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SUPPLEMENTARY MATERIAL TO

The RP-HPLC method for analysis of usnic acid as potential marker of herbal drugs-based formulations containing *Usnea barbata*

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In the first step, the active ingredients of the lozenge were characterized using GC/MS and GC/FID methods for essential oil, and an HPLC method in the case of *U. barbata* SCO_2, *S. scardica* and *O. hearcleoiticum* extracts. The HPLC methodology used in order to quantify the main components present in the extracts constituting the formulation, applying the external standard method, revealed that *U. barbata* SCO_2 extract was rich in usnic acid (980 mg g^{-1} dry extract), the presence of rosmarinic and *p*-coumaric acids, vitexin and luteolin (27.32, 7.53, 6.64, 1.55 mg g^{-1} of the dry extract, respectively) as the most abundant constituents in the *O. heracleoticum* extract, while the *S. scardica* extract contained in high amount ferulic and chlorogenic acid, vitexin-2″-*O*-rhamnoside, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside, apigenin and luteolin (59.3, 11.8, 7.0, 14.5, 6.4, 15.6 and 8.3 mg g^{-1} dry extract, respectively). In addition, the total phenolics, flavonoids and tannins contents, as well as the DPPH activity of the constituents of the lozenges were determined (Table S-I). The used HPLC methods[1-3] and methods for determination of DPPH activity, phenolics, flavonoids and tannins contents[4] were presented elsewhere.

In spite of this complex formulation, the preliminary efficiency research on antibacterial activity[5] revealed that the antibacterial activity should be ascribed to

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the SCO_2 extract of *U. barbata* (Old Man's Beard) and usnic acid as its main component (Table S-II).

Based on the presented results, we made an assumption, confirmed by performing the antibacterial investigation of the formulated lozenges that usnic acid could be considered as the carrier of activity against tested bacterial strains being responsible for the infections of upper respiratory tract (Table S-II).

TABLE S-I. DPPH activity, total phenolics, flavonoids and tannins in the components incorporated in investigated lozenges

Extract	$IC_{50} \pm SD$, μg ml^{-1}	Content of total phenolics ± *SD*, mg g^{-1}*	Content ± *SD*, %	
			Total flavonoids	Total tannins
U. barbata SCO_2 extract	322.0±7.5	0.6±0.1	/	/
S. scardica extract	31.5±0.4	188.5±12.9	0.4±0.0	5.7±0.0
O. heracleoticum extract	13.0±0.2	240.0±10.9	0.72±0.04	7.51±0.03
S. montana essential oil	1280.9±22.7	/	/	/
Lozenges	7.2±0.3	0.28±0.03	/	/
Trolox	5.9±0.3			
BHT	6.0±0.3	-		-

*mg gallic acid equivalents / g dry weight

TABLE S-II. Antibacterial activity of all single components, and their combination in the investigated lozenges

Bacterial strain	Minimal inhibitory concentration (*MIC*), μg ml^{-1}							
	U. barbata extract	*O. heraclaeoticum* extract	Usnic acid	The investigated lozenges	Gentamicin (Sigma)	Ampicilin (Sigma)		
Staphylococcus aureus ATCC 25923	40	1280	1280	320	10	160	<0.5	0.5
Staphylococcus aureus, clinical isolates	40	1280	1280	640	10	160		
MRSA ATCC 43300	40	1280	1280	640	5	320	4	-
MRSA, clinical isolates	40	1280	1280	640	5	320		
Enterococcus faecalis	10	>2560	2560	2560	2.5	1280	256	0.5

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