ELUCIDATING THE BIOACTIVE POTENTIAL OF MOUNTAIN GERMANDER (*Teucrium Montanum* L.) BY APPLYING FRACTIONATION OF PHENOLIC COMPOUNDS

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INTRODUCTION

Emerging from various health benefits and low-cost, the utilization of aromatic plants in the human diet and folk medicine has been integrated since ancient times. Hiding its undiscovered commercial potential among *Lamiaceae* species, Mountain Germander (*T.montanum* L.) represents an insufficiently explored plant with notable chemo- diversity. The popularization of this plant in ethnomedicine can be ascribed to the presence of various biologically active compounds, emphasizing polyphenols (1). According to research studies, the correlation between phenolic compounds and health benefits related to the prevention of cardiovascular diseases, neurodegenerative diseases, diabetes, colon cancer, etc., was established (2). In order to closely provide an insight into the phenolic composition of Mountain Germander, fractionation of phenolic compounds was conducted. Thus, this study could contribute to elucidation of phenolic characterization, important for further development of potential application of Mountain Germander in the pharmacological and food industry.

MATERIALS AND METHODS

In order to obtain free- soluble phenolics (FP), esterified phenolics (EP), glycosylated phenolics (EP) and insoluble- bound phenolics (IP), he fractionation of phenolic compoumds was conducted by Arruda et al. (2018), with some modifications. The general procedure for extraction and fractionation of phenolic compounds is represented on Sheme 1.

plant material (<450 µm)

ultrasound assisted extraction (15 min, 25°C) + mixing on magnetic stirrer (30 min, 25°C) — 3 x

1g of plant:20 ml water:metanol:acetone (6:7:7, v/v)

centrifugation (4 °C)

residue → addition of acetone → centrifugation (4 °C)

supernatant/ **inital extract (IE)**

vacuum evaporation + NaOH (5 M), HCl (4 M) + water

extraction liquid-to- liquid (1:1, v/v) diethyl ether:ethyl acetate (1:1, v/v) — 2 x

centrifugation (4 °C)

addition of anhydrous Na_2SO_4 + centrifugation (4 °C)

aquaeous phase

alkali hydrolysis 4M NaOH+20mM EDTA+2% (w/v) L-ascorbic acid; 2 hours, 25 °C

adjustment of pH with 4 M HCl+5 M NaCl

extraction liquid-to- liquid (1:1, v/v) diethyl ether:ethyl acetate (1:1, v/v) — 2 x

Centrifugation (4 °C)

addition of anhydrous Na_2SO_4 + centrifugation (4 °C)

organic phase

Glycosylated phenolics (GP)

Step marked with *

Cooling with the addition of NaCl for neutralisation

acid hydrolysis 4M HCl; 80 °C, 1h

aquaeous phase

addition of anhydrous Na_2SO_4 + centrifugation (4 °C)

Esterified phenolics (EP)

vacuum evaporation + dissolution in 80% methanol (v/v)

organic phase

alkali hydrolysis 4M NaOH+20mM EDTA+2% (w/v) L-ascorbic acid; 2h, 25 °C

adjustment of pH with 4 M HCl+5 M NaCl

extraction liquid-to- liquid (1:1, v/v) diethyl ether:ethyl acetate (1:1, v/v) — 2 x

Centrifugation (4 °C)

addition of anhydrous Na_2SO_4 + centrifugation (4 °C)

vacuum evaporation + dissolution in 80% methanol (v/v)

Insoluble-bound phenolics (IP)

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vacuum evaporation + dissolution in 80% methanol (v/v)

Free- soluble phenolics (FP)

Sheme 1. The flowchart describing the fractionation of phenolic compounds for Mountain Germander

RESULTS

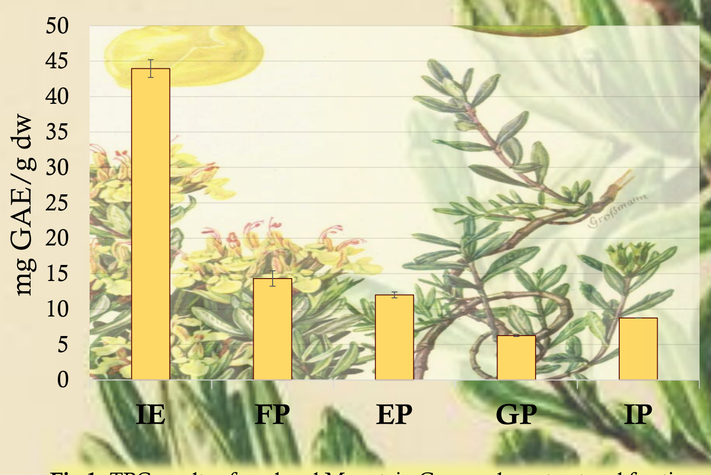

Fig.1. TPC results of analysed Mountain Germander extract and fractions

Phenolic compound (mg/g dw)	Extract/fraction				
	UTP	STP	GP	EP	NVP
echinacoside	7.05±0.03	-	-	-	-
verbascoside	4.94±0.09	1.87+0.30	-	-	-
***trans*- ferulic acid**	-	-	-	-	0.15±0.00
caffeic acid	-	-	0.91±0.11	3.09±0.52	1.50±0.00
3-O-caffeoylquinic acid	0.40±0.00	0.52±0.09	-	-	-
4-O-caffeoylquinic acid	0.29±0.02	0.32±0.06	-	-	-
***p*- coumaric acid**	-	-	0.52±0.06	1.38±0.22	1.59±0.00
4-hydroxybenzoic acid	0.05±0.00	0.32±0.06	-	-	-

Table 1. The identified polyphenols in obtained extract and fractions of Mountain Germander by HPLC

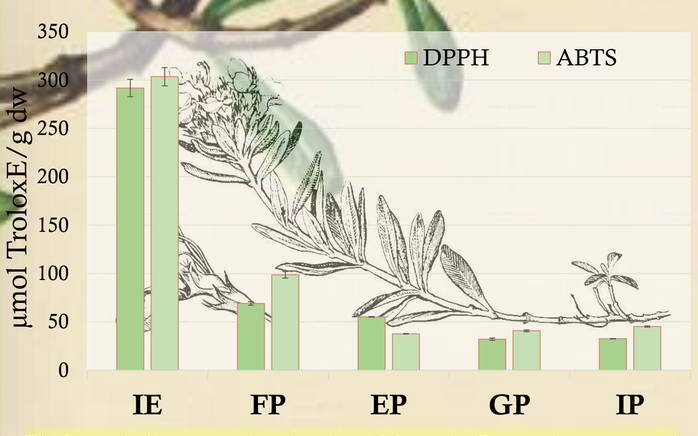

Fig.2. Antioxidant capacity of analysed Mountain Germander extract and fractions determined by DPPH and ABTS

CONCLUSIONS

- ✓ IE of Mountain Germander exhibited the highest TPC and antioxidant capacity
- ✓ Fractionaction of IE indicated similar content of FP, EP and IP, measured by TPC and antioxidant capacity
- ✓ Inital extract of Mountain Germander showed to be the notable source of phenylethanoid glycosides- echinacoside (7.05 mg/g dw) and verbascoside (4.94 mg/g dw)
- ✓ Caffeic esters were the most dominant in EP fraction (3.09 mg/g dw) as the consequence of the retained echinacoside and verbascoside in the aqueous phase of IE

REFERENCES

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