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Naděžda Vrchotová
Global Change Research Institute CAS: Ustav vyzkumu globalni zmeny Akademie ved Ceske republiky
Jan Bednář
Global Change Research Institute CAS: Ustav vyzkumu globalni zmeny Akademie ved Ceske republiky
Jan Tříska
triska.j@czechglobe.cz

Global Change Research Institute CAS: Ustav vyzkumu globalni zmeny Akademie ved Ceske republiky
https://orcid.org/0000-0003-3846-2158

Short Report

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Alpha-spinasterol in the roots of *Impatiens glandulifera*

Naděžda Vrchotová, Jan Bednář, Jan Tříska*

Global Change Research Institute, Czech Academy of Sciences, Bělidla 4a, 603 00 Brno, Czech Republic

*Corresponding author: Jan Tříska, e-mail: triska.j@czechglobe.cz

Abstract:

The roots of *Impatiens glandulifera* belong to plants, or to part of plants with a higher content of α-spinasterol. Chromatographic analysis of roots hexane extract using GC-MS revealed that α-spinasterol is the dominant substance in the freeze-dried roots of *Impatiens glandulifera* and its content reaches ca. 1 % wt. When the hexane extracts are concentrated, α-spinasterol crystals fall out in the form of tiny needles, so the isolation of this substance from the roots of *Impatients glandulifera* is very easy.

Keywords: *Impatiens glandulifera*, roots, α-spinasterol.

Introduction:

Impatiens glandulifera is an invasive plant in Europe, whose metabolites, especially phenolic compounds, show various biological properties; for example, phytotoxic properties (Vrchotová et al. 2011), repellent properties (Pavela et al. 2009), and plants contain also lipophilic components with antioxidant and cytotoxic abilities (Szewczyk et al. 2018). Of the lipophilic substances contained in *Impatiens glandulifera* just α-spinasterol among the sterols has interesting biological properties, but it does not occur in large quantities in plants. It got its name from spinach, where it was first identified by Collinson and Smedley-MacLean in 1931, its structure was further refined in other works (Hart and Heyl 1932, Obata et al. 1955). Alpha-spinasterol is chemically (3β,5α,22E)-stigmasta-7,22-dien-3-ol (syn. hitodesterol; bessisterol). The occurrence of α-spinasterol in various plants and its biological activities are summarized in Table 1.

Recently an overview of the all until then available important pharmacological properties of α-spinasterol was published by Majeed et al. (2022). Let's mention at least some of them to illustrate further possible developments in research regarding the biological properties of α-spinasterol. Jeong et al. (2004) described that α-spinasterol has inhibitory potency about 1,000 times higher than that of simvastatin, which is also administered in the treatment of diabetic nephropathy. A recent study showed that α-spinasterol could inhibit intestinal absorption of glucose with IC50 value of 8.6 µg/mL for inhibition activity of α-glucosidase, which was not significantly different from that of the standard α-glucosidase inhibitor, acarbose (Lawal et al., 2020). α-Spinasterol can be also used as a novel selective antagonist of transient receptor potential vanilloid 1 receptor (TRPV1) for the treatment of mental disorders such as depression and anxiety (Trevisan et al. 2012, Socała et al. 2016). In recent years, α-spinasterol has been among selected natural substances tested for antiviral activity against SARS-CoV-2 (Siddiqui et al. 2020; Zubair et al. 2021).

Table 1. The brief overview of plants containing α-spinasterol

Plants	Parts of plants	Activity and amount of α-spinasterol	Literature
Acacia auriculiformis	leaves	cholinesterase inhibitory effect	Lawal et al. 2020
Albizia schimperiana	stem bark	antimicrobial activity	Fufa et al. 2018
Amaranthus cruentus	seeds	sum of α-spinasterol and sitosterol = 50% of sum sterols	Czaplicki et al. 2012
Amaranthus spinosus	seeds		Billah et al. 2013
Amaranthus spinosus	whole plant	α-spinasterol; spinasterol-3-O-β-glucopyranoside	Tuyen et al. 2019
Anacardium occidentale	nuts		Wang et al. 2019
Angelica sinensis	rhizomes	are not autotoxic properties	Xin et al. 2019
Arachis hypogadea	nuts		Wang et al. 2019
Ardisia pyramidalis	leaves	suppressed blood wessel branching	Raga et al. 2017
Argania spinosa	oil	α-spinasterol about 30% of total phytosterols	El Kharrassi et al. 2018
Beta vulgaris	beet pulp	α-spinasterol obtained from its glucoside	Obata et al. 1955
Cactaceae spp. (columnar species)		inhibitory activity against COX-1, COX-2, *Helicobacter pylori*, HeLa	Salazar et al. 2020
Carya sp.	nuts		Wang et al. 2019
Castanea sp.	nuts		Wang et al. 2019
Citrullus colocynthis	leaves	α-spinasterol + dihydrospinasterol; aphicidal activity	Ahmed et al. 2020
Citrullus lanatus	seeds		Wang et al. 2019
Corulus sp.	nuts		Wang et al. 2019
Cucurbita sp.	seeds		Wang et al. 2019
Cucurbita maxima	flowers	anticarcinogenity activity	Villaseñor and Domingo 2000
Cucurbita pepo	seeds		Hrabowski et al. 2012
Filicium decipiens	stem bark	from 5 kg of dry material obtained 32 mg of α-spinasterol	Muthia et al. 2015
Gypsophyla trichotoma	roots		Krasteva et al. 2008

Impatiens balsamina	roots, leaves	antibacterial activity	Wang et al. 2011
Impatiens balsamina	roots		Panichayupakaranant et al. 1995
Impatiens glandulifera	root, seed, leaf		Szewczyk et al. 2018
Impatients noli-tangere	root, seed, leaf		Szewczyk et al. 2018
Juglans sp.	nuts		Wang et al. 2019
Koreana stewartia	leaves	glycosidic α-spinasterol	Lee et al. 2011
Macadamia sp.	nuts		Wang et al. 2019
Manilkara zapota	bark		Chunhakant and Chaicharoenpong 2019
Mautabea quianensis	stems	allelopatic activity agains common weeds of the Amazon region	Ripardo Filho et al. 2012
Medicago sativa	seeds		King and Ball 1939
Melandrium firmum	whole plants	prevent benign prostatic hyperplasia	Lee at al. 2014
Physospermum verticillatum	aerial parts	antibacterial activity	Boulacel et al. 2017
Phytolacca americana	roots	therapeut potencial of diabetic nephropathy	Jeong et al. 2004
Pistachia vera	nuts		Wang et al. 2019
Polygala sp.		antifungal activity	Johann et al. 2011
Prunus amygdalus	nuts		Wang et al. 2019
Pueraria lobata	roots	antitumor activity	Jeon et al. 2005
Pueraria mirifica	roots	antitumor activity	Jeon et al. 2005
Sheareria nana	aerial parts	α-spinasterol-3-O-β-D-glucoside	Meng et al. 2018
Spinacia oleracea	leaves	α-spinasterol = 0,010 % of dried material	Hart et al. 1932
Stegnosperma halimifolium	stems, leaves	antiproliferative activity in the cervical cancers, murine macrophage cancer cells	Meneses-Sagrero et al. 2017
pure compounds		antidepressant, anxiolytic effect	Socała and Wlaź 2016
pure compounds		antagonist TRPV1 , cyclooxygenase inhibitor, antiniciceptive, antidepressant	Fischer et al. 2020
pure compounds		α-spinasterol resembles the membrane behavior of cholesterol	Haralampiev et al.2017

The content of α-spinasterol is not very high in plants. Hart and Heyl (1932) states that the content of α-spinasterol is 0.010 % of dried materials of *Spinacia oleracea* leaves. Fernholz and Moore (1939) obtained from dehydrated alfalfa meal 0.02 % wt. of α-spinasterol and state that alfalfa meal is probably the most accessible source for this sterol. Wang et al. (2019) reports in his work the results of measuring the content of α-spinasterol in seeds and nuts of 13 plants. They found the most bound and free α-spinasterol in pumpkin seeds (0.024 % and 0.018 % of dry weight) and watermelon seeds (0.033 % and 0.017 % of dry weight). Kalač and Moudrý (2000) state that α-spinasterol constitutes 50 % of the sum of dry sterols in *Amaranthus* seeds. The main goal of this work was to determine the amount of α-spinasterol in freeze-dried roots of *Impatiens glandulifera* and to isolate this substance from the extract in the simplest possible way.

MATERIAL AND METHODS

Plant material.

The roots of *Impatiens glandulifera* (Royle) were collected in the vicinity of the city of České Budějovice (Czech Republic). The roots were cleaned, frozen and then lyophilized. After lyophilization, the material was stored at -18 °C.

Chemicals.

Hexane (Merck for GC/MS), tetracosane (Sigma-Aldrich), β-sitosterol (Sigma-Aldrich).

Preparation of the plant material.

Prior to extraction, the freeze-dried roots were ground to a very fine powder and then extracted with hexane. Two samples (A and B) were prepared, each weighing 0.1 g. 3 ml of hexane were added to the plant material, the extraction took place in the dark at room temperature for 1 hour with shaking. Then the extract was taken and the extraction with hexane was repeated four times more. Samples A1-A5 and B1-B5 were thus obtained. Hexane was evaporated from the fractions with nitrogen and the evaporates were dissolved in exactly 3 ml of hexane.

Sample preparation.

Samples 1A, 1B, 2A and 2B were injected directly: 200 µl of the sample was taken from the extract and 8 µl of the internal standard solution - tetracosane (C_{24}) with a concentration of 50 µg/ml was added to it. Samples 3A, 3B, 4A, 4B, 5A, 5B were blown dry with nitrogen and then diluted to 0.5 mL with hexane. Then 200 µl was taken into the insert, blown dry with nitrogen and diluted with 50 µl of hexane. Then 2 µl of the internal standard solution - tetracosane (C_{24}) with a concentration of 50 µg/ml were added.

GC-MS analysis.

GC–MS analyses were performed on a Finnigan GCQ instrument, column Rxi–5 ms (Restek Co., Bellefonte, PA, USA), 30 m×0.25 mm×0.25 μm. Injection volume 1 μl. Injection temperature 250 °C, splitless time 1.00 min., split flow 50 ml/min. Carrier gas flow (helium) 1 ml/min. Initial column temperature 120 °C, increased at a rate of 20 °C/min up to temperature of 260 °C, which was held for 2 minutes. It was then increased up to 310 °C at a rate of 3 °C/min. The temperature of 310 °C was maintained for 2 minutes. The samples were measured in full scan mode in the range of 50-750 amu from 3.00 - 28.5 minutes.

NMR data.

α-Spinasterol. 1H-NMR (CDCl3): δ (ppm) 5.16 (dd, 15.0, 8.9, 1H, H-22), 5.15 (bm, 1H, H-7), 5.02 (dd, 15.0, 8.9, 1H, H-23), 3.57 (m, 1H, H-3), 1.02 (d, 6.6, 3H, H-21), 0.85 (d, 6.4, 3H, H-27), 0.81 (t, 7.2, 3H, H-29), 0.80 (d, 6.0, 3H, H-26), 0.79 (s, 3H, H-19), 0.55 (s, 3H, H-18).

The 1H NMR spectrum was referenced to the solvent signal (CDCl3, 7.32 ppm). 1H and 2D experiments (COSY, HSQC, TOCSY) were measured on Varian INOVA 500 MHz spectrometer equipped with standard broadband probe at 25 °C. Only the dominant signals could be undoubtedly signed. The measured record matched the literature data of Ahmed et al. (2022).

RESULTS AND DISCUSSION

Alpha-spinasterol has already been extracted from many plants. Since the authors in the literature try to determine as many lipophilic metabolites as possible in the plant material, most extractions are relatively very complex, using a variety of solvents and successive extractions (petroleum ether, acetone, alcohol, chloroform, etc.) For obtaining α-spinasterol from fractions that are analysed on GC the following procedures were used. For example, Szewczyk et al. (2018) extracted different parts of *Impatiens glandulifera* plant material, the extraction method included nine steps (the plant material was air-dried). The authors do not report the yield of α-spinasterol per gram of material, but only the mixture of α-spinasterol and chondrillasterol in mass % of total fraction. This mixture of leaf extract constitutes 69 % w/w of total fraction. In the roots, they found significantly less of this mixture (9 % w/w), and 44 % w/w of β-sitosterol. In addition to *Impatiens glandulifera*, the authors also extracted lipophilic material from *Impatiens noli - tangere.* We analysed only the roots of *Impatiens glandulifera.* Ground freeze-dried roots of *Impatiens glandulifera* were extracted only with hexane. When repeatedly extracting the roots with hexane (Figure 1.), it is clear that we achieve 60-70% efficiency during the first extraction. The dependence of the yield on the number of extraction steps is given by an exponential function with a correlation coefficient of 0.9928 - see Fig. 1. To achieve the maximum amount of α-spinasterol, repeated extraction is necessary, but as can be seen from Fig. 1 triple extraction is completely sufficient for approx. 100% extraction of α-spinasterol. It can be seen from the chromatogram (Figure 2.)

that α-spinasterol is the dominant substance in the freeze-dried roots of *Impatiens glandulifera.* When the extracts are highly concentrated, α-spinasterol needles fall out.

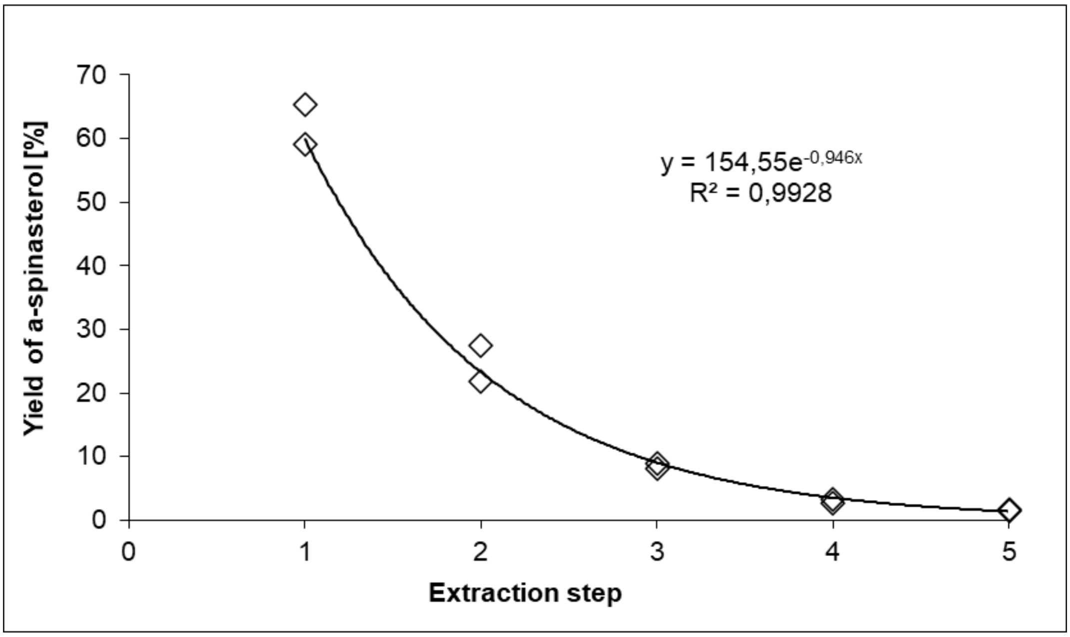

Fig. 1 Efficiency of α-spinasterol extraction using hexane

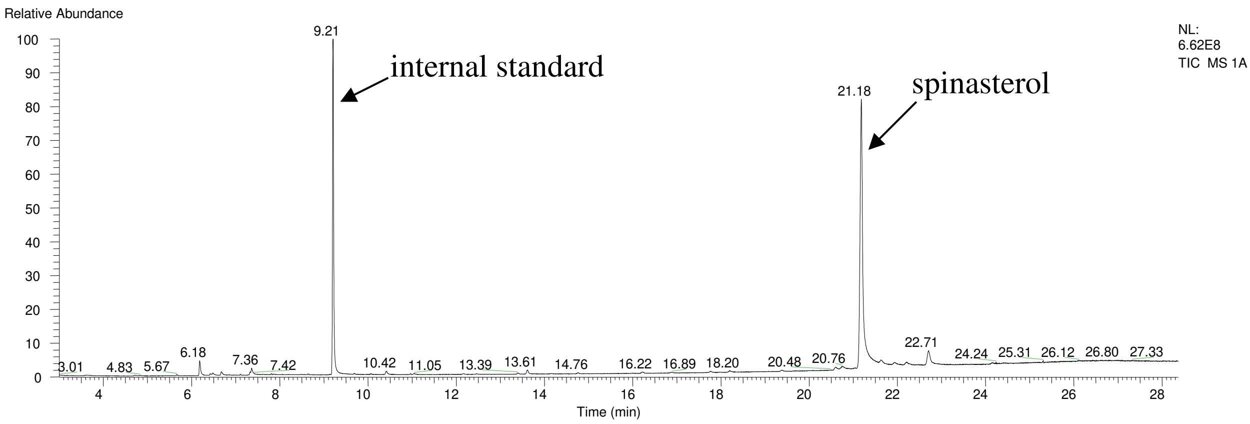

Fig. 2 TIC chromatogram of GC-MS separation of the sample A1.

The problem of broader application and use of α-spinasterol in the pharmaceutical industry or food industry lies in its low content in plants. Currently, there is available (albeit rare and expensive) oil from *Argania spinosa*, which contains, in addition to a whole range of tocopherols and fatty acids, also sterols, including α-spinasterol. Argan oil is mainly used in cosmetics.

We found that the concentration of α-spinasterol in lyophilized roots of *Impatiens glandulifera* is quite high: sample A 0.99 %, sample B 1.12 % of lyophilized materials and the extract is very pure. The results show that freeze-dried roots of *Impatiens glandulifera* are a relatively good source of α-spinasterol.

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Declarations

Confict of interest. The authors declare that there is no conflict of interest.

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