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## Geranylated homogentisic acid derivatives and flavonols from *Milium usangense*



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### 1. Subject and source

*Milium usangense* Chaowasku & Kessler, which is locally known as Ra-Khang-Hom, is an Annonaceae plant that was recently discovered in Thailand (Chaowasku and Keßler, 2013). The leaves of *M. usangense* were collected in Tak Province, Thailand, in 2010. A voucher specimen (Chaowasku 89) has been deposited at Nationaal Herbarium Nederland, Leiden University, The Netherlands.

### 2. Previous work

Previous chemical investigations of plants in this genus have revealed a number of different classes of natural products, including alkaloids (Harrigan et al., 1994; Hasan et al., 2000; Jumana et al., 2000a; Chen et al., 2002, 2003; Thanh Thuy et al., 2011), acetogenins (Jumana et al., 2000b; Connolly et al., 2003), terpenoids (Wu et al., 2001; Brophy et al., 2004; Thanh Thuy

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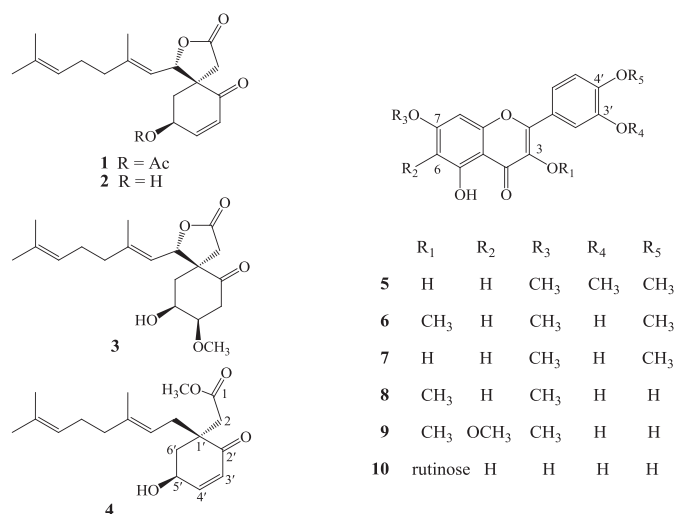


Fig. 1. Chemical structures of 1–10 of *Miliusa umpangensis*.

et al., 2011), flavonoids (Kamperdick et al., 2002; Huong et al., 2004, 2005; Thanh Thuy et al., 2011; Naphong et al., 2013), styryl compounds (Kamperdick et al., 2002; Huong et al., 2008), geranylated homogentisic acid derivatives (Kamperdick et al., 2002; Huong et al., 2004; Zhang et al., 2006), neolignans and lignans (Sawasdee et al., 2010, 2013a, 2013b), and other aliphatic and aromatic compounds (Huong et al., 2004; Lei et al., 2008). However, *Miliusa umpangensis* has not been chemically studied.

### 3. Present study

The dried and powdered leaves of *M. umpangensis* (199 g) were extracted with MeOH (7 × 3 L) to yield 46.8 g of the extract after removal of the solvent. This MeOH extract presented 80 and 60 percent inhibition against *Herpes simplex virus* types 1 and 2, respectively, at a concentration of 50 µg/mL. The MeOH extract was then separated through repeated column chromatography to obtain 10 pure compounds. These compounds were identified as (+)-miliusate (**1**, 12 mg) (Zhang et al., 2006), (+)-miliusol (**2**, 7 mg) (Zhang et al., 2006), (+)-miliusane I (**3**, 9 mg) (Zhang et al., 2006), methyl 2-(1'β-geranyl-5'β-hydroxy-2'-oxocyclohex-3'-enyl) acetate (**4**, 44 mg) (Mbah et al., 2004), 7,3',4'-trimethylquercetin (**5**, 12 mg) (Chen et al., 2009), ayanin (**6**, 10 mg) (Wang et al., 1989; Matsuda et al., 2002), ombuin (**7**, 85 mg) (Huong et al., 2005), quercetin 3,7-dimethyl ether (**8**, 66 mg) (Guerrero et al., 2002), chrysosplenol-D (**9**, 29 mg) (Ling et al., 2010), and rutin (**10**, 27 mg) (Slimestad et al., 2008) (Fig. 1) through a comparative analysis of their spectroscopic data (<sup>1</sup>H and <sup>13</sup>C NMR and ESI-MS) and optical properties with literature values. All of these compounds were evaluated to determine their inhibitory activity against HSV-1 and HSV-2 using the post-treatment method (Likhitwitayawuid et al., 2006). Compounds **8** and **9** showed weak activity against HSV-1 (IC<sub>50</sub> 94.7 and 86.8 µM, respectively) and HSV-2 (IC<sub>50</sub> 189.5 and 86.7 µM, respectively) compared with the positive control acyclovir (IC<sub>50</sub> 1.9 and 2.1 µM, respectively). The other compounds were devoid of activity.

### 4. Chemotaxonomic significance

The genus *Miliusa* Lesch. ex A. DC. (Annonaceae) comprises approximately 50 species distributed from the Indian subcontinent, southern China, and Southeast Asia to New Guinea and northern Australia (Chaowasku and Keßler, 2006).

Geranylated homogentisic acid derivatives (**1–4**) are members of a rare class of plant secondary metabolites. These C18 compounds, known as miliusanes, have an unusual spiro-lactone structure and have been previously identified in *Miliusa balansae* (Kamperdick et al., 2002; Huong et al., 2004) and *Miliusa sinensis* (Zhang et al., 2006). The presence of miliusanes in *M. umpangensis* and *M. balansae* strongly supports their classification as members of clade B (Chaowasku and Keßler, 2013). It should be noted that miliusanes have very limited distribution, and to date, their only occurrence outside *Miliusa* is in *Glossocalyx brevipes* Benth (Monimiaceae) (Mbah et al., 2004). Thus, miliusanes may be useful as a chemotaxonomic marker of *Miliusa*.

The analysis of flavonols **5–10** showed that the A ring of **9**, which is biogenetically derived from the acetate pathway, has four oxygenations. This 1,2,3,5-tetraoxygenated aromatic structure is also present in the flavanones and dihydrochalcones previously identified from *M. balansae* (Kamperdick et al., 2002; Huong et al., 2004, 2005) and *M. sinensis* (Thanh Thuy et al., 2011). Hence, flavonoids with this substitution pattern could also be considered as a marker for the genus *Miliusa*.

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