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Aconitum lycoctonum L.: Phenolic Compounds and Their Bioactivities

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Aconitum lycoctonum L.: Phenolic Compounds and Their Bioactivities

Abstract

The very first report on the phenolic composition of aerial parts of *Aconitum lycoctonum* L., a species belonging to the toxic *Aconitum* genus, is presented here. Aerial parts were subjected to the extraction with four different solvents and analyzed via LC-MS/MS for the content of phenolic acids and flavonoids. Furthermore, isolated extracts were tested for antimicrobial and antioxidant activities. Ethanolic extracts of both flowers and vegetative parts (leaves and stems) were found to be the richest in the phenolic compounds, following the water extracts. Ethanolic extract of flowers was very rich in flavonoid apigenin, while high levels of salicylic and 4-hydroxybenzoic acids were found in the same extract of leaves and stems. On contrary, water extract contained significant amounts of kaempferol and rosmarinic acid. All extracts showed potent antioxidant activity, which is correlated with the content of phenolics. The antimicrobial assay showed that all extracts, except aqueous, were quite potent against all microbial organisms tested.

Keywords

phenolic compounds; LC-MS/MS; antioxidant activity; antimicrobial activity; Aconitum lycoctonum L.

Cover Page Footnote

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Introduction

The genus *Aconitum* consists of more than 300 species that are mainly perennial herbs, distributed in the Northern Hemisphere (Singh *et al*. 2002). Due to the presence of diterpenoid alkaloids, the majority of the plants of this genus are found to be highly toxic (Wink 2009), although *Aconitum lycoctonum* had medicinal use in ancient times (Hartwich 1897). Main neurotoxins from this species are alkaloids aconitine, mesaconitine, and lycoctonine, found in plant tubers. They activate sodium channels which cause numbness in muscle and paralysis (Singh *et al*. 2002; Wink 2009).

Many of the *Aconitum* species are used for medicinal purposes as analgesic and anti-inflammatory medicaments (Aslam and Ahmad 2016). Scientific interest in this genus is mainly due to alkaloids present that are carriers of many pharmacological properties. Moreover, flavonoids as chemotaxonomic markers have also been studied, but there are only a few studies on their biological activities (Srivastava *et al*. 2010).

There is a need for more phytochemical studies to be carried out on this genus, thus, this study comprises the investigation of phenolic compounds and antioxidant and antimicrobial activities of the extracts of aerial parts of *Aconitum lycoctonum* L. (Figure 1). As phenolic compounds are a large group of plant secondary metabolites, we used four different solvents to ensure more comprehensive isolation of divergent compounds.

Material and methods

Samples of *Aconitum lycoctonum* L. were collected at Mt. Jahorina in Bosnia and Herzegovina (GPS coordinates: 43.707939, 18.580780), during the flowering stage in July 2017 (Figure 1). Above ground parts of five specimens with fully developed flowers were collected. Specimens were authenticated by the botanist Mr.sc. Aldin Boskailo, University Dzemal Bijedic, and deposited at the Herbarium of Department of Biology, Faculty of Science, University of Sarajevo, with the Voucher no. AL-356.

Inflorescence and vegetative parts (leaves and stems) were separated and air-dried for 7 days at room temperature (23 °C) in a shaded, well-ventilated laboratory. Dried samples were finely powdered in the mill (TissueLyser Qiagen; Retsch) and stored at +4 °C until use. Four 500 mg aliquots of powdered plant material were soaked in 12.5 mL of one each of the four solvents including petrol ether, chloroform, ethanol, or water, and sonicated for 30 min. The supernatant was removed and sediment was re-extracted. Petrol ether and chloroform extracts were evaporated to dryness and resuspended in dimethyl sulfoxide (DMSO).

UHPLC-MS/MS was performed on UltiMate™ 3000 liquid chromatographic system consisting of binary pumps, an autosampler, and a column thermostat coupled to a TSQ Quantum Access Max triple quadrupole mass

Figure 1. *Aconitum lycoctonum* L.

spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was performed on an Acquity BEHC18 (150 × 3.0 mm; 1.7 µm particle size) UHPLC column (Waters Corp., Milford, MA, USA) kept at 40°C. The mobile phase consisted of 10 mM formic acid in water (A) and acetonitrile (B). Target compounds (15 phenolic acids and 14 flavonoids) were separated using a binary gradient starting at 5% B for 0.8 min, increasing to 10% B in 0.4 min with an

isocratic run for 0.7 min, then increasing to 15% B for 0.5 min and isocratic run for 1.3 min, then increasing to 20% B for 0.3 min and isocratic for 1.2 min, then increasing to 25% B for 0.5 min with next increase to 35% B within 2.3 min, then increasing to 70% B for 2.5 min, then further increase to 100% B for 1 min, with an isocratic run for 1 min, and then back to 5% B for 0.5 min. Finally, the equilibration to the initial conditions took 3.3 min, with a total chromatographic run of 16 min. The flow rate was 0.4 mL/min and the injection volume 10 µL.

All analytes were detected in negative ionization mode ESI-. Multiple reaction monitoring (MRM) mode was used for their quantification. The MRM transitions are listed in the Table 1, together with their optimal collision energies and retention times. The spray voltage was 3 kV, and the vaporizer the ion transfer tube temperatures were 320°C.

Standard solutions of target compounds, purchased from Sigma Aldrich Company, Germany, were firstly prepared in methanol at 1 mM concentrations, and solutions were gradually diluted in the mobile phase (95% A, 5% B) to the working concentrations that ranged from 0.01 to 50 µM. Quantification was performed by an isotopic diluting method using *p*-coumaric acid-d_6 and salicylic acid-d_4.

2.2-diphenyl-1-picrylhydrazyl radical (DPPH) antioxidant capacity was evaluated for all four extracts and standards according to Meda *et al*. (2005). Antioxidant potential was evaluated according to the absorbance change and presented as a percentage of scavenged DPPH radical. Naringenin was used as a reference substance.

Agar well diffusion method was used to evaluate the antimicrobial activity of plant extracts and standards according to National Committee for Clinical Laboratory Standards (NCCLS, 1993). Each well contained 100 µL of extract or standard. Bacterial strains used in the analysis included Gram-positive: *Enterococcus faecalis* ATCC® 19433™, *Staphylococcus aureus* subsp. *aureus* ATCC® 6538™ and Gram-negative bacteria: *Salmonella abony* NCTC® 6017™, *Escherichia coli* ATCC® 8739™, and the yeast *Candida albicans* ATCC® 10231™. Bacterial strains were used as standardized inoculum of $5x10^5$ CFU/mL using McFarland standard.

Müller-Hinton and Sabouard medium were used for the cultivation of bacterial strains and yeast, respectively. Ampicillin was used as an antibiotic for bacterial strains and antimycotic nystatin for *Candida albicans*. Ethanol and DMSO were used as negative controls. The antimicrobial effect was expressed as a diameter of inhibition zone in mm reduced by the inhibition zone of negative controls if appropriate.

Results and discussion

Among 29 target compounds, 16 were identified and quantified in eight extracts of *A. lycoctonum*, i.e. 4-hydroxybenzoic acids, 5-hydroxycinnamic acids, and 7

Table 1. Retention times, MRM transitions, and limits of detection (LOD) and quantification (LOQ) of analyzed phenolic compounds.

#	Compound	Rt (min)	MRM	CE (V)	LOD (μM)	LOQ (μM)
1	GA	3.26 ± 0.04	169 > 69, 79, 125	25	0.04	0.10
2	ChA	5.16 ± 0.02	353 > 191	25	0.02	0.06
3	CATH	5.26 ± 0.09	289 > 109, 123, 203	25	0.01	0.04
4	4HBA	5.68 ± 0.02	137 > 65, 93	25	0.06	0.16
5	CA	6.24 ± 0.05	179 > 79, 134, 135	25	0.08	0.20
6	VA	6.37 ± 0.01	167 > 95, 108, 152	25	0.18	0.60
7	23DHBA	6.39 ± 0.05	153 > 109	25	0.07	0.22
8	3HBA	6.62 ± 0.05	137 > 65, 93	25	0.07	0.20
9	HESP	7.35 ± 0.01	609 > 271, 300	40	0.01	0.05
10	RUT	7.38 ± 0.03	663 > 415, 427	40	0.01	0.05
11	*p*CA-d6	7.52 ± 0.01	169 > 99, 125	25	0.02	0.08
12	*p*CA	7.61 ± 0.03	163 > 93, 119	25	0.02	0.08
13	SiA	8.14 ± 0.05	223 > 121, 149, 193	25	0.07	0.21
14	FA	8.21 ± 0.01	193 > 93, 134	25	0.06	0.15
15	QUEI	8.55 ± 0.04	447 > 271, 300	25	0.02	0.06
16	NARI	8.58 ± 0.01	579 > 151	40	0.02	0.06
17	SyA	8.83 ± 0.01	197 > 73, 135	25	0.08	0.20
18	RA	8.83 ± 0.02	359 > 161, 79	25	0.07	0.21
19	MYR	9.06 ± 0.03	317 > 137, 151, 179	25	0.02	0.06
20	SaA-d4	8.55 ± 0.03	141 > 69, 97	25	0.03	0.10
21	SaA	8.61 ± 0.02	137 > 65, 93	25	0.03	0.10
22	QUE	9.69 ± 0.09	301 > 107, 125, 151	25	0.02	0.06
23	MOR	10.40 ± 0.05	301 > 121, 151	25	0.02	0.06
24	API	10.99 ± 0.06	269 > 117, 151	25	0.07	0.21
25	*t*CA	11.00 ± 0.07	147 > 77, 103	25	0.34	0.90
26	NARE	11.04 ± 0.01	271 > 107, 119, 151	25	0.02	0.06
27	KAE	11.09 ± 0.09	285 > 93	40	0.02	0.06
28	CHR	12.20 ± 0.07	253 > 119, 143	40	0.08	0.20
29	PIN	12.27 ± 0.06	255 > 107, 171	40	0.08	0.20
30	GAL	12.32 ± 0.08	269 > 141, 195	40	0.08	0.20
31	CaA	13.57 ± 0.02	331 > 287	25	0.09	0.25

GA – gallic acid; ChA – chlorogenic acid; CATH – cathechin, 4HBA – 4-hydroxybenzoic acid; CA – caffeic acid; VA – vanilic acid; 23DHBA – 2,3-dihydroxybenzoic acid; 3HBA – 3-hydroxybenzoic acid; HESP – hesperidin, RUT – rutin; *p*Ca – *p*-coumaric acid; SiA – sinapic acid; FA – ferulic acid; QUEI – quercitrin; NARI – naringin; SyA – syringic acid; Ra – rosmarinic acid; MYR – myricetin; SaA – salycilic acid; QUE – quercetin; MOR – morin; API – apigenin; *t*CA – *trans*-cinammic acid; NARE – naringenin; KAE – kaemferol; CHR – chrysin; PIN – pinocembrin; GAL – galangin; CaA – carnosic acid.

flavonoids. Summarized results of UHPLC-MS/MS quantification of phenolics in four extracts with different polarity (each isolated from flowers, and leaves and stems) are presented in Figure 2, while Table 2 represents the content of each particular compound detected. As expected, non-polar extracts had very low concentrations of phenolics. Petrol ether extract of leaves and stems contained considerable levels, in comparison with other extracts, of 4-hydroxybenzoic and

chlorogenic acids, while both chloroform extracts were quite rich in the content of salicylic acid. Ethanolic extracts were very rich in phenolic compounds (276.35-336.71 μmol/g), i.e. flower extract contained a significant amount of flavonoids (Figure 2), with apigenin as the major representative (Table 2), while leaves and stems extract comprised the highest levels of hydroxybenzoic acids among all analyzed (Figure 2), with salicylic acid as the main one. Also, aqueous extracts contained noteworthy amounts of phenolic acids, as well as flavonoid kaempferol (Table 2), which was not detected in less polar extracts.

Few studies have dealt with the content of phenolic compounds in *Aconitum* sp. (Srivastava et al., 2010; Aslam and Ahmad, 2016) and there are none about A. *lycoctonum*. Kaempferol and quercetin derivatives are mainly found in other *Aconitum* species (Mariani et al., 2008). According to the results presented here, *A. lycoctonum* possess significant levels of apigenin, naringenin, naringin, and morin, while quercetin was not detected.

Aconitun lycoctonum extracts showed strong antioxidant potential, with IC_{50} values ranging from 37.91 up to 3717.24 μg/mL, for ethanolic flower extract,

Figure 2. The content of phenolic compounds in four different solvent extracts of *Aconitum lycoctonum*. F – flowers, LS – leaves and stems.

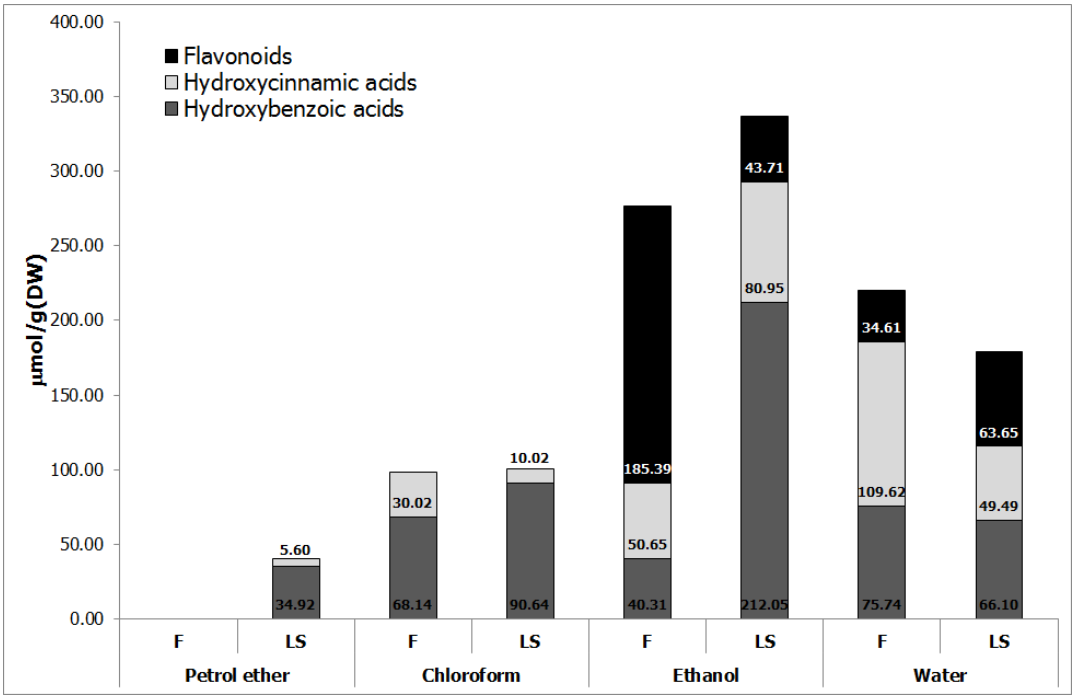

Table 2: Phenolic composition of the extracts of *Aconitum lycoctonum* detected in four different solvent extracts (μmol/g).

Compound	Petrol ether		Chloroform		Ethanol		Water	
	F	LS	F	LS	F	LS	F	LS
23DHBA	nd	nd	nd	nd	nd	nd	5.42 ±0.50	nd
4HBA	nd	34.92 ±2.24	42.16 ±0.84	57.10 ±3.69	11.52 ±0.03	98.27 ±2.03	28.09 ±3.19	15.73 ±1.69
SA	nd	nd	25.98 ±1.85	33.54 ±0.65	28.79 ±1.81	113.78 ±6.18	36.13 ±0.91	47.47 ±5.89
VA	nd	nd	nd	nd	nd	nd	6.10 ±0.04	2.90 ±0.42
CA	nd	nd	7.26 ±0.93	8.55 ±0.01	22.90 ±2.14	38.72 ±0.80	12.91 ±0.28	40.21 ±2.89
ChA	nd	5.60 ±0.31	1.45 ±0.16	1.47 ±0.12	0.42 ±0.02	0.54 ±0.04	11.25±0.52	7.66 ±0.87
*p*CA	nd	nd	21.31 ±3.64	nd	5.53 ±0.91	41.69 ±4.20	15.40 ±0.05	nd
FA	nd	nd	nd	nd	21.80 ±3.02	nd	2.06 ±0.24	1.62 ±0.25
RA	nd	nd	nd	nd	nd	nd	68.00 ±3.72	nd
API	nd	nd	nd	nd	88.45 ±7.56	nd	nd	nd
HESP	nd	nd	nd	nd	6.83 ±0.35	nd	nd	nd
KAE	nd	nd	nd	nd	nd	nd	31.34 ±3.80	38.08 ±0.45
NARE	nd	nd	nd	nd	28.10 ±0.70	nd	nd	nd
NARI	nd	nd	nd	nd	24.76 ±1.57	43.71 ±0.34	nd	nd
MOR	nd	nd	nd	nd	24.13 ±2.10	nd	3.27 ±0.30	25.57 ±1.88
RUT	nd	nd	nd	nd	13.12 ±4.02	nd	nd	nd

23DHBA – 2,3-dyhydrobenzoic acid; 4HBA – 4-hydroxybenzoic acid; SA – salycilic acid; VA – vanillic acid; CA – caffeic acid; ChA – chlorogenic acid; pCA – p-coumaric acid; FA – ferulic acid; RA – rosmarinic acid; API – apigenin; HESP – hesperidin; KAE – kaempherol; NARE – naringenin; NARI – naringin; MOR – morin; RUT – rutin; F – flowers; LS – leaves and stems; nd – not detected.

and petrol ether extracts of leaves and stems, respectively (Table 3). These values correlate with the content of phenolic compounds detected in analyzed extracts. This is the very first report on the antioxidant activity of *A. lycoctonum* with very few reports on the antioxidant activity of other members of the *Aconitum* genus. It was found that ethanolic extracts of *A. taipeicum* also possess strong antioxidant

activity measured in vitro (Xu and Guo, 2008), while the main carriers of antioxidant activity of methanolic extracts of *A. anthora* were quercetin, kaempferol, cloven, and robinin (Mariani et al., 2008).

Table 3: Antimicrobial and antioxidant activities of the extracts of *Aconitum lycoctonum*.

Extract		Antimicrobial activity (mm)					Antioxidant activity IC_{50} (μg/mL)
		S. aboni	*E. coli*	*E. faecalis*	*S. aureus*	*C. albicans*	
Petrol ether	F	nd	12.33 ±0.58	11.33 ±0.58	12.33 ±0.58	28.33 ±0.58	2660.00
	LS	12.33 ±0.58	16.00 ±1.73	16.00 ±3.61	11.67 ±0.58	22.00 ±1.73	3717.24
Chloroform	F	13.00 ±1.73	16.00 ±1.73	14.67 ±1.53	13.00 ±0.58	18.67 ±1.73	473.20
	LS	nd	13.67 ±0.58	14.00 ±1.00	12.33 ±0.58	18.67 ±1.15	274.81
Ethanol	F	12.67 ±0.58	nd	15.67 ±1.15	19.00 ±0.00	23.67 ±2.52	37.91
	LS	14.00 ±1.00	12.00 ±0.00	15.00 ±1.00	14.67 ±0.58	22.67 ±2.89	188.57
Water	F	nd	nd	nd	nd	nd	73.98
	LS	nd	nd	nd	nd	nd	41.12
Antibiotic/mycotic		17.00 ±1.41	14.33 ±2.83	19.33 ±0.71	34.33 ±2.83	19.67 ±1.15	-

F – flowers; LS – leaves and stems; nd – not detected.

Both ethanolic extracts showed potent antimicrobial activities, especially against *Candida albicans* (Table 2). The potency of ethanol extract is probably due to a high diversity of phenolic acids and flavonoids as well as their high concentrations with other extracts, especially salicylic acid (Monte et al., 2014; Karalija et al., 2020). The antimicrobial effect against *E. coli* and *S. aureus* can be attributed to the synergistic action of all compounds present in the extracts (Sanhueza et al., 2017). On contrary, the water extract showed no antimicrobial activity. This might be explained by the fact that water does not possess diffusing properties in agar medium. Numerous studies are describing that water extracts revealed low or no activity in comparison with other solvents (Gonelimali et al., 2018). To the best of our knowledge, there is only one report on antimicrobial properties of *Aconitum* species (*A. chasmanthum*), where a high concentration of the extract revealed relatively low inhibition or Gram-negative microorganisms (Anwar et al., 2003).

Conclusions

The phytochemical investigation of phenolic compounds from *Aconitum lycoctonum* is presented for the very first time. The phenolic composition of aerial

parts implies this species is a good source of pharmacologically important natural compounds. Moreover, the examination of the antioxidant and antimicrobial activities of the extracts confirms the fact this species, along with the other members of the genus, also has the potential to be used in therapeutic treatments.

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