading corresponding to drug treated sample}}{\text{Reading corresponding to control}} \times 100$$

The results are given in Table-3.

TABLE-3
EFFECT OF ETHYL ACETATE ISOLATE AGAINST
HYPOTONICITY INDUCED HAEMOLYSIS

Conc. of the drug (µg)	Prevention (%)	Conc. of the drug (µg)	Prevention (%)
15	82	65	74
25	85	75	64
35	85	85	60
45	82	95	53
55	79	105	45

RESULTS AND DISCUSSION

The fresh flowers of *Plumeria alba* have been found to contain kaemferol and its 4'-O-glucoside. The UV spectrum of the flavonol aglycone obtained form the diethyl ether fraction established two major peaks at 367 nm (band 1) and 266 nm (band 2) which showed a flavonol skeleton. A bathochromic shift of 49 nm on the addition of NaOMe revealed the presence of a free 4'-OH group in the B-ring. A shift + 57 nm on the addition of $AlCl_3$/HCl showed the presence of a free 5-OH in

the A-ring. The presence of a free OH at C-7 was ascertained by a shift of + 8 nm band 11 on the addition of NaOAc. The $AlCl_3$ spectrum was exactly same as that of ($AlCl_3$ + HCl) revealing the absence of catechol type of substitution in B-ring. The H_3BO_3 spectrum also confirmed it as there was only + 5 nm shift on the addition of NaOAc/H_3BO_3.

The (band 1) UV absorption of the glycoside is at 355 nm is indicative of flavonal skeleton. A bathochromic shift of only + 15 nm showed the absence of a free 'OH' group at C-4'. A comparison of band 1 absorption of the glycoside and the aglycone on the addition NaOMe reveals that there may be 4'-glycosylation in the flavonol. The $AlCl_3$/HCl spectrum showed + 45 nm shift ascertained a free 5-OH group. As there is no change in the absorption peaks between $AlCl_3$/HCl and $AlCl_3$. It is confirmed that there is no catechol type of substitution in B-ring. It was confirmed by H_3BO_3 spectrum. The presence of a free OH at C-7 is evident form + 14 nm band-II shift on the addition of NaOAc. Based on this pigments have been characterized as kaempferol and its 4'-glucoside.

The SRBC membrane stabilization effects of EtOAc fraction can be inferred from the data presented in Table-3 which showed a dose-dependent activity against haemolysis. It has maximal inhibition of hypotonicity (haemolysis) induced SRBC lysis at a concentration of 30 µg.

In general only a biphasic activity is observed in the drug, which is a common feature of the flavonoid glycoside found in literature. From these studies it can be inferred that this drug may exert its antiinflammatory activity stabilizing the lysosomal membrane.

Conclusion

The fresh flowers of *Plumeria alba* apocynaceae have been found to contain kaempferol and its 4'-O-glucoside. The flavanol and its glycoside have been duly characterized by choromatographic and hydrolytic studies as well as spectral technique. The isolation of a flavanol and its 4'-glucoside from the flowers of *P. alba* have considerable antiinflammatory activity.

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