Doctoral School of Engineering and Mathematics

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SUMMARY OF THE PhD THESIS

RESEARCH REGARDING THE VALORIZATION OF AUTOCHTHONOUS INVASIVE PLANTS FOR OBTAINING BIOACTIVE INGREDIENTS AND COLORANTS

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Keywords: ***Ailanthus altissima*, *Rhus typhina*, extraction, biologically active compounds, antioxidant activity, biological and cytotoxic properties, natural dyeing**

INTRODUCTION

The relevance and requirement of the topic

Even though textile industry uses large amounts of water and energy, it represents one of the most relevant and important industrial sectors. In recent years, scientific communities showed an increasing preference regarding the use of natural dyes which, although they produce faded colors that are often difficult to reproduce, these are safer for the environment and consumer health than synthetic dyes, which bring risks of pollution and development of serious diseases such as cancer. Various plant species, native or invasive, due to their content of pigments such as anthocyanins, flavonoids, carotenoids, betalains etc., represent sources of natural dyes. Widespread availability of invasive plants produced by their spread strategies and the presence of limited methods of effective control for them, has led to them being seen as an important resource of raw materials with uses in medicine and synthesis of nanoparticles, dyes and other products based on their highly bioactive compounds.

This doctoral thesis focused on two woody invasive species found in Romania, namely *A. altissima* (Tree of Heaven) and *R. typhina* (staghorn sumac), under the following aspects: the use of different conventional and modern extraction techniques, dosing of bioactive compounds of interest, optimization of process parameters with the aim of increasing the concentrations of these compounds, evaluation of antioxidant and antimicrobial activities, but also the dyeability of one of these species on a cellulosic textile substrate.

The leaves of *A. altissima* and/or fruits of *R. typhina* were used in extraction processes for which the following parameters were modified: harvesting season, the solvent used, extraction sequence, frequency, incident electric field strength, bead size, bead material, solvent/solid ratio or process time, in order to reduce the degradation of sensitive compounds and decrease resource consumption in increasing the extraction yield.

Novelty of the study

The original character of this thesis consists in the assessment of microwave extraction (MAE) efficiency, performed at low frequencies on leaflets of *A. altissima*, utilization of a sequential extraction technique, recommending the use of an environmentally friendly and polar solvent, optimization of the contents in bioactive compounds using a Box-Behnken experimental design, investigating the efficiency of bead-beating extraction for *R. typhina* fruits, *in vitro* evaluation of citotoxicity for the two invasive species on some cell lines previously unstudied for these plants and on liposomal membranes, testing the ecotoxicity of Tree of Heaven, but also in the valorizing of an aqueous extract from staghorn sumac fruits in the cotton dyeing, through conventional and modern techniques (exhaustion vs. ultrasonication) and meta-mordanting with less commonly used mordants, respectively citric acid and a mixture of iron sulfate and oxalic acid.

1. ANALYSIS OF THE CURRENT STATE OF KNOWLEDGE ON THE APPLICATIVE POTENTIAL OF INVASIVE PLANTS

1.1. General aspects regarding the selection of some raw materials based on invasive plants

Invasive plant species became a considerable threat to sustainability (Ellison et al., 2017) and ecosystem biodiversity (Li et al., 2024), due to their effective dispersal strategies (Mounger et al., 2021), allelopathic substances they secrete (Liu et al., 2021) and environmental changes caused by strong anthropogenic impacts (Argüelles & March, 2022), in Romania the management of their control being realized according to the procedure described in „EU Biodiversity Strategy for 2030” (Comisia europeană, 2020).

Even so their presence is associated with development of allergies (Lazzaro et al., 2018) or allergic bronchitis (Ellison et al., 2017), their high contents in bioactive compounds (Nguyen et al., 2023), the increased tolerance to heavy metals in soil (Afzal et al., 2023) and their compatibility with the principles of circular economy (Pušić et al., 2024), have led to the involvement of their raw material in numerous research studies that have confirmed their usefulness in products such as antimicrobial nanoparticles (Nguyen et al., 2023), biofuels (Gramauskas et al., 2023), biopesticides (Kozuharova et al., 2024) or natural dyes (Flax et al., 2021).

1.2. Description of analyzed invazive plants

1.2.1. Overview and invasive character of woody species Tree of Heaven (*Ailanthus altissima*) and staghorn sumac (*Rhus typhina*)

Ailanthus altissima (Mill.) Swingle (Tree of Heaven) is classified in Sapidales order, Simaroubaceae family (EPPO, 2024), and in Romania it is often mistaken with *Rhus typhina* (Crainic et al., 2019), which belongs to Anacardiaceae family from the same order (EPPO, 2024). Both plants are decidous wood species (Caramelo et al., 2021), that have been introduced in Romania as ornamental plants (Anastasiu și Negrean, 2007, Qu et al., 2020 b).

As invasive species, Tree of Heaven and staghorn sumac will be frequently found in heavily anthropized lands such as roadsides (McAvoy et al. 2012), areas near railways (Seiler et al., 2019) or abandoned and burned fields (Maschek et al., 2018, Seiler et al., 2019).

Tree of Heaven can adapt to extreme temperature conditions, up to −33°C (Caramelo et al., 2021), or to long periods of drought (Trošt Sedej et al., 2021).

1.2.2. Chemical composition

Both species *A. altissima* and *R. typhina* represent rich sources of bioactive compounds, such as flavonoids (Lai et al.,2014, Kim et al., 2016), alkaloids (Shao et al., 2023, Konarska et al., 2024), phenolic acids (Pangi et al., 2022, Lai et al., 2014), coumarins (Qiu et al, 2016, Duan et al., 2021), tannins (Dai et al., 2020, Zazharskyi et al., 2020) etc.

Ailanthone and shinjulactone represent compounds specific to the Simaroubaceae family, named quassinoids (Pavela et al., 2014), and altissimacoumarin H is a coumarin found in the fruits of *A. altissima* (Ni et al., 2019).

The antioxidant activity and prevailing phenolic acid for Tree of Heavan leaves can be different from a geographical region to another (Poljuha et al., 2017, Caramelo et al., 2021).

The fruits of staghorn sumac contain high amounts of vitamins (Zhang et al., 2022), essential acids (Wang & Zhu, 2017), anthocyanins and pyranoanthocyanins (Kirby et al., 2013, Wang & Zhu, 2017), but also of phenolic acids malic, citric and tartaric (Wang & Zhu, 2017, Arlandini et al., 2021).

1.2.3. Potentially allergic or toxic effects

The environmental conditions in which a plant species grows can influence its potential allergenic effects (Martí-Garrido et al., 2020). The pollen produced by both species *A. altissima* and *R. typhina* can cause serious allergies (Martí-Garrido et al., 2020, Qu et al., 2020 a).

The contact with sap, stems or leaves of those two plants can lead to dermatitis (Whiticar & Harvey, 2009, Schall & Davis, 2009, Seo et al., 2018). Regarding the contact with *A. altissima* sap, there were raported cases of myocarditis (Karalija et al., 2020).

The seeds oil of staghorn sumac was declared edible by National Health Commission of China (Zhang et al., 2022), but the smoke produced by its wood burning is toxic (Lazzaro et al., 2018).

Leaves of Tree of Heaven are toxic for animals to the point of lethality, goats being an exception (Bourke, 1996). If for *A. altissima* skin tests were performed to confirm allergies (Martí-Garrido et al., 2020), studies regarding the toxicity of staghorn sumac fruits were not applied on humans until now (Wang & Zhu, 2017).

1.2.4. Applicative character in medicine and industry

Both *A. altissima* and *R. typhina* proved to be valuable medicinal plants, traditional used in treating gastrointestinal issues (Lai et al.,2014, Wang et al., 2017), wounds or bleedings (Zhu et al., 2020, Opiyo et al., 2021), genital system problems (Tabassum et al., 2017, Zhu et al., 2020) and respiratory diseases (Lungu et al., 2016, Liu et al., 2019). Used as „nux vomica" (Pedersini et al., 2011), Tree of Heaven represented an important plant for homeopathy (Kowarik & Säumel, 2007).

Antiproliferative and antimalarial properties of Tree of Heaven (Yan et al., 2018, Knüsel et al., 2019) and antiseptic and neuroprotective effects of staghorn sumac (Liu et al., 2019, Naik et al., 2021) are currently demonstrated by scientific research.

Tree of Heaven and staghorn sumac proved strong phytotoxic (Trošt Sedej et al., 2021, McCoy et al., 2022) and insecticide (Kozuharova et al., 2022) actions, those being suitable for obtaining ecological pesticides.

Various extracts of those plants can be used for synthesizing nanoparticles with antimicrobial (Ivanuša et al., 2022), antidiabetic (Samad et al., 2024) or UV protection enhancing (Čuk et al., 2021) properties.

1.3. Literature review on the application of pre-treatments and extraction of compounds from targeted raw materials

1.3.1. Collection and drying of the raw material

Following documentation based on 60 articles indexed in Web of Science about *A. altissima*, respectively 26 papers about *R. typhina*, searched by key words such as „applications", „bioactive compounds", „biofuels", „allergens", etc., the following observations were outlined:

- ❖ The most studies regarding the applicative potential of the two invasive species were carried out in Asia and Europe (including Romania);
- ❖ The plant material was mainly collected in summer and autumn (leaves and seeds of *A. altissima* in September and leaves, fruits and seeds of staghorn sumac in August and October);

- The most studied part of the two plants is the leaf;
- Most studies utilized the plant material dried at room temperature or fresh, kept at –20°C until the moment of extraction;
- Often, the dried material was ground and sieved before storage;

1.3.2. Extraction of bioactive compounds

Regarding the extraction of bioactive compounds, there were observed the following aspects:

- Through the analyzed studies, the greater part of them focused on the identification and isolation of bioactive compounds from the two invasive species;
- Maceration was the most commonly used extraction method for Tree of Heaven and staghorn sumac, lasting between 30 min–10 days, and as a modern method popular in studies there was used the ultrasound-assisted extraction, for a time interval of 30 min–4 h;
- It was selected to prepare the extraction mixtures in the 1:10 ratio (substrate:solvent);
- The plant material was usually extracted only once, but in some cases the process was repeated 2-3 times;
- In different studies, at the end of extraction the supernatant was concentrated and resuspendend in various solvents such as distilled water or DMSO;
- The polar solvents were preferred in extractions of Tree of Heaven and staghorn sumac, especially methanol, ethanol and distilled water, but in some cases were also used non-polar solvents such as hexane, petroleum ether and chloroform;
- The supernatant separation was often done using filter paper, centrifugation or syringe filters ;

1.4. Utilization of natural pigments in ecological dyeing of textiles

The most important aspects to watch out for in the textile dyeing concerns the safety of using the obtained products and the impact of dyeing process on the environmental safety. These requirements are better fulfilled by natural dyes, which have an equally good coloring ability as synthetic dyes, while they utilize fewer resources (human resource, time) and are biodegradable, renewable and decrease the risks of developing allergies or other serious diseases.

Natural dyes have various origins, from simple organisms such as bacteria to invertebrates such as molluscs and insects. These can be also obtained from inorganic sources including clays or sulfur salts.

Traditional dyeing with plants was popular in Europe from ancient times and it was based on different compunds of color found in their structure: anthocyanins, betalains, carotenoids, flavonoids, tannins etc.

Because of the absence of regulatory standards for natural dyeing of textiles and its sustainable character, different sources of coloring and mordant substances were included in NODS („Natural Organic Dye Standard”) criteria (Karadag, 2023).

Natural dyes are not useful only in textile field, but also in medicine, research or in food industry. Nowadays, there is an increasing trend of encouraging the development of textile dyeing domain towards a more ecological, sustanaible direction, supporting the principles of circular economy, using renewable natural resources such as agricultural residues, vegetable waste and organic matter of different invasive plants.

2. ORIGINAL CONTRIBUTIONS ON THE INFLUENCE OF EXTRACTION PARAMETERS AND TECHNIQUES ON THE CONTENT OF BIOACTIVE COMPOUNDS FROM TREE OF HEAVEN (*A. ALTISSIMA*)

2.3.1. Microstructural peculiarities of raw material (leaves of *A. altissima*) observed by SEM microscopy

The SEM technique was used to obtain images of microstructures of Tree of Heaven leaves, fresh or dried at 50°C (collected in summer or autumn). In the investigated samples, the presence of epidermal elements such as protective (tector) bristles (Figures 1a,b,c și 2a) and stomata (Figures 1c, 2a), found with a higher density on the underside of leaflets, was highlighted. An overview of nectariferous glands found at the base of the abaxial face of the leaflets was captured, more details regarding these structures being included in the study of Poljuha et al. (2023). Drying and grinding of samples resulted in damage of the cell walls, with the appearance of cracks and a decrease in volume of the protective trichomes, while the poorly represented epicuticular wax layer was difficult to observe in the examined samples.

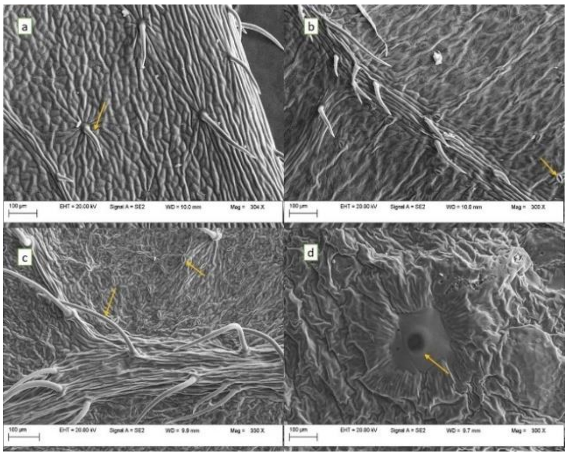

Figure 1: SEM images (300 ×) of fresh leaves of *A. altissima*: (a) – protective trichomes on the upper epidermis (b) – stomata and trichomes located on a vein in the upper epidermis; (c) – stomata and protection hairs located on the main vein in the abaxial side of the leaflet; and (d) – extrafloral nectraries at the base of the leaflet (observed on the underside of the leaflet).

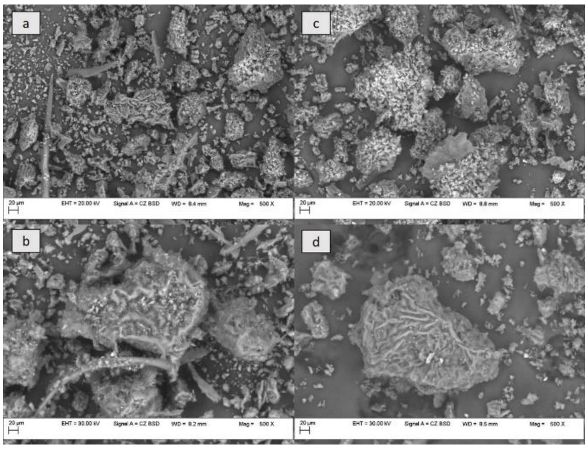

Figure 2: SEM images (500 ×) of *A. altissima* leaf powders: (a,b) – powder from summer leaves; (c,d) – powder from autumn leaves. There are presented elements such as: (a,b) – protective trichomes; (a) – stomata; (b,d) – large fragments of the leaf surface.

2.3.2. Study regarding the microwave irradiation efficiency on the extraction of bioactive compounds from leaves of *A. altissima*

2.3.2.1. Total polyphenols and tannins content and the antioxidant activity

After the irradiation of samples, their final temperature did not exceed 43°C. The results regarding the dosed amounts of bioactive compounds in the extract of the leaflets frozen or dried at 60°C, obtained at different process parameters, and their antioxidant activity, have been included in Table 1, previously published in Cocîrlea, M. D., Miclăuş, S., Oancea, S. (2022).

Table 1: Antioxidant activity (FRAP) and the content in total polyphenols (TPC) and tannins (TTC) for the ethanolic extracts of frozen and dried *A. altissima* leaves, obtained by MAE technique

Analyzed parameters	Control (untreated sample)	Exposure time (h)	Frequency (GHz)	Incident electric field strength (V/m)	Values for the irradiated samples
Extract from frozen leaves of *A. altissima*					
TPC (mg GAE/ 100 g DW)	1652.47±26.51	0.5	1.74	660	1741.39±21.16
		1	1.74	660	1684.86±42.85
		3	1.74	660	1744.86±22.73
		0.5	2.3	950	1690.89±35.73
		1	2.3	950	1768.01±34.26
		3	2.3	950	1880.18±15.41
TTC (mg CE/ 100 g DW)	401.43±5.71	0.5	1.74	660	464.50±2.74
		1	1.74	660	439.89±7.44
		3	1.74	660	448.12±9.06
		0.5	2.3	950	403.93±1.96
		1	2.3	950	469.11±9.55
		3	2.3	950	424.89±4.94
FRAP (mg AAE/ 100 g DW)	1566.21±28.95	0.5	1.74	660	1647.05±28.90
		1	1.74	660	1391.25±55.81
		3	1.74	660	1518.17±43.28
		0.5	2.3	950	1506.40±29.41
		1	2.3	950	1583.65±20.84
		3	2.3	950	1694.75±31.28
Extract from dried leaves of *A. altissima*					
TPC (mg GAE/ 100 g DW)	1158.35±19.55	0.5	1.74	660	1143.37±16.47
		1	1.74	660	1178.64±13.07
		3	1.74	660	1152.19±21.45
		0.5	2.3	950	1138.83±18.35
		1	2.3	950	1196.86±9.49
		3	2.3	950	1161.55±18.09
TTC (mg CE/ 100 g DW)	447.41±8.71	0.5	1.74	660	437.66±6.33
		1	1.74	660	442.81±6.41
		3	1.74	660	440.26±7.63
		0.5	2.3	950	463.23±6.62
		1	2.3	950	428.27±6.64
		3	2.3	950	432.94±8.97
FRAP (mg AAE/ 100 g DW)	857.96±21.31	0.5	1.74	660	847.65±26.81
		1	1.74	660	842.97±28.71
		3	1.74	660	871.46±19.27
		0.5	2.3	950	841.66±29.22
		1	2.3	950	877.86±21.23
		3	2.3	950	864.85±23.19

All obtained extracts presented a considerable antioxidant activity, supported by a high content in polyphenolic compounds, but a low amount of condensed tannins.

The type of plant material (frozen or dried) showed by Kruskal-Wallis analysis a significant influence on the mean polyphenol content ($p = 0.001725$) and FRAP antioxidant activity ($p = 0.001745$), with higher values for frozen material ($p = 0.0009^*$), according to Dunn's test.

Higher values of TPC, TTC and FRAP activity responses were obtained for frozen samples by irradiation, especially at 2.3 GHz frequency, compared to the values of non-irradiated samples.

2.3.2.2. Characterization of the extracts obtained by microwave irradiation, using ATR-FTIR technique

Using scientific publications (Deepika et al., 2017, Nandiyanto et al., 2019, Awwad & Amer, 2020), the absorbtion bands of the FTIR spectra obtained for frozen (Figura 3A) and dried (Figura 3B) material, were identified.

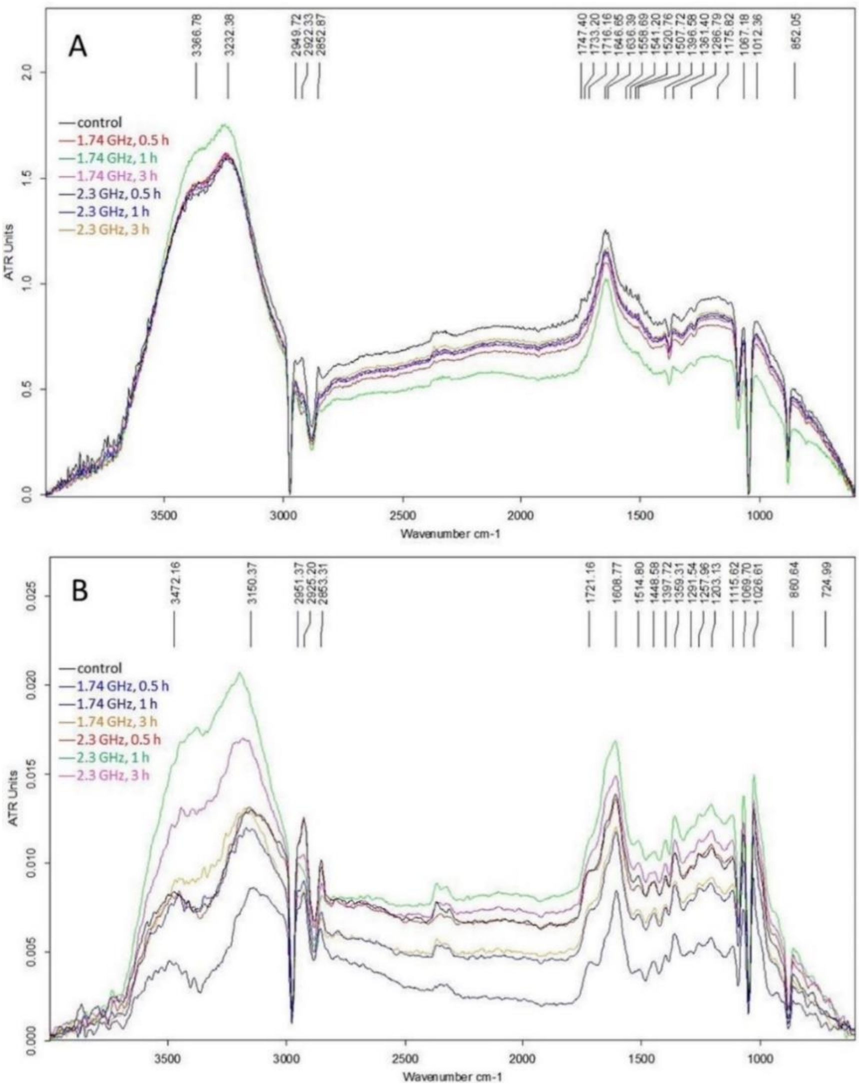

Figure 3: ATR-FTIR spectra of ethanolic extracts from *Ailanthus altissima* leaves, microwave irradiated și non-irradiated (control), prepared from frozen (A) or oven-dried (B) material

In general the resulting spectra for the two types of samples were similar. A shift and a clearer delimitation of the peaks at 3472 și 3150 cm^{-1} in the spectra of the dried leaves, compared to the intense peaks at 3366 şi 3232 cm^{-1} present in the spectra of frozen material, was raported probably due to the modification of some phenolic compounds (observation in agreement with the results of TPC dosage analysis) or to the more concentrated extracts obtained from dried material for the same solvent/solid ratio as for the frozen samples.

The chemical structure of the compounds was not significantly influenced by microwave irradiation at the tested frequencies (1.74 and 2.30 GHz), but the resulting mechanical action on the cell walls improved the extraction yield.

2.3.3. Experimental study regarding the impact of pretreatment and the collection season of the raw material, but also of the sequential extraction, on the bioactive content of *A. altissima* leaves

2.3.3.2. The impact of raw material pretreatment on the color changes, tested in the CIELAB system

Color changes were observed for frozen leaflets of Tree of Heaven and the ones dried at the room temperature (r.t.), 30°C and 50°C. In general pronounced differences of color were the result of chlorophyll degradation caused by drying processes. The extracts of frozen samples, found in yellow-yellow-green-greenish color range, presented a higher brightness (L*).

Reddish hue of dried powders can be associated with the presence of anthocyanins (ex. cyanidin) (Vidović et al., 2015), while the yellow one – with the presence of flavonoids and carotenoids (Carradori et al., 2020, Andonova et al., 2023). No significant changes in overall color difference were reported (Table 2) for the tested drying temperatures compared to frozen samples (for ΔE >3) (Pathare et al., 2013). Dried samples were more colorful (less white) and presented a subtler hint of yellow. The obtained results, included in Table 2, were published in **Cocîrlea, M. D.**, Soare, A., Petrovici, A. R., Silion, M., Călin, T., Oancea, S. (2024).

Table 2: Changing color parameters of extracts from dried samples (t.c., 30°C or 50°C), compared to extracts from frozen leaves, using the CIELAB system (brightness L*; red-green a*; yellow-blue b*; color differences ΔE).

Sample	Material processing	L*	a*	b*	ΔE	Whiteness index	Yellowness index
Acetonic extract from *A. altissima* leaves	2° field of view						
	Freezing	27.68±0.82	-43.90±0.05	44.01±0.07	-	-14.81	96.01
	Drying (r.t.)	0.65±0.11	0.46±0.04	0.83±0.16	67.54	-0.14	74.19
	Drying (30°C)	1.26±0.05	-0.58±0.07	1.80±0.04	66.00	-0.32	83.86
	Drying (50°C)	0.38±0.05	0.09±0.04	0.41±0.05	67.68	-0.06	63.04
	10° field of view						
	Freezing	26.63±2.66	-35.05±1.46	43.32±2.83	-	-14.81	96.01
	Drying (r.t.)	0.60±0.10	0.42±0.06	0.73±0.13	61.23	-0.14	74.19
	Drying (30°C)	1.16±0.28	-0.40±0.05	1.63±0.42	59.90	-0.32	83.86
	Drying (50°C)	0.36±0.17	0.15±0.06	0.36±0.07	61.44	-0.06	63.04

2.3.3.3. The total content of bioactive compounds (polyphenols, flavonoids, tannins, carotenoids) from extracts obtained by the sequential process

Following the sequential extraction and determination of bioactive compounds, it was observed that ethanol was a more suitable solvent for obtaining extracts with high bioactivity, regardless the type of samples (frozen, dried, collected in summer or autumn).

The results regarding the total content in bioactive compounds are presented in Table 3.

Table 3: The content in bioactive compounds of ethanolic and hexanic extracts from *A. altissima* leaves, obtained by sequential process

Pre-treatment type and harvesting season	Type of extract	TPC (mg GAE/ 100 g DW)	TTC (mg CE/ 100 g DW)	TFC (mg QE/ 100 g DW)	Carotenoids (mg beta-carotene/ 100 g DW)
Frozen (summer)	hexanic extract 1	18.42 ± 0.13	9.37 ± 1.51	1611.23 ± 30.00	2.62 ± 0.04
	ethanolic extract 2	3791.43 ± 4.76	233.68 ± 3.39	6169.3 ± 16.03	75.67 ± 0.58
	ethanolic extract 1	5272.62 ± 11.99	347.84 ± 1.01	9197.65 ± 53.43	57.11 ± 0.09
	hexanic extract 2	6.45 ± 0.15	33.54 ± 3.45	410.47 ± 12.90	33.83 ± 0.14
Frozen (autumn)	hexanic extract 1	9.91 ± 0.22	6.47 ± 0.59	298.59 ± 28.97	1.74 ± 0.01
	ethanolic extract 2	5789.12 ± 3.14	285.37 ± 7.59	6020.88 ± 35.90	84.98 ± 0.29
	ethanolic extract 1	7256.92 ± 13.65	485.89 ± 1.80	7998.83 ± 55.38	71.59 ± 0.14
	hexanic extract 2	34.41 ± 0.12	23.76 ± 0.13	619.93 ± 7.20	29.64 ± 0.06
Dried (summer)	hexanic extract 1	64.48 ± 0.15	123.76 ± 0.03	119.37 ± 0.75	70.33 ± 0.43
	ethanolic extract 2	5578.37 ± 0.37	990.35 ± 0.48	9176.74 ± 26.06	105.65 ± 0.54
	ethanolic extract 1	5378.42 ± 1.18	943.26 ± 0.90	7557.66 ± 26.29	108.18 ± 0.64
	hexanic extract 2	34.70 ± 0.02	36.45 ± 0.04	169.19 ± 8.02	61.32 ± 0.05
Dried (autumn)	hexanic extract 1	52.55 ± 0.08	58.59 ± 0.25	90.62 ± 1.18	50.92 ± 1.39
	ethanolic extract 2	6180.00 ± 15.83	598.42 ± 0.93	6565.38 ± 33.38	69.69 ± 0.23
	ethanolic extract 1	6026.31 ± 4.89	575.50 ± 0.10	7012.84 ± 21.08	55.60 ± 0.11
	hexanic extract 2	14.12 ± 0.02	43.90 ± 0.09	302.68 ± 23.34	44.84 ± 0.07

While frozen autumn samples presented the highest content in total phenolics (7256.92 ± 13.65 mg GAE/ 100 g DW), frozen summer leaflets gave the highest amount of flavonoids (9197.65 ± 53.43 mg QE/ 100 g DW). Dried summer samples were the best source of condensed tannins (990.35 ± 0.48 mg CE/ 100 g DW) and carotenoids (108.18 ± 0.64 mg beta-carotene/ 100 g DW).

Regarding the ethanolic extracts, it was observed a statistically significant higher TPC mean for summer samples reported to those obtained by leave harvested in autumn ($p < 0.05$), but a significant lower mean of condensed tannins for frozen samples compared to the dried samples ($p = 0.02$). In the case of flavonoids and carotenoids, the differences between the means were not significant between the categories of plant material (harvested in summer *vs*. in autumn, respectively frozen *vs*. dried).

Correlations between the analyzed response variables were significant and positive ($p < 0.05$), excluding the one between flavonoids and carotenoids ($p = 0.0517$).

The obtained results, published in the paper **Cocîrlea, M.D.**, Soare, A., Petrovici, A. R., Silion, M., Călin, T., Oancea, S. (2024), brought novelty regarding the variation of content in bioactive compounds from *A. altissima* leaves reported to the harvesting season (summer *vs*. autumn) and to the pre-treatment of raw materials (frozen *vs*. dried), also filling the gaps regarding the content in tannins that was less evaluated in studies.

2.3.3.4. Polyphenolic profile of ethanolic extracts depending on the collection season and pretreatment, determined by HPLC analysis

The polyphenolic profile for four ethanolic extracts of *A. altissima* (extraction order polar-non-polar), obtained by maceration technique, from frozen and dried leaflets, was investigated through HPLC-DAD technique, using 15 reference standards. The retention times (RT) for standards injected individually or in a mixture, but also for the identified polyphenols in the tested samples, were included in Table 4. Even so there were observed overlaps of peaks for vanillic acid and epicatechin, respectively for syringic acid and caffeic acid, when the mixture was injected, in the chromatograms of extracts their delimitation was clearly observed.

Table 4: The retention times (RT) for polyphenolic compounds identified in ethanolic extracts from *A. altissima* leaves

Sample / Polyphenols	Individually injected polyphenols	Polyphenols injected as a mixture	Samples			
			Frozen (summer)	Frozen (autumn)	Dried (summer)	Dried (autumn)
	Retention time (RT) (min)					
Gallic acid	10.97	10.84	10.73	10.97	10.71	10.71
Protocatechuic acid	13.98	13.90	–	14.04	13.97	13.99
Catechin	15.63	15.91	–	–	–	15.79
Vanillic acid	16.74	overlapped with epicatechin	16.64	16.79	16.6	16.65
Epicatechin	17.28	17.38	–	–	17.34	–
Caffeic acid	17.49	17.96	–	–	–	–
Syringic acid	18.09	overlapped with caffeic acid	–	–	–	–
Rutin	20.42	20.63	20.68	–	20.66	20.70
Ferulic acid	22.38	22.35	22.12	22.32	–	22.38
p-Coumaric acid	23.10	23.46	23.42	23.61	23.49	23.48
Hesperidin	24.01	24.03	24.32	–	24.36	24.38
Rosmarinic acid	26.33	26.69	26.46	–	26.57	26.53
Salicylic acid	29.31	29.61	–	–	–	–
Quercetin	34.72	34.65	–	–	35.07	35.22
Kaempferol	39.71	41.00	–	–	–	–

Note: ”–” undetected compound

The composition of dried samples was in general similar for the two seasons of harvestings, the difference being represented by replaceing of catechin in the autumn extracts with epicatechin in the summer extracts. In the case of frozen leaflets, in samples harvested in the summer were found more bioactive compounds than in the ones collected in autumn, such as rutin, hesperidin and rosmarinic acid. Quercetin was found only in samples with dried leaflets. The results included in Table 4 were published in **Cocîrlea, M. D.**, Soare, A., Petrovici, A. R., Silion, M., Călin, T., Oancea, S. (2024).

2.3.3.5. Antioxidant activity of extracts obtained by the sequential process

FRAP antioxidant activity of ethanolic extracts resulting from sequential maceration presented values between 5043.59–8522.58 mg AAE/100 g DW, while for DPPH activity the results were found in the range 79.11% – 94.50%. The values for hexanic extracts were much lower, FRAP activity being situated between 0.79–79.95 mg AAE/100 g DW, and DPPH antioxidant activity – between 1.85% and 44.45%.

The most powerful antioxidant activity for the methods of analysis performed, was recorded for the ethanolic extracts obtained from residue of: frozen autumn leaves for FRAP (8522.58 ± 73.86 mg AAE/ 100 g DW) and dried summer leaves for DPPH (94.50 ± 0.02%).

Wilcoxon test confirmed the presence of significant higher means for analyzed antioxidant activities of the extracts obtained on the use of ethanol versus hexane (p = 0.00016) (Figure 4). There were not reported significant differences of means for these response variables depending on the type of plant material used (harvested in summer *vs*. In autumn, frozen *vs*. dried).

The results obtained for the two antioxidant activities FRAP and DPPH were related by a strong positive correlation (p = 0.0006, r = 0.76).

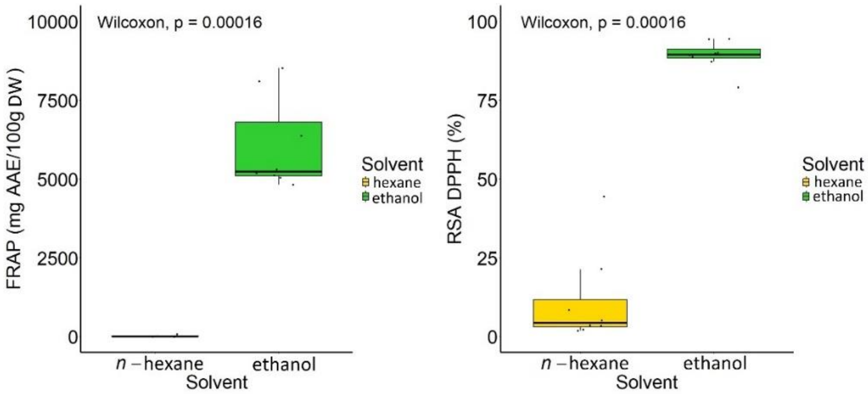

Figure 4: Boxplot representation of total antioxidant activity (FRAP and DPPH) depending on the solvent type

2.3.3.6. Statistical analysis of experimental data with regard to the efficiency of the microwave and sequential techniques for extraction of bioactive compounds

In the case of antioxidant activity FRAP, the analysis of GLM model indicated total phenolic content as significant predictor ($p = 9,61\times10^{-11}$, t = 20,65) for the extracts obtained by MAE technique, and in the case of extracts obtained by sequential maceration, its determined significant predictors were: TFC (for extracts from samples harvested in summer, frozen or used in the second phase of extraction), TPC (for extracts obtained by samples harvested in autumn, dried or used in the first phase of extraction). For the DPPH activity, the significant predictors for the extracts obtained by sequential technique were: TPC (fost extracts from leaflets collected in summer, dried or used in the second phase of extraction), the content in carotenoids (for the extracts made by leaflets collected in summer or used in the first phase of extraction), and TFC (for the extracts obtained by frozen plant material).

Extraction efficiency for frozen leaflets or collected in summer increased when ethanol was used as solvent in the sequential technique, while for autumn or dried samples the use of the polar solvent was recommended for the second extraction phase.

2.3.3.7. Evaluation of antimicrobial activity of the ethanolic extracts

The four ethanolic extracts from leaflets of *Ailanthus altissima* obtained by sequential technique (extraction order: polar to non-polar), presented as a result of *in vitro* antimicrobian tests inhibitory effects on bacterial strains of *Staphylococcus aureus* (clinical isolate) (8-10 mm) and *Enterococcus faecalis* (ATCC 29212) (8-10 mm). The results regarding the antimicrobial activity, presented in Table 5, were published in **Cocîrlea, M. D.**, Soare, A., Petrovici, A. R., Silion, M., Călin, T., Oancea, S. (2024).

Regarding fronzen samples, the extracts from summer leaflets inhibited more bacterial strains. In the case of dried samples, the extracts from autumn leaflets inhibited six out of ten bacterial strains, the maximum number of sensitized pathogenic strains by this plant in the present study.

Tested antifungal activity on a *Candida albicans* strain was absent.

Diameters of inhibition zones determined for the four observed extracts on *E. coli*, *E. faecalis*, *S. aureus* and *S. pyogenes* strains were below the lower limit of diameters determined by standard antibiotics such as ampicillin, gentamicin or penicillin (CLSI, 2022, EUCAST, 2023).

Table 5: Diameter of inhibition (mm) for the growth of microorganisms in the presence of ethanolic extracts from *A. altissima* leaves

Sample / Microbial strain	Inhibition diameter (mm)			
	Frozen (summer)	Frozen (autumn)	Dried (summer)	Dried (autumn)
Staphylococcus aureus (ATCC 25923)	–	–	8,0 ± 0,1	8,0 ± 0,1
Staphylococcus aureus (izolat clinic)	8,0 ± 0,1	8,0 ± 0,1	10,0 ± 0,2	10,0 ± 0,2
Streptococcus pyogenes (ATCC 19615)	–	–	–	8,0 ± 0,1
Enterococcus faecalis (ATCC 29212)	8,0 ± 0,1	9,0 ± 0,2	9,0 ± 0,2	10,0 ± 0,3
Bacillus subtilis (ATCC 6633)	–	–	–	8,0 ± 0,1
Salmonella enterica (ATCC 13076)	–	–	–	–
Pseudomonas aeruginosa (ATCC 27853)	–	–	–	–
Escherichia coli (ATCC 25922)	9,0 ± 0,2	–	–	10,0 ± 0,2
Enterobacter aerogenes (ATCC13084)	–	–	–	–
Candida albicans (ATCC 10231)	–	–	–	–

"-" = lack of inhibitory activity

2.3.3.8. Experimental research regarding the environmental and health risks, realized in the perspective of practical applications of extracts from leaves of *A. altissima*

Toxicity tests *in vitro* performed on the ethanolic extract from dried autumn leaflets indicated ecotoxic and cytotoxic effects with a trend of general increasing as the applied dose of the analyzed extract was higher. Thus, the growth and germination indices for the wheat and tomatoes seeds were the strongest affected by the concentration of 1000 µg/mL, the toxic effect on *Artemia salina* larvae was developed in 3h after the exposure of 2000 µg/mL concentration, the highest rate of hemolysis (6.95 ± 1.04%) was observed for the maximum tested concentration of 1000 µg/mL, the MTS test indicated for HGF, HepG2 and MeWo cell lines a low level of cytotoxicity for the highest amount of extract at which those were exposed (500 µg/mL), and the larger amount of released carboxyfluorescein caused by the rupture of liposomal membrane was 6,83% for a fraction of 200 µg extract.

3. ORIGINAL CONTRIBUTIONS REGARDING THE INFLUENCE OF EXTRACTION PARAMETERS AND TECHNIQUES ON THE CONTENT IN BIOACTIVE COMPOUNDS FROM STAGHORN SUMAC (*R. TYPHINA*)

Concerning the bioactivity of *R. typhina* fruits, an experiment aimed at building an Box-Behnken experimental design by which the values of three extraction parameters to be optimized, so the responses of some bioactive compounds of interest to be increased. The levels of parameters (low, high and center) were included in Table 6, and the conditions resulting from the experimental design for a number of 15 experimental runs are presented in Table 7. These data were published in **Cocîrlea, M. D.**, Simionescu, N., Călin, T., Gatea, F., Badea, G. I., Vamanu, E., Oancea, S. (2024).

Table 6: Selected process parameter levels for Box-Behnken design

Variable [unit]	Symbol	Level		
		Low (−1)	Center (0)	High (+1)
Solvent concentration [%]	X1	40	55	70
Time of ultrasonic extraction [min]	X2	10	20	30
Solvent/solid ratio [*v/w*]	X3	10/1	15/1	20/1

Table 7: Experimental conditions for the extraction of bioactive compounds from *R. typhina* fruits, based on obtained Box-Behnken design

Experimental run	Solvent concentration (%)	Time of ultrasonic extraction (min)	Solvent/solid ratio (*v/w*)
1	40	10	15/1
2	70	10	15/1
3	40	30	15/1
4	70	30	15/1
5	40	20	10/1
6	70	20	10/1
7	40	20	20/1
8	70	20	20/1
9	55	10	10/1
10	55	30	10/1
11	55	10	20/1
12	55	30	20/1
13	55	20	15/1
14	55	20	15/1
15	55	20	15/1

3.3.1. Microstructural peculiarities of raw material (fruits of *R. typhina*), observed by SEM microscopy

Microstructure of fresh and dried drupes of *R. typhina*, whole or ground, was visualized by SEM microscopy and there was highlighted the presence of numerous acicular trichomes on the outer surface of fruits (Figure 5e) and their insertion sites on larger particles from the ground and sieved powder (Figure 5b). Powder consisting in non-uniform particles contains fragments of the seed coat. SEM image of a seed of staghorn sumac is presented in Figure 5f, and a cross section through the seed coat can be observed in Figure 5c. Authors such as Ali & Al-Hemaid (2011) explained that the ornamentation and microstructural composition of different seeds can help in identification of various plant species or families.

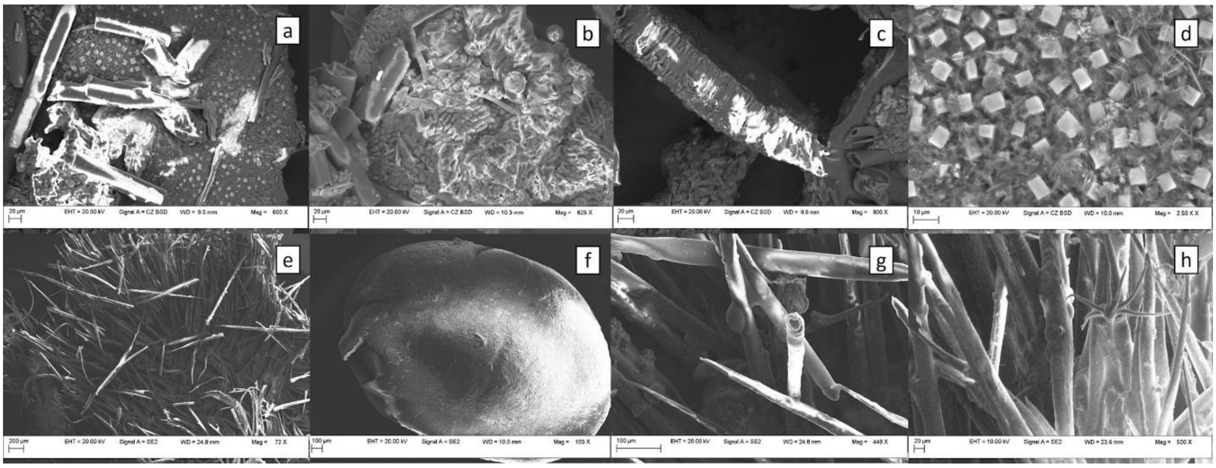

Figure 5: SEM images of *R. typhina* fruits, obtained at various magnifications (72 ×, 2,5 K ×): (a,b) fresh fruit homogenate; (c) ground dried fruits; (d,f) seed; (e, g, h) dried fruit (whole).

3.3.2. The influence of ultrasound-assisted and bead-assisted extraction process and parameters used on the content in bioactive compounds and antioxidant activity of *R. typhina* fruits

Two modern extraction techniques, respectively ultrasound-assisted extraction (UAE) and bead-beating extraction (BBE), were utilized in order to obtain products with high biological activity. The contents of the following categories of compounds were measured: anthocyanins (TAC), total polyphenols (TPC), condensed tannins (TTC), flavonoids (TFC) and carotenoids, the antioxidant activities being subsequently analyzed by FRAP and DPPH analyzes.

Ultrasound-assisted extraction (UAE)

a. The content in bioactive compounds

The extractions performed by UAE technique were realized based on experimental conditions found in Table 7. The 55% solvent concentration presented the highest results for the majority of measured bioactive compounds when the extraction lasted 30 min. Most of the compounds were extracted in higher amounts from more diluted mixtures (solvent/solid ratio of 20/1 (*v/w*)), but anthocyanins were more effectively isolated from concentrated mixtures (10/1 (*v/w*)). TAC values were the only ones increased for the shortest extraction time used (10 min).

The application of both Kruskal-Wallis and Dunn's tests led to observations such as: TAC and TFC means were significantly higher at 55% solvent concentration compared to 70% ($p = 0.0032$*, respectively 0.0440), the content in polyphenols, flavonoids and carotenoids was higher for the 20/1 (*v/w*) raport than for 10/1 (*v/w*) (p = 0.0008*, p = 0.0171*, p = 0.0085*). The mean value of TTC was significantly higher when the extraction lasted 30 min compared to 10 min (p = 0.0136*) (Figure 6).

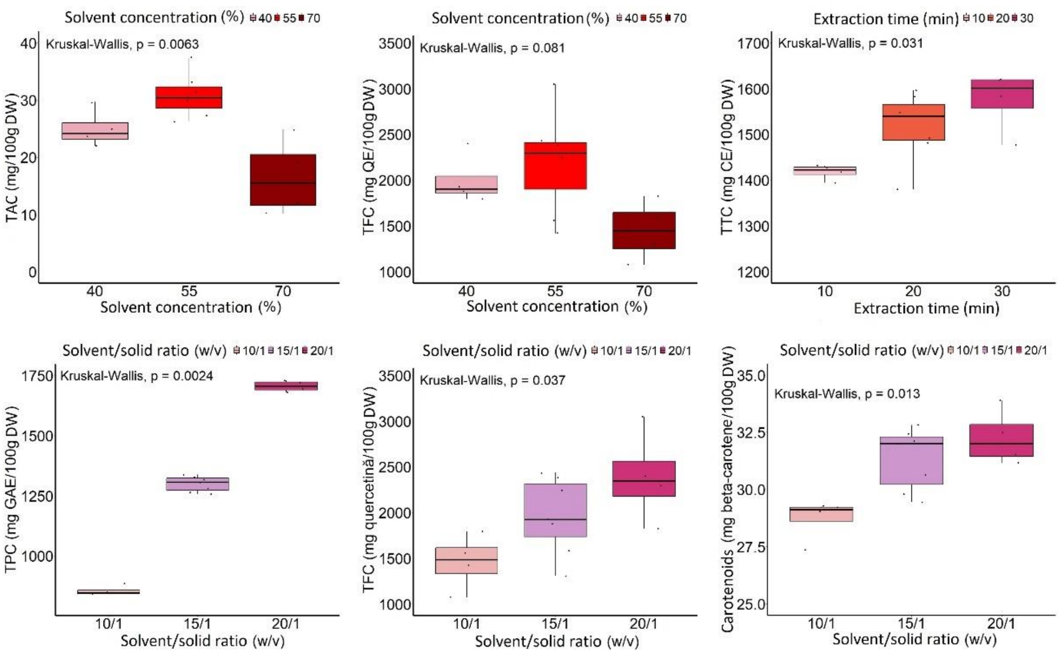

Figure 6: Boxplot representations of TAC, TPC, TTC, TFC from staghorn sumac fruits reported to the UAE parameters (solvent concentration, extraction time and solvent/solid ratio)

b. Evaluation of antioxidant activity

The results of FRAP antioxidant activity for the 15 extractions performed by UAE technique were in the range 106.817 ± 2.687 – 910.749 ± 4.572 (mg AAE/ 100 g DW), while the values for DPPH activity were between 84.422 ± 0.089 – 91.257 ± 0.059 %. For the both investigation methods of total antioxidant activity, the solvent/solid ratio of 20/1 gave the best results, regardless of the extraction time. Kruskal-Wallis analysis and Dunn's test indicated higher means when the ratio of 20/1 (*v/w*) was used compared to 10/1 (*v/w*) (p = 0.0008*) (Figure 7).

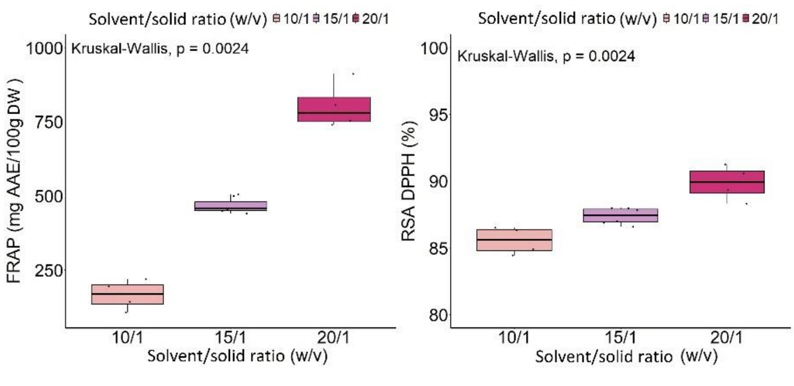

Figure 7: Boxplot representations of antioxidant activity for *R. typhina* fruits, extracted through UAE technique, depending on the solvent/solid ratio

Bead-beating extraction (BBE)

a. The content in bioactive compounds

Optimal extraction conditions identified for the UAE technique, using a Box-Behnken design, indicated a solvent concentration of 61.51% and a solvent/solid ratio of 20/1 (*v/w*), which were then used in the BBE extraction technique. In its case, the varied parameters were: extraction time (5 min *vs.* 10 min), bead size in mm (1.4, 2.4 *vs.* 2.8) and the material type of the bead (ceramics *vs.* metal).

The highest values of TAC were found when ceramic beads of 2.8 mm were used, while the content in tannins increased in the extraction with metal beads of 2.4 mm.

The utilization of Kruskal-Wallis and Dunn's tests showed the following observation: TAC mean was the lowest when beads of 2.4 mm were used compared to the ones of 2.8 mm (p = 0.0428), TTC mean increased in samples where metal beads of 2.4 mm were used reported to the control (p = 0.045*, respectively 0.0089*), and the mean of the content in carotenoids was higher when metal beads (p = 0,0057 *) or beads with the size of 2.4 mm and 2.8 mm (p = 0.0113 *, respectively p = 0.0280) were utilized, in comparison with the control (Figure 8).

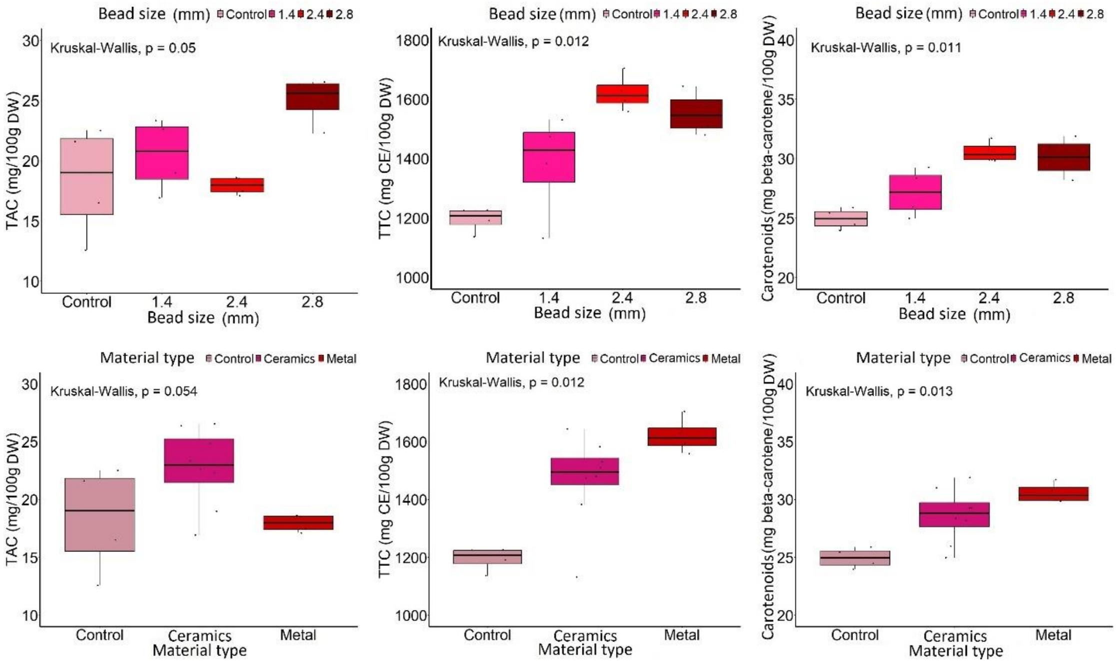

Figure 8: Boxplot representations of TAC, TPC, TTC, TFC from staghorn sumac fruits, reported to the BBE parameters (bead size, material type of the bead)

b. Evaluation of antioxidant activity

The values of FRAP antioxidant activity measured for the extracts obtained by BBE technique were in the range of 702.25 ± 0.75 and 885.03 ± 1.64 mg AAE/ 100 g DW, while the DDPH activity had values between 83.26 ± 0.42 – 93.32 ± 0.04 (%).

FRAP antioxidant activity was higher in the extracts for which beads 1.4 mm were used, while for DPPH activity, the 2,8 mm beads were more efficient.

Kruskal-Wallis analysis and Dunn's test highlighted a higher mean of FRAP activity at an extraction time of 5 min (p = 0.0230 *) compared to 10 min, but also in the case of 1.4 mm beads reported to those of 2.4 mm size (p = 0.0180 *), respectively for the ceramic ones compared to the metal beads (p = 0.0432). Even so, DPPH activity was significantly higher in the extraction with beads of 2.8 mm compared to the ones of 2.4 mm (p = 0.0347) (Figure 9).

Due to the fact that the extraction time of 5 minutes significantly influenced only FRAP antioxidant activity mean, this value was used as being optimal for this parameter subsequently subjected to the antimicrobial analysis and *in vitro* toxicity tests.

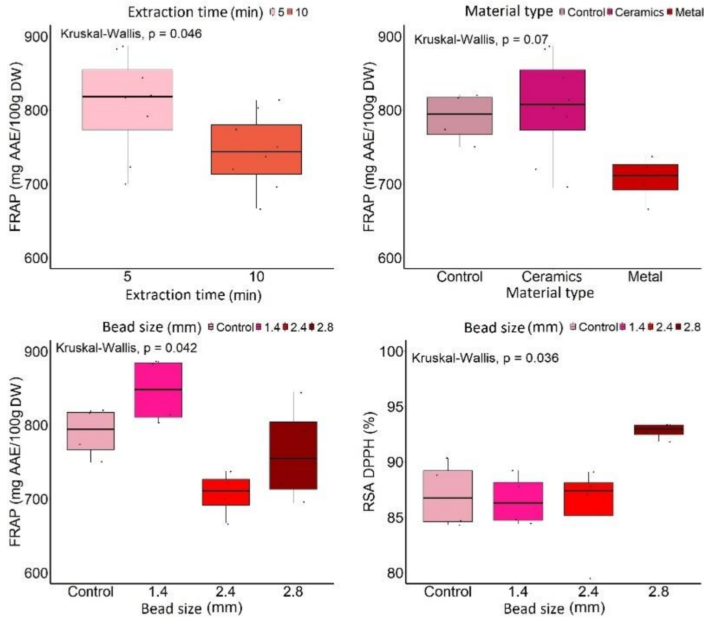

Figure 9: Boxplot reprezentations of the antioxidant activities for *R. typhina* fruits, extracted through BBE technique, reported to extraction time, bead size and material type of the bead

3.3.3. Polyphenolic profile of the ethanolic extract BBE, determined by HPLC analysis

The analysis of polyphenolic profile of the BBE extract from *R. typhina* fruit, obtained in optimized conditions (61.51% hydroalcoholic solution, 2.8 mm ceramic beads, 20/1 solvent/solid ratio, extraction time of 5 min), through HPLC-DAD technique allowed the focus on the presence of two phenolic acids and a flavonoid with high biological significance, but also their quantification (Table 8).

The predominant compound in the obtained extract was *p*-coumaric acid (17.62 ± 0.59 µg/mL), followed by ferulic acid (8.91 ± 0.74 µg/mL) and myricetin (8.89 ± 0.23 µg/mL), with close values of their concentrations.

Table 8: Polyphenolic compounds identifed by HPLC analysis in the ethanolic extract from *R. typhina* fruits

Polyphenolic compound	Concentration (µg/mL)
p-Coumaric acid	17.62 ± 0.59
Ferulic acid	8.91 ± 0.74
Myricetin	8.89 ± 0.23

3.3.4. Antimicrobial activity of the ethanolic extract BBE

The extract from drupes of staghorn sumac obtained by BBE technique (61.51% solvent concentration, 20/1 (*v/w*) solvent/solid ratio, extraction time of 5 min, ceramic beads of 2.8 mm, concentrated to half the initial volume) presented growth inhibitory effects on six tested bacterial strains (three Gram-pozitive species and three Gram-negative species). The results included in Table 9 were published in **Cocîrlea, M. D.**, Simionescu, N., Călin, T., Gatea, F., Badea, G. I., Vamanu, E., Oancea, S. (2024).

The largest diameters for the zones of inhibition produced by the presence of the extract were identified in the case of *Streptococcus pyogenes* (20 mm) and *Salmonella enterica* (12 mm) strains. The diameters observed for the other affected pathogenic species (*Bacillus subtilis*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Salmonella* group D (clinical isolate)) were below 8 mm as size. The antifungal effect on *C. albicans* and *Saccharomyces cerevisiae* strains was absent.

Table 9: Zones of inhibition (mm) for pathogenic microorganisms in the presence of the ethanolic extract from *R. typhina* fruits

Category	Microbial strain	Inhibition zone (mm)
Gram-positive bacteria	*Bacillus subtilis* ATCC 6633	8
	Enterococcus faecalis ATCC 29212	8
	Enterococcus faecalis VRE ATCC 51299	–
	Staphylococcus aureus ATCC 25923	–
	Streptococcus pyogenes ATCC 19615	20
Gram-negative bacteria	*Acinetobacter baumannii* ATCC 19606	–
	Citrobacter freundii ATCC13316	–
	Escherichia coli ATCC 25922	–
	Klebsiella aerogenes ATCC 13048	–
	Klebsiella pneumoniae ATCC 13883	8
	Pseudomonas aeruginosa ATCC 27853	–
	Salmonella enterica ATCC 13076	12
Fungus	*Candida albicans* ATCC10231	–
	Saccharomyces cerevisiae ATCC9763	–
Clinical and food-borne bacterial isolates		
Gram-positive bacteria	*Staphylococcus aureus* (clinical isolate)	–
	Staphylococcus aureus (food isolate)	–
	Streptococcus anginosus (clinical isolate)	–
	Streptococcus anginosus group F (clinical isolate)	–
	Streptococcus mutans (clinical isolate)	–
Gram-negative bacteria	*Salmonella* group D (clinical isolate)	8

3.3.5. Optimization of UAE extraction technique conditions of bioactive compounds from *R. typhina* fruits by Response surface methodology (RSM)

The aim of this section was the optimization of the extraction parameters for UAE technique (solvent concentration (X_{Sc}), extraction time (X_T) and solvent/solid ratio (X_R)), using the Box-Behnken design, so that the values of TAC (Y1), TPC (Y2) and antioxidant activities FRAP (Y6) and DPPH (Y7) to be as high as possible, but TTC value (Y3) – the lowest. The obtained results, presented in Table 10, were published in **Cocîrlea, M. D.**, Simionescu, N., Călin, T., Gatea, F., Badea, G. I., Vamanu, E., Oancea, S. (2024).

The optimization analysis indicated as suitable values for the independent variables included in the study, reported to the maximization and minimization requirements for the selected bioactive compounds and total antioxidant activities, the following: 61.51% solvent concentration, extraction time of 10 min and solvent/solid ratio of 20/1 (*v*/*w*).

The composite desirability of 0.7719 frame the results of the optimization analysis as being acceptable for the investigated responses, visualized as a whole or individually.

Table 10: Optimal UAE extraction values resulting from optimization of the analyzed parameters

Response	Aim	Predicted response	Desirability	Composite desirability	Optimal values for factors
Y1	Maximization	29.2151	0.7010	0.7719	X_{Sc} = 61.5152 %; X_T = 10 min; X_R = 20/1 (*v*/*w*);
Y2	Maximization	1698.0266	0.9547		
Y3	Minimization	1430.4662	0.7896		
Y6	Maximization	848.0085	0.9220		
Y7	Maximization	88.2253	0.5565		

The perspective plots, created with the aim of observing the simultaneous influence of two parameters of extraction (the third one is considered to be constant) on the measured response variables, showed a trend of increase for the contents in anthocyanins and tannins in the prepared extracts with the 20/1 (*v/w*) ratio, for medium or low mean concentrations of the solvent (Figure 10 (I, III a)). The extraction time decrease and the increase of extract concentration (10/1 (*v*/*w*)) lead to raisings of results for TAC (Figure 10 (I b)). As the extraction ratio increased (a mixture more dilluted), the content in total polyphenols and tannins were higher at longer process times (Figura 10 (II–III b)). Also in the context of a longer extraction, lower results for TAC, TPC and TTC were observed when a more concentrated hidroalcoholic solution was utilized (Figure 10 (I–III c)).

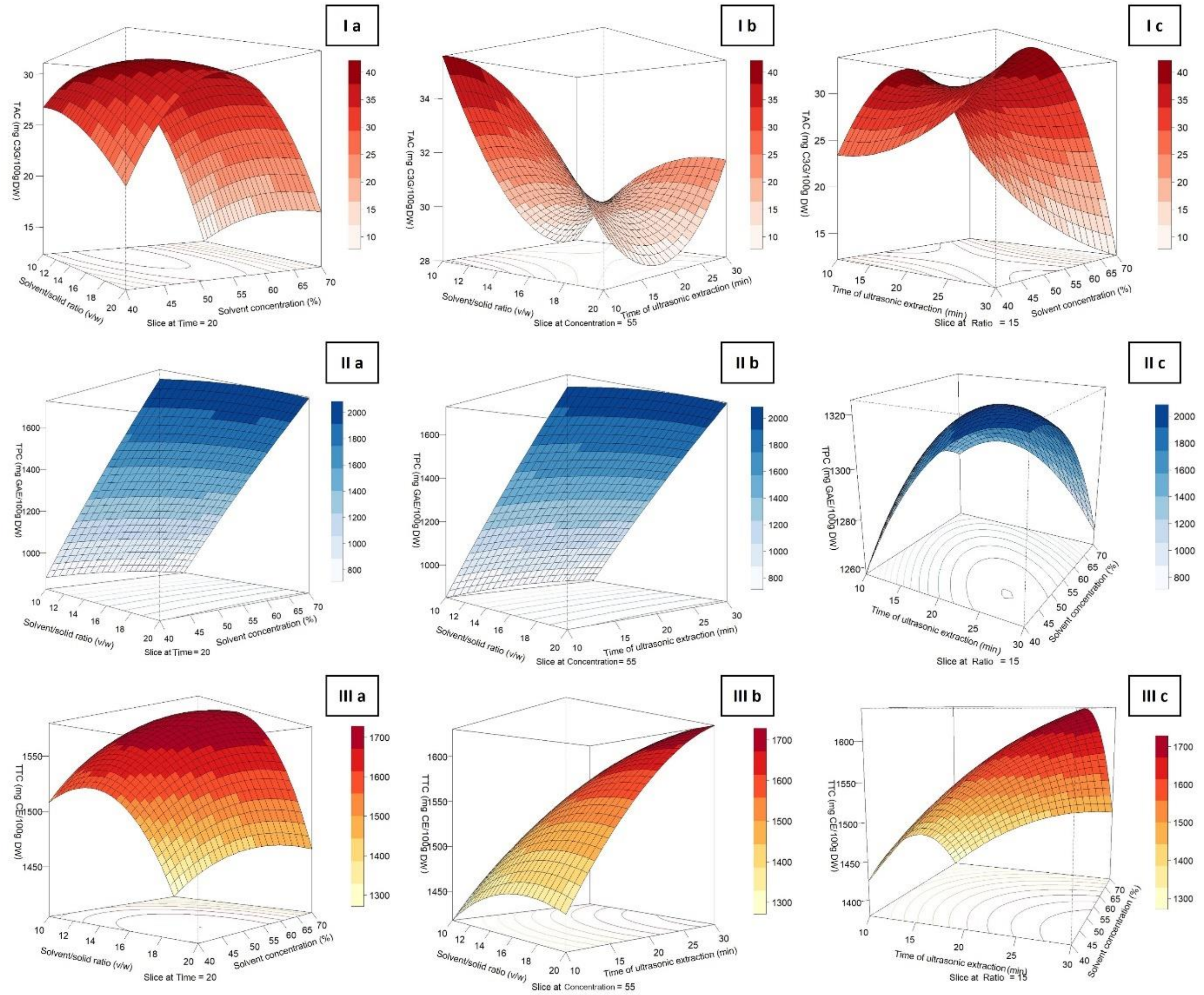

Figure 10: 3D plots of the response surface showing the effects: solvent/solid ratio and solvent concentration (I–III a), solvent/solid ratio and time of extraction (I–III b), time of extraction and solvent concentration (I–III c), on I – TAC, II – TPC, III – TTC.

FRAP antioxidant activity decreased as the extraction time of a mixture prepared with a more concentrated solvent increased, while for DPPH activity the effect of these conditions was the opposite, causing its growth (Figure 11 (I–II c)). As the solvent/solid ratio increased, the application of longer extraction times provided higher values of DPPH activity, while the utilization of these conditions caused the decrease of FRAP activity (Figure 11 (I–II b)).

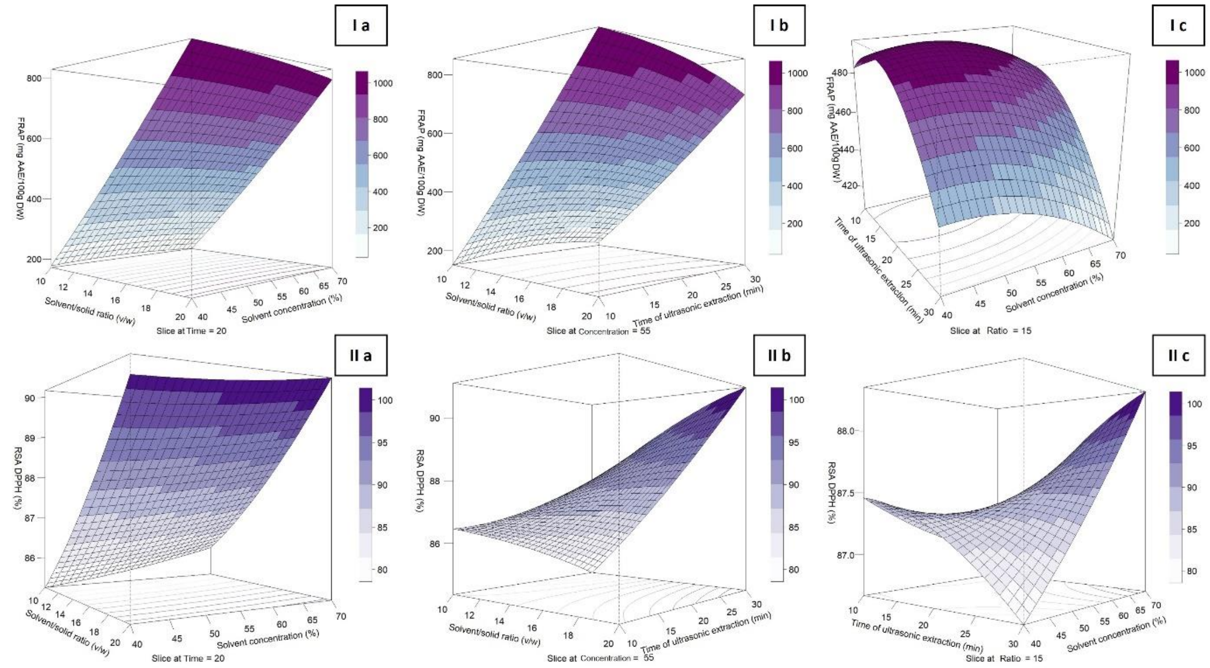

Figure 11: 3D plots of the surface response showing the effects: solvent/solid ratio and solvent concentration (I–III a), solvent/solid ratio and time of extraction (I–III b), time of extraction and solvent concentration (I–III c), on I – TAC, II – TPC, III – TTC.

3.3.6. Comparative analysis on extraction efficiency of bioactive compounds from fruits of *R. typhina* through UAE and BBE techniques

Using separately the results obtained in the staghorn sumac fruit extractions by UAE and BBE techniques, there were calculated two types of mean values: a general one, including all the values, regardless the applied experimental conditions, and a second mean, more specific, covering only the values for the extracted that were obtained at an process time of 10 min. The observation of general mean indicated that the best results for TAC, TFC and the content in carotenoids were obtained by UAE extraction, while the mean values for TPC, TTC and FRAP were raised in BBE extraction. Regarding the mean calculated using the values obtained for 10 min of extraction, BBE technique produced values higher for the four response variables: TPC, TTC, FRAP, DPPH, and the content in carotenoids was similar with the one of the extracts obtained by UAE method. Thus, bead-beat extraction was preferred because in the case of both types of means, it gave the highest content of total polyphenols and FRAP antioxidant activity, and added the advantages of a more powerful thermal stability of the mixture and a lower risk of contamination between samples, compared with the ultrasound-assisted extraction.

Analysis of the relationship between obtained experimental data for *R. typhina* extracts through GLM liniar models

Analysis of GLM liniar models for the ultrasound-assisted extraction showed the variable TPC as a significant predictor in the models of total antioxidant activities FRAP and DPPH ($t = 10{,}389$; $p = 2{,}37 \times 10^{-7}$ ***, respectively $t = 6{,}594$; $p = 1{,}73 \times 10^{-5}$ ***). In the case of bead-beating extraction, the significant preditor for FRAP activity was the content in carotenoids (t

= −4,647; p = 0,00046 ***), while TAC was the significant predictor for DPPH activity (t = 1,999; p = 0,0655).

3.3.7. Experimental research regarding the cytotoxic potential of the *R. typhina* fruit extract

In vitro analysis of the potential toxic effects of staghorn sumac fruits (obtained by BBE technique performed under the coditions: 61.51% ethanolic solution, ceramic beads of 2.8 mm, 20/1 (*v/w*) solvent/solid ratio, extraction time of 5 min) showed an increase of the toxic effect when the concentration of tested extract was higher. The highest rate of hemolysis was recorded at the highest tested concentration (1000 μg/mL), with a value of 19.85 ± 1.30%, while the highest citotoxicity was observed for the concentration of 500 μg/mL (the highest from the tested ones) on HepG2 cells, with a viability rate of 62% after 24 h of incubation.

4. APPLIED RESEARCH REGARDING UTILIZATION OF THE EXTRACT FROM FRUITS OF STAGHORN SUMAC (*R. TYPHINA*) IN THE ENVIRONMENTALLY-FRIENDLY DYEING OF TEXTILE FABRICS

4.2. Classic and modern dyeing strategies for textile fabrics

Chemical mordants can be divided into two categories: the ones that make the colors darker, respectively the ones that make it lighter (Singh et Singh, 2018). Tannic and citric acids, through the presence of more hidroxyl and carboxyl groups, play an important role for bio-mordants able to increase the affinity of the cellulose textile substrate for dyeing particles (Ontiveros-Ortega et al., 1998, Darmawan et al., 2024). It was observed that iron sulfate can provide green shadows to the treated material (Singh & Singh, 2018).

As dyeing techniques utilized nowadays in studies, can be listed: sonication method (Periyasamy, 2022), dyeing by boiling (the most common) (Hossain, 2023), dyeing with specific appliances/machinery (Ain et al.,2024), microwave-assisted dyeing (Yameen et al., 2023), etc.

4.3. Results regarding ecological dyeing of cotton fabrics with the extract from *R. typhina* fruits

In the aqueous extract from *R. typhina* drupes prepared for the dyeing of cotton textile samples (maceration at the room temperature, for 24 h, in the dark, in distilled water at an initial temperature of 40 °C), was identified the presence of a content in anthocyanins of 18.29±1.35 mg cyanidin-3-O-glucoside/ 100 g DW.

4.3.1. Chromatic characterization and testing the wash and dry rubbing resistence of cotton samples dyed with the extract from *R. typhina* fruits

Cotton samples were dyed with the extract from fruits of *R. typhina* by conventional technique of exhaustion and the modern method of ultrasonication, in the presence of solutions of chemical and ecological mordants, with concentrations of 3% or 5% (meta-mordanting). As control samples were used cotton samples undyed and dyed in the absence of mordanting process. The results for 22 experimental variants of ecological dyeings with the natural extract obtained from staghorn sumac fruits, regarding specific color parameters and color fastness at water and dry rubbing, were published in **Cocîrlea**, M.D., Coman, A., Popovici L.F., Oancea S., Coman D (2024).

The brightness of dyed samples was decreased by treating of textile substrate with mordants, reported to untreated samples, regardless the mordant type (conventional or modern) or the concentration of its solution. In the case of exhaustion, for the most of the tested mordants, concentration of 3% decreased stronger the brightness (in the case of iron sulfate (II) and copper sulfate, concetration of 5% produced the lowest brightness). Regarding the ultrasonication technique, the highest concentration used (5%) gave the lowest values for ΔL* parameter, an exception being represented by citric acid for which the darkest color were found at the concentration of 3%. The darkest samples were obtained through the treatment of cotton with iron sulfate.

In general, the color's proximity to the red region of the colorimetric spectrum decreased compared to untreated samples through the tannic acid and chemical mordants utilization, but slightly increased through the treatment of samples with citric acid. The samples treated with $FeSO_4$ presented values of b* coordinate with a trend towards the blue region.

Color changes reported through the values of Δa and Δb indices, between the dyed samples, mordanted and untreated with mordant, are presented in Figures 12–13.

White samples of cotton showed after dyeing with extract from fruits of staghorn sumac shades in the pink-light purple zone, varied depending on the mordant solution used to fix the color.

Color differences higher (over 6 units) was found for solutions of 3% and 5% $FeSO_4$, $CuSO_4$ 5% (exhaustion) and the mixture of 5% iron sulfate and 4% oxalic acid (ultrasonication).

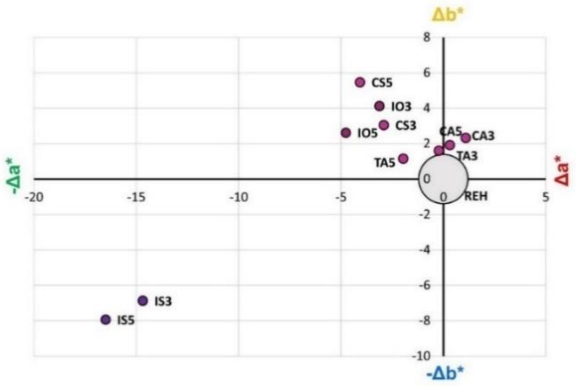

Figure 12: Distribution of color characteristics for samples dyed and mordanted simultaneous, through exhaustion technique

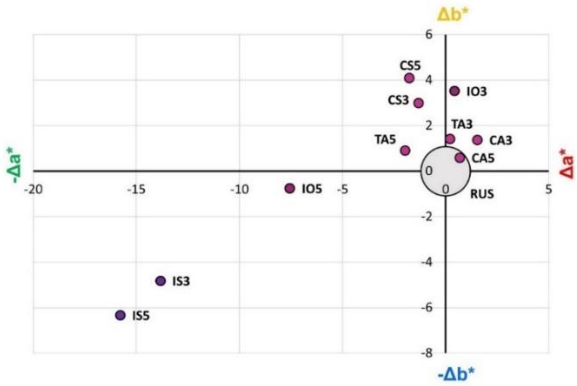

Figure 13: Distribution of color characteristics for samples dyed and mordanted simultaneous, through sonication technique

General color differences were lower in the case of US dyeing, the process temperature of 40°C contributing to this aspect. Analyzing the values of hue difference (ΔH*), it was observed that samples treated with bio-mordants (3-5%) showed pink-orange shades, similar to the untreated samples, while in the case of iron sulfate the color tone was directed towards greenish-blue shades. Regarding the saturation of samples, these presented a higher mean value in the case of dyeing by ultrasonication technique.

The treatment of cellulosic textile material with ecological mordants decreased with 1-2 units the resistence at water compared to samples mordanted with classical mordants. Regarding the dry rubbing fastness, there were not observed significant differences between the two categories of mordants used. Between the ecological mordants, 5% citric acid solution offered the highest resistence at rubbing for the dyed cotton, similar to the one obtained for the iron sulfate (5%) and the mixture of FeSO4 (3-5%) with oxalic acid.

4.3.2. Characterization by ATR-FTIR technique of cotton samples dyed with extract from *R. typhina* fruits

In the Figures 14–15 are presented the ATR-FTIR absorbance spectra for control cotton samples (undyed) and dyed with extract of *R. typhina* (mordanted or untreated), delimited according to the dying methos used: exhaustion or ultrasonication, which can bring to the fore the functional groupings from the extract of staghorn sumac, which were imprinted in the structure of the cellulosic fibre.

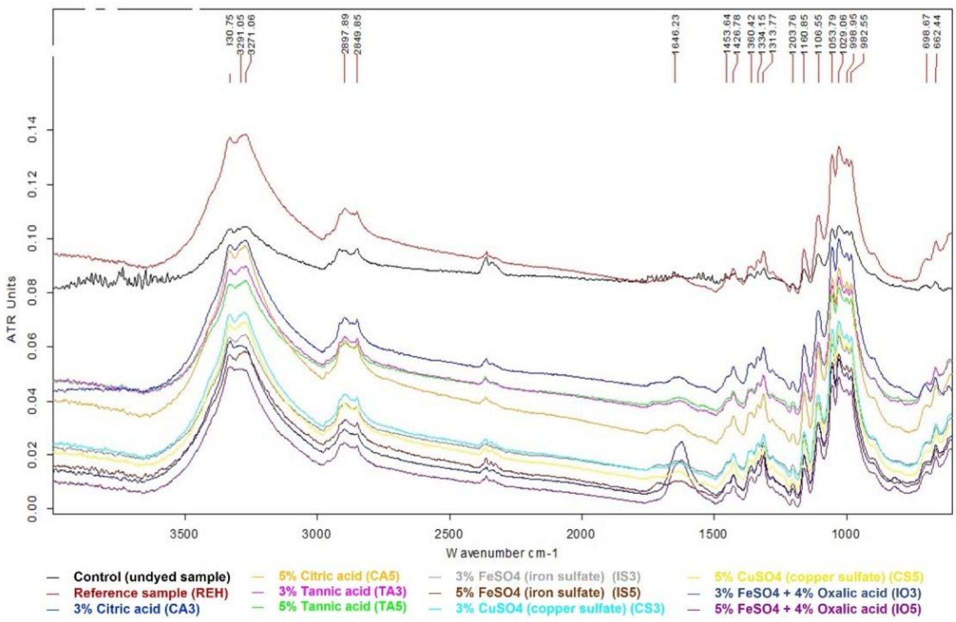

Figure 14: ATR-FTIR spectra for cotton samples dyed by exhaustion, with aqueous extract from *R. typhina* fruits, in the presence and absence of treatment with mordants

The presence of stretching vibrations of an alcoholic or phenolic hydroxyl group (−OH), specific to the cellulosic structure of cotton fiber or the possible existence of tannins found in the staghorn sumac extract, was indicated through the absorbtion peaks at 3271.06 cm^{-1} (EH) and 3270.06 cm^{-1} (US). Another absorbtion peak specific to cellulose was observed at 1028 cm^{-1}, corresponding to a C−O−C pyranosis ring. The spectral region under 1000 cm^{-1} was considered the unique zone of the sample („fingerprint area”) (Zhang et al., 2023). The chemical structure of dyes samples hasn’t variated considerably in relation to the utilized technique (exhaustion vs. ultrasonication), but ultrasonication kept more similar with the control sample the structure of cellulosic material. The most powerful impact on the samples structure was determined by the treatment with mixtures with iron sulfate (3-5%) and oxalic acid 4%.

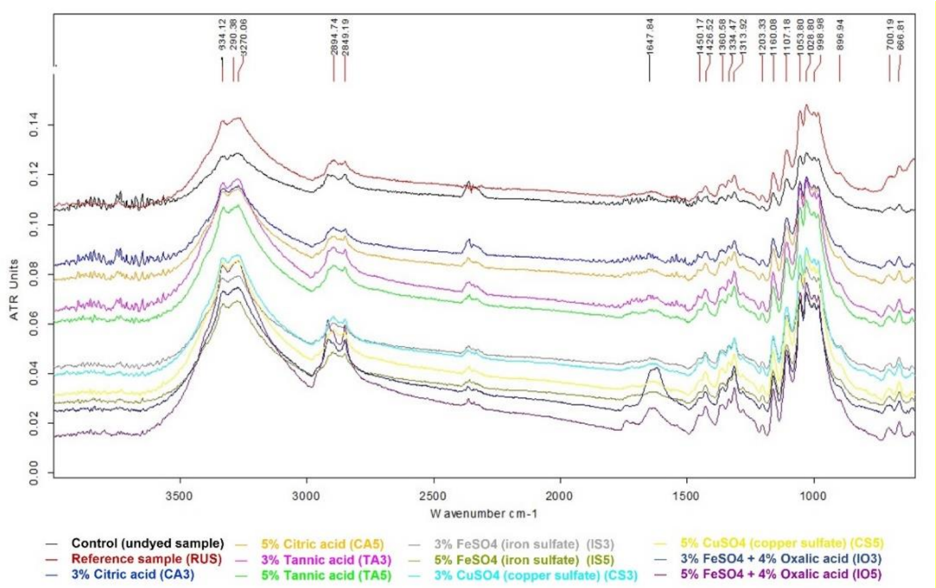

Figure 15: ATR-FTIR spectra of cotton samples dyed by ultrasonication with aqueous extract from *R. typhina* fruits, in presence and absence of treatment with mordants

GENERAL CONCLUSIONS, PERSONAL CONTRIBUTIONS, RECOMMENDATIONS AND RESEARCH PERSPECTIVES

As a result of analyzed studies, performed experimental methodology and statistical interpretation of obtained data, there were outlined more final conclusions, presented as follows:

- Based on the documentation of specialized literature, leaves of *A. altissima* and fruits of *R. typhina* presented a wide utility for more industrial domains (cosmetic, medical, pesticide production etc.), due to their content in valuable bioactive compounds, their biological properties being confirmed by the test results obtained in the experimental research of the doctoral thesis.
- SEM images caught morphological elements specific to leaves (stomata, trichomes) in the fresh and dried samples of *A. altissima*, but also cracks and decreases in the volume of trochomes, as a result of drying process performed at 50°C and grinding; on the surface of staghorn sumac fruits there were observed numerous coverage hairs, with the visibility of their places of insertion on the more voluminous particles in the sieved powders.
- Extractions of bioactive compounds were realized using classical (simple maceration or magnetic stirring) and unconventional techniques (microwave-assisted extraction (MAE), ultrasound-assisted extraction) (UAE) and bead-beating extraction (BBE)).
- Microwave irradiation of frozen Tree of Heaven leaves, exposed to radiation with 2.3 GHz frecvency, gave a better extraction yield for TPC (1690.89±35.73– 1880.18±15.41 mg GAE/100g DW) and FRAP antioxidant activity (1506.40±29.41– 1694.75±31.28 mg AAE/100g DW) compared to the one obtained by simple maceration (1652.47±26.51 mg GAE/100g DW, respectively 1566.21±28.95 mg AAE/100g DW), ATR-FTIR analysis confirming the absence of important structural modifications. Even so, drying leaflets at 60°C produced a decrease of values for the response variable raported to samples with frozen material.
- Application of a sequential extraction (polar–non-polar, non-polar–polar), showed that the bioactive substances from frozen leaflets of *A. altissima* are better concentrated when ethanol is used as first solvent in extraction (for dried powders, a pre-extraction in hexane can improve the extraction yield).
- CIELAB analysis indicated the absence of important color changes (ΔE) between the frozen material and those dried at the room temperature, 30°C and respectively 50°C, but the dried samples presented more reddish hues and drecreases of yellowness index.
- Ethanol was a more suitable solvent than hexane for the extraction of *A. altissima* leaves, regardless of sample type or the applied pre-treatment (summer or autumn, frozen or dried).
- The highets value of TPC and antioxidant activity FRAP were identified for frozen autumn material, and the highest content in tannins, flavonoids and carotenoids – for the dried summer material (extracts where ethanol was used as first solvent). The DPPH antioxidant activity was higher in the extracts from summer samples, dried at 50°C.
- HPLC analysis reported the presence of ten phenolic compounds in the ethanolic extract from autumn Tree of Heaven leaflets, and the gallic, *p*-coumaric and vanillic phenolic acids were identified in all four tested extracts (ethanolic extracts from summer or autumn, frozen or dried, leaves). In the case of *R. typhina* fruits, *p*-coumaric acid was identified and dosed (17.62 ± 0.59 μg/mL), along with ferulic acid (8.91 ± 0.74 μg/mL) and myricetin (8.89 ± 0.23 μg/mL).

- The extract from dried autumn leaves of *A. altissima* inhibited the growth of six from ten tested microbial strains, determining a diameter of inhibition zone of 10.0 ± 0.2–10.0 ± 0.3 mm for the patogenes *S. aureus* (clinical isolate), *E. coli* (ATCC 25922) and *E. faecalis* (ATCC 29212). The extract from *R. typhina* fruits (BBE, 5 min, 20/1 (*v/w*), 61,51%, 2.8 mm ceramic beads, concentrated to half) presented the most powerful inhibitory effect on *S. pyogenes* (ATCC 19615) (20 mm) and *S. enterica* (ATCC 13076) (12 mm) strains development. In the case of both plants, the potential antifungal effect was absent.
- The highest hemolytic activity of tested extracts from Tree of Heaven and staghorn sumac was recorded for the concentration of 1000 µg/mL (6.95%, respectively 19.85%). The only toxic effect was found in the case of 500 µg/ mL concentration for the extract of staghorn sumac, on the HepG2 cell line (viability of 62% after 24 h).
- Regarding the ultrasound extraction of the compounds from staghorn sumac fruits, performed according to a Box-Behnken design, it was observed that the highest value of TAC was present as an extraction time of 10 min, a 10/1 (*v/w*) ratio and 55% solvent concentration, while for the other response variables (TPC, TTC, TFC and carotenoids) the extraction performed at 30 min, 20/1 (*v/w*) ratio and 55% solvent concentration gave better results. The FRAP antioxidant activity was higher at shorter extraction times (10 min), but DPPH activity – at longer times (30 min).
- Regarding the extraction of staghorn sumac drupes through BBE technique, the mean of FRAP antioxidant activity was significantly higher for beads with 1.4 mm diameter than for 2.4 mm beads, while the metal beads meaningfully favorized the extraction of carotenoids compared to the samples where beads were not used.
- Bead-beating extraction technique (BBE) is recommended in the case of staghorn sumac fruits, producing at a similar extraction time with UAE technique, close or even higher mean values for the dosed bioactive compounds and measured antioxidant activities, without bringing the issue of thermal degradation of sensititve compounds or contamination between samples.
- The cotton dyeing with extract from staghorn sumac fruits, containing an amount of anthocyanins of 18.29±1.35 mg cyanidin-3-O-glucoside/ 100g DW, in general produced pink-violet shades.
- In the case of staghorn sumac fruits, the tested ecological mordants (tannic and citric acids) presented a good fixing capacity of the extracted dyes in the cotton fabrics, without strongly changing the shades of samples, but increasing the fastness of color reported to the one of samples untreated with mordants.
- Regardless of whether the dyeing was made by exhaustion or ultrasonication, iron sulfate make the color darker up to blue-green shades. The biggest difference of color ($\Delta E > 6$ units) and the lowest brightess were identified for the sample dyed with a mixture of 5% Fe_2SO_4 and 4% oxalic acid, through ultrasonication technique.
- Citric acid conserved better the pink-violet shades of the dyed samples; tannic acid slightly changed the hues to pink-yellow.
- 5% concentration of the bio-mordants solutions were more efficient in increasing the fastness at water and dry rubbing of samples compared to those treated with solutions of 3% concentration, but not as stronger as the classical mordant solutions did;
- Significant positive correlations were identified between Δa^* and ΔC^* (positive) and ΔE^* and ΔL^* (negative).
- Leaves of *A. altissima* and fruits of de *R. typhina* showed an important applicative potential, though the polyphenolic compounds contained by them, their demonstrated antioxidant and antimicrobial activities, and the low cytotoxic and/ or ecotoxic effects, the good dyeing capacity in the presence of ecological mordants and staghorn sumac drupes, but also an increased efficiency in the ethanolic extraction of compounds with biological activity from Tree of Heaven leaves, ethanol being a solvent environmental-friendly.

ELEMENTS OF ORIGINALITY (PERSONAL CONTRIBUTIONS)

The present doctoral thesis brought contributions in theoretical, experimental and applicative terms, regarding the extracts from leaves and fruits of some invasive species of global concern, but also for Romania, which present characteristics that make them suitable for different industrials sectors, and especially for sustainable processing of textiles. Through the developed elements of originality, there are included:

- Enrichment of the informational base regarding the bioactive character and optimal conditions of extraction of the collected organic matter for the investigated plants;
- Identification of the efficiency of the classical and modern extraction techniques, for the selected species, on their content in substances with biological activity, antioxidant properties and the capacity of dyeing textile materials (cotton);
- The application on non-conventional technique of extraction by microwaves (MAE), at low frequencies, on leaflets of Tree of Heaven, in order to identify the most suitable experimental conditions;
- Evaluation of the influence, in the context of a sequential extraction, of harvesting season and solvent (polar *vs.* non-polar), to find out the best conditions to prepare an extract from leaves of Tree of Heaven;
- The use of a Box-Behnken experimental design in order to simultaneously optimize the content in various antioxidant compounds, for the ultrasound-assisted extraction of *R. typhina* fruits;
- Evaluation of bead-beating extraction (BBE) in obtaining of extracts rich in polyphenols from staghorn sumac fruits and identification of the most advantageous conditions of extraction;
- Investigation through ATR-FTIR technique of the differences in composition for the extracts from Tree of Heaven leaves, obtained by two different techniques: conventional and unconventional;
- Contributions regarding the utilization of the extract from *R. typhina* drupes in the dyeing process of cotton fabrics, involving classical (exhaustion) and modern (ultrasonication) techniques, in the presence of absence of traditional meta-mordanting, with chemical or ecological mordants, evaluated through the determinations of CIELAB characteristics and of fastness at water and dry rubbing, in accordance with the specific standard tests.

RECOMMENDATIONS AND FUTURE RESEARCH DIRECTIONS

The main study directions designed to complete the information obtained by the reaserch reported in the present doctoral thesis, refer to:

- Utilization of other sustainable resources of bioactive compounds (other invasive plant, food waste or residues from technological processes), that could be subjected to the developed experimental procedure;
- The use of the extracts obtained in optimized conditions for making qualitative and environmental-friendly products;
- Deepening the applicative research regarding the extract from *R. typhina* fruits on other textile fabrics, or using other ecological mordants, and also the involvement of extract from Tree of Heaven in this process, individually or in a mixture with the extract of staghorn sumac;

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