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EVALUATION OF SOME BIOCHEMICAL PARAMETERS OF RAW OF *ARTEMISIA* SPP. (ASTERACEAE BERCHT. & J. PRESL.)

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This study was aimed to investigate the biochemical composition of plant raw material of *Artemisia* spp. in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv) at both budding and flowering stages. It was investigated the following species of *Artemisia* L. genus: *A. abrotanum* L., *A. annua* L., *A. argyi* H. Lev. & Vaniot, *A. austriaca* Jacq., *A. japonica* Thunb., *A. ludoviciana* Nutt., and *A. maritima* L. The content of dry matter determined by measuring till constant weight, the total content of reducing sugars by Bertrand method, tannins with indigo carmine, titratable acidity by titration with sodium hydroxide, and ascorbic acid with 2.6-dichlorophenol-indophenol, and the content of carotene on spectrophotometer with Kalosh petrol. At the stage of budding content of dry matter was from 26.72 (*A. annua*) to 48.63 (*A. maritima*) %, the content of reducing sugars from 5.11 (*A. austriaca*) to 8.93 (*A. maritima*) %, the titratable acidity from 2.06 (*A. abrotanum*) to 3.52 (*A. japonica*), the tannin content from 2.77 (*A. abrotanum*) to 5.1 (*A. ludoviciana*) %, ascorbic acid content from 11.65 (*A. argyi*) to 37.68 (*A. japonica*) mg%, and the content of carotene from 0.09 (*A. ludoviciana*) to 0.56 (*A. abrotanum*) mg%. At the stage of flowering, dry matter in raw was from 31.64 (*A. annua*) to 42.74 (*A. austriaca*) %, the content of sugars from 6.8 (*A. austriaca*) to 8.23 (*A. annua*) %, titratable acidity from 2.8 (*A. abrotanum*) to 4.66 (*A. annua*) %, tannin content from 4.22 (*A. austriaca*) to 6.36 (*A. annua*) %, the ascorbic acid content from 12.93 (*A. abrotanum*) to 65.18 (*A. annua*) mg%, and carotene content from 0.14 (*A. austriaca*) to 0.22 (*A. annua*) mg%. Also, at the period of budding very strong correlation was between titratable acidity and tannin content ($r = 0.824$), moderate correlation between dry matter and sugars content ($r = 0.581$). At the stage of flowering determined a very strong correlation between sugars and tannin content ($r = 0.890$), titratable acidity and tannins ($r = 0.957$), titratable acidity and ascorbic acid content ($r = 0.999$), tannins and ascorbic acid content ($r = 0.966$). In the M.M. Gryshko National Botanical Garden of the NAS of Ukraine just plants of *A. abrotanum*, *A. annua*, and *A. austriaca* passed in the period of flowering. Obtained data can be used for the deep further biochemical and pharmacological study.

Keywords: *Artemisia*, biochemical composition, correlation

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Introduction

Artemisia L. genus belongs to Asteraceae Bercht. & J. Presl. family and concludes more than 200 species (Isani et al., 2019). Species of *Artemisia* spp. exhibited antioxidant, antimicrobial, and anti-inflammatory activities (Pavithra et al., 2018; Moacă et al., 2019). The secondary metabolite artemisinin from *A. annua* L. a unique sesquiterpene lactone demonstrated antimalarial properties (Knudsmark et al., 2014; Czehowski et al., 2019). *A. annua* is an important medicinal plant widely used in Africa for the treatment of malaria and other diseases (Chukwurah Nkachukwu et al., 2014). Also, this species has been used for centuries in Traditional Chinese Medicine. Among 600 phytochemicals identified in *A. annua*, the most dominated are sesquiterpenes, flavonoids, and coumarins (Isani et al., 2019). Some reviews demonstrated the antioxidant activity of *A. annua* but *A. ludoviciana* had a higher value of this parameter (Lutgen, 2018). Also, the antimicrobial activity of *A. abrotanum* described against gram-positive and gram-negative bacteria and *Candida albicans* (Ivashchenko et al., 2014).

Among secondary metabolites of *A. nilagarica* organs determined alkaloids (the most in shoot buds), saponins (the most in stems), steroids (the most in roots), phenols (the most in leaves), etc. (Nganthoi et al., 2016). Powder of *A. annua* content gross energy 3,876.7 kcal/kg, total fat 3.04%, cellulose 27.61%, ash 8.90%, amino acids (aspartic acid 1.77%, glutamic acid 1.74%, leucine 1.32%, threonine 1.26%, arginine 1.02%, rest of amino acids was less 1% of content) (Panaite et al., 2018).

This study aimed to determine the peculiarities of biochemical compound accumulation in raw of different species of *Artemisia* L. genus in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Material and methodology

Biological material

In this study investigated following species of *Artemisia* L. genus: *A. abrotanum* L., *A. annua* L., *A. argyi* H. Lev. & Vaniot, *A. austriaca* Jacq., *A. japonica* Thunb., *A. ludoviciana* Nutt., *A. maritima* L. Plants collected from the experimental collection of Department of Cultural Flora in M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG) at the stage of budding and flowering during 2019–2020. Biochemical analyses were conducted in the laboratory of Department Cultural Flora of M.M. Gryshko National Botanical Garden. All investigated plants are perennial.

Biochemical analyses

Dry matter determination

Plant samples were dried in drying oven at the 105 °C till constant weight in aluminum boxes. Results are given in percentages (Hrytsajenko et al., 2003).

The total content of sugars determination

The total content of sugars was investigated by Bertrand's method in water extracts. 4 g of fresh mass mixed and homogenized with distilled water (approximately 50 ml) in the 100 ml test-tubes and heated in the water bath at 70 °C during 15–20 min. After cooling in the obtained mixtures added 1 ml of the phosphate-oxalate mixture. After this was added 1.5 ml of lead acetate. The obtained mixture brings to the mark (100 ml) with water. After filtration from obtained solution took 50 ml and mixed with 8 ml of 20% HCl (at the 70 °C in a water bath for 5 min) and after cooling was neutralized by 12% NaOH and brought to the mark by distilled water (100 ml). 3 ml of obtained solution mixed with 6 ml of Fehling's solution reagent (6 min boiling in the water bath). Obtained mixture analyzed for the total content of sugars. Results are given by percentages (Hrytsajenko et al., 2003).

The total content of ascorbic acid

Determination of ascorbic acid content conducted by method offered by K. Murri. 2 g of fresh mass mixed with 50 ml of 2% oxalic acid. Obtained mixture put into the dark for 20 min. Content of ascorbic acid of obtained extracts determined by a 2.6-dichlorophenol-indophenol method that based on the reduction properties of ascorbic acid. Obtained results expressed in the mg% DW (Hrytsajenko et al., 2003).

The total content of carotene

The concentration of total carotene determined according to Pleshkov (1985) using extraction with rubber solvent (petrol). 1 g of absolutely dried raw mixed with 20 ml of Kalosha petrol for 2 hours. After this obtained filtrate measured spectrophotometrically at the wavelength 440 nm at the Unico spectrophotometer. Obtained results expressed in mg% DW.

The total content of tannins

The content of tannins was determined with indigo carmine as an indicator (Yermakov et al., 1972). 5 g of fresh mass mixed with distilled water (approximately 50 ml) in 100 ml taste-tubes. Obtained mixture heated in the water bath at 70 °C for 2 hours. After cooling, adding water to the 100 ml and following filtration 10 ml of filtrate used for determination of the total content of tannins. This procedure used 700 ml distilled water and 25 ml of 1% solvent of indigo carmine. Obtained results expressed in %.

The total content of organic acids

The total content of organic acids determined with phenolphthalein and results calculated with a malic acid coefficient (Krishchenko, 1983). 10 ml of filtrate (the same procedure described for the determination of total content of tannins) titrated with 1 N solvent of NaOH in presence of phenolphthalein. Obtained results expressed in percentages.

Statistical analysis

The mean values of three replicates and the standard deviation are given. Data submitted with ANOVA and differences between means compared using Tukey-Kramer test ($\alpha = 0.05$). Correlation analysis performed using Pearson's criterion.

Results and discussion

Our investigation of biochemical composition and antioxidant activity of plant extracts of representatives of Asteraceae, among which *Artemisia dracunculus* L., *Rhaponticum carthamoides* Willd., *Serratula coronata* L., *Scorzonera hispanica* L., *Silphium* spp. have studied (Korablova and Rys, 2012; Andrushchenko et al., 2018; Vergun et al., 2018; Ivashchenko et al., 2019; Rakhmetov et al., 2019; Vergun et al., 2019).

We found the content of dry matter for plants of *Artemisia* spp. from 26.72 (*A. annua*) to 48.63 (*A. maritima*) % at the stage of budding (Figure 1). Sugars are the most important regulators of many physiological processes such as photosynthesis, seed germination, flowering and processes under abiotic stresses (salt, drought, and cold stresses) (Sami et al., 2016). The study of *Triticum aestivum* showed that tolerant genotypes elevated reducing sugars, while susceptible plants had decline sugar content (Khan and Naqvi, 2012). The content of reducing sugars was from 5.11 (*A. austriaca*) to 8.93 (*A. maritima*) %. The titrable acidity values of investigated plants determined from 2.06 (*A. abrotanum*) to 3.52 (*A. japonica*) %.

One of the most important secondary metabolites tannins plays an important role in the growth of plants and act as protective compounds. They characterized by antimicrobial, antihelmintic and protein bypassed effects (Hassanpour et al., 2011). The above-ground part of plants identified tannin content from 2.77 (*A. abrotanum*) to 5.1 (*A. ludoviciana*) % at the stage of budding (Figure 1).

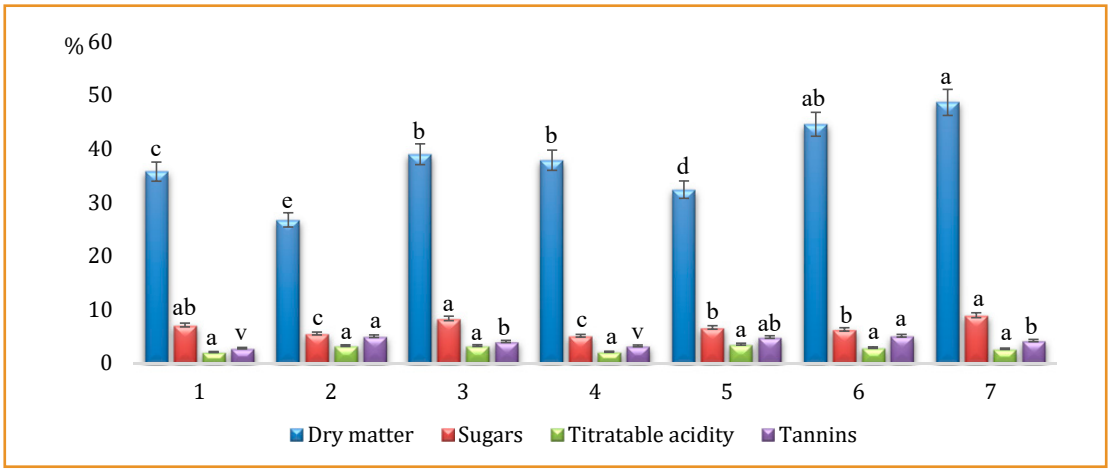

Figure 1 The content of dry matter, reducing sugars, tannins, and titrable acidity in the plant raw material of *Artemisia* L. species at the period of budding
1 – *A. abrotanum*; 2 – *A. annua*; 3 – *A. argyi*; 4 – *A. austriaca*; 5 – *A. japonica*; 6 – *A. ludoviciana*; 7 – *A. maritima* (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD))

According to Iqbal et al. (2012), the content of carbohydrates was 8.3%, fat 6.09%, fiber 14.2%, total tannins 0.61%. Tannins are naturally occurring polyphenol compounds that form complexes with proteins (Singh et al., 2012). As reported Lutgen (2018), the content of

tannins in different organs of various species of *Artemisia* from Turkey, Iran, Algeria varied but leaves of *A. annua* had a higher content of tannins in approximately 10 times. Singh et al. (2012) determined the content of tannins expressed on gallic acid equivalent to 30.44 mg/g in the water extract. The dry matter of *A. annua* powder in an investigation of Panaite et al. (2018) was 88.30%. Also, we determined the accumulation of ascorbic acid and carotene concentration in the above-ground part of the investigated plants (Figure 2).

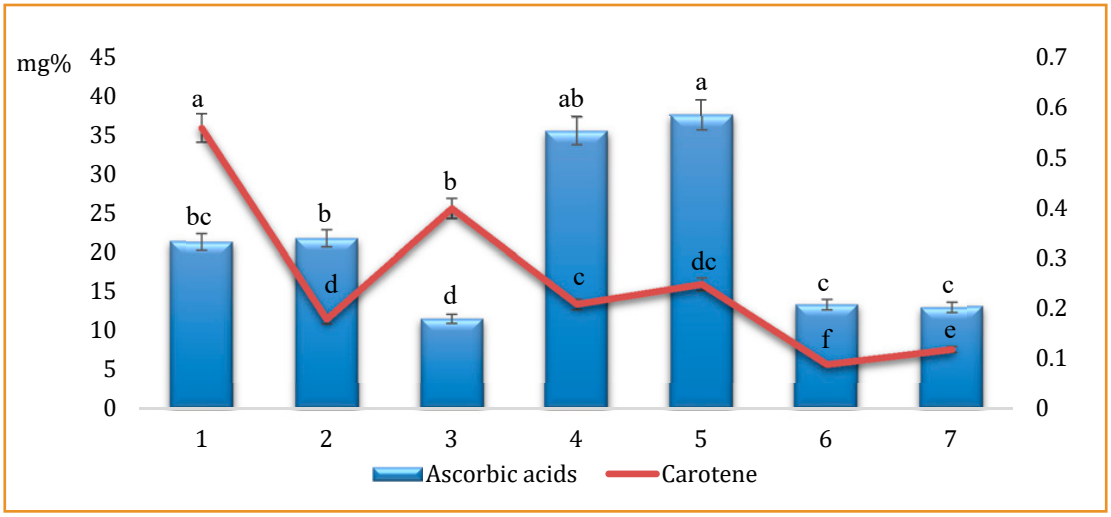

Figure 2 The content of ascorbic acid and carotene in the plant raw material of *Artemisia* L. species at the period of budding
1 – *A. abrotanum*; 2 – *A. annua*; 3 – *A. argyi*; 4 – *A. austriaca*; 5 – *A. japonica*; 6 – *A. ludoviciana*; 7 – *A. maritima* (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD))

Ascorbic acid content at the stage of budding was from 11.65 (*A. argyi*) to 37.68 (*A. japonica*) mg%. We identified the content of carotene from 0.09 (*A. ludoviciana*) to 0.56 (*A. abrotanum*) mg%.

Smoylovska et al. (2010) found the content of ascorbic acid in *A. absinthium* during vegetation 0.035–0.113% (its matches 35–113 mg%). The most content of ascorbic acid determined in the full spring vegetation and budding stage.

Observations on plant growth showed that three species only passed to the next stage of growth (flowering): *A. abrotanum*, *A. annua*, and *A. austriaca*. We found that the content of dry matter of three investigated plant species at the stage of flowering was from 31.64 (*A. annua*) to 42.74 (*A. austriaca*) % (Figure 3).

Content of sugars in our study was from 6.8 (*A. austriaca*) to 8.23 (*A. annua*) %, titrable acidity from 2.8 (*A. abrotanum*) to 4.66 (*A. annua*) %, and tannin content from 4.22 (*A. austriaca*) to 6.36 (*A. annua*) %. It should be noted that dry matter content, sugars content and titratable acidity for *A. abrotanum* at the stage of flowering was approximately the same as at the stage of budding, while the content of tannins at the stage of flowering increased 1.7 times. The

values of these investigated parameters for *A. annua*, *A. austriaca* increased in the stage of flowering comparing with a budding stage.

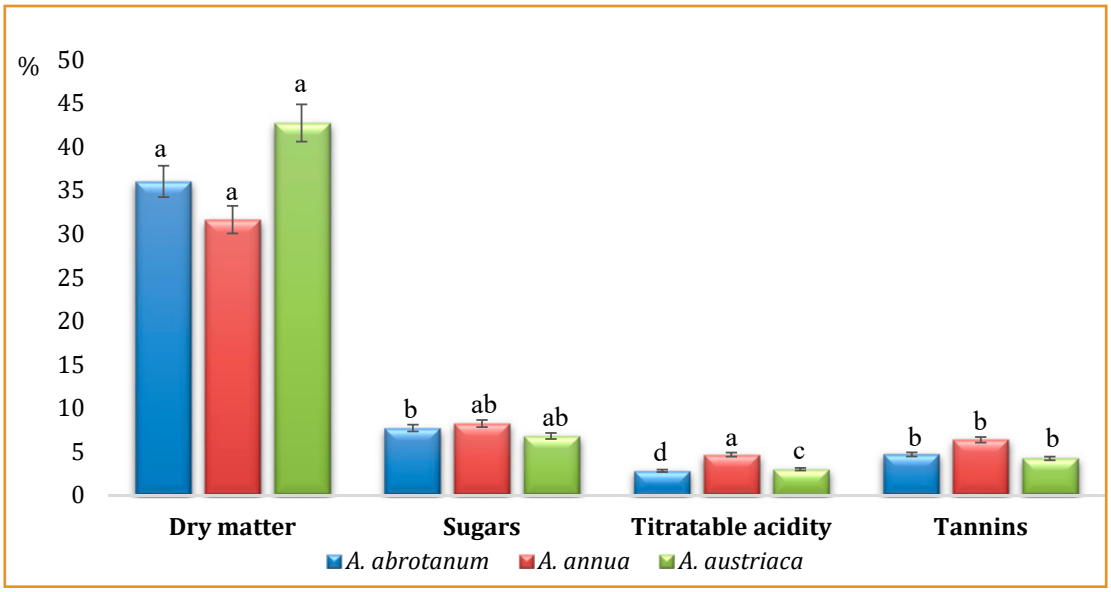

Figure 3 The content of dry matter, reducing sugars, tannins, and titrable acidity in the plant raw material of *Artemisia* L. species at the period of flowering (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD))

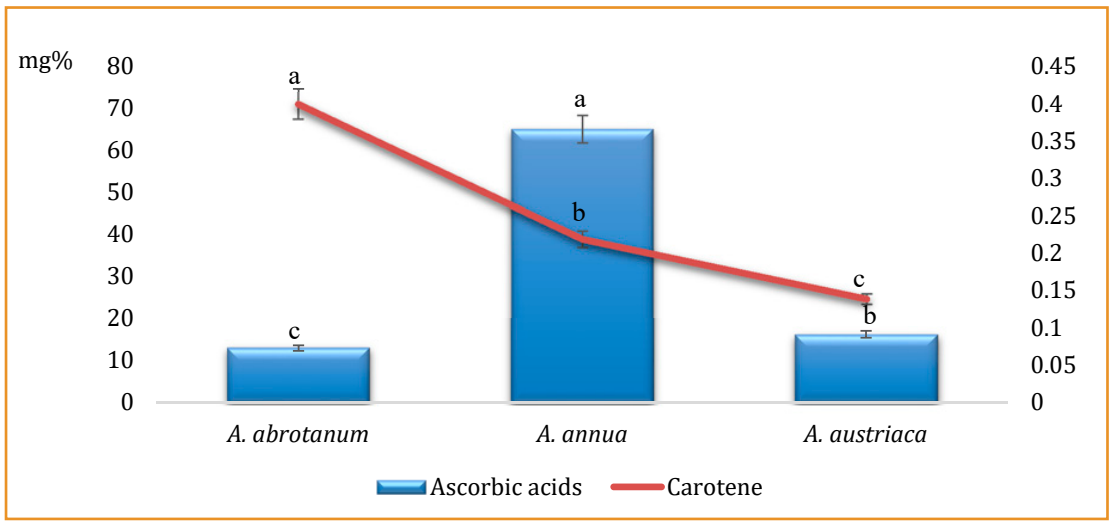

Figure 4 The content of ascorbic acid and ß-carotene in the plant raw material of *Artemisia* L. species at the period of flowering (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD))

The ascorbic acid content in raw of investigated species at the stage of flowering was from 12.93 (*A. abrotanum*) to 65.18 (*A. annua*) mg% (Figure 4). At the stage of flowering carotene accumulated in the range from 0.14 (*A. austriaca*) to 0.22 (*A. annua*) mg%. Content of ascorbic acid and carotene decreased from the budding stage to flowering for *A. abrotanum* and *A. austriaca*, while for *A. annua* increased. Taking into account previous studies with *A. dracunculus*, the highest content of ascorbic acid found in the leaves at the stage of spring vegetation up to 730 mg%, the least content found in stems of this species at the stage of flowering 24.6 mg% (Korablova, 2003).

We also conducted a correlation analysis between the accumulation of investigated compounds in two stages of growth (Table 1). We found that at the period of budding very strong correlation was between titratable acidity and tannin content (r = 0.824), moderate correlation between dry matter and sugars content (r = 0.581), a weak correlation between sugars and titratable acidity (r = 0.127), and very weak correlation was between carotene and ascorbic acid content (r = 0.023).

Table 1 Correlation analysis between investigated parameters of *Artemisia* spp.

Parameter	Dry matter	Sugar content	Titratable acidity	Tannin content	Ascorbic acid content
Budding					
Sugar content	0.581*	1			
Titratable acidity	-0.311*	0.127*	1		
Tannin content	-0.094	-0.081	0.824*	1	
Ascorbic acid content	-0.527*	-0.622*	-0.053*	-0.142	1
ß-carotene content	-0.299	0.202*	-0.278	-0.690	0.023*
Flowering					
Sugar content	-0.999	1			
Titrable acidity	-0.743*	0.720*	1		
Tannin content	-0.905*	0.890	0.957*	1	
Ascorbic acid content	-0.764	0.741*	0.999*	0.966*	1
ß-carotene content	-0.413	0.445*	-0.302	-0.013*	-0.272

Note: significant according to the t-test (p <0.05)

At the stage of flowering determined a very strong correlation between sugars and tannin content (r = 0.890), titratable acidity and tannins (r = 0.957), titratable acidity and ascorbic acid content (r = 0.999), tannins and ascorbic acid content (r = 0.966). Strong correlation found sugars and titratable acidity (r = 0.720), sugars and ascorbic acid content (r = 0.741). The moderate correlation found between sugars and carotene (r = 0.445). Rest relations determined as negative correlated.

Conclusions

Taking into account obtained data of the biochemical composition of *Artemisia* L. spp., it should be noted that in the M.M. Gryshko National Botanical Garden of the NAS of Ukraine just plants of *A. abrotanum*, *A. annua*, and *A. austriaca* passed in the period of flowering. Investigation of the biochemical composition of *Artemisia* species showed a very strong correlation between titratable acidity and tannin content in both budding and flowering stages, sugars and tannin content and ascorbic acid with tannin content and ascorbic acid with titratable acidity. The most content of dry matter and sugars at the budding stage was maximal in raw of *A. maritima*, titratable acidity and ascorbic acids in raw of *A. japonica*, tannin content in raw *A. ludoviciana*, carotene content in raw of *A. abrotanum* (as well as in flowering stage). At the flowering stage, the high content of dry matter found in raw *A. austriaca*, the total content of sugars, tannins, ascorbic acid, and titratable acidity in raw *A. annua*. Obtained data can be used for the deep further biochemical and pharmacological study.

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