Phytochemical Investigations on Couropita Guianensis

KEYWORDS

Couropita guianensis, Lecythidaceae, quercetin, and quercitrin

INTRODUCTION

Couropita guianensis, known by several common names, is a deciduous tree in the family Lecythidaceae, which also contains the Brazil nut (Bertholletia excelsa). It is native to the rainforests of Central and South America. It is cultivated in many other places. Couropita guianensis. Aubl. (Syn.) Couratari pedicellaris. Rizine, Lecythis bracteata. Wild Pekea, Couroupita juss. Ex Dc popularly known as nagalingam in tamil is of the order Ericales. C. Guianensis tree is known as Cannon ball tree, a native to the rainforest of central and south America. It is widely planted in tropical and subtropical botanical gardens.

The methanolic extract of C. Guainensis root possesses potential anxiolytic activity (through its action on GABA / benzodiazepine receptors) and has therapeutic potential in the treatment of CNS disorders and provides evidence at least at a preclinical level. C. guianensis, is endowed with curative properties including anti-fungal, anti-biotic, anti-septic, analgesic, anti-malaria, stomach-ache, tooth-ache, scabies, gastritis, bleeding piles, dysentery and scorpion poison. The petals of C. guainensis is found to contain cyanidin and delphinin 3 – glucoside. In order to find additional information about C. guainensis its ingredients have been isolated and investigated hereunder.

EXTRACTION AND FRACTIONATION:

Fresh pinkish white flowers of C. guainensis collected from sidhhdar koil of Nagapattinam district was extracted with 85% EtOH under reflux. The specimen for C. guainensis is kept at Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph ’s college (Campus), Tiruchirapalli- 620 002, the specimen number being SA 013. The alc. extract was successively fractionated with pet. ether (60 – 80⁰ C), peroxide free EtOAc fractions did not yield any isolable material.

RESULTS AND DISCUSSION

The flowers of C.guainensis have been found to contain rutin (quercetin 3- O- rutinoside). The free aglycone from the EtOAc fraction was taken up in Me2CO and left in an ice- chest for two days when a pale yellow solid separated. It came out as pale yellow plates on recrystallisation, m.p 187 - 189⁰ C, yield 0.1% and developed a greenish – brown colour with alc. Fe3+, formed yellow precipitate with basic lead acetate solution and reduced ammonical AgNO3, but not Fehling’s solution. Its Rf values are given in table I – 1. It responded to Wilson’s boric acid, Molisch’s and Gibb’s tests. But did not answer the Horhammer – Hansel test. It had nm 259, 266sh, 299sh, 359; (+NaOMe) 272, 327, 410; (+AICI3) 275, 303sh, 433; (+AICI3 – HCI) 271, 300, 364s, 402; (+NaOAc) 271, 325, 393 ; (+NaOAc / H2BO3) 262, 298, 387. It was identified as rutin and the identity confirmed by Co- and mixed – PC and m.m.p with an authentic sample of Wrightia tinctoria.

The residue from EtOAc fraction was found to contain rutin (quercetin 3- O- rutinoside). The free aglycone from the EtOAc fraction was taken up in Me2CO and left under chilled conditions for a few days when an yellow solid was obtained. Its colour reactions, chromatographic behaviour and UV spectral data were identified as quercetin.

IDENTIFICATION OF THE AGLYCONE: (quercetin)

The residue from the Et2O fraction of the hydrolysate was taken up in Me2CO and left under chilled conditions for a few days when an yellow solid was obtained. Its colour reactions, chromatographic behaviour and UV spectral data were identified as quercetin.

IDENTIFICATION OF THE SUGAR: [glucose and rhamnose]

The filtrate after the removal of the aglycone was neutralized with BaCO3. The concentrated filtrate when examined by paper chromatography gave Rf values corresponding to those of glucose and rhamnose. The running properties of the glycoside were also in favour of a bioside. The identity of the sugars was confirmed by comparison with authentic samples of glucose and rhamnose.

PARTIAL HYDROLYSIS OF THE GLYCOSIDE:

The glycoside was subjected to partial hydrolysis by treatment with 10% formic acid in cyclohexane. The resulting solution was extracted with EtOAc and subjected to PC. The Rf values of the EtOAc fraction agreed with those of quercetin – 3- O-glucoside (isoquercitrin). The sugar obtained after the partial hydrolysis of glycoside was found to be rhamnoside. The Rf values are indicated in table I - 2. On this basis it can be concluded that glucose is directly linked to the aglycone moiety.

RESULTS AND DISCUSSION

The flowers of C. guainensis have been found to contain rutin (quercetin 3- O- rutinoside). The free aglycone from the Et2O
fraction could be characterised as quercetin. The structure has been confirmed by comparing it with an authentic sample isolated from Calophyllum inophyllum.

The UV spectrum of the glycoside showed two absorption maxima at 359 nm (band I) and 259 nm (band II). A bathochromic shift of 51 nm observed in band I if its NaOMe spectrum indicates the presence of a free –OH group at C-4'. The AlCl₃ – HCl spectrum of the glycoside showed four absorption maxima indicating a free –OH group at C- 5 which is further supported by a bathochromic shift of 43 nm in its NaOAc – H₂BO₃ spectrum. Further, a bathochromic shift observed in the MeOH spectrum (band I) of the aglycone obtained after hydrolysis of the glycoside as compared to that of the glycoside suggests that the site of glycosylation could be at C- 3 which is also supported by the fact that the glycoside did not respond to the Horhammer – Hansel test whereas the aglycone did.

In the ¹H – NMR spectrum of the glycoside (400 MHz, DMSO – d₆, TMS) (fig I -5) the signal at δ7.97 ppm (d, J= 9 Hz) and 7.56 ppm (J= 6 Hz) correspond to the protons at C-2' and C-6' respectively. The proton at C-5' appears at δ6.84 ppm (d, J=8Hz) whereas those of C-6 and C-8 resonate respectively at δ6.19 ppm (d, J=1.7Hz) and 6.69 ppm (d, J=2.0 Hz) the 5–OH proton appears at δ12.61 ppm of the glucose resonates at δ5.4 ppm (J=8Hz) while that of H-1” of rhamnose at δ4.57 ppm (d, J= 4 Hz) [8]. The signal appearing in the range of δ0.8 – 1.1 ppm correspond to the C- 6” protons (methyl protons of rhamnose) and is clearly reminiscent of the presence of rutinoside. Had it been a neohesperidoside where the linkage is 1 – 2, the corresponding signal would have appeared at δ 1.1 – 1.3 ppm. The rest of the sugar protons appear in the range of δ 3.0 ppm -3.8 ppm [10].

Supporting evidence for the structure of the flavonoid glycoside is provided by the ¹³C-NMR (100 MHz, DMSO – d₆, TMS) (fig I – 3) spectral data. A complete assignment of the various signals is provided in table I (I – 8). A bathochromic shift observed in the MeOH spectrum (band I) of the aglycone obtained after hydrolysis of the glycoside as compared to that of the glycoside suggests that the site of glycosylation could be at C- 3 which is also supported by the fact that the glycoside did not respond to the Horhammer – Hansel test whereas the aglycone did.

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