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Comparison of the profiles of non-glycosylated triterpenoids from leaves of plants of selected species of genus Dioscorea

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ABSTRACT

Remarkable qualitative and quantitative differences in non-glycosylated triterpenoid profiles of twelve *Dioscorea* spp. leaves were demonstrated with the use of GC–MS/FID analysis. The total content of tetracyclic triterpenoids and their esters ranged from 397 μg/g of dry leaf weight in *D. bulbifera* to 762 μg/g d.w. in *D. discolor* and 777 μg/g d.w. in *D. alata.* Three main phytosterols, i.e. campesterol (**1**), sitosterol (**2**) and stigmasterol (**3**) were found in extracts from all analyzed species in total amount ranging from 316 μg/g in *D. bulbifera* to 676 μg/g of dry leaf weight in *D. hispida*, with either sitosterol (**2**) or stigmasterol (**3**) as predominant in the profile. Extracts from *D. hispida* and *D. purpurea* leaves were distinguished from the others by particularly high amount of campesterol (**1**). In the majority of the species, except for *D. caucasica*, other tetracyclic triterpenoids were found: cycloartanol (**4**), 24-methylenecycloartanol (**5**) and cycloeucalenol (**6**). Less common steroids, stigmastan-3-en-6β-ol (**7**) and ergosta-7,22-dien-3β-ol (**8**) were detected in *D. japonica.* The significant content (992 μg/g) of pentacyclic triterpenoids of ursane, oleanane, taraxastane and taraxerene (friedooleanane)-type carbon skeletons, i.e. α-amyrin (**9**), β-amyrin (**10**), taraxasterol (**11**) and taraxerol (**12**), respectively, was found in *D. caucasica*. The obtained results supplement the knowledge of biochemical diversity of Dioscorea genus.

1. Introduction

Dioscorea is a monocotyledonous genus of over 600 species in the family Dioscoreaceae, native throughout the warm temperate and tropical regions of the world. The name *Dioscorea* was given after the ancient Greek physician and botanist Dioscorides (Burkill, 1960; Ayensu, 1972). The genus is considered as one of the most primitive angiosperms being well diversified at the end of cretaceous period, approximately 75 million years ago (Dutta, 2015). The *Dioscorea* plants are tuberous lianas, growing to 2–12 m or more tall. The leaves are spirally arranged, mostly broad heart-shaped. Several species, known as yams, are important agricultural crops in tropical regions, grown for their large tubers. Many of these are toxic when fresh, but can be detoxified and eaten (Lazima et al., 2016). Different ethnic groups in Africa, Asia, Latin America and Oceania have been using yams as a source of food due to their high starch content and caloric value but also to cure certain ailments (Dutta, 2015). The genus still holds importance for global food security since seven to ten species of *Dioscorea* (e.g. *D. alata*, *D. cayennensis*) are cultivated on a large scale as important crop food for over 100 million people, and approximately fifty species are wild-harvested (Price et al., 2016). Currently yams are farmed on about 5 million hectares in about 47 countries in tropical and subtropical regions of the world for food, pharmaceutical products and ornamental purposes (Cornet et al., 2007; Parvatha Redy, 2015). Some species has become invasive, e.g. *Dioscorea bulbifera* introduced to Florida (Lake et al., 2015). Use of *Dioscorea* species in traditional medicines, Ayurveda and Traditional Chinese Medicine, is documented to at least 2000 BC (Dutta 2015; Tang et al., 2015; Lazima et al., 2016; Price et al., 2016). Two main classes of bioactive compounds are found in many *Dioscorea* species: alkaloids (e.g. dioscorine) and steroidal saponins (e.g. dioscin). Alkaloids are highly toxic and some species such as *D. dumetorum* and *D. hispida* Dennst. have primarily been used for their poisonous properties (Price at al., 2016). Nevertheless, starch from the tuber of *D. hispida* is edible and can be consumed after removing dioscorine by soaking in flowing water (Lazima et al., 2016). The most

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characteristic metabolites in Dioscorea genus are steroidal saponins, derivatives of diosgenin, that has been used in the pharmaceutical industry as the starting material for the synthesis of pregnenolone-derived steroids, e.g. cortisone, contraceptives, anabolic agents (Price et al., 2016; Yang et al., 2016; Zhang et al., 2016). While edible yams should lack high amounts of saponins, the abundance of these compounds is important for those species which serve as raw material for the isolation of diosgenin derivatives (Vendl et al., 2006). Around 15 species of *Dioscorea* are currently used as a source of diosgenin. Over-harvesting has threatened populations of several rare *Dioscorea* species (Price et al., 2016).

The majority of studies on *Dioscorea* plants have focussed on the tubers or rhizomes due to their importance as food or as a source of pharmaceutical precursors, neglecting the foliage as waste (Vendl et al., 2006; Price et al., 2016). However, utilising *Dioscorea* foliage as a renewable source of any natural product could be of economical and ecological interest since some cultivated species generate substantial above-ground biomass each annual growth cycle. Moreover, the determination of the chemical composition of leaves of different species could supplement the current knowledge of the biochemical diversity of Dioscorea genus. Recently, the metabolite profiling of polar extracts from leaf and petiole material of a diverse collection of *Dioscorea* has been performed for "more holistic biochemical understanding of the economically, nutritionally and medicinally important yet understudied genus *Dioscorea*" (Price et al., 2016). The obtained data allowed species discrimination and comparison with phylogenetic relationships and morphological traits. Additionally, the potential of foliage material as a renewable source of shikimic acid was demonstrated.

It had been frequently reported that the content of steroidal saponins was negligible in *Dioscorea* spp. leaves by comparison to tubers (Vendl et al., 2006; Lin et al., 2009). In turn, only few studies had been done on triterpenoids present in free or esterified forms (Savikin-Fodulovic et al., 1998; Vendl et al., 2006; Zhang et al., 2016). Therefore, the aim of this study was the qualitative and quantitative analysis of non-glycosylated triterpenoids occurring in leaves of 12 species of *Dioscorea.*

2. Results and discussion

2.1. Identification of triterpenoids occurring in leaves of Dioscorea *spp*

GC–MS analysis of the fractions containing steroids and neutral triterpenoids obtained from diethyl ether extracts of leaves of different *Dioscorea* plants revealed the presence of three typical sterols characteristic for higher plants, i.e. campesterol (**1**; 24*R*-ergost-5-en-3β-ol), sitosterol (**2**; stigmast-5-en-3β-ol) and stigmasterol (**3**; 22*E*-stigmasta-5,22-dien-3β-ol). These compounds were identified in all extracts, however, the intensity of their peaks on GC-FID chromatograms displayed that the quantitative ratios of these three phytosterols were not identical in all analyzed species.

In the majority of the extracts two other tetracyclic triterpenoids were identified: cycloartanol (**4**; 9β,19-cyclolanostan-3β-ol) and 24-methylenecycloartanol (**5**; 24-methylene-9β,19-cyclolanostan-3β-ol). Cycloartanol (**4**) was not found only in the extracts obtained from leaves of *D. caucasica* and *D. discolor.* The occurrence of cycloartanol (**4**) and 24-methylenecycloartanol (**5**) is typical for plants synthesizing and accumulating significant amounts of steroids. In all analyzed *Dioscorea* extracts excluding *D. caucasica* and *D. hispida*, another much less common tetracyclic triterpenoid was identified, i.e. cycloeucalenol (**6**; 4,14-dimethyl-9β,19-cycloergost-24(28)-en-3β-ol). Two other steroids, stigmastan-3-en-6β-ol (**7**) and ergosta-7,22-dien-3β-ol (**8**) were found in extracts of *D. japonica,* the former in both samples of this species from Japan (two localities: Tokyo, Tokyo Prefecture and Tanzawa Mountains, Kanagawa Prefecture) whereas the latter exclusively in the extract of leaves of *D. japonica* collected in Tanzawa Mountains. The

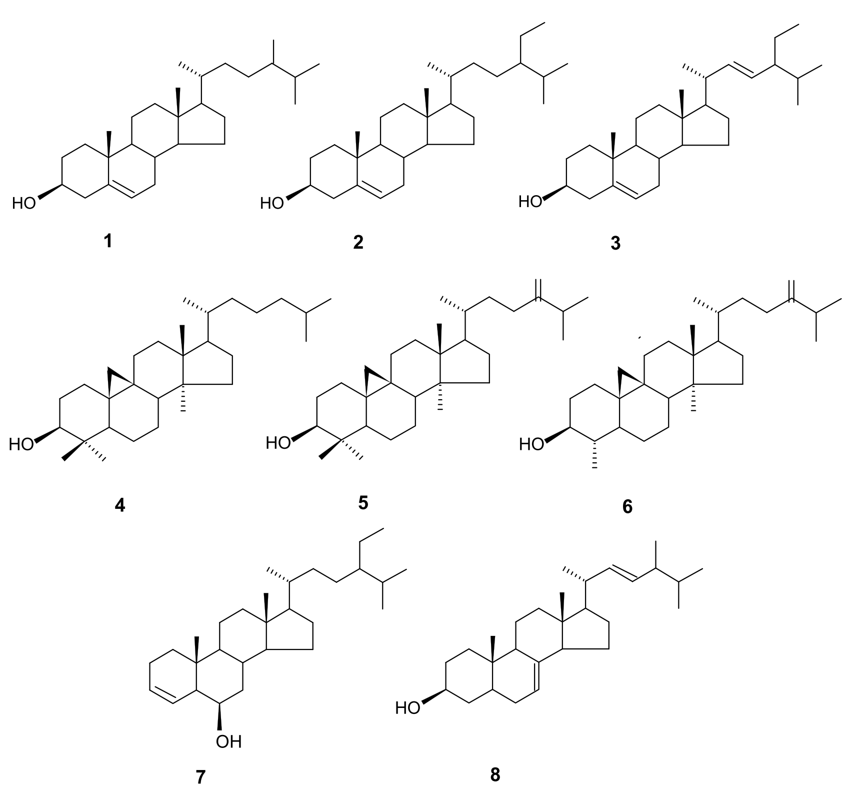

Fig. 1. Chemical structures of tetracyclic triterpenoids identified in *Dioscorea* spp. leaf extracts: campesterol (**1**), sitosterol (**2**), stigmasterol (**3**), cycloartanol (**4**), 24-methylenecycloartanol (**5**), cycloeucalenol (**6**), stigmastan-3-en-6β-ol (**7**) and ergosta-7,22-dien-3β-ol (**8**).

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