CHAPTER IV

Rare Quercetinbioside from the roots of

Boerhaavia erecta L.
CHAPTER IV

Rare Quercetinbioside from the roots of

Boerhaavia erecta L.

INTRODUCTION

Nyctaginaceae Juss is a family comprising of 30 genera and about 290 species, distributed in tropical and subtropical regions. Revision of this taxon in India accommodates 6 genera and 15 species. Boerhaavia L is a genus of some 40 herbaceous species represented by 5 in India. Boerhaavia diffusa L (syn B repens L var diffusa Hook f, B procumbens Banks ex Roxb, B repanda) is the reputed drug Punamava, mentioned in all indigenous systems of medicine as a diuretic in the treatment of oedema and is recognized in Indian Pharmacopoeia. The drug is also used in several parts of Asia and Africa and a variety of therapeutic properties are attributed to this drug plant. In the ancient Ayurvedic literature, two varieties of this drug source (one with white and the other with red flowers) have been recognized by Vaghbaha (300-400 AD) besides a third rare blue flower variety (in Rajanighantu) for therapeutic purposes. As another plant, Tnanthema portulacastrum L (Aizoaceae) closely resembled this literature description, punamava had to get listed among the "Controversial Indigenous Herbal Drug of India". Three closely similar species B diffusa, B erecta (B punamava) and T portulacastrum and a morphologically distinct B repanda have been marketed and utilized as punamava in different parts.
of India. Though the dispute over the identity of the true source of punarnava prevailed for a long time, today, only *B. diffusa* is recognized as the drug plant *punarnava* and *T. portulacastum* as *Varshabhu*.\(^{19}\) As the plant is endowed with a wide variety of therapeutic utility in the ancient Indian literature,\(^{21-23}\) countless number of articles have appeared on the pharmacognosy, chemistry, pharmacology and therapeutic utility of *B. diffusa* commencing from the pioneering works.\(^{22,23}\) These have been documented and reviewed,\(^{13,14,24-27}\) especially hepatoprotective, adaptogenic, antifibrinolytic, anti-inflammatory, diuretic and antiviral activities. Recent contributions include the isolation and characterisation of sterol and rotenoids from the roots\(^{28}\) and flavonoids from the whole plant.\(^{29}\)

Aq. extract of *B. caribeae*\(^{30}\) is found to have anti-HIV activity and is used in the treatment of lung and liver diseases in Bolivia, while the roots of *B. coccinea* is a Brazilian traditional drug (*pega-pinto*) for the treatment of liver, loins and urinary diseases.\(^{31}\) Rotenoids\(^{31,32}\) and chromone\(^{32}\) have been isolated and characterised from *B. coccinea*. From the literature, it may be inferred that roots of *B. repanda* Willd (syn. *B. chinensis* L.) also has been adorned with all the therapeutic claims of *punarnava* and was referred to as *punamava*. The therapeutic efficacies\(^{33}\) have also been assessed for antileucorrhoeal, anti-spermatorrhoeal,\(^{35}\) antihelmintic\(^{36}\) activities.

*B. erecta* L. (syn. *B. punamava* Saha & Krishn. *Spec. nov.*)\(^{3,4,37-39}\) is a small, erect or decumbent, puberulous annual, with ribbed stems, ovate leaves, white or pinkish white flowers and non-glandular fruits. Anthocarp obconic, truncate at apex glabrous. It is an exotic herb common in Malaysia, West Indies and tropical
America, possibly Africa and perhaps found its way to India with food grains imports\textsuperscript{38} some 35-40 years back. Subsequently got naturalized in waste places, gardens and roadsides. The leaves are cooked and eaten as spinach in some parts of the American tropics and the amino acid content of the aerial portions determined.\textsuperscript{14} It is one among the 24 medicinal plants used as decoction in traditional medicine of skin diseases in the Sahelien area of Niger and assessed to possess antiparasitic and antifungal activities.\textsuperscript{40} The only record of chemical examination of the secondary metabolites of \textit{B. erecta} is the isolation of quercetin and isoquercitrin from the aerial parts.\textsuperscript{41} Though there is an indication\textsuperscript{42} of other chemical analysis, enquiries with the author\textsuperscript{43} and the Institution\textsuperscript{44} concerned made it negative. This chapter deals with the isolation and characterisation of an extremely rare quercetin bioside, quercetin 3-[2'-glucosyl] rhamnoside from the root barks of \textit{B. erecta}. 
PRESENT WORK

Air dried root barks of *B. erecta* were exhaustively extracted with CHCl₃ and MeOH in succession. The MeOH extract, which indicated the presence of flavonoids was concentrated *in vacuo* and separated using a column of Sephadex LH 20, by elution with 95% aq. MeOH. Two fractions, I and II, containing flavonoids of different composition were collected, concentrated and kept in the ice chest. Fraction I yielded an yellow solid, labelled as compound A and fraction II another yellow solid, labelled as compound B. Both these compounds were recrystallised from MeOH.

Characterisation of Compound A

(3,5,7,3',4'-pentahydroxyflavone – quercetin)

Compound A, yellow needles, C₁₅H₁₀O₇, mp 305-306° gave yellow colour with NH₃, Na₂CO₃, pink with Mg-HCl and olive green with Fe³⁺. It was yellow under UV and UV/NH₃ and had Rf (Table IV 1A) characteristic of flavonoid aglycone and λ₁max (MeOH) 256, 267sh, 301sh and 370nm. A characteristic batochromic shift in band II of NaOAc spectrum with decomposition of band I suggested free 3,7 and 4'-OH groups. NaOMe spectrum with fast decomposition showed the presence of 3,4'-OH. A batochromic shift of 57 nm in band I of AlCl₃ / HCl spectrum was indicative of the presence of 3 and/or 5-OH groups. A hypsochromic shift of 30 nm in band I of AlCl₃ / HCl spectrum compared to AlCl₃ spectrum indicated c-dihydroxy
in ring B, which was further supported by 18 nm bathochromic shift of band I in NaOAc-H₃BO₃ spectrum (refer Experimental). Further the ¹H-NMR spectrum showed signals for five OH protons at δ 12.22, 10.20, 8.94, 8.72 and 8.50 ppm besides giving the characteristic chemical shift and splitting pattern expected for the five aromatic protons at 6,8,2',5' and 6' (refer Experimental). The compound yielded a penta acetyl derivative with mp 198-199° and a penta methyl ether mp 150-151°, agreeing with the values reported.⁴⁶-⁴⁸ These properties led to the identification of the compound B as 3,5,7,3',4'-pentahydroxyflavone (quercetin) (IV) and the identity was further confirmed by its ¹³C-NMR spectrum and direct comparison with an authentic sample.⁴⁷

Characterisation of Compound B

(Quercetin 3-0-α [2"-0-β-D glucopyranosyl]-L-rhamnopyranoside)

Compound B, C₂₇H₃₀O₁₆, gave all colour reactions characteristic of a flavonol glycoside. It appeared purple under UV, changing to yellow under UV / NH₃. It had λ⁰₉₉ (MeOH) 257, 264sh, 303sh, 352nm and 2N acid hydrolysis yielded an aglycone, indistinguishable in Rf, UV absorption characteristics and ¹H-NMR pattern with compound A and therefore confirmed as quercetin. The two sugars obtained as part of the hydrolytic products were identified as D-glucose and L-rhamnose by comparison with authentic sugars, including co-PC. Partial hydrolysis with 1% H₂SO₄ resulted in the formation of quercetin 3-0-rhamnoside as one of the products, indicating the rare nature of the glycoside. The UV absorption characteristics of compound B was typical of a 3-0-glycosylated quercetin with the
rest of the hydroxyl groups free to effect characteristic shifts in the $\lambda_{\text{max}}$ with diagnostic shift reagents.$^{48}$

The FAB mass spectrum of compound B displayed a pseudomolecular ion peak at m/z 611 [(M+H$^+$)] and characteristic fragment ion peaks at 499 [611-glucosyl residue] and 303 [Aglycone + H$^+$]. This neatly established the nature of compound B to be quercetin, glycosylated with the rare glucosyl rhamnose disaccharide. PMR signals were also consistent with the presence of quercetin, glucose and rhamnose moieties. The free hydroxyl protons at 5,7,3' and 4' positions of quercetin generated resonance signals at 12.6, 10.8, 9.2 and 9.7 ppm respectively and thereby implied the site of glycosylation as 3-OH. The conspicuous rhamnosyl-CH$_3$ protons resonance at 1.06 ppm and the broad singlet at 5.38 ppm corresponding to H-1" of rhamnose together with the doublet of H-1'" of glucose at 4.38 ppm and their coupling constants$^{48}$ substantiated the inference already made. The aromatic $^{13}$C resonances of compound B corresponded to the general impression of the quercetin moiety. The characteristic and discriminating signal at 106.092 ppm would arise only for the anomeric carbon of terminal glucose while the corresponding signal for the non-terminal glucose$^{49}$ occurred at 101.5 ppm. Similarly, the resonance at 81.139 ppm was characteristic of the ipso carbon downfield shift (70-77 ppm) which normally arose for rhamnose but not for glucose.$^{48-51}$ Meticulous correlation of the other $^{13}$C resonances also agreed with the reported values for quercetin 3-O-$\alpha$(2"-O-$\beta$-D-glucopyranosyl)-L-rhamnopyranoside, (II) one of the Ginkgo flavonoids characterised earlier,$^{51}$ unequivocally established the structure of compound B.
RESULTS AND DISCUSSION

The systematic analysis of the flavonoid content of the air dried root barks of B. erecta resulted in the isolation of the ubiquitous flavonol quercetin and its rare 3-O-glucosylrhamnoside. The structures of both the compounds were determined after thorough analysis of the informations gathered from chemical and spectral studies. The PC mobilities in solvents of varying polarities, UV fluorescence and absorption maxima indicated the compounds to be quercetin and quercetin 3-O-glycoside. Acid (2N HCl) hydrolysis of the glycoside released glucose and rhamnose (identified by PC) and the aglycone co-migrated with quercetin and was indistinguishable in spectral characteristics from it. Partial hydrolysis (1% H2SO4) resulted in the formation of quercetin 3-O-rhamnoside as one of the products, indicating the glucose moiety to constitute the terminal sugar of compound B. The enzyme β-glucosidase failed to effect the hydrolysis of compound B and this provided sufficient evidence to show that glucose was not connected directly to the flavonol moiety. Further evidence for the interglycosidic linkage of this rare bioside was provided by the FAB Mass spectral data. The appearance of signals at m/z 611 [M+H\(^{+}\)], 449 [611-glucosyl residue] and 303 [Aglycone + H\(^{+}\)], clearly indicating the end sugar to be glucose.

Comparison of the UV absorption maxima of the glycoside and the aglycone, in presence of diagnostic shift reagents, suggested 3-O-substitution of quercetin. The hydroxyl proton resonances displayed by both the compounds were
almost similar, composed of the signals corresponding to the 5,7,3' and 4'-OH system. The absence of a singlet around \( \delta = 8.72 \text{ ppm} \), integrating for one proton in the glycoside, compared to its aglycone (Compound A) was conspicuous and strongly supported the 3-O-glycosylation. Otherwise, the aromatic proton resonances of both the compounds were similar, as can be seen from the 'H-NMR spectra, thus confirming the identical nature of both the aglycones. The presence of the sugar, rhamnose, was distinctly clear from the CH\(_3\) resonance at \( \delta = 1.06 \text{ ppm} \) (d, 3H). The single proton doublet resonance at \( \delta = 4.38 \text{ ppm} \) with \( J = 7.2 \text{ Hz} \) correlated to the terminal \( \beta\)-D-glucose anomic proton, while that at \( \delta = 5.38 \text{ ppm} \) (d, 1H, 2.5 Hz) conformed to that of \( \alpha\)-L-rhamnose directly attached to quercetin.

\(^{13}\text{C-NMR} \) spectrum of the compound B was in close agreement with the rare 3-O-\( \alpha\) [\( \beta\)-D-glucosyl (1\( \rightarrow \)2)] L-rhamnosyl quercetin, previously isolated from \textit{Ginkgo biloba}. The (1\( \rightarrow \)2) linkage of glucorhamnosides could be distinguished by the presence of the rhamnose C-4 resonance at \( \delta \sim 71-72 \text{ ppm} \), a diagnostic signal which is largely unaffected by glycosylation at C-2.

In all, about seven examples of the rare biosidic combinations were available in literature, viz., 4'-methoxy-8-prenylkaempferol 3-O-glucop(1\( \rightarrow \)2) rhamnoside, \(^{52}\) K 3-O-rhamnoside 7-O-\( \beta\)-[6''-feruloylgluco-(1\( \rightarrow \)3) L-rhamnoside], \(^{53}\) K 3-O-glucop (1\( \rightarrow \)4) rhamnoside, \(^{54}\) K 3-O-glucop (1\( \rightarrow \)2) rhamnoside, \(^{51}\) K 3-O-[6''-p-coumaroylgluco (1\( \rightarrow \)2) rhamnoside], \(^{51}\) hordenine-0-\( \alpha\)-L-[6-o-t-cinnamoyl-\( \beta\)-glucosyl (1\( \rightarrow \)4) rhamnoside] (an alkaloid) and 8-prenylkaempferol 3-O-\( \alpha\)-L-[\( \beta\)-D-glucosyl (1\( \rightarrow \)4) rhamnoside]-7-O-\( \beta\)-D-glucoside. \(^{57}\) Occurrence of rare sugars are not
uncommon in Boerhaavia\textsuperscript{29} and even a rare flavone, 6,5'-dimethoxy 5,7,3'-
trihydroxyflavone has been isolated from \textit{B. diffusa}.\textsuperscript{56}

The isolation of quercetin 3-0-glucorhamnoside from \textit{B. erecta} is reported for
the first time and it is the second reported occurrence of this compound in nature.
The compound has been reported earlier as a novel one from the leaf extracts of
\textit{G. biloba}. This leaf extract containing flavonoids (24\%) and terpenes (16\%)
ginkgolides and bilobolides] are claimed to increase peripheral and cerebral blood
flow\textsuperscript{59} and are prescribed for therapeutic purposes\textsuperscript{60} in cerebral impairment, multiple
vascular infarcts, psycho-behavioural symptoms etc. An attempt to investigate
similar therapeutic effects of \textit{B. erecta} may be worthy since the flavonol glycoside
isolated is one of the flavonoids available in \textit{G. biloba}. Communities of Sahelian
area (Niger) traditionally use the plant against skin disease and was assessed to
possess antiparasitic and antifungal activities.\textsuperscript{40} Several polyphenolics and
flavonoids, including the 3-0-glucoside and arabinoside of quercetin have been
shown to exhibit antifungal\textsuperscript{61} and antiparasitic activities.\textsuperscript{62} Hence, the reported
activities of \textit{B. erecta} may be attributed, at least partly, due to the presence of the
compound isolated.
EXPERIMENTAL

Air dried barks (300 g) of *B. erecta* roots, collected from Pondicherry (the voucher specimen authenticated by Dr. Nair, V.J., Dy. Director, Botanical Survey of India, Coimbatore, India) was exhaustively extracted with CHCl₃ and MeOH in succession. The MeOH extract, which indicated the presence of flavonoids was concentrated under reduced pressure and separated using a column of Sephadex LH 20 by elution with 95% aq. MeOH. Two fractions, I and II, containing flavonoids of different composition were collected, concentrated under reduced pressure and kept in the ice chest. Fraction I yielded an yellow solid, labelled compound A, which was further purified by washing with petroleum ether and recrystallised from Me₂CO and fraction II yielded another yellow solid, labelled compound B, which was washed with petroleum ether and Et₂O and recrystallised from MeOH.

**Compound A**

(Quercetin)

Yellow needles (Me₂CO) mp 305-306°, C₁₅H₁₀O₇, pink colour with Mg-HCl, olive green with Fe³⁺ and yellow with alkalis (NH₃, Na₂CO₃ and NaOH). It appeared yellow under UV as well as under UV / NH₃.
UV (λ_max nm)

<table>
<thead>
<tr>
<th>Solution</th>
<th>Wavelengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>256, 267sh, 301sh, 370</td>
</tr>
<tr>
<td>+NaOAc</td>
<td>257sh, 272, 300, 389 (dec)</td>
</tr>
<tr>
<td>+NaOAc + H₃BO₃</td>
<td>260, 302sh, 388</td>
</tr>
<tr>
<td>+NaOMe</td>
<td>245sh, 321, 412 (dec)</td>
</tr>
<tr>
<td>+AlCl₃</td>
<td>272, 304sh, 332, 457</td>
</tr>
<tr>
<td>+AlCl₃ + HCl</td>
<td>265, 301sh, 328, 427</td>
</tr>
</tbody>
</table>

Rf (Table IV 1), ¹H-NMR (Table IV 2 and Fig IV 1) and ¹³C-NMR (Table IV 3 and Fig IV 2)

**Acetylation of Compound A**

(Quercetin penta acetate)

Compound A (15 mg) was dissolved in a few drops of C₅H₅N and treated with 2 mL of Ac₂O. It was kept at room temp for 24 h and poured into crushed ice kept for 3 h and filtered. The solid when recrystallised from EtOAc-petrol gave colourless needles, mp 198-199°

**Methylation of Compound A**

(Quercetin pentamethylether)

Compound A (20 mg) was dissolved in 10 mL of dry Me₂CO, added to a mixture of 1 mL Me₂SO₄ and 1 g anhydrous K₂CO₃ and refluxed for 36 h at 70°. The reaction product was cooled, filtered, washed with Me₂CO. The residue from
Me₂CO was added to cold water. The colourless solid formed was filtered, washed with cold water, dried and recrystallised from MeOH to yield colourless needles mp 150-151°.

**Compound B**

(Quercetin 3-O-glucosylrhamnoside)

Yellow needles (MeOH), C₂₇H₃₀O₁₆, gave pink colour with Mg-HCl, olive green with Fe³⁺, yellow with alkalis and violet in Molisch’s test. It appeared purple under UV, changing to yellow under UV / NH₃.

**UV (λ max nm)**

<table>
<thead>
<tr>
<th></th>
<th>MeOH</th>
<th>+NaOAc</th>
<th>+NaOAc + H₃BO₃</th>
<th>+NaOMe</th>
<th>+AlCl₃</th>
<th>+AlCl₃ + HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>257, 264sh, 303sh, 352</td>
<td>265, 334sh, 373</td>
<td>261, 367</td>
<td>270, 332sh, 394</td>
<td>275, 300sh, 325sh, 423</td>
<td>271, 296sh, 353, 394</td>
</tr>
</tbody>
</table>

Rf (Table IV 1A), ¹H-NMR (Table IV 2 and Fig IV 3) and ¹³C-NMR (Table IV 3 and Fig IV 4).

MS (FAEI, m/z, relative intensity as %) 611 [(M + H)⁺, 56], 499 [611-glucosyl 17] and 303 [Aglycone + H⁺, 56] (Fig IV 5).
**Acid hydrolysis of Compound B**

(Quercetin, D-glucose, L-rhamnose)

To a solution of compound B (20 mg) in hot aq MeOH (5 mL) and equal volume of 4N HCl was added and the mixture refluxed at 100° for 3 h. Usual working up yielded an yellow solid, mp 313-315°C and indistinguishable from compound A in all aspects. The sugars in the aqueous layer were identified as D-glucose and L-rhamnose by Rf and co-PC with authentic samples (Table IV 1B).

**Partial hydrolysis of Compound B**

(Quercetin, quercetin 3-0-rhamnoside, D-glucose, L-rhamnose)

Compound B (20 mg) in 5 mL aqueous MeOH was mixed with 1% H$_2$SO$_4$ (5 mL) and kept at 28°C for 1 h. The hydrolytic products were found to be quercetin, quercetin 3-0-rhamnoside, D-glucose and L-rhamnose, determined adopting the usual procedure.
REFERENCES

3. Kumar, G R (1987), in *Flora of Tamil Nadu, India, Series 1 Analysis*, vol 2 (eds A N Henry, G R Kumar and V Chitra), Botanical Survey of India Coimbatore, India, p 188
7. Datta, S C and Mukerji, B (1952), *Pharmacognosy of Indian Leaf Drugs* Government of India Press, Calcutta, India, p 78
11 Kirtikar, K R and Basu, B D (Reprinted 1984), Indian Medicinal Plants vol III
   Bishen Singh Mahendra Pal Singh, Dehra Dun, India, p 2045
   Medicinal Plants vol 1, Orient Longman Ltd, India
   of India vol 1, Indian Council of Medical Research, New Delhi, India, p 139
   Publications & Informations Directorate (NISCOM), New Delhi, p 174
   Res 27, 178
17 Chakravarty, H L (1939-40), Ann Rep Bot Soc Bengal 11
18 Chopra, R N (1940), Indian J Med Res 28, 475
19 Vaidya, B G (1972), J Res Indian Med 7(2), 69
20 Pant, P C and Joshi, M C (1993), J Res Edn Indian Med 12, 27
21 Sivarajan, V V and Balachandran, I (1994), Ayurvedic Drugs and their Plant
   Sources, Oxford & IBH Publishing Co Pvt Ltd, India, p 387
22 Ghoshal, L M (1910), Food and Drugs, 80
23 Chopra, R N, Ghosh, S, Ghosh, B N and De, P (1923), Indian Med Gaz 58,
   203
24 Nagarajan, S and Jain, H C (1981), Bibliography, Med Aromatic Plants
   Abstr 3, 645

122
Chatterjee, A and Pakrashi, S C (1991), Treatise on Indian Medicinal Plants vol 1, Pub & Informn Directorate (NISCOM), New Delhi, India p 76

Rastogi, R P and Mehrotra, B N (1990-1998), Compendium of Indian Medicinal Plants (ed R P Rastogi) CDRI, Lucknow and P I D (NISCOM) CSIR), New Delhi, vol 1, p 60, 2, 103, 3, 98, 4 111 and 5 132


Singh, S P (1979), Indian J For 2, 370


Nair, N C (1967), J Bull Bot Surv India 9, 283


41 Subramanian, S S and Ramakrishnan, S (1965), *Indian J Pharmacy* 27 41


43 Hemadri, K, *Personal Communication* dated 16 1 1995

44 Khanna, P N, *Personal Communication* dated 1 2 1996


49 Markham, K R., Geiger, H and Jaggy, H (1992), *Phytochemistry* 31 1009


52 Oshima, Y O., Okamoto, M and Hikino, H (1987), *Heterocycles* 26 935

124

54 Yamasaki, K *et al* (1977), *Tetrahedron Letters* 1231


56 Li, Y S. and Liu, Y L. (1990), *Phytochemistry* **29**, 3311


60 Krishnan, P V V (ed) (2000), *CI MS 70*, 194


TABLE IV 1A

$R_f$ values of the flavonoids from *B. erecta*
$R_f \times 100$ (Whatman No.1, ascending, 28 ± 2°C)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>H$_2$O</th>
<th>5% HOAc</th>
<th>15% HOAc</th>
<th>50% HOAc</th>
<th>n-BAW</th>
<th>Phenol</th>
<th>Forestal</th>
<th>t-BAW</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Quercetin</td>
<td>-</td>
<td>01</td>
<td>04</td>
<td>30</td>
<td>80</td>
<td>40</td>
<td>48</td>
<td>73</td>
</tr>
<tr>
<td>B. Q 3-glucorhamnoside</td>
<td>40</td>
<td>42</td>
<td>54</td>
<td>70</td>
<td>50</td>
<td>55</td>
<td>76</td>
<td>64</td>
</tr>
</tbody>
</table>

n-BAW: n-BuOH : HOAc : H$_2$O, 4:1:5, upper layer
Phenol: Water saturated phenol
Forestal: HOAc : HCl : H$_2$O, 30:3:10
t-BAW: t-BuOH : HOAc : H$_2$O, 3:1:1
<table>
<thead>
<tr>
<th>Sugars</th>
<th>n- BAW</th>
<th>Phenol</th>
<th>t-BAW</th>
<th>EPW</th>
<th>n- BEW</th>
<th>BBPW</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucose</td>
<td>22</td>
<td>40</td>
<td>41</td>
<td>17</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>L-rhamnose</td>
<td>43</td>
<td>60</td>
<td>63</td>
<td>37</td>
<td>42</td>
<td>45</td>
</tr>
</tbody>
</table>

n-BAW : n-BuOH : HOAc : H₂O, 4:1:5, upper layer
Phenol : Water saturated phenol
t-BAW : t-BuOH : HOAc : H₂O, 3:1:1
EPW : EtOAc : C₅H₅N : H₂O, 10:4:3
BEW : n-BuOH : EtOH : H₂O, 4:1:4
BBPW : C₈H₈ : n-BuOH : C₅H₅N : H₂O, 1:5:3:3
Table IV 2

$^1$H-NMR Chemical Shifts of the Compounds isolated from *B. errecta*
(400 MHz, TMS int. std.)

<table>
<thead>
<tr>
<th>Proton position</th>
<th>$\delta$, ppm (multiplicity, integration, J)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compound A</td>
</tr>
<tr>
<td>OH-3</td>
<td>8.72 (s, 1H)</td>
</tr>
<tr>
<td>5</td>
<td>12.22 (s, 1H)</td>
</tr>
<tr>
<td>7</td>
<td>10.20 (s, 1H)</td>
</tr>
<tr>
<td>3'</td>
<td>8.50 (s, 1H)</td>
</tr>
<tr>
<td>4'</td>
<td>8.94 (s, 1H)</td>
</tr>
<tr>
<td>H-6</td>
<td>6.22 (d, 1H, 2.2 Hz)</td>
</tr>
<tr>
<td>8</td>
<td>6.37 (d, 1H, 2.2 Hz)</td>
</tr>
<tr>
<td>2'</td>
<td>7.75 (d, 1H, 2.2 Hz)</td>
</tr>
<tr>
<td>5'</td>
<td>6.91 (d, 1H, 8.4 Hz)</td>
</tr>
<tr>
<td>6'</td>
<td>7.59 (d, 1H, 8.3 Hz)</td>
</tr>
<tr>
<td>1''</td>
<td>-</td>
</tr>
<tr>
<td>1'''</td>
<td>-</td>
</tr>
<tr>
<td>6'''</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table IV 3

**$^{13}$C-NMR Chemical Shifts of Compounds isolated from *B. erecta* (100 MHz; DMSO-$d_6$)**

<table>
<thead>
<tr>
<th>Moiety</th>
<th>C positions</th>
<th>Compound A δ ppm</th>
<th>Compound B δ ppm</th>
<th>R 3-O-glucorhamnoloside* δ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>2</td>
<td>146.35</td>
<td>156.33</td>
<td>159.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>135.81</td>
<td>133.20</td>
<td>136.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>175.66</td>
<td>177.27</td>
<td>179.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>160.98</td>
<td>161.11</td>
<td>163.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>98.47</td>
<td>98.60</td>
<td>100.1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>164.02</td>
<td>163.96</td>
<td>166.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>93.55</td>
<td>93.50</td>
<td>94.9</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>156.54</td>
<td>156.54</td>
<td>158.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>103.25</td>
<td>103.87</td>
<td>105.9</td>
</tr>
<tr>
<td></td>
<td>1'</td>
<td>122.58</td>
<td>121.51</td>
<td>122.9</td>
</tr>
<tr>
<td></td>
<td>2'</td>
<td>115.11</td>
<td>116.18</td>
<td>117.0</td>
</tr>
<tr>
<td></td>
<td>3'</td>
<td>144.81</td>
<td>144.64</td>
<td>146.5</td>
</tr>
<tr>
<td></td>
<td>4'</td>
<td>147.37</td>
<td>148.30</td>
<td>149.9</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>115.46</td>
<td>115.13</td>
<td>116.5</td>
</tr>
<tr>
<td></td>
<td>6'</td>
<td>120.33</td>
<td>121.08</td>
<td>122.9</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>1&quot;</td>
<td>100.64</td>
<td></td>
<td>100.9</td>
</tr>
<tr>
<td></td>
<td>2&quot;</td>
<td>81.14</td>
<td></td>
<td>81.5</td>
</tr>
<tr>
<td></td>
<td>3&quot;</td>
<td>70.46</td>
<td></td>
<td>70.3</td>
</tr>
<tr>
<td></td>
<td>4&quot;</td>
<td>71.75</td>
<td></td>
<td>71.9</td>
</tr>
<tr>
<td></td>
<td>5&quot;</td>
<td>69.90</td>
<td></td>
<td>70.5</td>
</tr>
<tr>
<td></td>
<td>6&quot;</td>
<td>17.62</td>
<td></td>
<td>17.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>1&quot;</td>
<td>106.09</td>
<td></td>
<td>106.3</td>
</tr>
<tr>
<td></td>
<td>2&quot;</td>
<td>75.79</td>
<td></td>
<td>74.1</td>
</tr>
<tr>
<td></td>
<td>3&quot;</td>
<td>76.34</td>
<td></td>
<td>76.5</td>
</tr>
<tr>
<td></td>
<td>4&quot;</td>
<td>70.28</td>
<td></td>
<td>69.4</td>
</tr>
<tr>
<td></td>
<td>5&quot;</td>
<td>73.97</td>
<td></td>
<td>76.9</td>
</tr>
<tr>
<td></td>
<td>6&quot;</td>
<td>60.91</td>
<td></td>
<td>60.7</td>
</tr>
</tbody>
</table>

*Reference spectrum, K-3-O-glucorhamnoloside*46.50
Fig IV 2 $^{13}$C NMR spectrum of Quercetin
Fig IV 4  $^{13}$C NMR spectrum of Quercetin 3-gluconorhamnoside
R = H : Quercetin

R = β-D-glucosyl (1→2) α-L-rhamnosyl : Quercetin 3-O-β-D glucosyl (1→2) rhamnoside

β-D-glucosyl (1→2) α-L-rhamnosyl