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PHENOLIC ACIDS AND FREE RADICAL SCAVENGING ACTIVITY OF *ALCHEMILLA JUMRUKCZALICA* PAWL.

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ABSTRACT

Keywords:

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Comparative study of free radical scavenging activity and phenolic acid content of Bulgarian endemic *Alchemilla jumrukczalica* and the commercial herbal - *Alchemilla vulgaris complex* was carried. The methanol extracts of studied samples showed significant antiradical activity. The concentration of the extracts needed for 50% inhibition of DPPH was found to be 12,09 µg/ml and 19,62 µg/ml for *A. jumrukczalica* and *A. vulgaris complex*, respectively. Ten free and seventeen bonded phenolic acids were identified and quantified by performed of gas chromatography mass-spectrometry (GC-MS). Genticic, protocatechuic, salicylic and trans-cinnamic acids were represented in the greatest quantity among the identified phenolic acids. This is the first detailed analysis on qualitative and quantitative composition of phenolic acids in *Alchemilla* species.

INTRODUCTION: In the course of phytochemical studies of the high mountain medicinal plants from Bulgaria in particular *Alchemilla* species¹, the Bulgarian endemic *Alchemilla jumrukczalica* (Rosaceae) was investigated. No phytochemical studies on *Alchemilla jumrukczalica* have been reported. *Alchemilla vulgaris complex* is the commercial herbal - mixture of *Alchemilla* species, herb with well documented medicinal application².

The extract of *A. vulgaris complex* was used as positive control. Species of genus *Alchemilla* have been studied mainly for the content of flavonoids, triterpenes, tannins and total phenols³⁻⁶. With regard to phenolic acids, we only had data for the content of ellagic and gallic acids in *Alchemilla* species^{7, 8}. Phenolic compounds are considered free-radical scavengers^{9,10}. That is why the objectives of present study were to;

- 1) Compare the antioxidant activity of the extracts of the studied species, and

- 2) Identify and quantify phenolic acids in these extracts that may contribute to antioxidant activity.

Plant Material: The plant material of *Alchemilla jumrukczalica* was collected in Central Balkan. A voucher specimen of *A. jumrukczalica* (SOM 165680) has been deposited at the Herbarium of Institute of Biodiversity and Ecosystem Research.

Preparation of Extracts: Air dried leaves of *A. jumrukczalica* and *A. vulgaris complex* was extracted with 80% methanol threefold. After evaporation of the solvent the received extracts were used for evaluation of antiradical activity and content of phenolic acids.

Antiradical Assay: The antioxidant activity of the methanol extracts was evaluated by the scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) radicals^{11,12}.

The extracts showed significant antiradical activity with IC_{50} values of 12, 09 $\mu\text{g/ml}$ and 19, 62 $\mu\text{g/ml}$ respectively for *A. jumrukczalica* and *A. vulgaris complex*. Obtained values are comparable with those of commercial antioxidant butylated hydroxytoluene (BHT) - 12.65 $\mu\text{g/ml}$ and syringic acid - 4.40 $\mu\text{g/ml}$.

Phenolic Acid Analysis: Free and bonded phenolic acids were extracted with ethyl acetate from acidulated methanol-water extract of plant materials before and after thermal hydrolysis. Ten free and seventeen bonded phenolic acids were identified and quantified by performed of gas chromatography mass-spectrometry (GC-MS). The extracts of the both

samples contain free phenolic acids in comparable quantities while the amount of bonded phenolic acids was higher in the extract of *A. Jumrukczalica*. Among the identified free phenolic acids gentisic, protocatechuic, salicylic and caffeic acids are represented in the greatest quantity. Salicylic, protocatechuic, caffeic, trans-cinnamic, gentisic and vanilic acids were the major bonded phenolic acids in the studied extracts (**Table 1**). The presence of such a variety of phenolic acids largely explain the high free radical scavenging of the extracts, taking into account that this type of compounds carry a high antioxidant properties^{9,10}.

TABLE 1: FREE AND BONDED PHENOLIC ACID OF *A. JUMRUKCZALICA* PAWL. AND *A. VULGARIS* COMPLEX EXTRACTS

Phenolic Acids	Parameters		<i>A. jumrukczalica</i>	<i>A. vulgaris complex</i>
	RT (min)	RI	Mean \pm SD ($\mu\text{g/g DW}$)	Mean \pm SD ($\mu\text{g/g DW}$)
Free Phenolic Acids				
β -phenylpyruvic acid TMS	15,84	1447,9	0,91 \pm 0,22	0,96 \pm 0,24
Salicylic acid TMS	16,61	1479,9	3,09 \pm 0,71	2,99 \pm 0,72
Trans-cinnamic acid TMS	17,25	1506,8	1,23 \pm 1,67	1,14 \pm 0,69
Vanilic acid TMS	22,29	1729,8	1,55 \pm 0,48	1,59 \pm 0,49
Gentisic acid TMS	22,67	1747,5	4,64 \pm 0,74	5,12 \pm 0,72
Protocatechuic acid TMS	23,54	1787,3	4,46 \pm 0,63	4,72 \pm 0,66
Syringic acid TMS	25,08	1864,4	1,69 \pm 0,56	1,81 \pm 0,38
p-coumaric acid TMS	25,74	1897,6	1,08 \pm 0,59	1,12 \pm 0,61
Caffeic acid TMS	29,99	2123,1	2,48 \pm 0,69	2,78 \pm 0,68
Sinapic acid TMS	31,55	2210,3	1,27 \pm 0,62	1,63 \pm 0,63
Bonded Phenolic Acids				
Benzoic acid TMS	9,93	1199,9	7,41 \pm 1,18	6,18 \pm 1,23
β -phenylpyruvic acid TMS	15,85	1448,1	10,06 \pm 1,73	9,17 \pm 1,77
Salicylic acid TMS	16,63	1480,0	54,44 \pm 1,61	52,58 \pm 1,64
Trans-cinnamic acid TMS	17,27	1507,0	46,64 \pm 1,76	51,97 \pm 1,71
m-hydroxybenzoic acid TMS	19,04	1581,8	6,96 \pm 0,61	5,51 \pm 0,66
p-hydroxybenzoic acid TMS	19,25	1590,5	7,72 \pm 0,58	7,31 \pm 0,56
β -resorcylic acid TMS	21,02	1671,0	5,95 \pm 0,54	6,18 \pm 0,64
Vanilic acid TMS	22,31	1730,2	24,71 \pm 1,43	23,08 \pm 1,49
Gentisic acid TMS	22,64	1748,0	35,19 \pm 1,67	32,94 \pm 1,62
Mandelic acid TMS	23,32	1777,4	6,18 \pm 0,58	5,75 \pm 0,53
Protocatechuic acid TMS	23,56	1787,7	51,37 \pm 1,86	52,54 \pm 1,79
Syringic acid TMS	25,11	1864,9	16,48 \pm 0,88	15,78 \pm 0,86
p-coumaric acid TMS	25,76	1897,9	13,94 \pm 0,79	10,07 \pm 0,64
Gallic acid tms	26,44	1933,4	7,19 \pm 0,48	6,73 \pm 0,47
3, 4, 5-trimethoxymandelic acid tms	28,81	2057,7	6,39 \pm 0,41	5,31 \pm 0,42
Caffeic acid tms	30,01	2123,6	34,18 \pm 1,52	32,77 \pm 1,55
Sinapic acid tms	31,57	2210,7	10,06 \pm 1,05	11,92 \pm 1,09

In conclusion, the present study is the first detailed report of qualitative and quantitative composition of phenolic acids in *Alchemilla* species. The methanol extracts of *A. jumrukczalica* and *A. vulgaris complex* showed significant antiradical activity. No significant difference was found in the content of phenolic acids and antiradical activity of studied samples. These results revealed the extract of *A. jumrukczalica* as potential source of antioxidant activity.

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