SUPPLEMENTARY MATERIAL

Tephrosia apollinea seed: a new rich source of essential polyunsaturated fatty acids, tocopherols, sterols, and squalene

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ABSTRACT

Tephrosia apollinea is a legume species, native to southwest Asia and northeast Africa, rich in bioactive flavonoids (hydrophilic compounds). *T. apollinea* seeds were not considered previously as a potential source of lipophilic compounds such as: essential fatty acids, tocopherols, sterols, and squalene, hence, the present study were performed. The oil yield in *T. apollinea* seeds amounted to 11.8% dw. The *T. apollinea* seed oil was predominated by the polyunsaturated fatty acids - linoleic (26.8%) and α-linolenic (22.7%). High levels were recorded also for oleic (27.6%) and palmitic (14.9%) acids. Four tocopherols and one tocotrienol, with the domination of γ-tocopherol (98%) were identified in *T. apollinea* seed oil. The β-sitosterol (59%), Δ5-stigmasterol (21%) and campesterol (9%) were detected as main sterols in *T. apollinea* seed oil. The total content of tocochromanols, sterols, carotenoids and squalene in the *T. apollinea* seed oil was 256.7, 338.1, 12.5 and 1103.8 mg/100 g oil, respectively. *T. apollinea* seeds oil, due to the high concentration of lipophilic bioactive compounds can find a potential application in the food, cosmetic and pharmaceutical industry.

Keywords: *Tephrosia apollinea* seed oil; Polyunsaturated fatty acids; Tocopherols; Carotenoids; Phytosterols; Squalene.

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Experimental part

Reagents

n-Hexane, tert-butyl methyl ether, 2-propanol, methanol (HPLC grade), 5α-cholestane (≥97%, GC) were purchased from Sigma-Aldrich (Steinheim, Germany). Homologues (α, β, γ and δ) of tocopherol and tocotrienol (> 95%, HPLC) were obtained from LGC Standards (Teddington, Middlesex, UK), respectively. The Sylon BTZ and fatty acid methyl ester mix were received from Supelco (Bellefonte, PA, USA) and Supelco (Steinheim, Germany), respectively.

Plant material

The *T. apollinea* seed pods were collected in December, 2016 from plant growing naturally in Raipur region, Chhattisgarh, India (21.25°N 81.63°E). The *T. apollinea* with the specimen number "51656" was authenticated botanically by the professor Khageshwar Singh Patel. The seed pods were dried on the sun until the seeds reached moisture content below 10%. The seeds were manually removed and stored in polyethylene bags at -20 ± 1 °C until sending via courier to Latvia. Received seeds were freeze dried for 24 h using a freeze-dry system (FreeZone, Labconco, Kansas City, MO, USA) to remove maximally the residues of water. The seeds were randomly separated into three batches (40 ± 10 g of each batch) before were milled with a MM 400 mixer mill (Retsch, Haan, Germany). Dry weight basis (dw) for the obtained powder was measured gravimetrically.

Oil extraction

Oil was extracted with n-hexane by applying a vortex mixer REAX top (Heidolph, Schwabach, Germany) and ultrasonic bath Sonorex RK 510 H (Bandelin electronic, Berlin, Germany) according to an earlier introduced method (Górnaś, Rudzińska, et al. 2014).

Fatty acid determination

The fatty acid composition of the grape seed oil was estimated using gas chromatography (GC) according to AOCS (2005). Detailed description of the applied instruments, including the columns, gases flow, detectors (FID and MS) as well as standards and procedures has been reported previously (Górnaś, Rudzińska, et al. 2016).

Tocopherol and tocotrienol homologues determination

The oil sample was diluted in 2-propanol, mixed, filtrated through a nylon syringe filter (0.22 μm) and directly injected on a RP-HPLC system (Górnaś 2015). Tocopherol and tocotrienol homologues were identified by the HPLC system (Shimadzu, Kyoto, Japan) consisting a PFP (pentafluorophenyl) column (provides separation of isomers β and γ) and fluorescence detector according to the previously validated chromatographic conditions (Górnaś, Siger, et al. 2014).

Determination of total carotenoids

Total carotenoids content in *T. apollinea* seed oil diluted in n-hexane was measured spectrophotometrically at 450 nm with a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) and using the molar extinction coefficient for all-trans-β-carotene (ε = 139049) (Górnaś, Rudzinska, et al. 2016).

Sterols and squalene determination

Plant sterols were determined according to AOCS (1997) using GC system. Detailed description of the applied instruments, including the columns, gases flow, detectors (FID and MS) as well as standards and procedures has been reported previously (Górnaś, et al. 2016).

Statistical Analysis

The results were presented as means ± standard deviation (*n*=3) from three independent batches.

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Table S1. Fatty acid composition (%), sum of SFA, MUFA and PUFA (%), and FAs ratios of *T. apollinea* seed oil

Saturated fatty acid (SFA)				
C16:0	C18:0	C20:0	C22:0	ΣSFA
14.86 ± 0.12	2.96 ± 0.14	1.42 ± 0.03	2.49 ± 0.04	21.7
Monounsaturated fatty acid (MUFA)				
C18:1	C20:1			ΣMUFA
27.58 ± 0.53	1.02 ± 0.08			28.6
Polyunsaturated fatty acid (PUFA)				
C18:2	C18:3 *n*-6	C18:3 *n*-3		ΣPUFA
26.79 ± 0.45	0.17 ± 0.02	22.72 ± 0.25		49.7

Values are expressed as the mean ± standard deviation (n = 3).

Table S2. Minor lipophilic compounds (tocopherols, tocotrienols, carotenoids, sterols and squalene) (mg/100 g oil) of *T. apollinea* seed oil

Tocopherols and tocotrienols					
α-T	β-T	γ-T	δ-T	γ-T3	Total Ts+T3s
1.3 ± 0.1	0.1 ± 0.0	251.6 ± 6.2	3.4 ± 0.1	0.2 ± 0.1	256.7 ± 6.6
Sterols, carotenoids and squalene					
Campesterol	Campestanol	Δ5-Stigmasterol	β-Sitosterol	Δ5-Avenasterol	
30.7 ± 1.4	4.0 ± 0.3	72.5 ± 3.8	198.5 ± 9.8	16.0 ± 1.4	
α-Amyrin	Cycloartenol	Total sterols	Carotenoids	Squalene	
6.2 ± 0.2	10.3 ± 0.6	338.1 ± 17.5	12.5 ± 0.6	1103.8 ± 33.5	

Values are expressed as the mean ± standard deviation (n = 3). T, tocopherol; T3, tocotrienol.