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In vitro propagation of *Eranthis longistipitata* and its phytocomponent analysis

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Abstract. *Aimenova ZE, Matchanov AD, Abdirahimova SS, Esanov RS, Sumbembayev AA, Duissebayev SE. 2024. In vitro propagation of* Eranthis longistipitata *and its phytocomponent analysis. Biodiversitas 25: 4171-4178. Eranthis longistipitata* is an endemic plant of Central Asia. The available literature indicates the great potential of *Eranthis* plants for use in various branches of pharmacology and medicine. The medical use of this plant is limited by its short flowering period and narrow range of growth. In this paper, we present the results of a study on the micropropagation of this species. As a result of the selection of the modification medium supplemented with benzylaminopurine, kinetin and Indolyl-3-butyric acid we obtained calluses from *E. longistipitata*. Since some of the most valuable biologically active substances in *E. longistipitata* are flavonoids such as rutin, isorhamnetin, and hyperoside, we investigated their presence in the calluses via High-performance liquid chromatography. The elemental composition was established for the first time via the Inductively Coupled Plasma (ICP) method, and the antioxidant activity was investigated via the Ferric Reducing Antioxidant Power (FRAP) assay. Toxicity was assessed via the brine shrimp assay. Extracts of *E. longistipitata* leaves, including those obtained with chloroform, ethyl acetate, and ethanol, were prepared to investigate their antioxidant and cytotoxic properties. The elemental composition analysis of callus tissue revealed the presence of potassium at a concentration of 19.516 mg/10 g and sulfur at 17.802 mg/10 g, with no trace of heavy metal ions or toxic elements detected. The study of the antioxidant activities of the leaf extracts revealed that the ethanol extract presented the highest capacity at a concentration of 1 mg/mL. The toxicity analysis of the leaf extracts revealed no toxicity toward the *Artemia salina* larvae used in the experiment.

Keywords: Antioxidant activity, endemic, elements, flavonoids, micropropagation, toxicity, winter aconite, western Tien Shan

INTRODUCTION

The plants of the genus *Eranthis* are endemic perennial tuberous ephemerids (an ecological group of plants with a short vegetation period), psychromesophytes (plants growing mainly on cold and moist soils), and semigeliophytes (this ecological group includes those plants that are adapted to life in places with good sunlight but are resistant to shading) (Park et al. 2019).

The most studied and widespread species are *Eranthis hyemalis* and *Eranthis stellata*. In contrast, most species of this genus are narrowly endemic and have small geographical areas, which makes comprehensive studies of *Eranthis* plants difficult. The available literature indicates the great potential of *Eranthis* plants for use in various branches of pharmacology and medicine. Recent studies using phylogenetic analyses have shown that the organismal evolution of *Eranthis* plants could be related to the formation of three great Asian plateaus (*Eranthis* seeds are not expected to disperse over long distances or through physical barriers) and indicated that *Eranthis* serves as a critical biological model for inferring the geological history of Asia (Xiang et al. 2021).

Eranthis longistipitata (Figure 1) is an endemic plant of Central Asia (Erst et al. 2019). Its principal place of growth is on the slopes of the western Tien Shan. In our earlier investigation of the leaves and tubers of *E. longistipitata*, several important flavonoids (e.g., rutin, apigenin and gallic acid) were identified (Aimenova et al. 2023). The biological activities of rutin include antioxidant, cytoprotective, vasoprotective, neuroprotective, anticarcinogenic, and cardioprotective effects (Ganeshpurkar and Saluja 2017).

Since this plant is native to the western Tien Shan region, which is shared by five bordering countries, namely, Kazakhstan, Uzbekistan, Kyrgyzstan, Turkmenistan, and China, studies have been conducted on this plant within these states. For example, the chemical composition of *E. longistipitata* leaves found in the Republic of Kyrgyzstan has been determined. More than 160 compounds have been identified, with 72 of these compounds belonging to a specific class (Erst et al. 2022).

The highest concentrations of flavonoids and fatty acids were detected. Coumarins and furochromones found in *E. longistipitata* are of particular interest, as these substances possess pronounced anti-inflammatory antioxidant, and antitumor activities. In addition, chromones with antioxidant properties against superoxide anions have also been identified in *E. longistipitata* leaves.

Reproduction of *E. longistipitata* is challenging because of the difficulty in obtaining seeds and the complex tuber

structure. Additionally, the short growing season further complicates the process. *E. longistipitata* seeds also have specific germination and embryo development characteristics that are associated with deep and complex morpho-physiological dormancy, which requires preliminary cold stratification at temperatures below 10°C to overcome. This fact contributes to the stability of the species under difficult climatic conditions, such as those found in the subalpine and alpine regions of the western Tien Shan.

Data on the *Eranthis* species propagation by biotechnological methods are still scarce. Seed reproduction of *Eranthis* species is limited by the morphophysiological dormancy of seeds owing to underdeveloped embryos. Recent research revealed that peduncles with undeveloped buds are the most efficient as the primary explants of *E. stellata* (cultivation under conditions t: 17°C, lightening mode-dark for prevention phenolic oxidation, nutrient medium -MS+ 5 µM 6-benzylaminopurine) (Erst and Erst 2020). It is reported that for setting up *E. tanhoensis* callus culture, it is necessary to use etiolated tuber seedlings for cultivation in the MS+ growth regulators 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine (Filonova and Mitrenina 2020). In the same study (Tipirdamaz and Gömürgen 2000) the application of the low temperature (+4°C) and exogenous gibberellic acid treatment on the germination of *E. hyemalis* was applied. Recently, research concerning *E. longistipitata* in vitro seed germination (Erst and Erst 2019) has reported that a combination of cold stratification and embryo culture methods is necessary to overcome the dormancy of seeds. Nevertheless, it is necessary to find an alternate method for large-scale propagation and to investigate the chemical composition and treatment effects of *E. longistipitata.*

MATERIALS AND METHODS

Plant material

The *E. longistipitata* plants were collected during the flowering-fruiting period in March 2024. To search for *E. longistipitata* in the Kazakh part of the western Tien Shan, we conducted geobotanical expeditions in the territory of the UNESCO Biosphere Reserve (World Heritage)-Aksu-Jabagly Nature Reserve. During these expeditions, three locations with *E. longistipitata* populations were identified: Taldy-Bulak Gorge (42º25’12N, 70°28’28E); Zhetimsay Gorge (42º24’41N, 70°32’41E) and the Irsu Valley (42º21’33 N, 70°22’28E). The populations found in the Irsu River valley differed in their abundance and morphometric characteristics (stem height, number of leaves and number of sepals). This result may be because this geographical area is higher than the other two areas (1,454 m above sea level) and is also characterized by increased humidity levels and longer light periods. At all three locations of *E. longistipitata* growth, the savanna vegetation type prevails.

Eranthis longistipitata populations from the Irsu Valley (42º21’33 N, 70˚22’28E) were harvested by hand and dug out with a knife. Undamaged, fully formed plants that were in a generative state (blooming as much as possible) were selected for collection. The dried seeds from mature plants (Figure 2) were randomly collected from at least 30 healthy plants in the Kazakh part of the western Tien Shan (Aksu Jabagly Reserve) and were used as explants.

Seed collection and explant preparation

The samples were pretreated with 2% alkali solution for 5-10 minutes with constant shaking and washed thoroughly with running tap water to remove the superficial dust particles as well as fungal and bacterial spores. They were then sterilized twice with sterile double distilled water and by 0.1% solution for 5 min of the fungicide "Prosaro" (Bayer CropScience Ltd, UK) inside the Laminar Air flow chamber. After sterilization, the seeds were exposed to 0.1% $AgNO_3$ solution for 1.5-3 minutes. Then, the seeds were immersed and rinsed two times in autoclaved distilled water to remove the growth inhibitors and to hasten the germination process (Gupta et al. 2020). The ability of seeds to remain viable for a long time and not proceed to germination is one of the most important adaptive properties of plants. In general, representatives of the Ranunculaceae family are characterized by a morphophysiological type of rest associated with underdevelopment of the seed embryo (Forbis and Diggle 2001). Cold stratification treatment was used to overcome seed dormancy in *E. longistipitata* seeds. The seeds were stratified by putting them in refrigerator (5°±1°C per 14 days) and control seeds (stored at room temperature and dry conditions for same period).

Figure 1. *Eranthis longistipitata* in the natural habitat in the Kazakhstan portion of western Tien Shan (Aksu Jabagly State Nature Reserve)

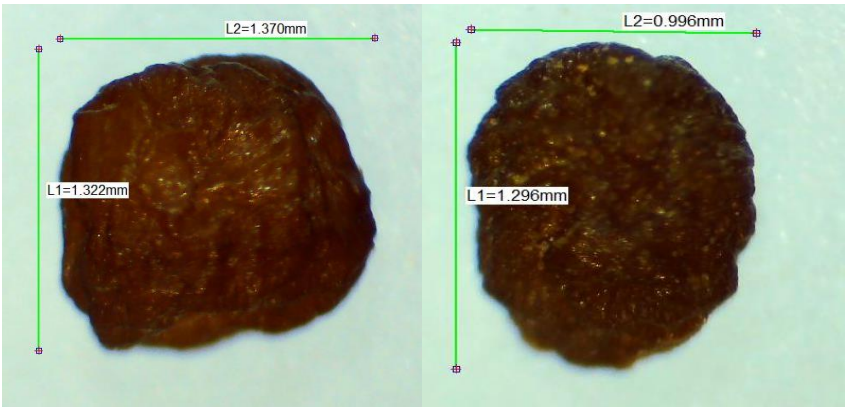

Figure 2. Seeds of *Eranthis longistipitata* under the microscope

The obtained material was inoculated into Murashige and Skoog media supplemented with Dichlorophenoxyacetic acid alone or in combination with benzylaminopurine, kinetin and Indolyl-3-butyric acid in different concentrations (0.1, 0.5 mg/L) were used to investigate its effects as multiplication media (Murashige and Skoog 1962). The induction of development was observed in the MS media without the addition of growth regulators. Semi-solid MS basal medium was adjusted to the desired pH 5.6-5.8 using HCl or NaOH before autoclaving at 121°C for 20 min. The culture room had a constant temperature of 25±2°C, under cool fluorescent light of about 5000 lux and a photoperiod of 16-h light and 8-h darkness. Visual observation of cultures was made weekly and the data were recorded.

Flavonoid analysis by High-Performance Liquid Chromatography

An Agilent 1260 High-performance liquid chromatography (HPLC) system (detector-diode matrix, ChemStation system, USA) was used to determine phenolic compounds via an Eclipse XDB column (5 microns, 4.6×250 mm), and t: 25°C was selected. The mobile phase of an aqueous solution of H_3PO_4 (0.1%) was 50-52%, 56 min (Van Beek 2002). The eluent flow rate was 1 mL/min. The detection wavelengths were 254, 320, and 381 nm, and the groups of phenolic substances were identified on the basis of their spectral characteristics (Erst et al. 2020). To identify the phenolic components in the *E. longistipitata* calluses, standard samples of salicylic and chlorogenic acids; quercetin; kaempferol; orientin (Sigma-Aldrich Chemie GmbH, Munich, Germany); gentisic and caffeic acids (Serva Heidelberg, Germany); and hyperoside and vitexin (FlukaChemie AG, Buchs, Switzerland) were used to identify the phenolic compounds in the extracts. The samples were analyzed twice.

Elemental analysis

A total of 0.1000 g of *E. longistipitata* callus culture was quantitatively transferred to a 3-mL Teflon autoclave. Three milliliters of concentrated nitric acid (HNO_3) and 2 mL of purified hydrogen peroxide (H_2O_2) were added. The autoclaves are closed and installed in a Berghof microwave oven (Speed WaveXpert or similar microwave oven). The decomposition command is used based on the finished program in the device interface. The number of autoclaves is indicated, while the device automatically controls the temperature and pressure inside them. A liquid crystal display controls the process information. The method was carried out under wet decomposition for 35-45 minutes under conditions of minimum temperature T (50°C), maximum temperature T (230°C), and maximum pressure R of 40 [bar] inside the autoclaves. The autoclaves are cooled to room temperature, and their liquids are quantitatively transferred to measuring flasks with a capacity of 50 or 100 mL (up to the mark). In this case, the autoclaves are washed 2-3 times and then topped with deionized water. The solution is mixed thoroughly, poured into an autosampler tube, and placed on an autosampler in a specific sequence. The program determines the position of each tube, the withdrawn mass, and the dilution coefficient (so that the device can automatically calculate the concentration). The mineralized solution is quantitatively analyzed via a PerkinElmer AVIO-200 inductively coupled plasma optical emission spectrometer (ICP-OES) (or a similar analog device) in comparison with a standard sample containing several macro- and microelements, heavy metal salts, and rare metals. The precision and standard deviation (RSD) of the analytical results is automatically calculated by recalculating the results based on the sample weights and dilution values at the end of the process.

Extraction

Three extracts of *E. longistipitata* (using chloroform, ethyl acetate, and ethanol) were prepared for antioxidant and toxicity assessments. Extracts were obtained via the standard extraction method on a Soxhlet apparatus. A total of 20 g of crushed raw material (leaves dried via the air-shade method) was placed in a sleeve and tank. 200 mL of extractant (chloroform/ethyl acetate/ethanol) were poured into a round-bottom flask, and a heating device was turned on to connect to the reverse refrigerator. After a 7-h extraction process, the resulting solution was distilled in a rotary evaporator, and 1.5-2 grams of the finished extract was obtained.

Antioxidant analysis via the FRAP assay

FRAP assay is based on the reduction of Fe^{3+} ions to Fe^{2+} by antioxidants via the K^3 antioxidant reduction reaction $[Fe(CN)_6]$ and is accompanied by the formation of yellow-colored $K_4[Fe(CN)_6]$. 0.25 mL of 0.2 M phosphate buffer (pH= 6.6) and 0.25 mL of 1% potassium hexacyanoferrate (III) solution were added to 0.1 mL of the studied substances in the 0.25, 0.5, 0.75, and 1.0 mg/mL concentration ranges. The reaction mixture was incubated for 20 minutes, and the reaction was stopped by adding 0.25 mL of 10% trichloroacetic acid solution. The mixture was subsequently centrifuged for 10 minutes (3000 rpm). The top 0.5 mL layer was mixed with 0.5 mL of distilled water and 0.1 mL of 0.1% $FeCl_3$.The optical density was measured at 700 nm. The antioxidant activities of the samples were compared with the antioxidant activity of gallic acid. Dilution was performed using 1 mg of the substance per 1 mL of solvent. Each sample was tested in three parallel experiments. The dilutions were carried out at 20±2°C during the natural light period. The concentrations of the working solutions were 0.25, 0.5, 0.75, and 1 mg/mL.

Toxicity assessment via the brine shrimp assay

Toxicity assessment was performed via the survival method using *Artemia salina* crustaceans. The flask was filled with artificial seawater, and *A. salina* eggs were added. They were kept with a soft air supply for three days until the crustaceans hatched from the eggs. Compared with the control, the acute lethal toxicity criterion of the substance mixture was indicated by the death of 50% of the larvae or more in the experiment. Dilution was performed using 1 mg of the substance per 1 mL of solvent. Each sample was tested in three parallel experiments at a temperature of 20±2°C with a natural light period. The pH of the control artificial mixture ranged from 8.0-8.5. The *A. salina* larvae are up to 1 day old. The larval planting density was 20-40 samples per test tube. Actinomycin D was used as a control. The samples were tested at concentrations of 10, 5, and 1 mg/mL.

Statistical analysis

All samples were analyzed as two biological replicates with three technical replicates per treatment group. All analyses were performed using the software BIOSTAT 2009 program (Analyst Soft, Inc., USA). For in vitro propagation analysis the experiment was conducted using a completely randomized design. Two experiments were conducted separately for each type of explant. The mean of replications was used for statistical analysis. For each of four explants, the MS medium was supplemented with five concentrations. Mean separation was performed using Duncan's multiple range test at 0.05 probability level.

RESULTS AND DISCUSSION

The quality and viability of the formed seeds are essential criteria that must be considered before preparing them for short-term and long-term storage and before growing new plants from them. The leading indicators of seed viability are the percentage of germination and the parameter seed strength (germination energy). Owing to weather conditions, *E. longistipitata* seeds ripen from the second half of May to the middle of June. The seeds are kidney-shaped, black or brown, 1.4-1.2 mm long, and 1.3-9 mm wide (Figure 2).

Primary cultivation. The obtained *E. longistipitata* material (30 sterile seeds for each Petri dish) was transferred to modified nutrient media in seven different combinations (E2 = MS + 2.4-D0.1 + BAP0.1; E3 = MS + 2.4-D0.5 + BAP0.1; E4 = MS + 2.4-D0.1 + BAP0.5; E5 = MS + 2.4-D0.1; E6 = MS + 2.4-D0.5; E7 = MS + BAP0.5 + Kn 0.4 + IBA0.4; E8 = MS + BAP0.1 + Kn0.5 + IBA0.5м) and control medium. From the 30 seeds planted on nutrient media, only 13-15 germinated (E1≈5, E3≈5, E7≈3). From the 30 seeds planted on nutrient media, germination was observed, on average, at 20 seeds (±21-23), contaminated samples were removed from the dishes. Control seeds without cold stratification treatment did not germinate at all. Weekly visual observation of cultures revealed first germination after 14 days, and a callus formed after 25-30 days.

With in vitro germination, the proportion of customarily germinated seeds was significantly lower than that under nonsterile conditions. When any of these combinations of sterilizing agents are used, anomalies in seedling development occur. This decrease in germination percentage may be due to the high toxicity of the sterilizing substances and their negative effects on the seed embryo.

For representatives of the Ranunculaceae family, the degree of differentiation of the embryo at the time of dissemination may be different. Despite extensive knowledge of the morphology of embryos in species of this family (Engel 1995), data on the growth requirements and rest disorders of embryos for this family are still insufficient. Studies have shown that some species of the Ranunculaceae family do not have an additional mechanism that prevents the growth and germination of seeds (Baskin and Baskin 1994). However, in the seeds of most representatives of this family, a special physiological mechanism delays the growth of the embryo even under optimal conditions. Cold stratification is often the most effective way to disturb rest in the Ranunculaceae family, which grows in temperate and alpine climates (Walck et al. 2000). In our experiment, the absence of callus formation in seeds that have not undergone cold stratification was also noted. According to research (Vandelook et al. 2009), the required temperature for seed stratification should be less than 10°C. In our study, this condition was met, the temperature was 5±1°C.

Callus cultures obtained from the seeds of *E. longistipitata* were inoculated into experimental nutrient media E1-E6 for development and regeneration. The first changes in the explants' response to the hormone were evident after two weeks, with significant differences observed at different concentrations (Table 1).

The data in Table 1 show that E3 and E6 media are the most optimal media for the development of *E. longistipitata* calluses. E7 and E8 media are experimental nutrient media for *E. longistipitata* plant regeneration (Table 1, Figure 3).

Regeneration of *E. longistipitata* and callus formation was observed in E7 and E8 media (Figure 3). These media differ from the others by the presence of benzylaminopurine, kinetin и Indolyl-3-butyric acid. 6-Benzylaminopurine is the most widely used adenine-based cytokinin as a supplement in plant growth media such as Murashige and Skoog and other media. Also, it is known as a first-generation cytokinin that influences plant growth and development, setting blossoms and stimulating fruit richness by stimulating cell division (Imelda et al. 2008). Kinetin is also a cytokinin growth factor inducing cell division, it is often used in plant cell and tissue culture for induction of callus formation (in conjunction with auxin) and to regenerate shoots from callus (with lower auxin concentration), it exists naturally in the DNA of almost all organisms, including human cells, and various plants (Duszka et al. 2009). Indolyl-3-butyric acid is an auxin plant growth regulator that can be absorbed by the roots, stems, leaves and fruits of plants. It can induce the differentiation of root tissue, stimulate hair roots, and increase the number of root systems. They promote cell elongation and regulate the growth of various organs and tissues, improve the absorption and operation of nutrient elements, and regulate vegetative growth and reproductive growth. For *E. hyemalis*, seed treatment at low temperatures in combination with gibberellic acid contributed to earlier germination (1 month earlier than in the control) (Tipirdamaz and Gömürgen 2000). In research (Erst and Erst 2019), it was detected that gibberellic acid seed treatment did not foster embryo development. In our studies, it was also found that the maturation of *E. longistipitata* seeds was observed without gibberelic acid treatment, but in combination with benzylaminopurine, kinetin and Indolyl-3-butyric acid and pre-cold stratification.

Table 1. Process of development and regeneration of *Eranthis longistipitata* calluses

Nutrient media options, mg/L		Six weeks		Eight weeks	
		Callus color	Callus length (cm)	Callus color	Callus length (cm)
E1	Control	White and yellow	0.3-0.5	yellow and brown	0.5-0.7
E2	MS+2.4-D*0.1+ BAP**0.1	White and green	0.6-0.8	white and green	0.8-1.2
E3	MS +2.4-D0.5+ BAP0.1	White and green	0.6-1	white and green	**1.5-1.8**
E4	MS+ 2.4-D0.1+ BAP0.5	White and green	0.5-0.8	white and green	0.8-1.4
E5	MS+ 2.4-D0.1	White and yellow	0.3-0.5	white and yellow	0.6-0.9
E6	MS+ 2.4-D0.5	White and yellow	0.6-0.9	white and yellow	**0.8-1.8**
E7	MS+BAP0.5+Kn*** 0.4 +IBA****0.4	White and yellow	0.6-0.9		0.8-1.4
E8	MS+BAP0.1+Kn0.5+IBA0.5м	White and yellow	0.8-1		0.8-1.6

Notes: *D-Dichlorophenoxyacetic acid; **BAP-benzylaminopurine; ***Kn-kinetin; ****IBA-Indolyl-3-butyric acid

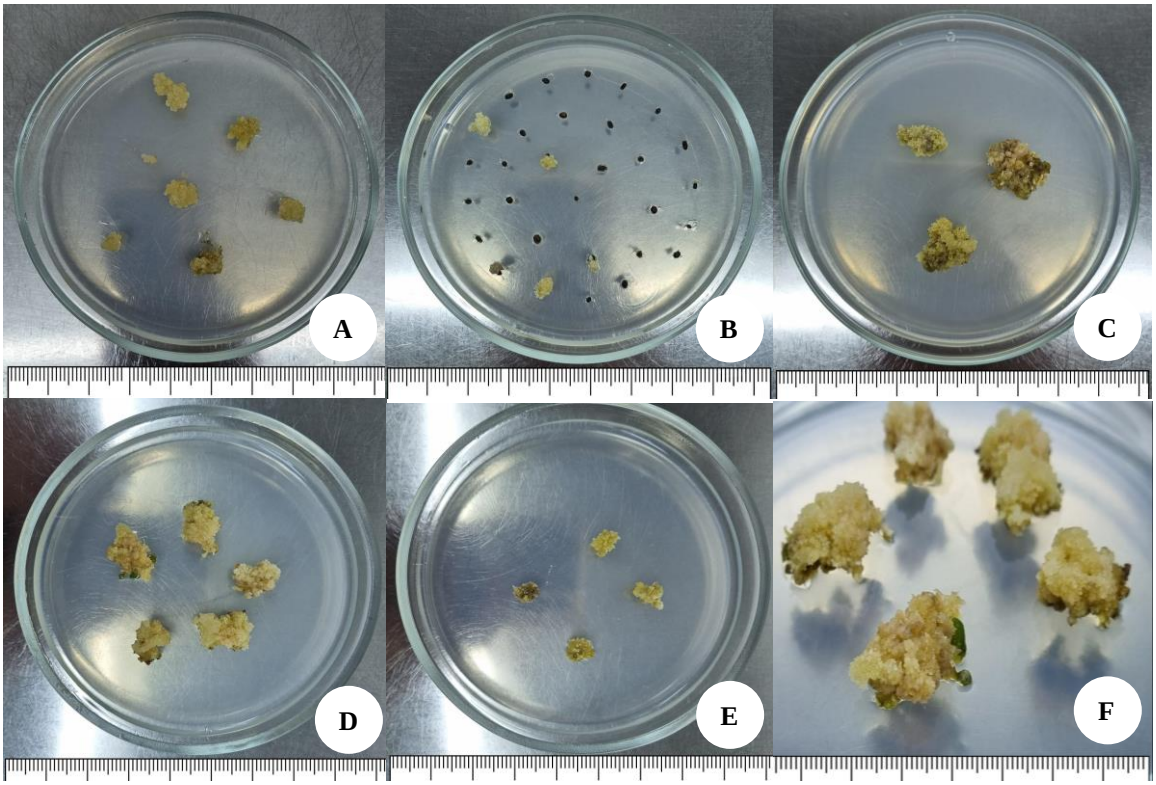

Figure 3. *Eranthis longistipitata* callus formation. A-C: E7 medium; D-F: E8 medium

Flavonoids are indispensable components in various fields of nutraceuticals, medicine, cosmetology, and pharmacology because of their antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties, as well as their ability to modulate critical functions of cellular enzymes (Panche et al. 2016). The next stage of our research was to determine the flavonoid contents in the resulting regenerated *E. longistipitata* plants (Table 2). In our previous studies (Aimenova et al. 2023), we determined the flavonoids contents in the leaves and tubers of *E. longistipitata* growing in their natural habitat.

Since the flavonoids present in *E. longistipitata* have antioxidant, antitumor, and anti-inflammatory activities, the presence of this group of biologically active substances in regenerated plants is important. Using the HPLC method, we established the presence of flavonoids in intact plants (Table 2). Valuable flavonoids such as rutin, apigenin, isorhamnetin, hyperoside, and quercetin are found in the calluses of *E. longistipitata*. The maximum values of rutin (488.676 mg/100 g), isorhamnetin (291.564 mg/100 g), were detected. Rutin has diverse biological properties, including antioxidant, anti-inflammatory, and antimicrobial effects and has a modulatory effect on the inflammatory response. Rutin is an effective therapeutic agent for mitigating and combating neuroinflammation and oxidative stress (Kessas et al. 2024). Isorhamnetin ameliorates cisplatin-induced acute kidney injury (Wang et al. 2024), inhibits the activity of pneumolysin from *Streptococcus pneumoniae* (Zou et al. 2023), and reduces oxidative stress, inflammation, and apoptosis (Abudalo et al. 2024).

Currently, the need for preparations containing trace elements has increased significantly. Trace elements in plants can stimulate the action of their biologically active compounds (Shkolnik 2012). The elemental compositions of plants are species-specific, depend on many environmental factors, and can vary widely. The amounts of absorbed substances depend on the growing conditions and the ion concentrations in the medium. Since *E. longistipitata* is an endemic medicinal plant, the determination of elements in its laboratory analog is a necessary step in its standardization as a medicinal plant. In this context, the next step of our research was to determine the contents of elements in *E. longistipitata* callus culture (Table 4). The amounts of absorbed substances depend on the growing conditions and the ion concentrations in the medium. Regenerated *E. longistipitata* plants were grown on modified nutrient media, the basis of which was Murashige and Skoog nutrient medium. The elemental composition of this nutrient medium is shown in Table 3.

Table 4. The elemental composition of the *Eranthis longistipitata* callus culture

Element	Content (mg/10 g)
K	19.516
S	17.802
Ca	3.405
P	6.500
Na	5.963
Mg	1.559
B	0.413
Fe	0.325
Mn	0.224
Cu	0.109
Li	0.097
Zn	0.093
Al	0.063
Se	0.057
Sr	0.046
Ni	0.037
Mo	0.028
V	0.014
Te	0
Sb	0
Sn	0
As	0
Pb	0
Cd	0
Ag	0
Hg	0
Co	0
Ba	0
Cr	0

Table 2. The amounts of flavonoids in *Eranthis longistipitata* calluses (mg/100 g)

Sample	Rutin	Apigenin	Isorhamnetin	Hyperoside	Hyperoside
E. longistipitata callus	488.676	55.542	291.564	63.994	35.321

Table 3. The elemental composition of the MS medium

Components	Content (mg/L)	Elements content	
		Mass fraction (%)	mg/L
KNO_3	1900	K-38.7	735.3
KH_2PO_4	170	K-28	48.8
KI	0.83	K-76.4	0.6
Na_2-EDTA·$2H_2O$ ($C_{10}H_{14}O_8N_2Na_2·2H_2O$)	37.3	Na-12.3	4.6
$CaCl_2·2H_2O$	440	Ca-27.3	120.1
$MgSO_4·4H_2O$	370	Mg-9.9	36.6
$FeSO_4·7H_2O$	27.8	Fe-20.1	5.6
$MnSO_4·4H_2O$	22.3	Mn-24.6	5.5
$ZnSO_4·7H_2O$	8.6	Zn-22.7	1.9
$Na_2MoO_4·2H_2O$	0.25	Na-19.0	0.05
		Mo-39.6	0.1
$CoCl_2·6H_2O$	0.025	Co-24.8	0.07
$CuSO_4·5H_2O$	0.025	Cu-25.5	0.01

The analysis of the elemental composition of *E. longistipitata* calluses revealed the presence of potassium (K) and sulfur (S) in significant quantities. Potassium, in particular, was found to be present in the highest amount, with a concentration of 19.516 mg per g. Potassium is known to have a high level of mobility, allowing it to be readily recycled between older and younger plant organs. It is typically concentrated in areas of the plant that are undergoing rapid growth and have an active metabolism, such as meristematic tissues, young roots, leaves, and shoots. It also helps plants resist abiotic stress in the environment (Johnson et al. 2022). Sulfur management is an important issue in plant nutrition. Sulfur promotes the formation of chlorophyll and enhances the development of the root system and the formation of seeds. It also increases the ability of plants to withstand stress and tolerate extreme weather conditions, such as decreases or increases in temperature, drought, and heavy rainfall (Astolfi et al. 2021). No heavy or toxic elements were detected in the regenerated plants. These studies confirm that *E. longistipitata* explants grown via micropropagation can be used as raw medicinal plant materials.

The antioxidant activity of *E. longistipitata* is due to the presence of flavonoids. Previously, studies on the antioxidant activity of *Eranthis cilicica* tubers have been conducted (Kuroda et al. 2009). The antioxidant activity of this plant is attributed to the presence of chromones in its tubers. Chromones have been found to be present in a relatively limited number of higher plant species in the Ranunculaceae family. We investigated the antioxidant activity of *E. longistipitata* leaf extracts in three different solvents, namely, chloroform, ethyl acetate, and ethanol, at various concentrations (0.25, 0.5, 0.75, and 1 mg/mL). We tested the antioxidant activity of *E. longistipitata* explants via the FRAP method, which is based on the ability of antioxidants to suppress the oxidative effects of reaction particles generated in the reaction mixture.

Table 5 shows the amount of antioxidants present in the various working solutions of *E. longistipitata* (using chloroform, ethyl acetate, and ethanol). At all concentrations tested, these solutions had lower average antioxidant activity than gallic acid. The maximum antioxidant activity was found at 1 mg/mL. Among the three solvents, the ethanol extract of *E. longistipitata* had a slight advantage in terms of qualitative antioxidant activity. There is a direct correlation between an increase in solution volume and the increase in antioxidant activity.

Since *E. longistipitata* is a medicinal plant and a potential source of raw medicinal plant material, it is necessary to study its toxicity. In particular, studies of the obtained explant plants are necessary. This study used a method based on the survival rate of the marine shrimp *Artemia salina*. This analysis is the most convenient because of its simplicity and speed. The LC50 (lethal concentration) value was calculated as the ratio of the percentage of *A. salina* mortality to the logarithm of the solution concentration (Table 6).

The acute toxicity was determined by measuring the adverse effect of various concentrations and types of *E. longistipitata* working solutions on brine shrimp *A. salina* growth, survival and mortality under intermittent flow-through conditions. The results (Table 6) were found after 24 hours to be in such a way that the chloroform, ethyl acetate, and ethanol extracts of *E. longistipitata* do not show cytotoxicity (larval mortality is 4-22%, which was negligible), in comparison with the drug actinomycin D, whereas the mortality rate of actinomycin D is 63-96% (as negative control solvent).

Table 5. Antioxidant activity of *Eranthis longistipitata* working solutions

Samples	The value of optical density at a concentration (mg/mL)			
	0.25	**0.5**	**0.75**	**1.0**
Gallic Acid (GA)	1.5609	1.6392	1.6612	1.7322
Chloroform extract (1)	0.3630	0.4165	0.4923	0.5590
Ethyl acetate extract (2)	0.3281	0.4244	0.5061	0.6455
Ethanol extract (3)	0.3344	0.4586	0.6134	0.7743

Table 6. Results of *Eranthis longistipitata* extract toxicity

Test substances	Concentration, mg/mL	Number of larvae in control		Number of larvae in sample		% of surviving larvae in control	% of surviving larvae in sample	Mortality rate, A,%
		Live	**Dead**	**Live**	**Dead**			
Actinomycin D	10	24	1	0	22	96	0	96
	5	24	1	1	25	96	4	92
	1	24	1	9	18	96	33	63
Chloroform	10	24	1	23	1	96	96	0
extract	5	24	1	23	0	96	96	0
	1	24	1	24	1	96	96	0
Ethylacetate	10	24	1	17	6	96	74	22
extract	5	24	1	28	0	96	96	0
	1	24	1	27	0	96	96	0
Ethanol extract	10	24	1	25	2	96	92	4
	5	24	1	23	1	96	96	0
	1	24	1	23	1	96	96	0

In minimum concentration of 1 mg/mL the was no mortality and as the concentration increased from 5 mg/mL and 10 mg/mL the mortality was about 22% (ethyl acetate extract) and 4% (ethanol extract). No neurotoxic effects of the chloroform, ethyl acetate, and ethanol extracts of *E. longistipitata* on *A. salina* larvae were detected. The results of the toxicity assessment of the prepared working solutions of *E. longistipitata* have confirmed the absence of hazardous compounds in the composition of this plant.

In conclusion, this study highlights effective medium composition for micropropagation of *E. longistipitata*. It is selective medium containing benzylaminopurine, kinetin, and Indolyl-3-butyric acid. As a result of an HPLC study of the flavonoid content in the resulting *E. longistipitata* callus, the presence of rutin (488.67 mg/100 g) and isorhamnetin (291.564 mg/100 g) was established. A study of the elemental composition of *E. longistipitata* callus culture showed a maximum content of K (19.516 mg/10 g) and S (17.802 mg/10 g) and the absence of heavy or toxic elements. The antioxidant activity of three extracts of *E. longistipitata* (using chloroform, ethyl acetate, and ethanol) showed a lower average antioxidant activity than gallic acid. However, an increase in the activity of ethanol extract with an increase in its concentration was found. *E. longistipitata* solutions possess no toxicity against test microorganisms (including neurotoxicity). *E. longistipitata* could potentially be a new source of medicinal ingredients.

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REFERENCES

Abudalo R, Gammoh O, Altaber S, Bseiso Y, Qnais E, Wedyan M, Oqal M, Alqudah A. 2024. Mitigation of cisplatin-induced cardiotoxicity by Isorhamnetin: Mechanistic insights into oxidative stress, inflammation, and apoptosis modulation. Toxicol Rep 12: 564-573. DOI: 10.1016/j.toxrep.2024.05.003.

Aimenova ZE, Matchanov AD, Esanov RS, Sumbembayev AA, Duissebayev SE, Dzhumanov SD, Smailov BM. 2023. Phytochemical profile of *Eranthis longistipitata* Regel from three study sites in the Kazakhstan part of the Western Tien Shan. Biodiversitas 24 (11): 6031-6038. DOI: 10.13057/biodiv/d241124.

Astolfi S, Celletti S, Vigani G, Mimmo T, Cesco S. 2021. Interaction between sulfur and iron in plants. Front Plant Sci 12: 670308. DOI 10.3389/fpls.2021.670308.

Baskin CC, Baskin JM. 1994. Deep complex morphophysiological dormancy in seeds of the mesic woodland herb *Delphinium tricorne* (Ranunculaceae). Intl J Plant Sci 155 (6): 738-743. DOI 10.1086/297212.

Duszka K, Clark BF, Massino F, Barciszewski J. 2009. Herbal Drugs: Ethnomedicine to Modern Medicine. Springer, Berlin, Heidelberg. DOI: 10.1007/978-3-540-79116-4_20.

Engell K. 1995. Embryo morphology of the Ranunculaceae. In: Jansen U, Kadereit JW (eds). Systematics and Evolution of the Ranunculiflorae. Sprinnger, Vienna. DOI: 10.1007/978-3-7091-6612-3_21.

Erst AA, Erst AS. 2019. Features of in vitro seed germination of *Eranthis longistipitata*, an endemic plant of Central Asia. Plant Cell Biotechnol Mol Biol 20: 611-616. DOI: 10.18223/207.

Erst AA, Erst AS. 2020. In vitro propagation of *Eranthis stellata* (Ranunculacea), endemic species with narrow distribution in the Russian Far East, Northeast China and North Korea. Bot Pac J Plant Sci Conserv 9 (2): 121-125.

Erst AS, Chernonosov AA, Petrova NV, Kulikovskiy MS, Maltseva SY, Wang W, Kostikova VA. 2022. Investigation of chemical constituents of *Eranthis longistipitata* (Ranunculaceae): Coumarins and furochromones. Intl J Mol Sci 23: 406. DOI: 10.3390/ijms23010406.

Filonova MV, Mitrenina EY. 2020. Production of the callus culture of the endemic species *Eranthis tanhoensis* (Ranunculaceae). VII International Scientific Conference, Tomsk. [Russian]

Forbis TA, Diggle PK. 2001. Subnivean embryo development in the alpine herb *Caltha leptosepala* (Ranunculaceae). Can J Bot 79 (5): 635-642. DOI: 10.1139/b01-037.

Ganeshpurkar A, Saluja AK. 2017. The pharmacological potential of rutin. Saudi Pharm J 25: 149-164. DOI: 10.1016/j.jsps.2016.04.025.

Gupta N, Jain V, Joseph MR, Devi S. 2020. A review on micropropagation culture method. Asian J Pharm Res Dev 8: 86-93. DOI: 10.22270/ajprd.v8i1.653.

Imelda M, Wulansari A, Poerba YS. 2008. Shoot regeneration from leaf petioles of iles-iles (*Amorphophallus muelleri* Blume). Biodiversitas 9 (3): 173-176. DOI: 10.13057/biodiv/d090304.

Johnson R, Vishwakarma K, Hossen MS, Kumar V, Shackira AM, Puthur JT., Abdi G, Sarraf M, Hasanuzzaman M. 2022. Potassium in plants: Growth regulation, signaling, and environmental stress tolerance. Plant Physiol Biochem 172: 56-69. DOI: 10.1016/j.plaphy.2022.01.001.

Kessas K, Lounis W, Chouari Z, Vejux A, Lizard G, Kharoubi O. 2024. Benefits of rutin on mitochondrial function and inflammation in an aluminum-induced neurotoxicity rat model: Potential interest for the prevention of neurodegeneration. Biochimie 222: 1-8. DOI: 10.1016/j.biochi.2024.02.010.

Kuroda M, Uchida S, Watanabe K, Mimaki Y. 2009. Chromones from the tubers of *Eranthis cilicica* and their antioxidant activity. Phytochemistry 70: 288-293. DOI: 10.1016/j.phytochem.2008.12.002.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Pharm 15: 473-497. DOI: 10.1111/j.1399-3054.1962.tb08052.x.

Panche AN, Diwan AD, Chandra SR. 2016. Flavonoids: An overview. J Nutr Sci 5: e47. DOI: 10.1017/jns.2016.41.

Park SY, Jeon MJ, Ma SH, Wahlsteen E, Amundsen K, Kim JH, Suh JK, Chang JS, Joung YH. 2019. Phylogeny and genetic variation in the genus *Eranthis* using nrITS and cpIS single nucleotide polymorphisms. Hortic Environ Biotechnol 60: 239-252. DOI: 10.1007/s13580-018-0113-0.

Shkolnik MY. 2012. Trace Elements in Plants. Elsevier, New York.

Tipirdamaz R, Gomurgen AH. 2000. The effects of temperature and gibberellic acid on germination of *Eranthis hyemalis* (L.) Salisb. Seeds. Turk J Bot 24 (2): 6.

Van Beek TA. 2002. Chemical analysis of *Ginkgo biloba* leaves and extracts. J Chromatogr 967: 21-35. DOI: 10.1016/S0021-9673(02)00172-3.

Vandelook F, Lenaerts J, Jozef AVA. 2009. The role of temperature in post-dispersal embryo growth and dormancy break in seeds of *Aconitum lycoctonum* L. Flora Morph Distrib Func Ecol Plant 204 (7): 536-542. DOI: 10.1016/j.flora.2008.11.003.

Walck JL, Baskin CC, Baskin JM. 2000. Seeds of *Thalictrum mirabile* (Ranunculaceae) require cold stratification for loss of nondeep simple morphophysiological dormancy. Can J Bot 77: 1769-1776. DOI 10.1139/b99-149.

Wang L, Xie Y, Xiao B, He X, Ying G, Zha H, Yang C, Jin X, Li G, Ping L, Weng Q. 2024. Isorhamnetin alleviates cisplatin-induced acute kidney injury via enhancing fatty acid oxidation. Free Rad Biol Med 212: 22-33. DOI: 10.1016/j.freeradbiomed.2023.12.010.

Xiang KL, Erst AS, Yang J, Peng HW, Ortiz RDC, Jabbour F, Erst TV, Wang W. 2021. Biogeographic diversification of *Eranthis* (Ranunculaceae) reflects the geological history of the three great Asian plateaus. Proceed Roy Soc B 288: 20210281. DOI: 10.1098/rspb.2021.0281.

Zou Y, Wang H, Fang J, Sun H, Deng X, Wang J, Deng Y, Chi G. 2023. Isorhamnetin as a novel inhibitor of pneumolysin against *Streptococcus pneumoniae* infection in vivo/in vitro. Microb Pathogen 185: 106382. DOI: 10.1016/j.micpath.2023.106382.