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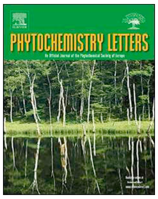

Phytochemical characterization of the Chinese endemic species *Stemona mairei* and five other *Stemona* species

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ABSTRACT

Comparative HPLC-PDA analyses of methanolic root extracts of the almost unstudied Chinese endemic species *Stemona mairei*, collected from nine locations throughout its natural habitat in northern Yunnan, led to the identification of the pyrrolo[1,2-α]azepine-type alkaloid protostemonine (**1**) as the main alkaloid and 3,4-dehydro-δ-tocopherol (**9**) as the main tocopherol derivative. Analysis of the five further Chinese *Stemona* species *S. sessilifolia*, *S. japonica*, *S. parviflora* and *S. shandongensis*, collected in adjacent locations, resulted in identification of eight *Stemona* alkaloids, with seven of them possibly derived from **1**. Additionally, the presence of three tocopherols was confirmed co-chromatographically with authentic samples. From *S. shandongensis*, the alkaloids stemocochinin (**7**) and croomine (**8**) were isolated and their structures confirmed by NMR and MS analyses. Additionally, the quantitative content of protostemonine (**1**) and the tocopherols **9** and **10** present in extracts were assessed. Concerning the tocopherols, *S. mairei* differs by the occurrence of chromenol derivatives from the other species which accumulate both chromenol and chromanol derivatives. Furthermore, the root extracts of *S. mairei* exhibited nematicidal activities against *Panagrellus redivivus*. These results are briefly discussed in a biological and chemotaxonomic context, respectively.

1. Introduction

The genus *Stemona* comprises of 33 species and is distributed in South East Asia and Northern Australia. Due to the ethnomedicinal importance in this area the roots from several species have been subject of phytochemical studies. In particular, roots of the species *S. tuberosa* Lour., of *S. sessilifolia* (Miq.) Miq. and of *S. japonica* (Blume) Miq. were analyzed for the accumulation and bioactivity of specific alkaloids considered as unique for this genus (e.g. Greger, 2006; But et al., 2012; Wang and Chen, 2014). These so called *Stemona* alkaloids are characterized by a pyrrolo[1,2-α]azepine nucleus usually linked to two γ-lactone moieties. Less common are the pyrido[1,2-α]azepine *Stemona* alkaloids (Kaltenegger et al., 2003; Kongkiatpaiboon et al., 2011; Mungkornasawakul et al., 2003). According to Greger (2006), three different skeletal types may be distinguished: Stichoneurine-, croomine-, and protostemonine type alkaloids, with the pyrido[1,2-α]azepine alkaloids being considered as arising from the protostemonine type alkaloids (Kaltenegger et al., 2003). All of these three types may be found in species of *Stemona*.

In comparison to the already mentioned species, far less attention was paid to other *Stemona* species which are not extensively used in traditional medicine. Only a few reports addressing the diversity of accumulated alkaloids within this genus were published within the last years from samples mainly collected in Thailand (Kongkiatpaiboon et al., 2011; Schinnerl et al., 2007). The stichoneurine-type alkaloid tuberostemonine and maistemonine were reported together with several derivatives from the endemic Chinese taxon *S. mairei* (H.Lév.) K.Krause (Fig. 1) (Cai and Luo, 2007; Lin et al., 1991b). The pyrido[1,2-α]azepine derivatives stemokerrine and oxystemokerrine were isolated from *S. kerrii* Craib (Kaltenegger et al., 2003; Kongkiatpaiboon et al., 2011). *Stemona sessilifolia* and *S. japonica*, are known for accumulation of protostemonine (**1**) and maistemonine (Li et al., 2007), whereas *Stemona parviflora* C.H. Wright yielded parvistemonine (Kaltenegger et al., 2003). Recently, several alkaloids together with the pyrrolo[1,2-

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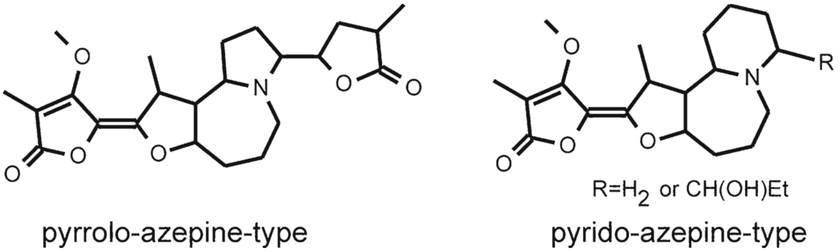

Fig. 1. Basic structures of pyrrolo- and pyrido[1,2-α]azepine type alkaloids.

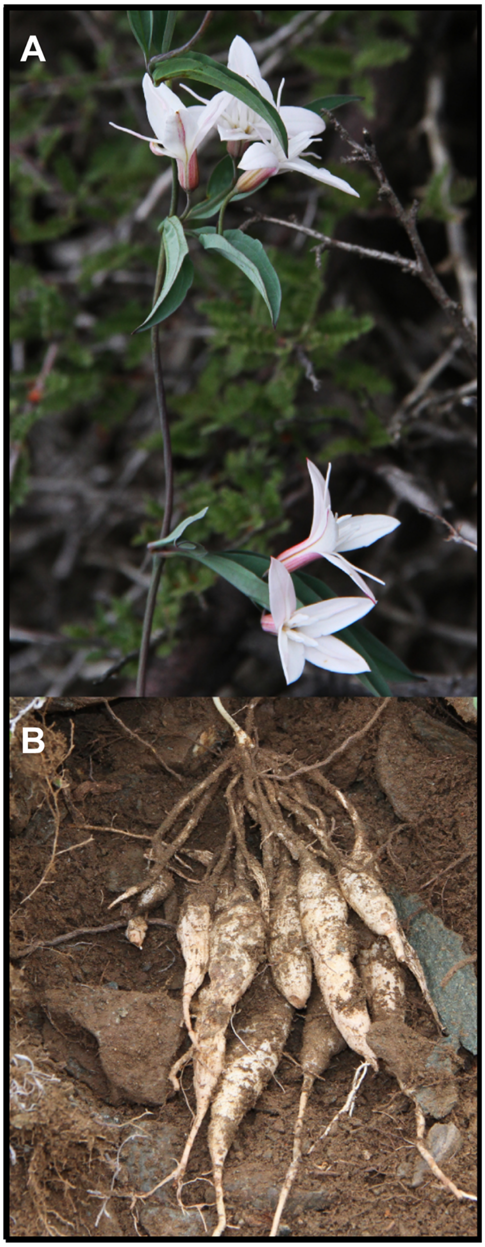

Fig. 2. *Stemona mairei* in its natural environment. The size of the flowers is approx. 3 cm (A) and the storage roots (B) are approx. 5–10 cm in length.

α]azepines protostemonine (**1**), stemofoline (**4**) and several other alkaloids were reported from this species (Huang et al., 2016).

In course of phytochemical studies within the genus *Stemona,* we focused on the diversification of alkaloid accumulation in different accessions of *S. mairei* (Fig. 2) since this species is not well studied yet. All of the samples were collected in their natural habitat in Southwest China. Additionally, several other species were analyzed for their alkaloid complement, among them *S. shandongensis* (Miq.) Miq., which has not been studied phytochemically so far. Apart from alkaloids, we also investigated the occurrence of anti-oxidative tocopherols since a constant co-occurrence of tuberostemonine and 3,4-dehydro-*δ*-tocopherol was reported for *S. tuberosa* recently (Chen et al., 2017). In order to confirm the supposed bioactivity of root alkaloids against herbivores, we performed an assay against the sour paste nematode *Panagrellus redivivus* L. Respective results are discussed in a biological and chemotaxonomic context.

2. Results

2.1. *Alkaloid pattern in the examined species*

Fifteen individuals out of six *Stemona* species were studied for their root alkaloid profile. In sum, eight *Stemona* alkaloids and three tocopherol derivatives could be identified (Fig. 3). Comparative HPLC profiling indicated the presence of various types of alkaloids in crude methanolic extracts obtained from air-dried roots. Of the alkaloids, protostemonine (**1**), a pyrrolo[1,2-α]azepine derivative, proved to be the predominant compound in most of the studied samples. However, *S. kerrii* diverged by the accumulation of stemocurtisine (**5**), a pyrido[1,2-α]azepine derivative, as the major alkaloid. The alkaloids **1**, **7** and **8** were isolated and identified from *S. shandongensis.* Their structures were elucidated on the basis of NMR and MS analyses, and the other alkaloid compounds were identified on the basis of co-chromatography with authentic samples from previous studies (Kaltenegger et al., 2003; Kongkiatpaiboon et al., 2011). Results are presented in Table 1; HPLC chromatograms of *S. mairei* are presented in Fig. 4, and those from the other species in Fig. 5, respectively.

2.1.1. *Stemona mairei*

This species was phytochemically studied for the first time in a broader sampling. Protostemonine (**1**), its isomer isoprotostemonine (**2**) and a yet unidentified protostemonine derivative (rt of 7 min) were detected in all of the nine accessions. Additionally, stemofoline (**4**) was found in five out of the nine samples. The stichoneurine-type alkaloid tuberostemonine and its derivatives as well as maistemonine could not be detected in these samples neither by TLC nor by HPLC analyses with combined UV-DAD and ELS detection, respectively. Hence, the obtained results contradict previous reports concerning these alkaloids (Cai and Luo, 2007). Explanations for that divergent results are difficult, possibly misidentification of the roots happened previously. Chemical variation within *S. mairei* might also be possible but results from other species collected in their natural habitat never showed co-occurrences of protostemonine- and stichoneurine-type alkaloids (Chen et al., 2017; Kongkiatpaiboon et al., 2011; Schinnerl et al., 2007).

2.1.2. *Stemona kerrii*

This species is characterized by the occurrence of stemocurtisine (**5**) as the main alkaloid (Fig. 5), with, **1**, **3** and **6** identified as minor compounds. The predominance of pyrido[1,2-α]azepine derivates was confirmed, whilst pyrrolo[1,2-α]azepine derivatives are only