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INVESTIGATION OF EXTRACTS OF *Amaranthus retroflexus* HERB, *Amaranthus hypochondriacus* HERB, AND CALLUS BIOMASS (cv. 'Lera') FOR TOTAL PHENOL AND FLAVONOID CONTENT

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Amaranthus is a pseudocereal plant with a high content of proteins and fatty acids in its seeds, and polyphenolic compounds and flavonoids in its leaves.

Aim. To investigate the influence of extractant type, hydromodule, extraction time, temperature, and extraction method on the extraction of polyphenolic compounds and flavonoids from *A. retroflexus* herb, *A. hypochondriacus* herb, and callus biomass.

Methods. The content of polyphenolic compounds and flavonoids was determined using a spectrophotometric method.

Results. The optimal extraction conditions for *A. retroflexus* herb were a 40% and 80% aqueous ethanol solution, a hydromodule of 1:10, an extraction temperature of 20 °C, and shaking at 100–200 rpm. For *A. hypochondriacus* callus biomass, the optimal conditions were a 40% aqueous ethanol solution and a hydromodule of 1:10. For *A. hypochondriacus* herb, the optimal conditions were a 50% aqueous ethanol solution, a hydromodule of 1:10, an extraction temperature of 30–40 °C, and magnetic stirring at 400 and 800 rpm for the maximum yield of flavonoids and polyphenolic compounds, respectively. Extraction was carried out for up to 270 h by static maceration.

Conclusions. Optimal extraction parameters were established to ensure maximum yields of polyphenolic compounds and flavonoids from *A. retroflexus* herb, *A. hypochondriacus* herb, and callus biomass.

Keywords: plant extract, callus biomass, polyphenolic compounds, flavonoids, maceration.

Traditional herbal medicines and preparations of plant origin are gaining increasing popularity. In Ukraine, as in the rest of the world, demand for medicines and consumer products enriched with plant secondary metabolites is increasing significantly. There is a growing demand for functional products of plant origin, for hygiene products, and cosmetic products based on natural ingredients. The stable, reproducible quality of plant raw materials used for medical and health purposes, in their natural form or for the manufacture of medicinal herbal preparations, is of paramount importance for the clinical efficacy and reproducibility of the positive effects observed in clinical studies. The safety of the plants and the medicinal products derived from them depends on the stability of their metabolite composition. Raw materials for the production of herbal medicines should come from a traceable and reproducible source [1].

The production of secondary plant metabolites, including their biosynthesis and bioconversion, can be achieved using in vitro cultivation technologies. Callus formation

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from primary explants is a well-established and effective cultivation method. Callus cultures can be subcultured to support organogenesis and embryogenesis, to establish cell suspension cultures in batch systems, or to be maintained as callus biomass.

In nature, callus is a wound tissue formed from proliferating cells in the injured area of an explant and induced by plant growth regulators. It typically consists of loose, compact, or hard, highly vacuolated cells that are partially differentiated and disorganized, often containing regions of small meristematic cell clusters [2].

Traditional herbal medicines and plant-derived preparations are gaining increasing popularity. In Ukraine, as in many countries worldwide, the demand for medicines and consumer products enriched with plant secondary metabolites is steadily increasing. There is also a growing demand for functional plant-based products, as well as hygiene and cosmetic products formulated with natural ingredients.

The stable and reproducible quality of plant raw materials used for medical and health purposes —either in their natural form or for the production of herbal medicinal preparations — is of paramount importance for clinical efficacy and for ensuring the reproducibility of therapeutic effects observed in clinical studies. The safety of medicinal plants and plant-derived products depends on the stability of their metabolite composition. Therefore, raw materials for herbal medicine production should originate from traceable and reproducible sources [1].

Classical ex situ plant cultivation methods require significant land resources, specialized personnel, and continuous maintenance. The success of such cultivation often depends on the length of the growing season and prevailing agroclimatic conditions. In vitro cultivation involves growing plants under sterile conditions on nutrient media. The success of this approach depends on the quality of the primary explant (a portion of the donor plant introduced into culture) and the composition of the nutrient medium [3].

Amaranthus is a pseudocereal that grows well in both temperate and tropical regions. Interest in this genus is driven by the high nutritional value of its seeds. In fact, *Amaranthus* is a promising crop that provides high-quality proteins, unsaturated fatty acids, and other valuable compounds at levels exceeding those found in conventional cereals such as maize or sorghum.

Over the past 15 years, several studies have investigated the content of phenolic compounds in amaranth seeds, as well as their functional and bioactive properties. For instance, hydroxycinnamic acids are associated with lipid stabilization and protection in grains. Information on the phenolic profile is important for evaluating the potential of *Amaranthus* as a dietary source of antioxidants [4].

Amaranthus retroflexus is a globally distributed species belonging to the family Amaranthaceae. Although it is often considered a weed due to its invasive nature and potential to reduce crop yields, *A. retroflexus* possesses significant nutritional and medicinal value. Modern scientific studies have demonstrated a rich phytochemical profile of this species, indicating its considerable potential health benefits [5]. The methanolic extract of *A. retroflexus* herb has been shown to contain protocatechuic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, and quercetin [6].

Amaranthus hypochondriacus seeds contain substantial amounts of protein, dietary fiber, and lipids, while extracts from its leaves and flowers exhibit strong antioxidant activity due to their high rutin content [7]. In addition, rutin, isoquercitrin, nicotiflorin, vanillic acid, ferulic acid, *p*-coumaric acid, 4-hydroxybenzoic acid, syringic acid, protocatechuic acid, caffeic acid, salicylic acid, gallic acid, and sinapinic acid have been identified in ethanol extracts of *A. hypochondriacus* leaves [8].

The flavonoid content of callus biomass of *A. tricolor* is influenced by the concentration of growth regulators in the culture medium. The addition of 2,4-D, NAA, or IAA significantly increases the total flavonoid content of callus cultures [9].

Materials and Methods

The objects of the study were callus biomass of *A. hypochondriacus* (cv. 'Lera'), obtained at the Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology of Lviv Polytechnic National University from environmentally friendly seeds of "Amarant Bio" (Cherkasy), as well as red phyto-tea of *A. hypochondriacus* (cv. 'Lera') "Amarant Bio" and *A. retroflexus* herb produced by the manufacturer "Soyuz Afghan" (Kropyvnytskyi).

The callus biomass of *A. hypochondriacus* was obtained in vitro on a modified Murashige

and Skoog medium supplemented with growth regulators: 0.5 mg/L naphthaleneacetic acid (NAA) and 3.0 mg/L 6-benzylaminopurine (BAP). Cultivation was carried out under a 16/8 h (light/dark) photoperiod, at an illumination of 2000 lux, a temperature of 26 ± 2 °C, and relative humidity of 60–70% for 50 days [10].

The average particle size of *A. retroflexus* herb, *A. hypochondriacus* herb, and callus biomass was 0.5 ± 0.1 cm.

The dependence of the content of polyphenolic compounds and flavonoids on the completeness of extraction from raw plant materials was investigated. Hydroalcoholic extracts were obtained under different conditions by varying the following parameters: extraction method, extractant concentration, solid-to-solvent ratio (hydromodule), temperature, and extraction time. Ethanol solutions of different concentrations (40%, 50%, 70%, 80%, and 96%) were used as extractants. Solid-to-solvent ratios of 1:10, 1:20, 1:50, and 1:100 were applied for extract preparation.

Hydroalcoholic extracts were obtained by maceration for 10 days, followed by filtration through paper filters into volumetric flasks. In each experimental series, only one factor was varied while all other parameters were kept constant. To evaluate the effect of extraction time, maceration was performed in 70% ethanol at a hydromodule of 1:10 over 10 days, with sampling every 48 hours. To study the effect of temperature, extraction was carried out using 40% ethanol at a hydromodule of 1:10 for 4 hours at 20, 30, and 40 °C.

To determine the optimal extraction method for secondary metabolites, extraction with 40% ethanol at a hydromodule of 1:10 for 4 hours was performed using different techniques: static maceration, magnetic stirring at 400, 600, and 800 rpm, and shaking at 100, 200, and 250 rpm. After extraction, all samples were filtered through paper filters.

Quantitative determination of polyphenolic compounds was performed spectrophotometrically using a UNILab 108UV spectrophotometer with the Folin–Ciocalteu reagent, and results are expressed as gallic acid equivalents (GAE). Total flavonoid content was determined spectrophotometrically using aluminum chloride, and results are expressed as quercetin equivalents. Each sample was analyzed in five replicates [11].

For selected samples, dry residue was determined. A 0.2 mL aliquot of each extract was weighed to ±0.0001 g using an analytical balance. Samples were dried at room temperature (24 ± 2 °C) to evaporate the solvent, leaving only the dry mass of the phytocomplex. Samples were weighed every 24 hours until constant mass was achieved.

Results and Discussion

Within the framework of this study, a preliminary quantitative assessment of polyphenolic compounds and flavonoids was performed using spectrophotometric methods as an initial screening stage.

After analyzing extracts of *A. retroflexus* herb, it was found that the highest total yield of extracted metabolites was obtained using 96% ethanol; however, only low amounts of polyphenolic compounds and flavonoids were detected in these extracts. The most efficient extraction of polyphenolic compounds from *A. retroflexus* herb was achieved using a 40% aqueous ethanol solution, whereas the highest yield of flavonoids was obtained with an 80% aqueous ethanol solution.

For *A. hypochondriacus* callus biomass, the highest total metabolite content was obtained using a 40% aqueous ethanol solution, including significant amounts of both polyphenolic compounds and flavonoids. The highest flavonoid content in callus extracts was observed in the 50% aqueous ethanol extract.

The optimal extractants for maximum metabolite extraction from *A. hypochondriacus* herb were 50% and 70% aqueous ethanol solutions, while the lowest metabolite yields were obtained using 96% ethanol. Polyphenolic compounds and flavonoids were most efficiently extracted using 40% and 50% ethanol solutions, which can be explained by the physicochemical properties of these secondary metabolites, particularly their increased solubility in more polar solvents.

Table 1 presents the results of the study on the content of polyphenolic compounds, flavonoids, and dry residue in *A. retroflexus* herb extract (ARH), *A. hypochondriacus* herb extract (AHH), and *A. hypochondriacus* callus biomass extract (AHC), depending on the concentration of the aqueous ethanol solution.

The extractant plays an important role in the extraction of secondary metabolites, as it must readily penetrate cell walls, selectively dissolve target compounds, and facilitate their release from plant material. For efficient extraction and reduced processing time, the extractant should be selective, chemically

Table 1. **Content of secondary metabolites in *Amaranthus* extracts obtained using different aqueous ethanol mixtures**

Extractant	Content of secondary metabolites. mg/ml								
	Polyphenols			Flavonoids $\times 10^{-5}$			Dry residue		
	ARH	AHC	AHH	ARH	AHC	AHH	ARH	AHC	AHH
40%	0.6482 ±0.0005	0.2314 ±0.0009	0.8543 ±0.0002	0.3149 ±0.0002	0.0261 ±0.0004	0.2893 ±0.0002	0.0198 ±0.0001	0.0342 ±0.0002	0.0240 ±0.0001
50%	0.5522 ±0.0005	0.1529 ±0.0007	1.0568 ±0.0002	0.2284 ±0.0005	0.0285 ±0.0005	0.2894 ±0.0002	0.0139 ±0.0001	0.0302 ±0.0001	0.0272 ±0.0001
70%	0.3818 ±0.0005	0.0851 ±0.0002	0.7938 ±0.0005	0.3096 ±0.0005	0.0033 ±0.0002	0.1356 ±0.0003	0.0223 ±0.0001	0.0301 ±0.0002	0.0272 ±0.0002
80%	0.3698 ±0.0007	0.0522 ±0.0007	0.6795 ±0.0002	0.3811 ±0.0006	0.0122 ±0.0007	0.2698 ±0.0002	0.0124 ±0.0001	0.0059 ±0.0001	0.0206 ±0.0002
96%	0.3825 ±0.0002	0.1455 ±0.0002	0.1916 ±0.0005	0.0437 ±0.0002	0.0099 ±0.0002	0.0011 ±0.0003	0.0238 ±0.0002	0.0083 ±0.0001	0.0110 ±0.0001

and pharmaceutically inert, of low toxicity, and volatile. Aqueous ethanol mixtures of different concentrations exhibit varying degrees of polarity, and the selection of an optimal concentration enables the selective extraction of secondary metabolites from plant raw materials [12].

To obtain extracts of *Amaranthus caudatus* leaves with a high content of polyphenolic compounds and flavonoids, a 50% aqueous ethanol solution is typically used. It has been reported that such extracts contain 101.63 mg/g of polyphenols and 54.75 mg/g of flavonoids (expressed as quercetin equivalents) [13].

In 70% aqueous ethanol extracts of leaves of *A. cruentus*, *A. retroflexus*, and *A. hybridus*, polyphenolic compound contents of 4.31, 5.81, and 6.75 mg/mL, respectively, were detected [14], which is lower than that reported for the 70% ethanol extract of *A. hypochondriacus* herb. The chemical composition of plant extracts is influenced by geographical location, soil composition, plant growth conditions, and cultivar variability [14].

For example, in a 60% aqueous ethanol extract of *A. tricolor* callus biomass, flavonoid contents ranging from 2.94 to 3.42 mg/g were observed depending on the composition of the culture medium [15], which is higher than that reported for *A. hypochondriacus* callus biomass.

The study of *A. retroflexus* herb, *A. hypochondriacus* herb, and *A. hypochondriacus* callus biomass extracts demonstrated that a decrease in the amount of extractant relative to the raw material leads to an increase in the content of polyphenolic compounds and flavonoids. It can therefore be concluded that the optimal plant material-to-extractant ratio is 1:10 when using a 70% aqueous ethanol solution. The highest flavonoid content was observed in the *A. hypochondriacus* herb extract at a hydromodule of 1:30.

The results of the study of 70% ethanol extracts obtained by maceration for 10 days at plant material-to-extractant ratios of 1:10, 1:30, 1:50, and 1:100 are presented in Table 2.

The appropriate hydromodule is an important parameter for balancing the extraction efficiency of phenolic compounds and flavonoids. According to mass transfer principles, a higher solid-to-solvent ratio increases the concentration gradient, thereby enhancing the diffusion rate of compounds from plant material into the solvent [16]. A decrease in extractable compound levels at higher solid-to-solvent ratios suggests that exceeding the optimal ratio may lead to dilution effects or inefficient mass transfer during extraction [17]. For optimal extraction of secondary metabolites from amaranth herb, the literature recommends a hydromodule of 1:10 [14, 18], whereas for callus biomass a significantly higher hydromodule of 1:500 has been reported [15].

A study evaluating the optimal extraction time of secondary metabolites from *A. retroflexus* herb and *A. hypochondriacus* callus biomass demonstrated that the content of polyphenolic compounds, flavonoids, and total extractives increases with extraction time for both plant material and callus cultures. The results are presented in Table 3.

Table 2. **Content of secondary metabolites in 70% ethanol extracts of *Amaranthus* species using different hydromodules**

Hydromodule	Content of secondary metabolites, mg/ml								
	Polyphenols			Flavonoids $\times 10^{-5}$			Dry residue		
	ARH	AHC	AHH	ARH	AHC	AHH	ARH	AHC	AHH
1:10	0.3818 ±0.0005	0.0851 ±0.0002	0.7938 ±0.0002	0.3096 ±0.0002	0.1049 ±0.0004	0.1356 ±0.0002	0.0223 ±0.0002	0.0301 ±0.0001	0.0272 ±0.0002
1:30	0.2954 ±0.0002	0.0648 ±0.0002	0.4374 ±0.0002	0.1553 ±0.0001	0.0221 ±0.0002	0.1559 ±0.0002	0.0072 ±0.0001	0.0022 ±0.0001	0.0097 ±0.0001
1:50	0.2759 ±0.0002	0.0558 ±0.0002	0.1978 ±0.0001	0.0901 ±0.0003	0.0059 ±0.0004	0.0874 ±0.0001	0.0049 ±0.0002	0.0018 ±0.0002	0.0078 ±0.0001
1:100	0.2264 ±0.0003	0.0131 ±0.0005	0.1164 ±0.0002	0.0492 ±0.0001	0.0049 ±0.0002	0.0276 ±0.0001	0.0008 ±0.0001	0.0004 ±0.0001	0.0044 ±0.0002

Table 3. **Content of secondary metabolites in 70% ethanol extracts of *Amaranthus* species depending on extraction time**

Extraction duration, h	Content of secondary metabolites, mg/ml					
	Polyphenols		Flavonoids $\times 10^{-5}$		Dry residue	
	ARH	AHC	ARH	AHC	ARH	AHC
48	0.3562 ± 0.0002	0.0541 ± 0.0005	0.2642 ± 0.0002	0.0171 ± 0.0005	0.0170 ± 0.0002	0.0006 ± 0.0002
136	0.4756 ± 0.0004	0.0551 ± 0.0002	0.3153 ± 0.0002	0.0477 ± 0.0005	0.0171 ± 0.0001	0.0031 ± 0.0002
184	0.5809 ± 0.0002	0.0595 ± 0.0005	0.3411 ± 0.0002	0.0623 ± 0.0002	0.0202 ± 0.0001	0.0056 ± 0.0002
232	0.5934 ± 0.0009	0.0722 ± 0.002	0.3653 ± 0.0007	0.0783 ± 0.0014	0.0246 ± 0.0002	0.0073 ± 0.0001
270	0.658 ± 0.0015	0.1204 ± 0.0005	0.3821 ± 0.0009	0.1049 ± 0.0004	0.0657 ± 0.0001	0.0094 ± 0.0001

The extraction duration plays a crucial role in maximizing the recovery of metabolites from plant materials. Prolonged extraction times may increase yield; however, they can also lead to oxidation, hydrolysis, or polymerization of bioactive compounds [19]. One study demonstrated that after 270 h of extraction, the content of secondary metabolites continues to increase, suggesting the potential for further improvement by extending extraction time. Nevertheless, such prolonged extraction is not economically feasible. Jamiołkowska et al. extracted *A. cruentus*, *A. hypochondriacus* × *hybridus*, *A. retroflexus*, and *A. hybridus* herbs by maceration for 72 h; however, the completeness of polyphenolic compound extraction from the raw materials was not evaluated [14].

A study was conducted to determine the content of polyphenolic compounds, flavonoids, and total extractives in 40% aqueous ethanol extracts of *A. retroflexus* and *A. hypochondriacus* herbs obtained by maceration at 20 ± 1 °C, 30 ± 1 °C, and 40 ± 1 °C. The results are presented in Table 4. The highest content of polyphenolic compounds and flavonoids from *A. retroflexus* herb was observed at 20 °C. For *A. hypochondriacus* herb, the highest extraction of polyphenolic compounds was achieved at 30 °C, while the highest flavonoid content was obtained at 40 °C. For both studied raw materials, the highest dry residue was observed at 30 °C.

Extraction temperature can significantly influence both the yield and stability of extractable compounds. Elevated temperatures increase solubility, diffusion rates, and

Table 4. **Dependence of secondary metabolite content in 40% aqueous ethanol extracts of *Amaranthus* on temperature (4 h extraction)**

Temperature, °C	Content of secondary metabolites, mg/ml					
	Polyphenols		Flavonoids $\times 10^{-5}$		Dry residue	
	ARH	AHH	ARH	AHH	ARH	AHH
20	0.6482 ± 0.0002	0.8543 ± 0.0005	0.3149 ± 0.0002	0.2893 ± 0.0001	0.0198 ± 0.0001	0.0240 ± 0.0001
30	0.4537 ± 0.0004	0.9059 ± 0.0002	0.1771 ± 0.0002	0.3477 ± 0.0005	0.0211 ± 0.0001	0.0302 ± 0.0001
40	0.6044 ± 0.0002	0.8717 ± 0.0002	0.2253 ± 0.0002	0.4680 ± 0.0005	0.0187 ± 0.0002	0.0262 ± 0.0001

mass transfer processes, thereby enhancing extraction efficiency. Flavonoids and phenolic acids are generally extracted more efficiently at moderate temperatures (40–60 °C); however, temperatures above 80 °C may lead to their degradation [19].

Extraction temperature also affects different subclasses of polyphenols: more polar phenolic compounds are typically extracted at lower temperatures, whereas less polar compounds are more efficiently extracted at higher temperatures. Depending on the plant species, polyphenolic compounds may occur in free form within the cell or be bound to proteins or polysaccharides. Heat treatment can facilitate the disruption of these interactions and thereby increase extraction yield. Since different plant species contain varying proportions of free and bound phenolics, the optimal extraction temperature must be determined experimentally for each species [20].

To isolate secondary metabolites from plant raw materials, various extraction methods based on diffusion and mass transfer processes are used. For the preparation of amaranth extracts, the literature recommends the use of magnetic stirring or shaking techniques [13–15, 18]. In this study, both a magnetic stirrer and a shaker were applied.

For *A. retroflexus* herb extracts, an increase in stirring speed led to a higher yield of extracted metabolites; however, the maximum content of polyphenolic compounds and flavonoids was observed at shaking speeds of 200 rpm and 100 rpm, respectively. The use of agitation during extraction increased the yield of extractable substances by 1.1–1.4 times compared with static maceration. The results of the study on the content of polyphenolic compounds, flavonoids, and dry residue in herb extracts at different stirring speeds are presented in Table 5.

For optimal extraction of metabolites from *A. hypochondriacus* herb, magnetic stirring is recommended. The highest content of polyphenolic compounds and extractives was observed at 800 rpm, while the highest flavonoid content was obtained at 400 rpm. The levels of polyphenols and flavonoids in *A. hypochondriacus* herb extracts obtained by shaking were lower than those obtained by maceration. The results of the study on the content of polyphenolic compounds, flavonoids, and dry residue in *A. hypochondriacus* herb extracts depending on stirring speed are presented in Table 6.

Traditional extraction methods such as maceration, Soxhlet extraction, and hydrodistillation have been widely used for obtaining secondary metabolites from plant materials. However, these methods have significant limitations, including prolonged extraction times, high solvent consumption, thermal degradation of thermolabile compounds, and limited selectivity. In addition, maceration may lead to co-extraction of unwanted compounds, thereby reducing the purity and bioactivity of the final extract [19].

Increasing stirring speed during dynamic maceration enhances the penetration of the extractant into plant material, thereby improving mass transfer rates. For the extraction of polyphenolic compounds from pomegranate peel, the optimal stirring speed was reported to be 500–1000 rpm. Similarly, for *Rhamnus alaternus* leaves, an optimal stirring speed of 518 rpm was determined during dynamic maceration [21], whereas for *A. retroflexus* it was lower.

Table 5. **Dependence of secondary metabolite content in 40% aqueous ethanol extracts of *A. retroflexus* herb on stirring speed**

Extraction	Content of secondary metabolites, mg/ml								
	Polyphenols			Flavonoids $\times 10^{-5}$			Dry residue		
	1	2	3	1	2	3	1	2	3
With a magnetic stirrer	0.3578 ± 0.0002	0.3897 ± 0.0004	0.4875 ± 0.0002	0.0959 ± 0.0003	0.1150 ± 0.0002	0.2847 ± 0.0002	0.0212 ± 0.0001	0.0216 ± 0.0001	0.0223 ± 0.0002
With a shaker	0.4385 ± 0.0002	0.7579 ± 0.0009	0.3779 ± 0.0009	0.4218 ± 0.0007	0.2024 ± 0.0002	0.0601 ± 0.0005	0.0219 ± 0.0002	0.0239 ± 0.0001	0.0207 ± 0.0002
Maceration	0.2963 ± 0.0002			0.2599 ± 0.0005			0.0180 ± 0.0002		

Note: 1 — the lowest speed; 2 — medium speed; 3 — the highest speed.

Table 6. **Dependence of secondary metabolite content in 40% aqueous ethanol extracts of *A. hypochondriacus* herb on stirring speed**

Extraction	Content of secondary metabolites, mg/ml								
	Polyphenols			Flavonoids $\times 10^{-5}$			Dry residue		
	1	2	3	1	2	3	1	2	3
With a magnetic stirrer	0.9463 ± 0.0004	0.8719 ± 0.0002	0.9474 ± 0.0002	0.5105 ± 0.0002	0.3282 ± 0.0005	0.3255 ± 0.0002	0.0178 ± 0.0002	0.0271 ± 0.0001	0.0289 ± 0.0001
With a shaker	0.5257 ± 0.0002	0.6418 ± 0.0005	0.3381 ± 0.0004	0.4511 ± 0.0002	0.3182 ± 0.0002	0.1870 ± 0.0002	0.0286 ± 0.0001	0.0224 ± 0.0001	0.0242 ± 0.0001
Maceration	0.6671 ± 0.0002			0.3226 ± 0.0009			0.0224 ± 0.0002		

Conclusions

The influence of extractant type, hydromodule, extraction time, temperature, and extraction method on the efficiency of polyphenolic compound and flavonoid extraction from *A. retroflexus* herb, *A. hypochondriacus* herb, and callus biomass was investigated.

Polyphenolic compounds from *A. retroflexus* herb and *A. hypochondriacus* callus biomass were most efficiently extracted using a 40% aqueous ethanol solution, whereas those from *A. hypochondriacus* herb were best extracted with a 50% aqueous ethanol solution. The highest flavonoid yields were obtained using 80%, 40%, and 50% aqueous ethanol solutions, respectively.

The optimal hydromodule for the extraction of both polyphenols and flavonoids was 1:10; an increase in the solid-to-solvent ratio led to a decrease in the content of extracted metabolites.

The content of polyphenolic compounds, flavonoids, and total extractives increased with extraction time; however, 270 h cannot be considered the final extraction endpoint, although such prolonged extraction is not practically feasible.

The optimal extraction temperature for *A. retroflexus* herb was 20 °C, while for *A. hypochondriacus* herb the highest yield of polyphenols was obtained at 30 °C and that of flavonoids at 40 °C.

The highest yields of polyphenolic compounds and flavonoids from *A. retroflexus* herb were achieved during dynamic maceration with shaking at 100–200 rpm. For *A. hypochondriacus* herb, the highest polyphenol content was obtained using magnetic stirring at 800 rpm, whereas the highest flavonoid content was observed at 400 rpm.

In future studies, it is recommended to identify metabolites in the extracts using HPLC and to evaluate their biological activity in order to confirm their potential for practical applications.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

K.I. Hutsko: study conceptualization, extraction procedures, experimental work, and data analysis. R.O. Petrina: methodology development and result analysis. All authors contributed to manuscript drafting, critical revision, and final approval of the manuscript.

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ДОСЛІДЖЕННЯ ЕКСТРАКТІВ ТРАВИ *Amaranthus retroflexus*, ТРАВИ ТА КАЛУСНОЇ БІОМАСИ *Amaranthus hypochondriacus* (СОРТ «ЛЕРА») НА ВМІСТ ЗАГАЛЬНИХ ФЕНОЛІВ ТА ФЛАВОНОЇДІВ

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Amaranthus — це псевдозлакова рослина, насіння якої містить значний вміст протеїнів та жирних кислот, а листя — поліфенольних сполук та флавоноїдів.

Мета. Дослідити вплив типу екстрагенту, гідропрофілю, часу екстракції, температури та методу екстракції на екстракцію поліфенольних сполук та флавоноїдів з трави *A. retroflexus*, трави та калусу *A. hypochondriacus*.

Методи. Визначення кількості поліфенольних сполук та флавоноїдів проводили спектрофотометричним методом.

Результати. Оптимальними умовами екстракції для трави *A. retroflexus* є 40% та 80% водно-етанольна суміш, гідропрофіль 1:10, температура екстракції 20 °С, перемішування на шейкері зі швидкістю 100–200 об/хв. Оптимальними умовами екстракції для калусної біомаси *A. hypochondriacus* є 40% водно-етанольна суміш, гідропрофіль 1:10. Оптимальними умовами екстракції для трави *A. hypochondriacus* є 50% водно-етанольна суміш, гідропрофіль 1:10, температура екстракції 30–40 °С, перемішування на магнітній мішалці зі швидкістю 400 та 800 об/хв для максимального виходу флавоноїдів та поліфенольних сполук відповідно. Екстракція, яку проводили методом статичної мацерації, тривала понад 270 годин.

Висновки. Було визначено оптимальні параметри екстракції, які забезпечують високий вихід поліфенольних сполук та флавоноїдів для трави *A. retroflexus*, трави та калусу *A. hypochondriacus*.

Ключові слова: рослинний екстракт, калусна біомаса, поліфенольні сполуки, флавоноїди, мацерація.

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