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Hylotelephium Spectabile (Boreau) H. Ohba x *Telephium* (L.) H.Ohba Leaf and Flower Extracts: Composition, Antioxidant and Antibacterial Activity

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Abstract: Composition, antioxidant and antibacterial activities for methanol extracts, and their hexane, ethyl acetate and butanol fractions of the fresh leaf and flower of *Hylotelephium spectabile* x *telephium* were studied for the first time. The extracts contain mostly quercetin and kaempferol glycosides, as confirmed by the composition of hydrolysates whose main components were quercetin and kaempferol. Evaluations of antioxidant activity were done by the following assays: DPPH, ABTS and total reducing power assay (Fe^{3+} to Fe^{2+}). Additionally, total flavonoid content and total phenols were determined. The antioxidant capacity of ethyl acetate flower fraction was very close or even higher than capacity of used standard antioxidants. Presented results qualify this species as a potential new source of antioxidant substances. Ethyl acetate fraction of leaf showed moderate bactericidal activity against *P. aeruginosa, S. typhimurium, S. aureus*, and *B. subtilis.*

Keywords: *Hylotelephium spectabile*; flavonoids; antioxidant and antibacterial activity.

1. Plant Source

Hylotelephium spectabile (Boreau) H. Ohba x *telephium* (L.) H. Ohba, a taxon of hybrid origin is grown as an ornamental and medicinal plant across Serbia. It is known by the common names: *debela koka* (fat han), *ranjenik* (wounded), and *kravlje vime* (cow's teat). Plant is very popular in Serbian folk medicine; people consume fresh leaves as a salad to regulate stomach acidity and to prevent bleeding [1].

The upper ground parts of cultivated *H. spectabile* x *telephium*, were harvested in the blossoming phase in October 2012. Voucher specimens No. 6853 is deposited in the Herbarium collection of the Faculty of Science and Mathematics, University of Niš (HMN).

2. Previous Studies

To our knowledge (SciFinder) no data on the chemical composition, antioxidant and antibacterial activities of *H. spectabile* x *telephium* methanol extract.

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3. Present Study

Extraction was carried out by a known procedure [2]. Yields of methanol extracts of fresh leaves and flowers were the same and amounted 2.7%. The yields of hexane, ethyl acetate and butanol fractions (relative to methanol extract) for leaves were: 11.5%, 18.1% and 44.1% (respectively).The yields of hexane, ethyl acetate and butanol fractions (relative to methanol extract) for flowers were: 25.0%, 19.1% and 29.0% (respectively).

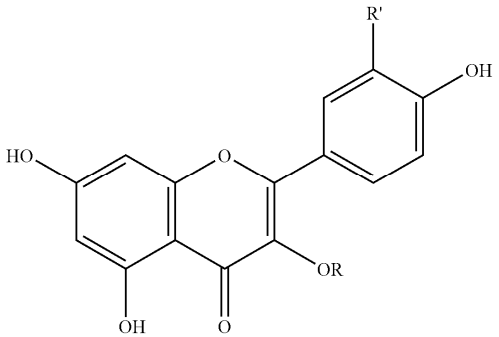
Hydrolysis of methanol extract, ethyl acetate and butanol fractions was performed by a known procedure [3].

For HPLC analysis all samples were dissolved in a methanol to obtain a final concentration of 1.0 mg mL^{-1} for standards and hydrolysates, and 5.0 mg mL^{-1} for extracts and fractions. Prior to injection, the sample solutions were filtered through a 0.45 μm membrane PTFE filter (Rotilabo-Spritzenfilter 13 mm, Roth, Karlsruhe, Germany). HPLC analysis was performed on an Agilent, Zorbax Eclipse XDB-C18, 5 μm, 4.6×150 mm column, by using a liquid chromatograph (Agilent 1200 series), equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies), a quaternary pump, an online vacuum degasser, an auto sampler and a thermostated column compartment, at a flow-rate of 0.5 mL min^{-1}. Gradient elution was performed by varying the proportion of solvent A (0.27 M formic acid in water,) and solvent B (methanol) as follows (v/v): initial 70 % A; 0-5 min, 70-30 % A; 5-20 min, 30-10 % A; 20-25 min. The column temperature was 25 ºC. The injected volume was 5 μL. The spectra were acquired in the range 190–400 nm and chromatograms plotted at 254 and 350 nm (See Supproting Information). Identification was based on retention time and coinjection of commercial standards (quercetin and kaempferol, Sigma Aldrich) and previously isolated and identified compounds (quercetin and kaempferol 3-*O*-rhamnosides and 3-*O*-glucosides from methanol extracts of examined plant).

Antioxidant assays (DPPH, ABTS, Total reducing power assay Fe^{3+} to Fe^{2+}, determination of total phenolic and flavonoid content) were performed as described previously [4-7], respectively.

The *in vitro* antibacterial activity of the samples against a panel of laboratory control strains belonging to American Type Culture Collection Maryland, USA: Gram-positive bacteria *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhimurium* ATCC 14028 was determined using the disk diffusion assay recommended by National Committee for Clinical Laboratory Standards [8].

Results of the examination of composition, antioxidant and antibacterial activity of *H. spectabile* x *telephium* methanol leaf and flower extracts and, their hexane, ethyl acetate and butanol fractions and, their hydrolysates are shown in the Tables 1 and 2, and Figures 1-4.



Quercetin (**1**): R = H, R' = OH
Quercitrin (**2**): R = α-L rhamnosyl, R' = OH
Isoquecitrin (**3**): R=β-D-glucosyl, R' = OH
Kaempherol (**4**):R = H, R' = H
Afzelin (**5**): R = α-L rhamnosyl, R' = H
Astragalin (**6**): R = β-D-glucosyl, R' = H

Figure 1. Main constituents of *H. spectabile* x *telephium* extracts and their hydrolisates

Quercetin and kaempferol 3-*O*-glucosides and 3-*O*-rhamnosides were identified as major extracts components (Figure 1 and Table 1). Quercetin and kaempferol 3-*O*-glucosides dominate in the leaf extracts while corresponding 3-*O*-rhamnosides prevail in the flower extracts (Table 1). In all samples predominant aglycone is quercetin. Relative ratio quercetin: kaempherol ranges from 3.0 for methanol flower hydrolisate to 1.7 for butanol leaf hydrolisate. Presence of flavonoids not detected in any of the hexane fraction (Figure 2D).

Table 1. Content of main constituents of examined extracts as determined by HPLC.

Sample[a]	Peak area (%)					
	QGlu[b]	KGlu[b]	QRha[b]	KRha[b]	Q[b]	K[b]
Ml/Mhl	15.6/ND	11.7/ND	3.1/ND	ND/ND	ND/18.0	ND/10.0
Mf/Mhf	9.2/ND	2.8/ND	27.2/ND	8.6/ND	ND/41.7	ND/13.7
El/Ehl	22.5/ND	14.7/ND	2.2/ND	ND/ND	ND/40.2	ND/19.6
Ef/Ehf	8.5/ND	13.4/ND	21.56/ND	19.3/ND	ND/20.5	ND/11.4
Bl/Blh	19.9/ND	9.9/ND	ND/ND	ND/ND	ND/34.3	ND/20.5
Bf/Bhf	14.2/ND	4.3/ND	2.4/ND	ND/ND	ND/38.1	ND/16.2

[a] Ml, leaf methanol extract; Mhl, hydrolysed leaf methanol extract; Mf, flower methanol extract; Mhf, hydrolysed flower methanol extract; El, ethyl acetate fraction of leaf methanol extract; Ehl, hydrolysed ethyl acetate fraction of leaf methanol extract; Ef, ethyl acetate fraction of flower methanol extract; Ehf, hydrolysed ethyl acetate fraction of flower methanol extract; Bl, butanol fraction of leaf methanol extract; Bhl, hydrolysed butanol fraction of leaf methanol extract Bf, butanol fraction of flower methanol extract; Bhf, hydrolysed butanol fraction of flower methanol extract.

[b]QGlu, quercetin 3-*O*-glucoside (isoquercitrin); KGlu, kaempferol 3-*O*-glucoside (astragalin); QRha, quercetin 3-*O*-rhamnoside (quercitrin); KRha, kaempferol 3-*O*-rhamnoside (afzelin); Q, quercetin; K, kaempferol.

ND- not detected

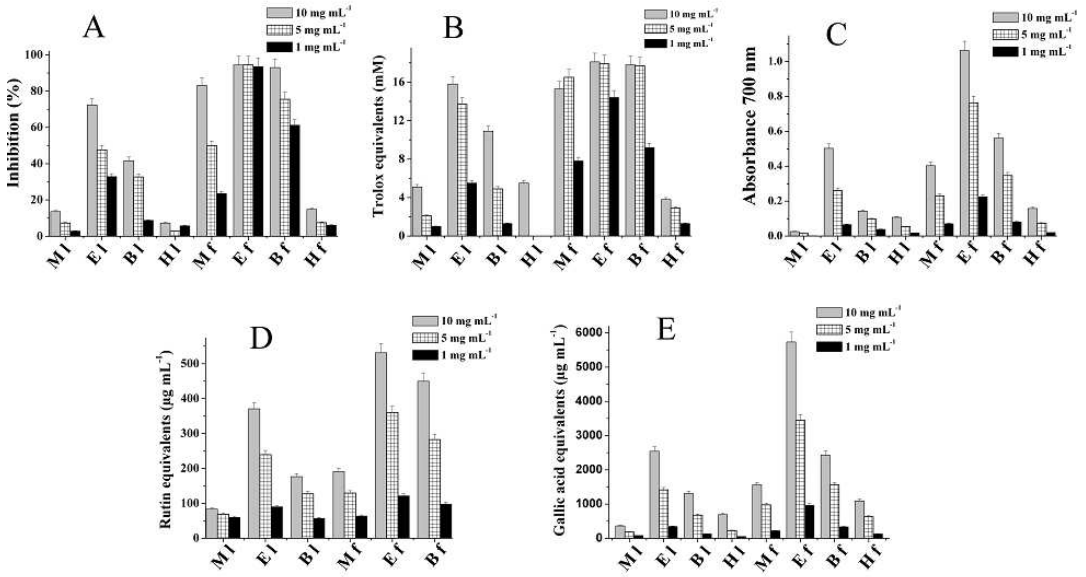


Figure 2. Antioxidant activities of *H. spectabile* x *telephium* extracts: MeOH leaf and flower extracts (Ml and Mf), respectively and their hexane (Hl, Hf), ethyl acetate (El, Ef) and butanol (Bl, Bf) fractions. A- DPPH; B- ABTS; C- Total reducing power; D- Content of flavonoids, and E- Content of phenolics

Regarding the antioxidant activity obtained results indicate that leaf samples have lower activity then corresponding flower samples (Figure 2). Higher samples concentration cause a greater value of the tested activities with the exception of two cases: DPPH assay for hexane leaf fraction and ABTS assay for methanol flower extract. It is important to note that in all three concentrations ethyl acetate flower fraction showed a very high and nearly the same activity against DPPH (around 90%, that is almost double of the activity of rutin and BHT, and in the range of activities of ascorbic acid, gallic acid, its propyl ester and quercetin) (Figures 2A and 4A). Mf, Ef and Bf at concentrations of 10 and 5 mg per mL showed approximately equal activity to $ABTS^{+\cdot}$ as standard antioxidants at a concentration of 1 mg per mL (Figures 2B and 4B). Total reducing power of Ef at concentrations of 10 and 5 mg per mL was higher or equal to reducing power of examined standards, even at a concentration of one mg per mL it was close to reducing power of trolox (Figures 2C and 4C).The order of reducing power of the samples to Mo^{6+} was the same as exhibited activity against Fe^{3+} (Figures 2C and 2E).

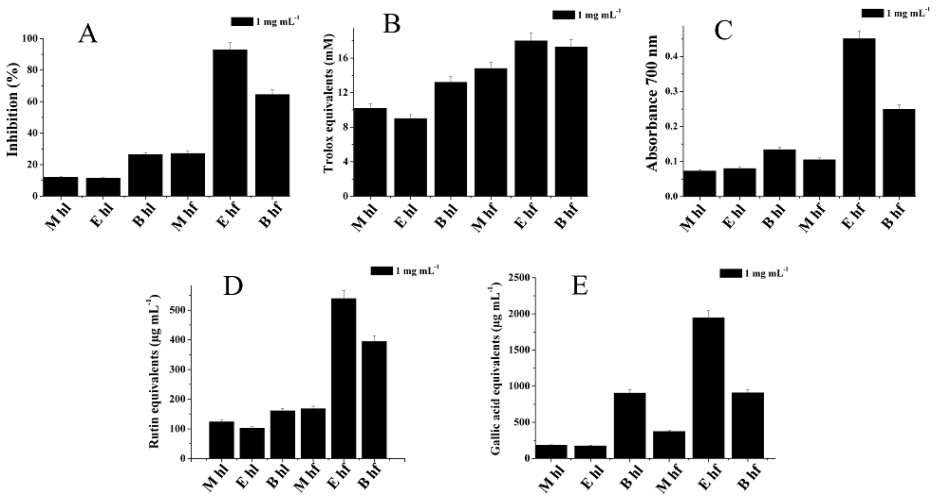


Figure 3. Antioxidant activities of *H. spectabile* x *telephium* leaf and flower extract hydrolysates: MeOH (Mhl, Mhf), EtOAc (Ehl, Ehf) and BuOH (Bhl, Bhf) (respectively). A- DPPH; B- ABTS; C- Total reducing power; D- Content of flavonoids, and E- Content of phenolics

The antioxidant activity of hydrolysates (at concentration of 1mg per mL) was greater or approximate to the activities of the corresponding initial fractions at the same concentration (Figures 2 and 3) with the exception of activity against DPPH radical for leaf EtOAc fraction and its hydrolysate.

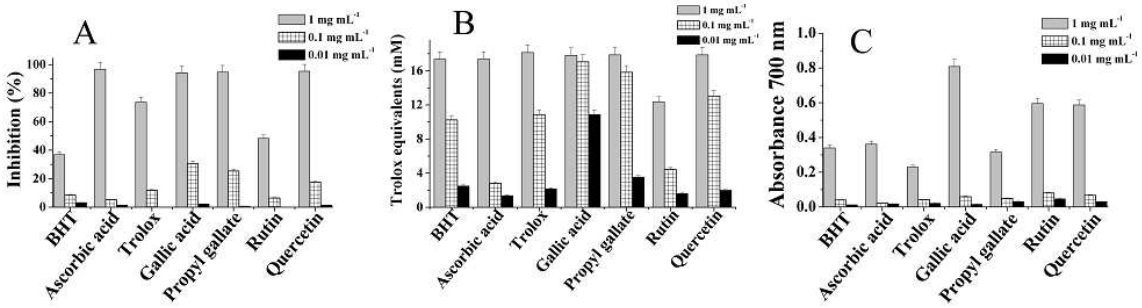


Figure 4. Antioxidant activities of standards (BHT, ascorbic acid, trolox, gallic acid, propyl gallate, rutin, quercetin). A- DPPH; B- ABTS; C- Total reducing power.

In concentration of 1 mg per mL EtOAc fraction and its hydrolisate were more active toward DPPH radical than the commercial antioxidants, BHT, trolox and rutin at the same concentration (Figures 3A and 4A)**.** Both of the above mentioned samples were more active than the rutin in the experiment with $ABTS^{+\bullet}$ (Figures 3B and 4B). Hydrolisate of EtOAc flower fraction possess higher potential to reduce Fe^{3+} to Fe^{2+} than BHT, ascorbic acid, trolox and propyl gallate (Figures 3C and 4C). Leaf EtOAc fraction showed moderate (width of the inhibition zone without diameter disk from 5 to 10 mm [9]) bactericidal activity against *P. aeruginosa, S. typhimurium, S. aureus*, and *B. subtilis*. The same is true for flower EtOAc fraction activity against *S. aureus*. Weak bactericidal activity exhibited BuOH leaf fraction against tested microorganisms excluding *B. subtilis*, which is not affected at all (Table 2).From the below results it seems that quercetin and kaempferol 3-*O*-rhamnosides are better antioxidants than corresponding 3-*O*-glucosides. The reverse is true for antibacterial activity. *Hylotelephium spectabile* x *telephium* flowers are better source of antioxidants than its leaves. In traditional medicine in Serbia, more frequently are used leaves because of their availability during the entire period of the plant life as flowers are only available during the short flowering period. Additionally, their mass representation is significantly lower than leaves mass representation in the aerial parts of the plant.

Table 2. The antibacterial activity of the extracts and fractions of *H. spectabile* x *telephium* (diameters of growth inhibitions zones are measured in mm, including diameter of disk 9 mm).

	Microorganism				
	P. aeruginosa	*E. coli*	*S. typhimurium*	*S. aureus*	*B. subtilis*
Samples[c]	C[a]±SD[b]/S[a]±SD[b]	C[a]±SD[b]/S[a]±SD[b]	C[a]±SD[b]/S[a]±SD[b]	C[a]±SD[b]/S[a]±SD[b]	C[a]±SD[b]/S[a]±SD[b]
Ml	-/-	-/-	-/-	-/12.1±0.2	-/-
El	16.1±0.2/-	13.0±0.2/-	15.0±0.3/-	14.0±0.3/-	17.1±0.4/-
Bl	10.0±0.1/-	10.0±0.1/-	10.0±0.1/-	12.0±0.2/-	-/-
Hl	-/-	-/-	-/-	11.0±0.2/-	11±0.2/-
Mf	-/-	-/-	-/-	-/-	-/-
Ef	-/-	-/-	-/-	14±0.3/-	-/-
Bf	-/-	-/-	-/-	-/-	-/-
Hf	-/-	-/-	-/-	-/-	-/-
A1[d]	24.0±0.5/-	17.0±0.5/23.0±0.5	18.0±0.2/20.0±0.3	23.0±0.4/-	23.0±0.4/-
A2[d]	17.0±0.2/-	24.0±0.6/31.0±0.7	23.0±0.4/32.0±0.8	26.0±0.4/-	30.0±0.6/-
MeOH[e]	-/-	-/-	-/-	-/-	-/-

[a]**C**, bactericidal zone; **S**, bacteriostatic zone.
[b]**SD**, standard deviation (each test was performed in triplicate).
[c]Used mass on all disks of each plant sample were 0.3 mg (30 µL of extract solution at concentration 10 mg mL^{-1}) (**Ml**, leaf methanol extract; **El**, ethyl acetate fraction of leaf methanol extract; **Bl**, butanol fraction of leaf methanol extract; **Hl**, hexane fraction of leaf methanol extract; **Mf**, flower methanol extract; **Ef**, ethyl acetate fraction of flower methanol extract; **Bf**, butanol fraction of flower methanol extract; **Hf**, hexane fraction of flower methanol extract). [d] Used masses on disk of antibiotics were 10 µg for Streptomycin (**A1**) and 30 µg for Chloramphenicol (**A2**).
[e]Solvent, methanol.
- absence of activity.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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