Polyphenolic Constituents of the Flowers of *Berberis aristata*

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BERBERIS aristata DC. (Berberidaceae) is an erect spinous shrub with golden yellow flowers, is used in indigenous system of medicine[1]. Berberine hydrochloride, an alkaloid of this plant, is known for various biological activities[2,3]. The present study reports the isolation and characterisation of five polyphenolic compounds from the flowers of *B. aristata*.

The aqueous alcoholic extract of the flowers of *B. aristata* furnished on cc, followed by pc in some cases, *E*-caffeic acid, quercetin, chlorogenic acid, meratin and rutin. These were characterised by spectral analyses, chemical correlation and by comparison with authentic samples. The detailed uv absorption spectra (MeOH) and with diagnostic reagents as well as ^{13}C nmr spectra showing complete assignment for chlorogenic acid have been provided for the first time.

The medicinal properties of *B. aristata* may be due to the presence of flavonoids and chlorogenic acid[4,5]. Chlorogenic acid identified for the first time in *B. aristata* and in Berberidaceae is an important secondary metabolite from the point of view of chemotaxonomy[6,7]. This compound has been earlier identified in plants belonging to 26 other families making Berberidaceae the latest addition to the list[6]. The presence of quercetin and its glycoside is a common feature of the order Ranunculales comprising Berberidaceae, Nymphaceae and Ranunculaceae. In the absence of sufficient data on the flavonoids of Berberidaceae, it is not possible to draw any meaningful inference on the interrelationship of the three families of this order; however, the absence of C-glycosyl flavones in *B. aristata* in Berberidaceae is indicative of its deviation from the other two families.

Experimental

Fresh flowers (500 g) of *B. aristata*, collected from Lovedale near Ooty of Nilgiri hills, were refluxed with rectified spirit (3×3 dm^3) and concentrated under reduced pressure. The aqueous alcoholic concentrate was kept in a refrigerator. The solid deposited was dissolved in minimum quantity of MeOH and chromatographed on a column of SiO_2 and eluted with $CHCl_3$–MeOH in varying proportions. $CHCl_3$–MeOH (9 : 1) eluates yielded a mixture of compounds A and B which were further separated by ppc (50% HOAc; R_f 0.81 and 0.31 respectively).

Compound-A, $C_9H_8O_4$, m.p. 210–12°; blue under uv changing to deep blue with NH_3; λ_{max} (MeOH) 214, 286 and 315 nm, was found to be *E*-caffeic acid by co-chromatography and superimposable ir spectra with an authentic sample from *Tabebuia rosea*[8].

Compound-B, $C_{15}H_{10}O_7$, m.p. 313–15° d; positive tests for flavonol[9]; λ_{max} (MeOH) 257, 270sh, 302sh and 370 nm, was identified as quercetin by co-chromatography and superimposable ir spectra with an authentic sample from *Aglaia roxburghiana*[8].

The $CHCl_3$–MeOH (3 : 1) fraction gave a mixture of compounds C, D and E which on further cc over SiO_2 yielded compound C ($CHCl_3$: CH_3COCH_3; 3 : 1 eluates) and a mixture of D and E ($CHCl_3$: CH_3COCH_3; 1 : 1 eluates) which were finally separated by ppc (H_2O; R_f 0.46, 0.18).

Compound-C gave colourless needles, $C_{16}H_{18}O_9$, m.p. 198–200°; blue fluorescence under uv light changing to yellow green with uv/NH_3; deep blue with Fe^{3+}; rosy red with phenol-red reagent; λ_{max} (MeOH) 243, 305sh and 329; (+NaOH) 265, 300sh and 380; (+$AlCl_3$) 260sh, 310sh and 358; (+$AlCl_3$/HCl) 240, 300sh and 327 nm; ^1H nmr spectra showing peaks corresponding to those reported earlier[9]; ^{13}C nmr (67.89 MHz, DMSO-d_6, TMS as internal standard) δ 175.00 (C-7), 166.13 (C-1′), 148.21 (C-7′), 145.92 (C-6′), 145.08 (C-3′), 125.63 (C-4′), 121.62 (C-9′), 115.82 (C-2′), 114.49 (C-5′/8′), 114.32 (C-5′/8′), 73.72 (C-1), 70.74 (C-5), 70.62 (C-3) 68.40 (C-4), 36.95 (C-2) and 36.45 (C-6); EI and FAB-ms data were in good agreement with the earlier report[5]. Hydrolysis with 2*N* NaOH yielded *E*-caffeic acid and quinic acid (1 : 1) identified by co-pc and hplc (R_t=16.16, phenyl Bondapakcolumn, gradient elution with 5% HOAc–MeOH, 25°). Thus compound-C was characterised as chlorogenic acid (3-*O*-caffeoylquinic acid) which was further confirmed by co-chromatography with an authentic sample from *Apocynum venetum* L. Var. *basikurumon*[6].

Compound-D gave yellow needles, $C_{27}H_{30}O_{17}$, m.p. 183–85°; λ_{max} (MeOH) 259, 266sh and 358 nm. The R_f value and other properties were characteristic of flavonol glycoside[10]. Acid hydrolysis yielded quercetin and glucose in 1 : 2 ratio. The compound was identified as meratin (quercetin-3-*O*-diglucoside) by co-chromatography and ir spectral comparison with an authentic sample from *A. roxburghiana*[8].

Compound-E, $C_{27}H_{30}O_{16}$, m.p. 190–92°, showed λ_{max}, R_f and other properties very similar to compound-D. Hydrolysis yielded quercetin, glucose and rhamnose in 1 : 1 : 1 a ratio. It was identified as

rutin (quercetin-3-*O*-rutinoside) by direct comparison with an authentic sample from *A. roxburghiana*[8].

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References

1. "The Wealth of India, Raw Materials", C. S. I. R., New Delhi, 1948, Vol. I, p. 178.
2. "Medicinal Plants of India", I. C. M. R., New Delhi, 1976, Vol. I, p. 131.
3. R. K. HADLER, N. C. NEOGI and R. S. RATHOR, *Indian J. Pharm.*, 1979, **2**, 26.
4. V. CODY, E. MIDDLETON and J. B HARBORNE, "Plant Flavonoids in Biology and Medicine", Alan R. Liss, New York, 1985
5. A. SAKUSHIMA, S HISADA, S. NISHIBE and H. BRANDENBERGER, *Phytochemistry*, 1985, **24**, 325.
6. P. MOLGAARD, *Nord J. Bot.*, 1985, **5**, 203.
7. P. MOLGAARD and H. RAVN, *Phytochemistry*, 1988, **27**, 2411.
8. A. G. R. NAIR, R. GUNASEKARAN and B. S. JOSHI, *Indian J. Chem , Sect. B*, 1974, **12**, 979.
9. A. SAKUSHIMA, S. NISHIBE and S. HISADA, *Yakugaku Zasshi*, 1978, **98**, 1395.
10. T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, "The Systematic Identification of Flavonoids", Springer Verlag, New York, 1970.