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Seasonal dynamic of main classes phenolics and radical scavenging activity of invasive tree Ailanthus altissima extracts

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Abstract. Aerial herbal substances from *Ailanthus altissima* were the subject of the present study, which aimed to follow the dynamics in the accumulation of bioactive compounds with antioxidant potential during vegetation, namely polyphenols, flavonoids, phenolic acids, and tannins quantified by pharmacopoeial methods. Aqueous and ethanolic extracts from dry plant material (under reflux and heating at 200°C) and ethanolic extracts from fresh plant material (under vacuum) were prepared to determine the optimal conditions for the extraction process of the antioxidant active components. Radical scavenging activities (according to ABTS and DPPH in vitro assays) were found for all tested extracts. Flower buds and flower extracts demonstrated the best results, followed by leaves and stem bark, which correlated with the total polyphenols and flavonoid content found. Vacuum extracts of flowers collected at the beginning of flowering were distinguished by the strongest antioxidant potential (3272.28 ABTS mmol TE/g dw and 2125.67 DPPH mmol TE/g dw). The same type of extract is also suitable for stem bark (411.61 ABTS mmol TE/g dw) for which the collection period is the beginning and the end of the growing season. Ethanolic leaf extracts (collected at the beginning flowering stage) with the application of temperature treatment exhibited the best antioxidant potency (504.75 ABTS mmol TE/g dw and 404.72 DPPH mmol TE/g dw) for this plant substance. The results of the present study provide a good basis for future research on *Ailanthus altissima* intending to incorporate it into phyto-preparations with strong antioxidant activity.

Key words: *Ailanthus altissima*, antioxidant activity, extraction conditions, seasonal dynamic of phenolics.

Introduction

Recently, there has been increasing interest in the tree species *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae), which is foreign to the flora of Europe, incl. Bulgaria, but it has a high adaptive and reproductive capacity, which is why it is defined as highly invasive, with a great threat to the natural vegetation (Kowarik & Säumel, 2007; Petrova et al., 2013; Sladonja et al., 2015), but at the same time, it turns out to be a source of valuable bioactive compounds (Kožuharova et al., 2014; Filippi et al., 2019; Al-Hashumi et al., 2018; Caramelo et al., 2021; Li et al., 2021; Andonova et al., 2021a; 2021b; 2023). A look at the medicinal value and benefits to man outlines a more reasonable and useful approach to mastering its invasive nature. One line of research has focused on the species' antioxidant potential. Aissani et al. (2018) reported that extracts obtained from bark and wood (aqueous and methanolic) were rich in phenolic compounds (polyphenols, tannins, and flavonoids) and exhibited antioxidant activity (AOA). Studies on leaves and barks of *A. altissima* revealed the relationship that exists between the total content of phenolic compounds in them and the strength of AOA (Luís et al., 2012). The amounts of phenols in two different leaf extracts, as well as ABTS and DPPH activity, are of scientific interest to Lungu et al. (2016) as well. From the total phenolic and flavonoid contents according to Marinaş et al. (2017), the leaves and flowers of the species were identified as herbal substances rich in phenolic compounds. According to Mohamed et al. (2021), leaf parts would be a reliable antioxidant and cytotoxic product, based on the proven potent reducing power and antiproliferative potential. Albouchi et al. (2013) reported AOA in methanolic extracts of dried leaves and in fresh leaf parts remaining after essential oil distillation. Ethanol extracts of aerial parts of ailanthus (leaves, stem barks, and flowers) have an antioxidant effect determined by ABTS, DPPH, CUPRAC, and FRAP methods, proven in our previous study, where some of their phenolic components were also chromatographically identified – 6 flavonoids and 10 phenolic acids (Andonova et al., 2023). Other authors also focus on the contents in the leaves (Poljuha et al., 2017; 2022) and flowers (Marinaş et al., 2017) flavonoids and phenolic acids, as well as the AOA manifested by them. Compounds from root extracts exhibit potent DPPH radical potential (Shi et al., 2020). A strong AOA for the barks and a weaker one for the leaf parts of *A. altissima* was investigated by Tanasković et al. (2021). Its fruit extracts reduce oxidative stress damage, exerting a beneficial effect on the treated with H_2O_2 cells (Mo et al., 2021). A carotenoid fraction from stem barks (Zhelev et al., 2016) and wood-water extracts (Abidi et al., 2017; Aissani et al., 2018) also exhibit the indicated biological activity. As seen from the review, the invasive species *A. altissima* contains phytochemicals with antioxidant potential that are known to have multiple benefits for human health. Various scientific studies have analyzed the amounts of total phenolic compounds in *A. altissima*, but there is a lack of data regarding their accumulation during the growing season. Such information would be valuable and essential as it would indicate when is the most suitable time to collect the herbal substances.

All this motivates us to expand research in this direction and set the goal of the present study, namely to track the dynamics of accumulation of main classes of phenolic compounds (polyphenols, tannins, flavonoids, and phenolic acids) for three consecutive growing seasons, as well as to the optimal conditions for extracting the antioxidant active components from leaves, stem bark, flower buds, flowers and fruits (samaras) from *Ailanthus altissima*.

Materials and Methods

Plant material collection

The plant material (leaves, stem barks, flowers, flower buds, and fruits) of *A. altissima* (average samples of ten different individuals) was collected in three consecutive growing seasons (April to September of 2019 – 2021), in the region of the city of Plovdiv, park Lauta (42°08'19"N, 24°46'59"E). The species was identified in the Department of Botany and Biological Education of the University of Plovdiv and a herbarium specimen of it was deposited under number 063263 in the Herbarium of the University of Agriculture (SOA), Plovdiv, Bulgaria. To track the dynamics of the accumu-lation of the main classes of phenolic compounds, the materials were collected as follows: leaves and stem barks - monthly, flowers – in the phase of flower buds and full bloom, and fruits – before their full ripening. A part of the collected samples for

analysis were dried at room temperature (in a ventilated place without direct access to sunlight), ground with the help of an electric grinder, and stored in dark paper bags or suitable plastic banks. Another part was crushed fresh and used to obtain ethanolic extracts.

Preparation of herbal extracts

Extracts of dry plant material (under reflux). Dried and ground plant samples (2.5 g) were extracted with the appropriate extractant (water or 70% ethanol; at a hydro module of 1:20), under heating (200ºC), under a magnetic stirrer, for 30 min. The biomass was removed by filtering through filter paper with a pore size of 5-8 μm. Test extracts were prepared immediately before analysis.

Extracts of fresh plant material (under vacuum). Crushed plant samples (100 g) of fresh flowers, leaves, and stem barks were extracted with 1L ethanol (96% Carl Roth, Germany) for 10 days with periodic stirring. The extracts thus prepared were filtered (Whatman filter No. 1) and dried under vacuum (Buchi rotary vacuum evaporator, Rotavapor R-300) at 50°C and 97 mbar. Storage of samples before analysis was in glass vials, at 4°C.

Quantitative spectrophotometric methods (SPM) for determination of main classes of phenolic compounds

Total phenolic compounds (total polyphenols and tannins). Their determination is carried out according to the methodology of the European Pharmacopoeia (Ph. Eur. 10.0, 2019a). The analysis is based on a reaction with Folin & Chiocalteu's phenol reagent (Sigma Aldrich-Chemie GmbH, Switzerland), sensitive to reducing compounds, including polyphenols. The intensity of the resulting blue color of the reaction medium (solution of sodium carbonate, Rai-him, Plovdiv, Bulgaria) is linearly dependent on the amount of reducing substances and is measured spectrophotometrically at λ=760 nm, after 30 minutes of stabilization of the reaction. The method measures the percentage content of total polyphenols and those that are not adsorbed by hide powder when using an aqueous solution of pyrogallol as a standard. Distilled water is used as a compensating solution for spectrophotometric readings. The percentage of tannins expressed as pyrogallol is calculated according to a formula given in the Pharmacopoeia.

Total flavonoids. A method according to the Pharmacopoeia of Russia (XI edition, 1990) was applied, which accounts for the intensity of the color complex in the interaction of flavonoids in herbal substances with $AlCl_3$ (Rai-him, Plovdiv, Bulgaria). The measurement of the formed color complex was at wavelength λ=430 nm, after 30 min stabilization of the reaction medium. The quantitative content of the flavonoid components was calculated as quercetin equivalents in percentage.

Total phenolic acids. Their quantitative content was followed by the European Pharmacopoeia (Ph. Eur. 10.0, 2019b). The basis of the method was the spectrophotometric measurement of the formed color complex between hydroxycinnamic derivatives present in the plant sample and aqueous solutions of $NaNO_2$ (Valerus, Sofia, Bulgaria) and $Na_2MoO_4.2H_2O$ (99.5%, Valerus, Sofia, Bulgaria). The absorption measurement was immediately after mixing the reagents at the wavelength λ=505 nm. Also, in the third applied method, the quantitative values of the measured total phenolic acids were expressed as a percentage.

Assays for determination of antioxidant activity

DPPH assay. The antioxidant activity determination of extracts with the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma –Aldrich, Germany) scavenging activity assay was used procedure described by Thaipong et al. (2006) with slight modifications. Pre-prepared DPPH in methyl alcohol (anhydrous, LC, Macron, Poland) was added to the samples. In a blank sample, the same amount of water. The reaction mixtures were incubated at 37°C. The measurements were made spectrophotometrically at λ=517nm against methanol. A calibration curve with different Trolox concentrations (in the range of 0.045 – 0.9 mmol) was used to represent the results.

ABTS assay. Dissolved in water 2,2-azinobis (3)-ethylbenzothiazoline 6-sulfonic acid (ABTS, Sigma –Aldrich, Germany) and $K_2S_2O_8$ (≥ 99.0%, Sigma –Aldrich, Germany) are mixed and placed in the dark for 12 hours. A generated ABTS radical was diluted to methanol. The extracts were added to the ABTS solution and after incubation for 15 min at 37°C were measured spectrophotometrically at λ= 734 nm against methanol. The

water was used for the blank samples. The performance of the result was like Trolox equivalent antioxidant capacity (Re et al., 1999).

Statistical analysis

The data for phenolic compounds accumulation in different plant organs and respectively the antioxidant activities of the corresponding extracts was analyzed using the one-way ANOVA test. Means comparison was performed using Tukey's HSD test (Statistics Kingdom, 2017). Superscripts indicate significant differences between samples at a confidence level of $P \leq 0.01$. All measurements were performed in triplicate and presented as means with corresponding standard deviations (SD).

Results and Discussion

Quantitative spectrophotometric determination of total water-soluble polyphenols, tannins, flavonoids, and phenolic acids in aerial substances of A. altissima.

The dynamic of the accumulation of different classes of phenolic compounds in leaves and stem barks in three consecutive growing seasons (from April to September 2019-2021) was monitored, as well as their content in the generative organs - flower buds, flowers, and fruits. The results obtained are presented in Fig. 1 and Fig. 2. The quantitative data of the monthly determined total polyphenols in leaf substances showed maximum values in May - July (4.12 – 5.08% for 2020; 4.87 – 5.71% for 2019 and 5.39 – 6.72% for 2021), in the period of the most active photosynthesis (Fig. 1a). A well-defined peak at the end of the growing season (month of September – 1.19%, 1.24% and 1.78%) was observed for barks – Fig. 1e. Of the three analyzed generative parts, the richest in polyphenols were flower buds (over 5%), followed by flowers and samaras (Fig. 2a). All investigated parts in terms of the content of total polyphenols at the peak of their accumulation were arranged in the following descending order: flower buds< leaves< flowers< samaras< stem barks.

The amounts of tannins determined in the herbal substances of ailanthus also follow the arrangement mentioned above (Fig. 1b, f; Fig. 2b). It gives the impression that the tannins in the leaves showed a gradual increase during the growing season and reached maximum values in September (between 3.38% in 2020 and 4.16% in 2021), which were almost twice as high in comparison with the beginning of the season (Fig. 1b). Similar to total polyphenols, the amounts of tannins were highest in flower buds, i.e. at the beginning of flowering (3.74 – 4.42%), after which they decrease in flowers and fruits (about 2.6%). The barks of ailanthus accumulate tannins between 0.1 – 0.6%, the highest amount reported in all three years being towards the end of the vegetation period, in September (Fig. 1f).

Total flavonoids were best represented in the leaves of all the studied substances from ailanthus (Fig. 1c, g; Fig. 2c). The dynamics of the accumulation of this group of compounds showed higher amounts in the phase of young leaves and up to the initial phase of flowering. At the end of the growing season, in August and September, flavonoids decrease by almost half (3.48 – 1.85 for 2019; 2.7 – 1.74 for 2020, and 4.04 – 2.43% for 2021, respectively) (Fig. 1c). The content of flavonoids in the investigated generative organs was lower than that in the leaves and was in the following descending order: flowers>flower buds>samaras (Fig. 2c). Well-developed flowers showed about 1.7% flavonoids on average for three years, while in the case of fruits, it decreases to 1.1% on average. The considered group of compounds was very poorly represented (0.1 – 0.17%) in ailanthus bark (Fig. 1g).

The content of phenolic acids in the leaves varied between 1.4 and 2.5%, showing a gradual increase during the growing season, with a peak in the months of July-August (fruiting phase), followed by a decrease in September (Fig. 1d). In flower buds, flowers, and samaras, total phenolic acids range around 1% (maximum 1.4% in flower buds) - Fig. 2d. In the stem barks of ailanthus, the accumulation of phenolic acids varies slightly during the active season and ranges from 0.2 to 0.3% (Fig. 1h).

The review of the literature showed that mostly total polyphenols and flavonoids were determined, less often tannins, and those for total phenolic acids were missing. A detailed study of the role of the phenological phase in the accumulation of total polyphenols, flavonoids, phenolic acids, and tannins in aerial substances of *A. altissima* has not been reported so far.

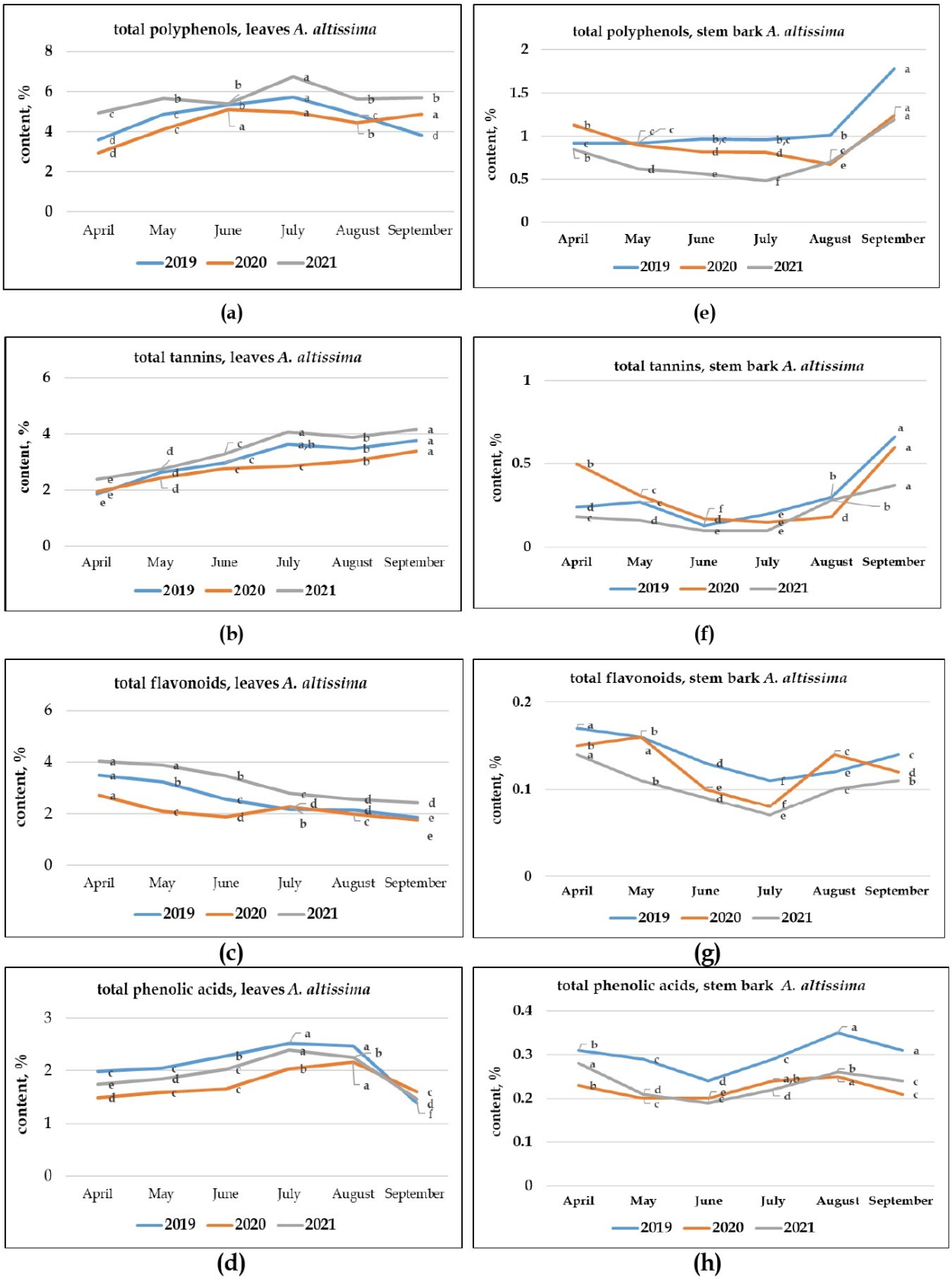

Fig. 1. Dynamic of accumulation of major classes' phenolic compounds in leaves (a, b, c, d) and stem bark (e, f, g, h) of *A. altissima* for tree vegetation seasons (2019-2021). The samples were analyzed in triplicate, and the statistically processed results were presented. Different letters indicate significant differences according to Tukey's test ($p < 0.01$).

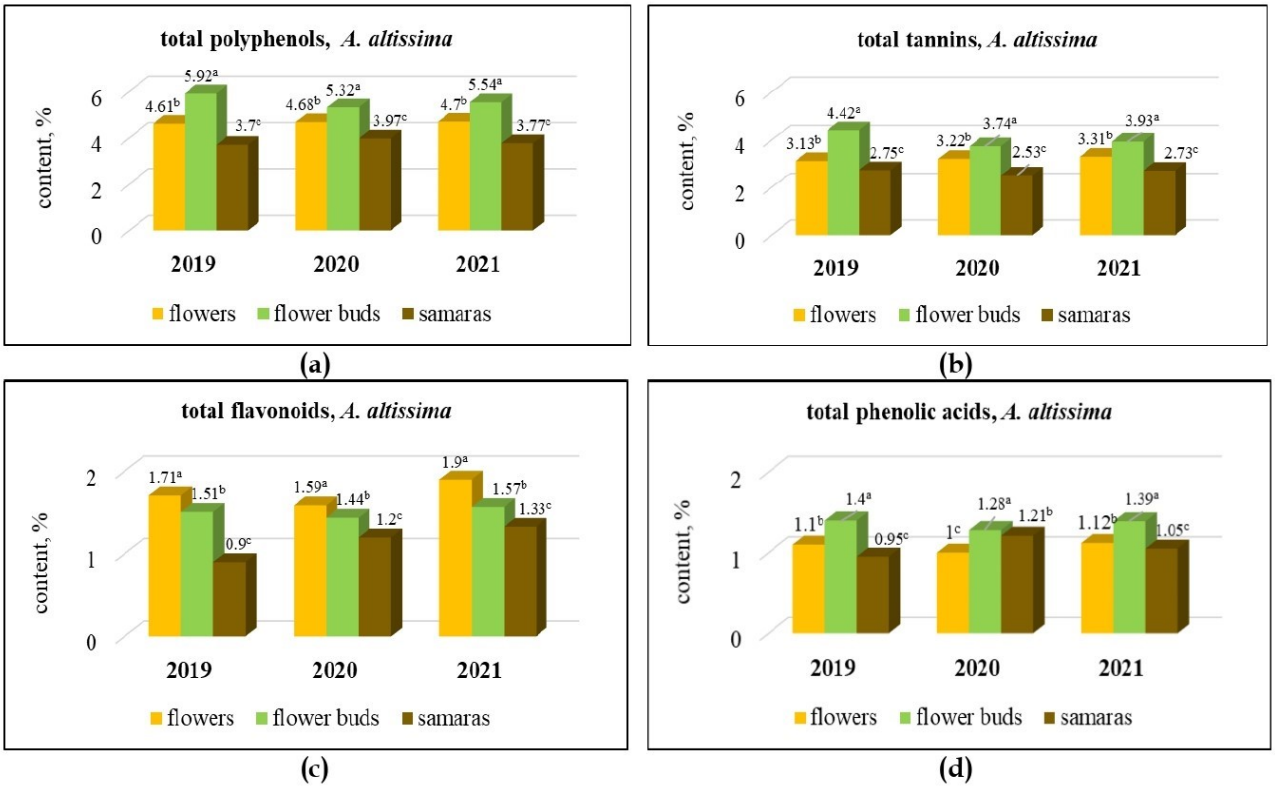

Fig. 2. Dynamic of accumulation of polyphenols (a), tannins (b), flavonoids (c), and phenolic acids (d) in generative organs of *A. altissima* for tree vegetation seasons (2019-2021). Samples were analyzed in triplicate and results were expressed as mean values.-Different letters indicate significant differences according to Tukey's test ($p < 0.01$).

Mohamed et al. (2021) investigated different leaf fractions of ailanthus and found that the ethyl acetate fraction was the richest in phenolics and flavonoids (551.72 mg GAE/g dw; 371.24 mg RE/g dw respectively). Close to these results are those obtained by Rahman et al. (2009) for certain total phenolics (122.53 mg GAE/g dw) in the leaf fraction obtained with the same solvent. Albouchi et al. (2013) also work with leaf substances, where for their methanolic extract the values of total polyphenols are 119.84 mg GAE/g dw, and for flavonoids – 0.97 mg QE/g dw. Unlike the last-mentioned authors, according to Poljuha et al. (2017) the values of the two phenolic groups were significantly higher (247 mg GAE/g dw and 57 mg QE/g dw for polyphenols and flavonoids, respectively). According to Jamous et al. (2015), the fruits (methanol extracts) contain more polyphenols (5.85 mg GAE/g dw) compared to the leaves (2.24 mg GAE/g dw), while the flavonoids in them are much lower and close in value (0.43 mg QE/g dw in fruits; 0.58 mg QE/g dw in leaves). For comparison our data showed that in a period of well-formed leaves, they content the higher values of total polyphenols and flavonoids then the fruits. The observed difference could probably be explained by the different solvents used (methanol in their case, water in ours) as well as the period of collection of these herbal substances. Aissani et al. (2018) investigated a higher content of total phenols in aqueous extracts of wood and bark (48.91 mg/g and 39.45 mg/g dw, respectively), as well as of total tannins – 28.03 mg/g and 17.27 mg/g dw (for wood and bark, respectively) compared to their methanolic extracts. The flavonoids in the aqueous extract again exceed those in the methanolic extract (31.20 mg/g in wood aqueous extract, and 25.89 mg/g in bark aqueous extract). Other studies prove a high content of total polyphenols and flavonoids in extracts of leaves, followed by stems and stalks, obtained using

different extractants (methanol, ethanol, acetone, and hydro-alcohol) (Luis et al., 2012). The total polyphenol content for leaves obtained by the cited authors was 2 to 3 times higher than for stems, and which was true for all solvents. In the present study, the polyphenols in the leaves have close values to those found by the above-cited author, but in the months of flowering and initial fruiting, they are several times higher (up to 14 times). Lungu et al. (2016) also found a high content of flavonoids and total polyphenols in two types of leaf extracts (reflux and ultrasound), to which compounds they attributed their good antioxidant capacity. Tanaskovic et al. (2021) studied the total polyphenols and tannins in 70% ethanolic dry extracts of barks and leaves of ailanthus, finding higher amounts in the barks (9 times) compared to those found in the leaves, in contrast to all the above-mentioned authors, including in the present study. High amounts of total polyphenols (of the order of 24.66 mg GAE/g dw for leaves and 17.94 mg GAE/g dw for flowers), as well as total flavonoids (6.20% for flowers and 7.39% for leaves as a percentage of total phenolic compounds) also report Marinaş et al. (2017). Their several times higher results compared to ours for leaves (1-4 times) and flowers (more than 3 times), could be explained by the different applied methodology, the different habitat conditions, and other factors.

Antioxidant activity assays

The radical scavenging activity of *A. altissima* extracts was investigated to trace the role of the plant material, the method of extraction, and the type of solvent used. ABTS and DPPH assays were applied to the obtained water and ethanol plant extracts from dry and fresh herbal substances. The results of the measured AOA of the different extracts from *A. altissima* are presented in Table 1. The data showed that all extracts exhibited free radical scavenging activity, which was more pronounced for the ABTS+ radical cation than for the free DPPH● radical. In the water extracts, the highest values were observed in flower buds (1548.76ABTS and 932.11DPPH mmol TE/g dw), followed by flowers (803.76ABTS and 721.61DPPH mmol TE/g dw), leaves and stem bark, and for the last substance the activity was about 18 times lower than that of flower buds. Ethanol extracts were found to have the same strongest activity for flowers and flower buds (about 1020ABTS mmol TE/g dw and 960DPPH mmol TE/g dw), 2 times lower for the leaves and again the lowest for the stem bark (Table 1.). As can be seen from the table, the values for stem bark do not differ from those obtained for aqueous extracts, while for leaves and well-developed flowers, the extractant matters (better effect for ethanolic ones).

In the case of vacuum extracts, the highest values were recorded for flower parts (by both methods). For stem bark (only by ABTS), compared with the other two types of extracts (Table 1.). The leaf vacuum extracts did not show a better antioxidant power than the water especially the ethanol samples of dry substances.

When comparing the three ways of obtaining the extracts, the most complete extraction of the antioxidant components from flower parts and stem barks of *A. altissima* was obtained with the vacuum extracts from fresh plant parts, while for the leaves - with extracts, subjected to high-temperature treatment and use of ethanol as a solvent, which allows for better extraction of biologically active substances.

The ability to neutralize free radicals (radical scavenging activity) of herbal substances of ailanthus is also reported by other scientific studies. Studies on leaf extracts and their fractions reported the highest for ethyl acetate (Rahman et al., 2009), and a moderate one for extracts obtained from stems and branches (Luís et al., 2012). Lungu et al. (2016) reported many times lower activity of leaf extracts from dried material (8.93 mmol TE, and 0.155 mmol TE for ABTS and DPPH, respectively), using a similar methodology (under reflux) and solvent 70% ethanol. This confirms, that the type of plant material (fresh or dry) and the extraction condition (with or without heat treatment), are important for the strength of the antioxidant activity. According to Poljuha et al. (2017) quantitative values for ABTS (51.8 mmol TE/100g dw) are relatively close to those for DPPH (48.8 mmol TE/100g dw) for the aqueous leaf extracts, which was also demonstrated in our analyses. Regarding the obtained stem barks, Tanasković et al. (2021) reported higher AOA of these plant parts compared to leaves. At the same time, however, in their studies, the ABTS method had higher values than DPPH. Higher AOA on all three applied by Aissani et al. (2018) test (ABTS,

DPPH, and IRC – iron reducing tests) shows an aqueous extract of the wood compared to that obtained from the bark of the plant species. Albouchi et al. (2013) demonstrated a different potency of AOA, where methanolic extracts of dried leaves showed many times lower activity compared to their hydrodistilled residues from fresh parts.

Table 1. *In vitro* antioxidant activity of *A. altissima* extracts. Samples were analyzed in triplicate and results were expressed as mean ± standard deviation. Statistical analysis is Tukey's test ($p < 0.01$).
[1] Trolox equivalent per gram of dry weight.

Plant Extract	ABTS-Assay, mmol TE/g dw[1]	DPPH-Assay, mmol TE/g dw
aqueous		
Leaf	399.59±4.00^{c}	361.92±1.13^{c}
Stem bark	81.78±1.51^{d}	52.19±2.51^{d}
Flower	803.76±2.24^{b}	721.61±15.0^{b}
Flower buds	1548.76±3.17^{a}	932.11±1.54^{a}
70% ethanol		
Leaf	504.75±1.66^{b}	404.72±1.66^{c}
Stem bark	80.26±2.01^{c}	52.54±1.60^{d}
Flower	1022.62±2.03^{a}	961.51±1.50^{a}
Flower buds	1018.62±2.21^{a}	953.16±2.53^{b}
under vacuum		
Leaf	392.01±1.53^{c}	52.96±1.00^{b}
Stem bark	411.61±2.53^{b}	53.19±2.05^{b}
Flower with flower buds	3272.28±2.13^{a}	2125.67±1.07^{a}

The existing close relationship between established levels of bioactive phenolic compounds and the potency of AOA has been pointed out by numerous authors who have worked on this plant species (Rahman et al., 2009; Luís et al., 2012; Poljuha et al., 2017; Andonova et al., 2023). The radical scavenging activity of leaf and flower extracts in the present study confirms the above relationship. When compared to our previous work on *A. altissima* (Andonova et al., 2023), where we used a different method of obtaining the extracts (triple extraction at 70°C), the biggest difference in ABTS scavenging activity was observed in the substances from stem barks (in the range of 2.6 - for water and ethanol extracts of dry substances, up to 13 times more - high values – for the vacuum extracts of fresh substances). In aqueous, ethanol, and vacuum extracts of flower parts in the present study, about 3.6 times higher ABTS assay values were obtained compared to our previous data, and about 1.3 times higher for leaf extracts. The greater extraction power of the antioxidant-active components in the present analysis indicates that the applied method with a single extraction of the herbal substances (at 200°C) is more suitable compared to the method applied at the triple extraction (at 70°C). High extraction temperature was indicated as more suitable also in the studies of Luís et al. (2012) and Lungu et al. (2016). Regarding the DPPH radical scavenging ability, no significant differences were found in the different ways of obtaining the extracts.

Conclusions

In conclusion, this is the first study on the seasonal dynamics (over three consecutive seasons) in the accumulation of major classes of phenolic compounds in herbal substances from *Ailanthus altissima*. Flowers and leaves accumulated the highest concentrations of bioactive components, and the most suitable period for their collection was the phase of initial flowering. This period can be longer for leaves - up to the fruiting phase. Expectedly, the appropriate collection period for stem bark occurs at the beginning or the end of the growing season. Samaras accumulated good amounts of phenolics before full ripening.

The radical scavenging potential was demonstrated for all ailanthus extracts tested by the ABTS and DPPH methods. Still, the effect was

strongest in flower buds and flowers, followed by leaves, which correlated with the total polyphenol and flavonoid contents. Ethanol was found to be a better solvent for compounds with antioxidant properties. For flower parts, better extraction of antioxidants occurs from fresh material and vacuum drying, while for leaves - from dried material, under temperature extraction.

The obtained results confirmed the significant antioxidant potential of herbal substances from *A. altissima* and clarified the period of their collection and the appropriate methods of obtaining the extracts to achieve a stronger effect.

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References

Abidi, A., Aissani, N., Sebai, H., & Khamsa, S. B. (2017). Anti-fibrotic and antioxidant effects of *Ailanthus altissima* wood aqueous extract against bleomycin-induced lung fibrosis in rat. *European respiratory journal*, (50)61, doi: 10.1183/1393003.congress-2017.PA4911.

Aissani, N., Jabri, M. A., Mabrouk, M., & Sebai, H. (2018). Antioxidant Potential and Antimicrobial Activity of *Ailanthus alfissima* (Mill.) Swingle Extracts against *Pseudomonas aeruginosa*. *Journal of Molecular Microbiology and Biotechnology*, 3(1), 3.

Albouchi, F., Hassen, I., Casabianca, H., & Hosni, K. (2013). Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of *Ailanthus altissima* (Mill.) Swingle leaves. *South African journal of Botany*, 87, 164-174. doi: 10.1016/j.sajb.2013.04.003.

Al-Hashumi, H., Al-Khero, N., & Al-daody, A. C. (2018). Investigation of some Carboxylic Acids and Phenolic Compounds of *Ailanthus altissima* Leaves and their its Effect on Italian Cupressus Seedlings Root Rot Fungi. *Rafidain Journal of Science*, 27, 8-18.

Andonova, T. G., Fidan, H. N., Slavov, I. Z., Stoyanova, A. S., & Dimitrova-Dyulgerova, I. Z. (2021a). Phytochemicals and Antimicrobial Potential of Dry Ethanol Extracts from *Ailanthus altissima* - An Invasive Plant Species for the Bulgarian Flora. *Ecologia Balkanica*, 13(2),161-171.

Andonova, T., Dimitrova-Dyulgerova, I., Slavov, I., Dincheva, I., & Stoyanova, A. (2021b). Volatile compounds in flowers, samaras, leaves and stem bark of *Ailanthus altissima* (Mill.) Swingle, growing in Bulgaria. *IOP Conference Series: Materials Science and Engineering*, 1031(1), 012087. doi: 10.1088/1757-899X/1031/1/012087.

Andonova, T., Muhovski, Y., Slavov, I., Vrancheva, R., Georgiev, V., Apostolova, E., Naimov, S., Mladenov, R., Pavlov, A., & Dimitrova-Dyulgerova, I. (2023). Phenolic profile, antioxidant and DNA-protective capacity, and microscopic characters of *Ailanthus altissima* aerial substances. *Plants*, 12(4), 920. doi: 10.3390/plants12040920.

Caramelo, D., Pedro, S. I., Marques, H., Simão, A. Y., Rosado, T., Barroca, C., Gominho, J., Anjos, O., & Gallardo, E. (2021). Insights into the bioactivities and chemical analysis of *Ailanthus altissima* (Mill.) Swingle. *Applied Sciences*, 11(23), 11331. doi: 10.3390/app112311331.

Filippi, A., Petrussa, E., Boscutti, F., Vuerich, M., Vrhovsek, U., Rabiei, Z., & Braidot, E. (2019). Bioactive Polyphenols Modulate Enzymes Involved in Grapevine Pathogenesis and Chitinase Activity at Increasing Complexity Levels. *International Journal of Molecular Sciences*, 20(24), 6357.

Jamous, R., Zaitoun, S., Husein, A., Qasem, I., & Ali-Shtayeh, M. (2015). Screening for biological activities of medicinal plants used in traditional arabic palestinian herbal medicine. *European Journal of Medicinal Plants*, 9(1), 1-13. doi: 10.9734/EJMP/2015/17429.

Kowarik, I., & Säumel, I. (2007). Biological flora of central Europe: *Ailanthus altissima* (Mill.) Swingle. *Perspectives in Plant Ecology, Evolution and Systematics*, 8(4), 207-237. doi: 10.1016/j.ppees.2007.03.002.

Kožuharova, E., Lebanova, H., Getov, I., Benbassat, N., & Kochmarov, V. (2014). *Ailanthus altissima* (Mill.) Swingle-a terrible invasive pest in Bulgaria or potential useful medicinal plant. *Bothalia journal*, 44(3), 213-230.

Li,X. ,Li,Y., Ma,S., Zhao,Q., Wu, J.,Duan, L. Xie, Y., & Wang, S. (2021). Traditional uses, phytochemistry, and pharmacology of *Ailanthus*

altissima (Mill.) Swingle bark: A comprehensive review. *Journal of Ethnopharmacology*, 275, 114121. doi: 10.1016/j.jep.2021.114121.

Luís, Â., Gil, N., Amaral, E., Domingues, F., & Duartea, P. (2012). *Ailanthus altissima* (Miller) Swingle: A source of bioactive compounds with antioxidant activity. *BioResources*, 7(2), 2105-2120.

Lungu, L., Savoiu, R., Manolescu, N., Farcasanu, C., & Popa, V. (2016). Phytotoxic and Antioxidant Activities of Leaf Extracts of *Ailanthus altissima* Swingle. *Revista de Chimie*, 67(10), 1928-1931.

Marinaş, C., Dinu, M., Ancuceanu, R., Hovaneţ, V., Oprea, E., Geană, I., & Lazăr, V. (2017). The phenols content and phytotoxic capacity of various invasive plants. *Romanian Biotechnological Letters*, 23(4):13887-13899. doi: 10.26327/RBL2017.61.

Mo, N., Cheng, F., Yang, Z., Shang, F., Liang, P., Shang, F., & Liu, Y. (2021). Antioxidant activity and the potential mechanism of the fruit from *Ailanthus altissima* Swingle. *Frontiers in Veterinary Science*, 8, 784898. doi: 10.3389/fvets.2021.784898

Mohamed, R., El-Wakil, A., Abdel-Hameed, S., El-Hashash, M., & Shemis, M. (2021). Evaluation of total phenolics, flavonoids, and antioxidant and cytotoxic potential of *Ailanthus altissima* (Mill.) Swingle leaves. *Journal of Reports in Pharmaceutical Sciences*, 10(1), 130-136. doi: 10.4103/jrptps.JRPTPS_7_21.

Petrova, A., Vladimírov-Hurban, V., & Georgiev, V. (2013). *Invasive alien species of vascular plants in Bulgaria*. Sofia, Bulgaria: Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 322 p. (in Bulgarian)

Ph. Eur. 10.0, 310 (01/2019a). *Methods in Pharmacognosy*. European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM). Strasbourg: Council of Europe.

Ph. Eur. 10.0, 1602-1603 (01/2019b). *Methods in Pharmacognosy*. European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM). Strasbourg: Council of Europe.

Pharmacopoeia of Russia XI edition, 323-325 (1990). Moscow, Russia: Medicine.

Poljuha, D., Sladonja, B., Šola, I., Dudaš, S., Bilić, J., Rusak, G., Motlhatlego, K., & Eloff, J. (2017). Phenolic Composition of Leaf extracts of *Ailanthus altissima* (Simaroubaceae) with Antibacterial and Antifungal Activity Equivalent to Standard Antibiotics. *Natural Product Communications*, 12(10), 1609 – 1612. doi: 10.1177/1934578X1701201021

Poljuha, D., Sladonja, B., Šola, I., Šenica, M., Uzelac, M., Veberič, R., Hudina, M., Famuyide, I.M., Eloff, J.N., & Mikulic-Petkovsek, M. (2022). LC–DAD–MS phenolic characterisation of six invasive plant species in Croatia and determination of their antimicrobial and cytotoxic activity. *Plants*, 11(5), 596. doi: 10.3390/plants11050596.

Rahman, A., Kim, E., & Kang, S. (2009). Antibacterial and antioxidant properties of *Ailanthus altissima* Swingle leave extract to reduce foodborne pathogens and spoiling bacteria. *Journal of food safety*, 29(4), 499-510. doi: 10.1111/j.1745-4565.2009.00172.x.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26(9-10), 1231-1237. doi: 10.1016/S0891-5849(98)00315-3.

Shi, W., Wang, Y., Yan, Z., & Huang, X. (2020). Studies on chemical constituents of *Ailanthus altissima* (Mill.) Swingle and their antioxidant activity. *Asian Journal Traditional Medicines,* 15(6), 320-327.

Sladonja, B., Sušek, M., & Guillermic, J. (2015). Review on invasive tree of heaven (*Ailanthus altissima* (Mill.) Swingle) conflicting values: assessment of its ecosystem services and potential biological threat. *Environmental management*, 56, 1009-1034. doi: 10.1007/s00267-015-0546-5.

Statistics Kingdom. (2017). One-Way ANOVA Calculator and Tukey HSD. Retrieved from: https://www.statskingdom.com/180Anova1way.html (October 30, 2023).

Tanasković, S., Gvozdenac, S., Kolarov, R., Bursić, V., Konstantinović, B., & Prvulović, D. (2021). Antifeeding and insecticidal activity of *Ailanthus altissima* and *Morus alba* extracts against gipsy moth (*Lymantria dispar* (L.), Lepidoptera, Lymantridae) larvae under laboratory conditions. *Journal of the Entomological*

Research Society, 23(3), 197-212. doi: 10.51963/jers.v23i3.1989

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of food composition and analysis*, 19(6-7), 669-675. doi: 10.1016/j.jfca.2006.01.003.

Zhelev, I., Georgiev, K., & Dimitrova-Dyulgerova, I. (2016). Carotenoid profile of *Ailanthus altissima* stem bark, in-vitro antioxidant and antineoplastic activities. *World Journal of Pharmaceutical Research*, 5(3), 1816-1825.

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