RESEARCH PAPER | Chemistry | Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Phytochemical Investigations on Couropita Guianensis

KEYWORDS: Couropita guianensis, Lecythidaceae, quercetin, and quercitrin

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ABSTRACT *Fresh flowers of Couropita guianensis have been analysed for their polyphenolic contents. It is found the flavonol glycoside quercitrin existing in the resyred fresh flowers of C. Guianensis. It has been characterised by means of modern physical methods like UV, H-1 nmr, C-13 nmr, chemical reactions, chromatographic techniques and hydrolytic studies,*

INTRODUCTION

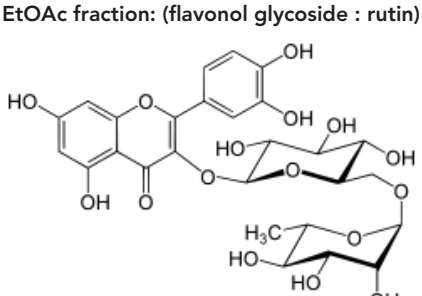
Couroupita guianensis, known by several common names, is a deciduous tree in the family Lecythidaceae, which also contains the Brazil nut (*Bertholletia excelsa*). It is native to the rainforests of Central and South America.[1] It is cultivated in many other places. *Couropita guianensis*. Aubl (Syn.) *Couratai pedicellaris*. Rizzine, *Lecythis bracteata*. Wild Pekea, *Couropita 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 [(1)]. It is widely planted in tropical and subtropical botanical gardens [(2)].

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 [(3)]. *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 [(4)]. The petals of *C. guainensis* is found to contain cyanidin and delphinidin 3 – glucoside [(5)]. In order to find additional information about *c. guianensis* its ingredients have been isolated and investigated hereunder.

EXTRACTION AND FRACTIONATION:

Fresh pinkish white flowers of *C. guianensis* collected from sidhdhar koil of Nagapattinam district was extracted with 85% EtOH under reflux. The specimen for *C. guianensis* the is kept at Rapinat Herbarium and Centre for Molecular Systematics, St. Josheph 's college (Campus), Tiruchirappalli- 620 002, the specimen number being SA 013. The alc. extract was concentrated *in vacuo*. The aq. extract of the flowers was successively fractionated with pet. ether (60 - 80 C), peroxide free Et_2O and EtOAc. The pet. ether and Et_2O fractions did not yield any isolable material.

EtOAc fraction: (flavonol glycoside : rutin)



The residue from EtOAc fraction was taken up in Me_2CO 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 - 89 C, yield 0.1% and developed a greenish- brown colour with alc. Fe^{3+} , formed yellow precipitate with basic lead acetate solution and reduced ammonical $AgNO_3$, but not Fehling's solution. Its R_f 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; +($AlCl_3$) 275, 303sh, 433; (+$AlCl_3$ – HCl) 271, 300, 364s, 402; (+NaOAc) 271, 325, 393 ; (+NaOAc / H_3BO_3) 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* [(6)].

HYDROLYSIS OF THE GLYCOSIDE:

The glycoside (0.05g) dissolved in hot aq. MeOH (2ml 50%) was hydrolysed with H_2SO_4 (5%) at 100 c for about 2h and the hydrolytic products identified as described below.

IDENTIFICATION OF THE AGLYCONE: (quercetin)

The residue from the Et_2O fraction of the hydrolysate was taken up in Me_2CO 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 $BaCO_3$. The concentrated filtrate when examined by paper chromatography gave R_f 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 [(7-8)]. The resulting solution was extracted with EtOAc and subjected to PC. The R_f 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 R_f 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.guianensis* have been found to contain rutin (quercetin 3- O- rutinoside). The free aglycone from the Et_2O

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

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 359nm (band I) and 259nm (band II). A bathochromic shift of 51nm observed in band I if its NaOMe spectrum indicates the presence of a free –OH group at C-4'. The $AlCl_3$ – 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 43nm in its $AlCl_3$ – HCl spectrum and positive response to Wilson's boric test. The presence of a –OH group at C-7 could be inferred from a bathochromic shift of 12nm (band II) on the addition of NaOAc. The presence of a catechol type of B – ring could be inferred from a bathochromic shift of 28nm (in band I) noticed in its NaOAc – H_3BO_3 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 – d_6, TMS) (fig I -5) the signal at δ7.97ppm (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 δ6.84ppm (d, J=8Hz) whereas those of C-6 and C-8 resonate respectively at δ6.19ppm(d, J=1.7Hz) and 6.69ppm (d, J=2.0 Hz) the 5 –OH proton appears at δ12.61ppm H-1" of the glucose resonates at δ5.4ppm (J=8Hz) while that of H -1"' of rhamnose at δ4.57ppm (d, J= 4 Hz) [9]. The signal appearing in the range of δ0.8 – 1.1ppm 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 – 1.3ppm. The rest of the sugar protons appear in the range of δ 3.0ppm -3.8 ppm [10].

Supporting evidence for the structure of the flavonoid glycoside is provided by the ^{13}C-NMR (100 MHz, DMSO –d_6, TMS) (fig I – 3)spectral data. A complete assignment of the various signals is provided in table (I – 8). Due to glycosylation, the signal of C-3 is shifted upfield by 2.60ppm. The downfield shift of the ortho related C-2 signal by 9.40ppm also confirms this [11] . The signal at δ104.0 ppm of C-10 is less intense due to the longer relaxation time of the quarternary carbon [12]. The signal of C-6"' of rhamnose at δ17.5ppm (not at δ20.90) and that of C- 6"' signal at δ67.9ppm (not at δ60.90) clearly shows that the glycoside is a 3 – O- rutinoside [13-14].
On this basis, the glycoside from the EtOAc fraction can be characterized as quercetin – 3 – O- rutinoside (rutin) in comparison with an authentic sample of rutin isolated from *Wrightia tinctoria*.

Acknowledgements:
The authors thank S.I.F., I.I.Sc. Bangalore, for its help in recording some of the nmr spectra of the isolates,

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