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Phytochemical Studies On Grevillea Robusta.a.cunn.exr.br

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ABSTRACT

Fresh flowers of Grevillea robusta, of Proteaceae has been collected from the slopes of the hills of Yelagiri, in Dharmapuri district. Kaempferol-5-O-β-glucoside a rare flavonol glycoside has been isolated from the fresh flowers of Grevillea robusta. The structure of which has been characterized by means of modern physical methods like UV, H-1 NMR, C-13 NMR, chromatographic techniques, chemical reactions and hydrolytic studies.

KEYWORDS :: Grevillea robusta, Proteaceae, Kaempferol, Kaempferol-5-O-β-glucoside

INTRODUCTION

Grevillea robusta, commonly known as the **southern silky oak** or **silky oak**, or **Australian silver oak**, is the largest species in the genus *Grevillea*of the family Proteaceae. It is not closely related to the true oaks, *Quercus*. It is a native of eastern coastal Australia, in riverine, subtropical and dry rainforest environments receiving more than 1,000 mm per year of average rainfall. **Grevillea robusta.** A.Cunn.exr. Br also known as savUku[1] in Tamil belongs to Proteaceae family. It is an Australian silk oak,commonly planted as ornamental in many warm–temperate and semi tropical climates. It is distributed in the tropical highlands of India[2]. It has been planted extensively as shade for tea and coffee[3-6]. It is frequently used as a wind break although opinions differ as to its wind firmness and branch – shedding tendencies [7]. Silk oak is an important honey tree in India where it is also regarded as a good fuel wood producer[8]. The tree produces an attractively figured easily worked wood,which was once a leading face veneer in world trade, where it was marketed as "lace wood". The wood contains an allergen that causes dermatitis for many people [9] . With a view to locate additional flavonoids the flowers of **G. robusta** have been investigated and the results were presented here under.

EXPERIMENTAL

EXTRACTIONAND FRACTIONATION The flowers of G.robusta collected from the slopes of hills of Yelagiri,Dharmapuri district, during May, were refluxed with MeOH (4x500 ml) under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate successively fractionated with benzene (3x250 ml), peroxide–free Et_2O (3x250 ml) and EtOAC (4x250 ml). No isolable material were obtained from the C_6H_6 and Et2O fractions.

EtOAc fraction (Kaempferol-5-O-β-glucoside):

The residue from EtOAC fraction afforded yellow crystals(on recrystallisation from MeOH) (m.p.184-86°C). It was soluble in acetone but insoluble in water, Et2O and CHCl3. It developed a red colour with Mg-HCl, brown colour with alc.Fe3+developed a yellow colour when viewed under UV light with and without NH_3. It answered the Horhammer-Hansel10 and Molisch's test[11] but did not respond to Wilson's boric acid test[12]. It had λ_{max}^{MeOH} nm

257, 271sh, 298sh, 306 sh,371sh; +NaOMe 290sh, 340, 436; +AlCl3 (with or without HCl) 272 sh, 316,362; +N_aOA_c 274,330,398 and (+Na_OA_c-H_3BO_3) 258,302 sh,330,384. It had Rf values of kaempferol 5-O-glucoside. The 1H-NMR of the glycoside also proved its presence.

HYDROLYSIS OF THE GLYCOSIDE:

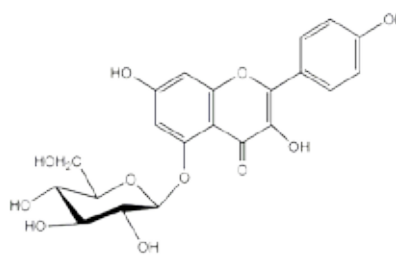
The glycoside(0.05g,0.2 mmole)was dissolved in hot aq.MeOH(2ml, 5%) and an equal volume of H_2SO4(7%)was added to it.The reaction mixture was refluxed under 100°C for 2 h,the excess of alcohol was distilled off from the hydrosylate and the resulting aq.solution was distilled with more water and left under chilled conditions for 2h.

IDENTIFICATION OF THE AGLYCONE(Flavonol-Kaempferol):

The residue from ether fraction of the hydrolysate was taken up in acetone and left under chilled conditions for few days. Yellow colour solid was obtained and its colour reactions,chromatographic behavior and UV spectral data were found to be similar to kaempferol.

IDENTIFICATION OF SUGAR(Glucose):

The aq.hydrolysate after the removal of the aglycone was cautiously neutralised with $Baco_3$ and filtered. The concentrated filterate on PC gave Rf values corresponding to those of glucose. The running properties of the glycoside was in favour of a monoside. The identity of the sugar was confirmed with an authentic sample of glucose.



RESULTS AND DISCUSSION

The flowers of **G.robusta** have been found to contain Kaempferol-5-O-β-glucoside. The UV spectrum of the aglycone exhibited two major peaks at 257 nm and 372 nm reveals a flavonol skeleton. A bathochromic shift of 64 nm (Band-I)noticed in its NaOMe spectrum indicated that the presence of a free OH at C–4'. The AlCl3 spectra(with or without HCl)showed only two peaks and a shoulder, indicating the absence of free-OH at C-5. The presence of a free –OH at C-7 was evident from the bathochromic shift of 17 nm (band II) on the addition of NaOAc. The AlCl3 spectrum was exactly the same as that of (AlCl3–HCl)revealing the absence of catechol type of substitution in B-ring.

The most significant clue for 5-glycosylation in the glycoside was its rapid rate of hydrolysis (hydrolysis was completed within 5 minutes). Another characteristic feature was its ability to adsorb strongly on to cellulose and it proved very difficult to elute it from chromatography. In the1H-NMR spectrum(300MHz,DMSOd6, TMS) of the glucoside obtained from EtOAc fraction ,the protons at C-6 and C-8 appear at δ 6.3 and 6.5 ppm respectively. The C-2' and C-6' protons appear as a doublet at δ 8.2ppm and the C-3' and C-5' protons at δ 6.9 ppm. The 1H-NMR of the glucose moiety appears at δ 5.4 ppm. The remaining glucosyl protons appear in the range δ 3.1 to 3.5 ppm. Supporting evidence for the structure of the glycoside was provided by ^{13}NMR (67.89 MHz,DMSOd 6 ,TMS)data and the complete assignmentof signals to various carbons is as given in Table1-2. As a result of glycosylation,the 5-OH,4-keto hydrogen bond is broken and this has aprofound effect on the electron density distribution in the molecule. The signal of C-4 appears at δ 166.7ppm instead of at δ 175.8 ppm as in the case of the corresponding aglycone. The signals of C-6 and C-8 appear downfield at δ 103.2 and 96.7 ppm respectively due to glyco-

sylation at C-5. It also cause an upfield effect on the corresponding meta carbon i.e,C-7. On this basis the identity of the pigment obtained from EtOAc fraction can be confirmed as Kaempferol 5-O-β-glucoside.

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