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Chemotaxonomic significance of polyoxygenated flavonoids from the leaves of *Micromelum minutum*

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1. Subject and source

Micromelum minutum (Forst. f.) Wight & Arn., (Syn. *M. pubescens* Blume) (Bengali name- Bankunch, Dulia) is a tall shrub found widespread in south-east Asia (Whitmore, 1972). The leaves were collected from the hilly areas of Chittagong, Bangladesh in June 1997 and were identified by Mr. Md. Yusuf, SSO, Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong. A voucher specimen for this collection is maintained at the Bangladesh National Herbarium under accession number, DACB 24349.

2. Previous works

A number of secondary metabolites occur in *M. minutum*, including alkaloids (Mester, 1983; Nakahara et al., 2002), 6-prenylcoumarins (Lamberton et al., 1967; Joshi et al., 1975; Das et al., 1984), 8-prenylcoumarins (Chatterjee et al., 1967; Tantivatana et al., 1983; Das et al., 1984; Tantishaiyakul et al., 1986; Rahmani et al., 1994; Ito et al., 2000), a polyoxygenated flavonoid, viz., 5,7-dihydroxy-3,4′,6,8-tetramethoxyflavone as a 7-O-ether with murrangatin (Das et al., 1984) and

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a dihydrocinnamic acid derivative (Rahmani et al., 1994). Our earlier investigation with *M. minutum* growing in Bangladesh revealed dihydromicromelin A and B, acetyldihydromicromelin A, hopeyhopin and 5,7-dihydroxy-3,4′,6,8-tetramethoxyflavone (Sohrab et al., 1999).

3. Present study

Dried and powered leaves (1.14 kg) were macerated with 3.0 L of 70% aqueous ethanol and the concentrated extract was partitioned successively with petroleum ether, carbon tetrachloride and chloroform. The carbon tetrachloride-solubles (1.5 g) were subjected to VLC over kieselgel 60H, eluted with a mixture of petrol and EtOAc, EtOAc and then with EtOAc-MeOH mixtures of increasing polarities to afford yellow needles (MeOH) of 5-hydroxy-3,4′,7,8-tetramethoxyflavone (11.2 mg) (Sarin and Seshadri, 1960) from fraction-7 and 5,7-dihydroxy-3,4′,8-trimethoxyflavone (20.3 mg) (Tandon and Rastogi, 1977) from fraction-9. The chloroform-solubles (0.87 g) were chromatographed on a silica gel column (particle size 0.063–0.200 mm; 70–230 mesh ASTM; Merck) using similar solvent systems with 73 fractions collected (each ca. 50 ml). The column fraction-4 afforded 12.3 mg of 5-hydroxy-3,4′,6,7,8-pentamethoxyflavone (Rashid et al., 1992) while preparative TLC of fractions 41 to 43 over silica gel PF_{254}, using Toluene-EtOAc-Acetic acid (90:10:few drops) as developing solvent, yielded trace amounts of coumarins: tomentin (Murray et al., 1975), scopoletin and marmesin (Murray et al., 1982). The structures of the isolated compounds were determined by high-field NMR and mass spectral analyses and confirmed by comparison with previously reported values.

4. Chemotaxonomic significance

According to the taxonomy proposed by Swingle (1948), the genus *Micromelum* is placed in the family Rutaceae, tribe Clauseneae that contains three subtribes, Micromelinae (*Micromelum*), Clauseninae (*Glycosmis*, *Clausena*, *Murraya*) and Merrilliinae (*Merrillia*). 5,7-Dihydroxy-3,4′,6,8-tetramethoxyflavone is the only flavonoid so far reported from *M. minutum* as free (Sohrab et al., 1999) or as a 7-O-ether with murrangatin (Das et al., 1984). Another compound of this type, 5-hydroxy-3,3′,4′,7,8-pentamethoxyflavone has been reported from *M. zeylanicum* (Bowen and Perera, 1982). Such polymethoxylated flavonoids are also known from *Murraya* sect. *Murraya* and *Merrillia* (Kong et al., 1988; Harborne, 1983). The present study describes the isolation of three further polymethoxylated flavonoids, viz., 5,7-dihydroxy-3,4′,8-trimethoxyflavone, 5-hydroxy-3,4′,7,8-tetramethoxyflavone and 5-hydroxy-3,4′,6,7,8-pentamethoxyflavone, which are new reports from the tribe Clauseneae. The isolation of these flavonoids from *M. minutum* is in line with expectations for this species.

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