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HEAVY METALS ANALYSIS, GCMS-QP QUANTIFICATION OF FLAVONOIDS, AMINO ACIDS AND SAPONINS, ANALYSIS OF TANNINS AND ORGANOLEPTIC PROPERTIES OF POWDER AND TINCTURE OF *ECHINACEA PURPUREA* (L.) AND *RHAPÓNTICUM CARTHAMOÍDES*

Aitbek Kakimov, Alibek Muratbayev, Klara Zharykbasova, Samat Amanzholov, Gulmira Mirasheva, Samat Kassymov, Asiya Utegenova, Madina Jumazhanova, Mohammad Ali Shariati

ABSTRACT
Medicinal plants are one of the main sources of vitamins, minerals salts, macro-and microelements, and other biologically active substances that have a health and protective effect on the human body. The current study was aimed to appraise the heavy metals contents in the powder materials of two medicinally important plants *Echinacea purpurea* (L.) and *Rhapónticum carthamoídes* collected from the Semipalatinsk nuclear test site using atomic absorption spectrophotometer. Flavonoids, saponins, amino acid contents quantification were done both in raw materials as well as tincture prepared from both plants via GCMS-QP 2010 Ultra chromatomass spectrometer. Further, tannins concentrations and organoleptic properties of the tincture were elucidated using previously reported standard procedures. In the current study, the concentrations of heavy metals were within the permitted range i.e. lead (0.0027 mg.100g^{-1}), cadmium (0.00012 mg.100g^{-1}), arsenic (ND), mercury (ND). In the crude powder, flavonoids were observed to be in the highest concentration in *E. purpurea* (L.) (5.5 ±0.20 mg.100g^{-1}), whereas, its concentration was 3.1 ±0.346 mg.100g^{-1} in *R. carthamoídes* powder. Tannin concentration was higher in *R. carthamoídes* (5.5 ±0.115 mg.100g^{-1}) and 3.1 ±0.46 mg.100g^{-1} in *E. purpurea*. Likewise, saponins concentrations were 4.1 ±0.40 mg.100g^{-1} and 5.6 ±0.17 mg.100g^{-1} in *E. purpurea* and *R. carthamoídes* powder respectively. Concentrations of these active metabolites in the resultant tincture were flavonoids (7.6 ±0.23), tannins (7.5 ±0.28), and saponins (8.5 ±0.16) mg.100g^{-1}. In the current study, we observed highest concentrations of these essential amino acids in the tincture including leucine/isoleucine (78.00 ±1.15 mg.100g^{-1}), histidine (14.00 ±1.44 mg.100g^{-1}), lysine (49.33 ±2.02 mg.100g^{-1}), methionine (18.66 ±2.90 mg.100g^{-1}), cystine (29.00 ±0.57 mg.100g^{-1}), phenylalanine (24.16 ±1.87 mg.100g^{-1}) and threonine (32.50 ±1.22 mg.100g^{-1}) respectively. The resultant tincture has a pleasant agreeable taste coupled with acceptable herbal flavor which are important organoleptic properties for any product.

Keywords: heavy metals; GCMS-QP; flavonoids; tincture; tannins; saponins

INTRODUCTION
Medicinal plants and their derived products are used by humans for a long for the management of various diseases **(Ovais et al., 2019; Ayaz et al., 2017a)**. Being natural, the majority of these products are safe and have comparatively fewer side effects. Though generally considered safe, natural products are sometimes contaminated with several hazardous heavy metals which can cause serious health consequences **(Ayaz et al., 2014; Khan et al., 2016)**.

Recent studies suggest that herbs contaminated with heavy metals might be potentially mutagenic, toxic, and carcinogenic if utilized for a prolonged time **(Schimmer et al., 1988; Higashimoto et al., 1993)**. In this regard, the World Health Organization (WHO) posted important recommendations regarding the use of herbal drugs. WHO recommends evaluating herbal agents for heavy metals contents before use to ensure their concentration is in the permitted range of human safety. In the East Kazakhstan region, one of the risk factors for disease, namely cancer, is the contamination of the environment, food raw materials, and foodstuffs with radioactive elements and heavy metals, including the consequences of the Semipalatinsk nuclear test site **(Kakimov et al., 2016a; Duyssembaev et al., 2014)**. Subsequently, contents of heavy metals including lead (Pb), cadmium (Cd), Arsenic (As), and mercury (Hg) were appraised via GC-MS in the current study.

Flavonoids are a highly assorted group of plant secondary metabolites having tremendous biological properties. They are predominantly present in green plants, vegetables, flowers, seeds, nuts, fruits, propolis, and honey. They are well-reputed for the management of numerous human disorders neurological disorders, microbial infections,

cardiovascular diseases, cancer, and inflammatory disorders **(Ahmad et al., 2015; Ayaz et al., 2019a; Ayaz et al., 2019b; Patel et al., 2018; Salaritabar et al., 2017)**. Dietary consumption of flavonoids and flavonoid-rich herbs are reported to exhibit significant beneficial effects on neurological health **(Sawikr et al., 2017)**. Thus scientific exploration of flavonoids contents of herbal products is the need of the day to signify their use in various disorders. Likewise, saponins represent another diverse group of pharmacologically active compounds that exist as triterpene or steroid glycosides. They are also reported to possess anti-cancer, anti-microbial, anti-diabetic, immunomodulating, anti-allergic, cardio-protective, hepatoprotective, and neuro-pharmacological potentials **(Lacaille-Dubois and Wagner, 2000; Thakur et al., 2011)**.

Echinacea *purpurea* L. also called purple coneflower (Figure 1a) belongs to the family Asteraceae and has a long history of use in traditional medicine in Europe, Australia, and North America **(Wills and Stuart, 1999; Stuart and Wills, 2000)**. Its medicinal uses include efficacy in bacterial respiratory infections, viral infections, immunomodulatory-

a) *Echinacea purpurea* L.

b) *Rhaponticum carthamoides*

Figure 1 *Echinacea purpurea* L. and *Rhaponticum carthamoides*.

anti-inflammatory effects, urinary tract infections **(Speroni et al., 2002; Stanisavljević et al., 2009; Luo et al., 2003)**. Roots and aerial parts of the plant are used in the preparation of food supplements. Secondary metabolites isolated from the plant including phenolics, flavonoids, alkaloids exhibit considerable anti-radical's properties **(Stanisavljević et al., 2009)**. Likewise, *Rhaponticum carthamoides* also called maral root or Russian leuzea (Figure 1b), belong to the family Asteraceae and is found in alpine meadows of Kazakhstan, Siberia, and cultivated throughout Russia as well as eastern Europe **(Kokoska and Janovska, 2009)**. Its root preparations are reported to exhibit useful effects on the learning and memory of rodents **(Mosharrof, 1987)**. *R. carthamoides* and its metabolites are immunomodulators, act as a stimulant and improve humoral immunity **(Azizov, 1997; Seifulla et al., 1993)**. It also improves working capacity of skeletal muscles and shows adaptogenic as well as anabolic properties **(Bespalov et al., 1992; Ramazanov, 2006; Winston, 2019)**. Numerous compounds including flavonoids, glycosides, and lactones are isolated from the plant and its essential oils. Plant extracts and secondary metabolites are reported to possess cardiovascular, CNS, immunomodulatory, antioxidant, anti-cancer, antimicrobial, and repellent properties **(Kokoska and Janovska, 2009)**. The current study was designed to prepare a tincture from these two medicinally important medicinal plants and quantitatively evaluate their heavy metals, flavonoids, amino acids, tannins, saponins contents, and organoleptic properties.

Scientific hypothesis

As the selected medicinal plants are collected from the Semipalatinsk nuclear test site in the East Kazakhstan region, so we hypothesize that there might be a higher concentration of toxic heavy metals in these plants. The use of these plants without heavy metals analysis could be hazardous to users. Further, we hypothesize that the selected plants might be a good source of essential amino acids and flavonoids which play a significant role in the prevention and management of various diseases. Agreeable taste and other organoleptic properties are important parameters for any herbal preparation to be used by patients. Subsequently, there was a need for the preparation of tincture which exhibits desirable organoleptic properties and can accommodate maximum bioactive ingredients for the management of various disorders.

MATERIAL AND METHODOLOGY

Samples

Plants collection and Processing

Experimental work was carried out departmental laboratories of Technology of food and processing industries, biotechnology and standardization, the scientific center of radioecological research" of the Shakarim State University of Semey, Kazakhstan and Research Institute of Biotechnology" of Kemerovo State University Russia. The selected plants were collected in the East Kazakhstan region, Katon-Karagai district - levsey, and Shemonaikha district- echinacea. *Echinacea purpurea* (L.) was sampled from the Shemonaikha area of the East Kazakhstan region and *Rhaponticum carthamoides* root from the Katon-Karagay area of East Kazakhstan region. The authenticity of medicinal plant raw materials was carried out by a

specialist biologist on macroscopic (external) and microscopic (anatomical) features under the requirements of General Pharmacopoeia Article 1.5.3.0003.15 "Technique of microscopic and microchemical research of medicinal plant raw materials and medicinal plant preparations".

Chemicals

Tincture preparation

For the preparation of a tincture, an aqueous solution of ethyl alcohol of different concentrations was used. It is recommended to use a 35% aqueous solution of ethyl alcohol to make a medicinal plant tincture. To prepare the tincture used pharmacopeia medicinal plants that are not poisonous: for one part of the plant mass used 5 parts of the extractant. The infusion temperature of the infusion was within 20 – 25 °C **(Kowalczyk et al., 2012)**.

Echinacea purpurea (L.) and *Rhapónticum carthamoídes* root were used as plant materials. To prepare the tincture, selected plant materials were shade dried and grinded to small particles of 3 – 5 mm size.

Powdered material of both plants (100 g each) was added to an aqueous solution of 35% ethyl alcohol at herb: extractant 1:5 ratios. The resultant mixture was loaded into a mesh basket, which was placed in a percolator. The percolator was equipped with a jacket, for the possibility of maintaining the temperature of maceration in the range from 20 to 30 °C for 24 hrs. Subsequently, the temperature of the percolator was increased up to 40 – 50 °C with continuous stirring for at least one hr. The solvent was removed and a fresh extraction solvent was added to the treated herbs in the ratio of the herb: extractant 1:1. Infusion with periodic mixing was carried out for 12 hrs and drained all the extract into an intermediate container. The solvent extracts were combined in the tank. To purify the resulting mixture of ballast substances was carried out via the process of sedimentation at a temperature of 5 – 8 °C for 5 days, then carry out the process of filtration of alcoholic infusion through a cotton-grass filter. The resulting tincture was stored in a dark glass jar (Figure 2).

Laboratory methods

Heavy Metals analysis

Heavy metal contents in the tincture were quantitatively analyzed using atomic absorption spectrophotometer following the previously reported standard procedure **(Ayaz et al., 2014; Khan et al., 2016)**. All chemicals and reagents used were of high-quality analytical grade. Plants powdered material 0.2 g was added to a crucible and ignited at 55 °C for six hrs using a muffle furnace. The resultant ash was digested via concentrated HCL 0.5 mL and evaporated using hot plate apparatus. A small quantity of double distilled water was added to the mixture followed by filtration and the final volume was increase to 30 mL using double distilled water. The solution formed was analyzed using Shimadzu single quadrupole GCMS-QP2010 SE gas chromatograph-mass spectrometer (Shimadzu Corporation, Japan) for the number of heavy metals according to the standards available in our laboratory including lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) following standard operating procedure and were compared with Permissible levels of Technical Regulations of the Customs Union in herbal preparation for human use.

Quantitative analysis of Flavonoids

Flavonoid quantification was determined by previously reported standard protocol **(Ayaz et al., 2017b; Pandey and Tripathi, 2014; Rajani Kanaki, 2008; Ab Halim et al., 2012)**.

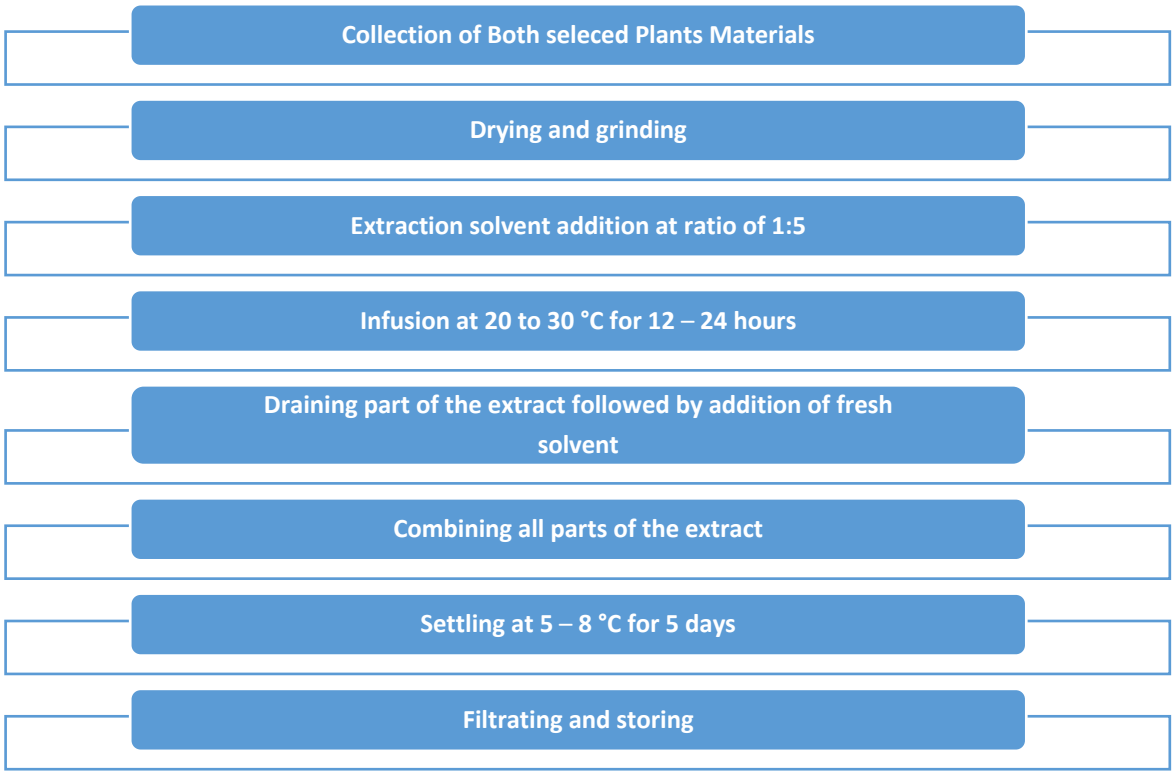

Figure 2 Systematic flow chart of the extraction process to prepare tincture.

Briefly, 1 g of powdered (1 mm) sample was added to 100 mL grinding flask and 30 mL of 70% alcohol was added to it. Flask was heated in a boiling water bath for 30 minutes. Subsequently, the flask was cooled to room temperature under a shower of cold water and filtered through a paper filter into a measuring flask with a capacity of 100 mL. The filtrate was flushed with 70% alcohol and added the alcohol to increase volume up to the mark of the flask. The obtained solution was named "solution A". Thereafter, 4 mL of "solution A" was transferred to a 25 mL flask and 2 mL of 2% solution of aluminum chloride in 95% alcohol was added to it, followed by the addition of 95% alcohol into the flask until the mark. The resultant solution was kept for 20 min and optical density was measured using a spectrophotometer at a wavelength of 410 nm in a 10 mm thick cuvette. For comparison solution, 4 mL of "solution A" was added to a flask, volume was increased to 25 mL and 1 drop of diluted hydrochloric acid was added to it. Finally, volume increased using 95% alcohol. Flavonoids (X) was quantified using the formula (1);

$$X = \frac{D \times 100 \times 100 \times 25}{330 \times m \times (100 - W)} \quad (1)$$

Where:
D – optical density of the tested solution; 330 – Specific absorption index of the complex with aluminum chloride at 410 nm; m – a mass of the sample (g); W – mass loss on drying (%).

Qualitative and quantitative analysis of Tannins

For the qualitative assessment of tannins content, powder material (300 mg) of the plants was used following the previously reported procedure **(Ejikeme et al., 2014)**. The required powder material was transferred to a test tube and boiled in a water bath for 30 minutes using a water bath. After boiling, the mixture was filtered through Whatman filter paper 42 and a few drops of ferric chloride (0.1%) were added to the filtrate. A brownish-green color was resulted indicating the presence of tannins. Subsequently, quantitative analysis was done **(Geetha and Geetha, 2014)**. In 500 mL of conical flask put 2 g of shredded and sieved through a sieve with a hole diameter of 3 mm sample and added 250 mL of boiling water to it. Then the flask was weighted using an electronic scale and boiled the contents with periodic stirring. Thereafter, the flask was placed at room temperature to get the contents cool and weighted again, freshly boiled water was added to fill the missing weight. About 100 mL of the resulting extraction was filtered into a conical flask of 200 – 250 mL through absorbent cotton so that particles of samples do not get into the flask. Subsequently, 25 mL of filtered solution was poured into another conical flask with a volume of 750 – 1000 mL, then 500 mL of water was added, 25 mL of indigo-sulphonic acid and titrated against potassium permanganate solution (0.02 $mol.L^{-1}$; reagent grade 99.5%, LLP "Nikitin", Semey, Kazakhstan) until golden yellow color appeared. At the same time, conducted a control experiment. The content of tannins (X) in percent converted to absolute dry raw materials, was calculated by the formula (2);

$$X = \frac{(V - V_1) \times 0.004157 \times 250 \times 100 \times 100}{M \times 25 \times (100 - W)} \quad (2)$$

Where:
V – the amount of potassium permanganate solution (0.02 $mol.L^{-1}$) used for titration of extraction (mL); V1 – the amount of potassium permanganate solution (0.02 $mol.L^{-1}$), used for titration in the control experiment (mL); 0.004157 – amount of tannins equivalent to 1mL of potassium permanganate solution (0.02 $mol.L^{-1}$) per tannin (g); M – sample weight (g); W – mass loss on drying (%); 250 – total extraction volume (mL); 25 – extraction volume took for titration (mL).

Amino acids contents

The amino acid composition was determined using GCMS-QP 2010 Ultra chromatomass spectrometer (Shimadzu Corporation, Japan), M-04-38-2009 test method (FR.1.31.2010.07015) **(Bashari et al., 2019)**. Plants sample weighing 1 g was transferred to a conical flask and a 1000 cm^3 extraction solution was added. The mixture was shaken for 60 min using a mechanical shaker and 100 cm^3 of supernatant liquid was transferred from it into a beaker. About 5.0 cm^3 of sulfosalicylic acid (reagent grade 99%, LLP "Kazbiohim", Almaty, Kazakhstan) was added to it and continued mixing with a magnetic mixer for 5 min. The mortar was filtered to remove the sludge. The pH of the obtained solution was adjusted to 2.20 using sodium hydroxide solution (assay percentage ≥98%, LLP "Kazbiohim", Almaty, Kazakhstan). The solution was transferred into a measuring flask and increased volume with a citrate buffer. The chromatography was performed following SOPs available for the equipment. It is important that the same amount (±0.5%) of standard solution and sample is entered into the column unless an internal standard is used (for chromatographic systems requiring a low sodium concentration) and unless the amino acid ratio in standard solutions and the sample should be as close as possible.

The amino acid content of the test sample, $g.kg^{-1}$, is calculated using the formula (3);

$$w = \frac{A_b \times c \times M \times V_b}{A_c \times m \times 1000} \quad (3)$$

Where;
A_b – amino acid peak area in hydrolyzate and extract; c – molar concentration of amino acid in standard solution, $mol.dm^{-1}$; M – amino acid molecular weight; V_b – total hydrolyzate volume or calculated total dilution volume of the extract cm; A_c – amino acid peak area in a standard solution.

Quantitative determination of saponins

Previously reported standard protocol was used to quantitatively analyze saponins content **(Ayaz et al., 2016; Zeb et al., 2014)**. The sample was milled to the size of the particles passing through a sieve with 1 mm diameter holes. About 0.5 g of the sample was packed in a chuck from filter paper and was placed in the extractor of the Soxhlet device. Chloroform extraction (reagent grade 99%, LLP LenReaktiv, Moscow, Russia) was discarded and the holder with the plant sample was dried. The sample was placed on the cartridge in a round bottom flask with a capacity of 100 mL. Subsequently, 50 mL of 90% alcohol (LLP "Nikitin", Semey, Kazakhstan) was poured in and heated with the back cooler in a boiling water bath for 1 hour. The

extraction was filtered through a dry paper filter into a distillation flask with a capacity of 200 mL, the rest on the filter is twice washed with 90% alcohol in portions of 5 mL. Extractions were combined and the solvent was distilled under vacuum drying.

The residue was dissolved in 20 mL of 96% alcohol (LLP "Nikitin", Semey, Kazakhstan) during heating, cooled, quantitatively transferred with 25 mL of 96% alcohol into a flask with 50 mL capacity, filled up with 96% alcohol till the mark. Two mL of the solution obtained was placed into a 50 mL conical flask, 8 mL of concentrated sulfuric acid (mass fraction of H_2SO_4, 93.6 – 95.6%, LLP "Nikitin", Semey, Kazakhstan) was added to it and stirred. After 30 min the optical density of the obtained solution was measured on the spectrophotometer SF-46 (Lomo Company, Russia) at a wavelength of 405 nm in a cuvette with a layer thickness of 10 mm. The mixture consisting of 2 mL of 96% alcohol and 8 mL of concentrated sulfuric acid was used for comparison. Content of saponins (X) in 1 g of raw material was calculated by the formula (4);

$$x = \frac{D \times m_0 \times 50 \times 10 \times 6 \times 2 \times 100 \times 100}{D_0 \times m \times 1 \times 2 \times 100 \times 25 \times 6 \times 100 \times (100 - W)} = \frac{D \times m_0 \times 20}{D_0 \times m \times (100 - W)} \quad (4)$$

Where:
D – optical density of the test solution; D_0 – optical density of Pharmacopoeia standard sample of Escin; m – sample weight, g; m_0 – the mass of Pharmacopoeia Standard Sample Escin (g); W – mass loss on drying (%).

Analysis of the organoleptic properties

Organoleptic and physicochemical indicators including consistency, appearance, taste, color, taste, and odor were analyzed in the obtained tincture following American Herbal Products Association (AHPA) guidelines for organoleptic analysis of herbal ingredients **(Upton et al., 2016; Dentali, 2013)**.

Statistical analysis

All experiments were performed in triplicate and results were expressed as mean ±SEM. One way ANOVA followed by multiple comparison Dunnett's test were applied for statistical differences among various study groups using GraphPad prism 7.0 software (USA). *p*-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Elevated levels of heavy metals particularly lead, cadmium, arsenic, and mercury which are usually acquired from the soil, household wastewater, irrigation systems used in agriculture, and other environmental sources. Being non-biodegradable these heavy metals present in herbal products can cause severe toxicological effects in humans if used chronically. Due to this reason, the WHO recommends that heavy metal contents of herbal products must be assessed before use to ensure that their concentration is within permitted limits. The majority of the heavy metals we assessed are non-essential elements and have no specific physiological function. Lead toxicity is manifested in the form of renal, hemopoietic, reproductive, and nervous system side effects **(Ara and Usmani, 2015; Papanikolaou et al., 2005)**. Likewise, chronic exposure to cadmium leads to cancer and toxicity of the respiratory, cardiovascular, CNS, urinary tract, and musculoskeletal system **(Rahimzadeh et al., 2017; Sun et al., 2020)**. Chronic exposure to arsenic skin lesions and cancer **(Ratnaike, 2003; Kakimov et al., 2016b)**. Mercury toxicity effect CNS, renal and gastro-intestinal system **(Bernhoft, 2012, Kuramshina et al., 2019)**. In the current study, we observed that the concentrations of heavy metals were within the permitted range i.e. lead (0.0027 mg.100g^{-1}), cadmium (0.00012 mg.100g^{-1}), arsenic (nondetectable), mercury (nondetectable) Table 1.

One of the mottoes beyond this analysis was contamination of the environment, food raw materials, and foodstuffs with radioactive elements and heavy metals as a result of Semipalatinsk nuclear tests and collection plants from that area.

Among the primary metabolites of medicinal plants are proteins, amino acids, chlorophyll, and some sugars, whereas secondary metabolites include phenolic compounds, flavonoids, alkaloids, saponins, tannins, terpenoids, and essential oils. The majority of these metabolites possess considerable therapeutic properties and medicinal plants are valued owing to the presence of these metabolites. Flavonoids exhibit strong anti-oxidant properties and thus ameliorate the progression of free radicals induced degenerative disorders **(Banjarnahor and Artanti, 2014; Rice-Evans and Packer, 2003; Imran et al., 2019)**. They are reported to inhibit the progression of tumors and halt cardiovascular diseases **(Rice-Evans and Packer, 2003; Hertog et al., 1993; Tijburg et al., 1997)**. Several medicinal plants rich in flavonoids demonstrate anti-inflammatory, anti-viral, hepato-protective, anti-allergic, neuroprotective, and anti-ulcer properties **(Rice-Evans and Packer, 2003; García-Lafuente et al., 2009, Akram et al., 2020)**. Saponins glycosides are present throughout the plant kingdom and contain diverse groups of compounds having steroidal aglycone and sugar molecules as basic components of their basic structure. Due to the diverse nature of compounds, this group of secondary metabolites is reported to exhibit numerous pharmacological properties including cytotoxic, antimicrobial, anti-oxidant, anti-diabetic, anthelmintic, antispasmodic, and anti-neuroplastic **(Yuan et al., 2010; Lu et al., 2005; Pal et al., 2009)**. Likewise, tannins are another important group of water-soluble, phenolic secondary metabolites that chemically belongs to proanthocyanidins **(Chung et al., 1998; Haslam, 1989)**. They possess anticancer, anti-viral, anti-inflammatory, anti-oxidant and enzyme inhibition properties **(Okuda et al., 1992; Scalbert, 1991)**.

In the crude powder, flavonoids were observed to be in the highest concentration in *E. purpurea* (L.) (5.5 ±0.20 mg.100g^{-1}), whereas, its concentration was 3.1 ±0.346 mg.10 g^{-1} in *R. carthamoides* powder. Tannin concentration was higher in *R. carthamoides* (5.5 ±0.115 mg.100g^{-1}) and 3.1 ±0.46 mg.100g^{-1} in *E. purpurea*.

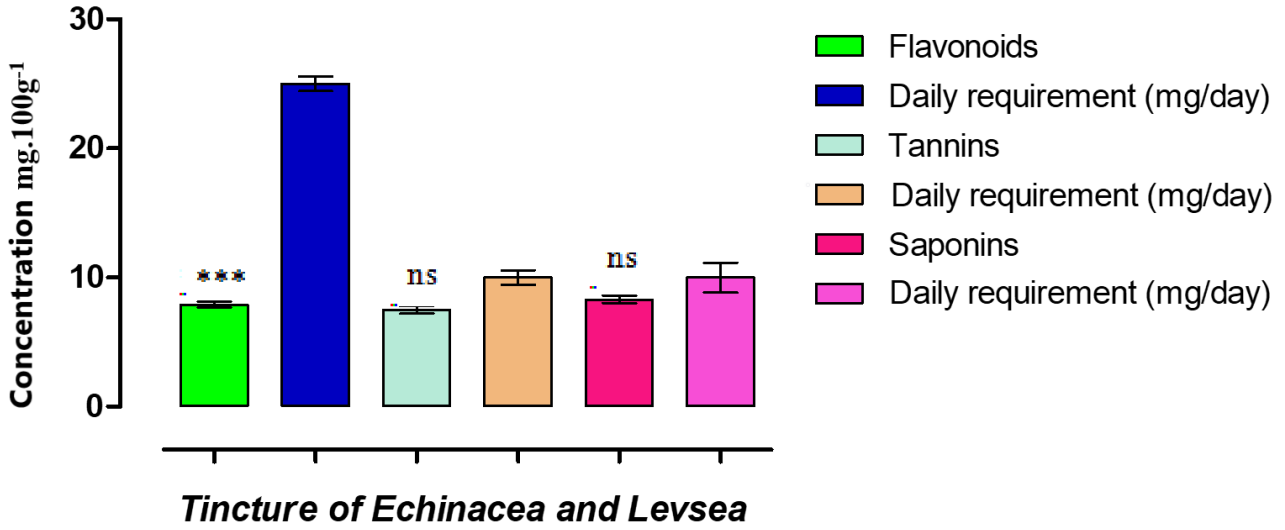

Figure 3 Concentrations of various phytochemicals including flavonoids, tannins and saponins in tincture prepared from selected plants.

Table 1 Concentration of heavy metals in tincture.

Heavy Metals	Concentration ($mg.100g^{-1}$)	Permissible levels of Technical Regulations of the Customs Union
Lead	0.0027	0.5
Cadmium	0.00012	0.03
Arsenic	ND	0.05
Mercury	ND	0.01

Note: Each value represents mean results of three independent experimental readings. ND – not detected.

Table 2 Chemical composition of medicinal herbs.

Name of medicinal herb	Group of Phytochemicals	$mg.100g^{-1}$	Daily human requirements (mg)
Rhapónticum carthamoídes	Flavonoids	3.1 ±0.346	25
	Tannins	5.5 ±0.115	10
	Saponins	5.6 ±0.17	10
Echinacea purpurea **(L.)**	Flavonoids	5.5 ±0.20	25
	Tannins	3.1 ±0.46	10
	Saponins	4.1 ±0.40	10

Note: Each value represents mean ± SEM of three independent experimental results.

Likewise, saponins concentrations were 4.1 ±0.40 $mg.100g^{-1}$ and 5.6 ±0.17 $mg.100g^{-1}$ in *E. purpurea* and *R. carthamoídes* powder respectively (Table 2).

To obtain the optimal ratio of medicinal plants in the composition at the first stage the chemical composition of the studied plants was determined. A mathematical simulation using MS Excel was performed to determine the ratio of levsea to echinacea. We enter data from Table 1 into MS Excel using a function;

$$F(x)= 0.5x_1+0.5x_2 \rightarrow const$$

Using the "Solution Finder" function of the solution we set limits. Based on the obtained mathematical model we obtain the ratio 1:1.

That is, it is established that to create a composition with a balanced composition of biologically active substances, we need a ratio of levsea and echinacea 1:1 respectively. The tincture was prepared using the above ratio of both plant powder materials and was assessed for the presence of flavonoids, saponins, and tannins. Concentrations of these active metabolites in the resultant tincture were flavonoids (7.6 ±0.23), tannins (7.5 ±0.28), and saponins (8.5 ±0.16) $mg.100g^{-1}$ (Figure 3). As discussed earlier, the plant powder as well as tincture consists of essential secondary metabolites and might be effective to use as a potential source of phytochemicals against various diseases.

Amino acids are highly abundant in medicinal plants and serve as a vital source of nutrition. Amino acids being the building blocks of proteins are grouped into essential and non-essential based on their synthesis inside the human body **(Kakimov et al., 2017; Garaeva et al., 2009)**. Briefly, an essential type of amino acid is synthesized only by medicinal plants whereas, a non-essential type of amino acid is prepared by both medicinal plants as well as humans **(Kumar et al., 2019)**.

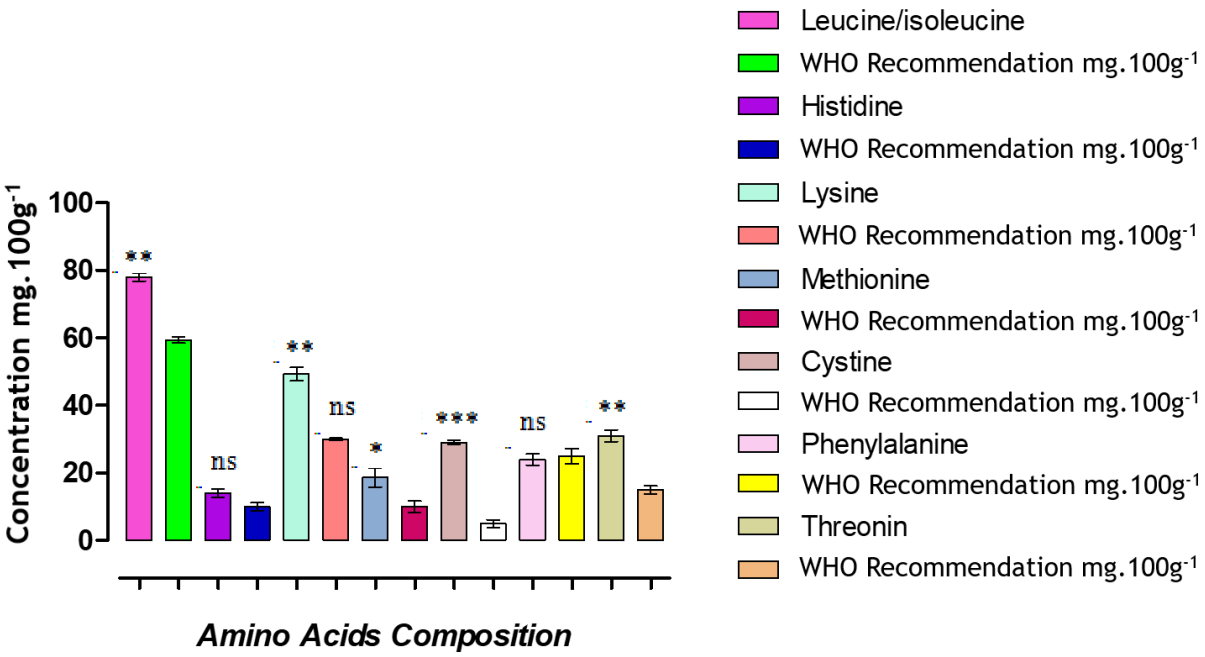

Figure 4 Results of Essential Amino acids quantification in the tincture of *E. purpurea* and *R. carthamoides.*

Table 3 Organoleptic indicators of tincture.

Indicator	Description/Observations
Consistency and appearance	Liquid, homogeneous, transparent without sedimentation.
Taste and odor	Pleasant taste and typical herbal smell
Color	Light brown, uniform across the entire mass

Due to the tremendous increase in the global population, there is a dire need for the development of alternative food sources. Among the alternative food sources, medicinal plants represent an important source of essential amino acids. These plants beside a source of nutrition are coupled to numerous pharmacological properties also which can be used both as a food source and therapeutic remedies **(Kumar et al., 2019)**.

In the current study, we observed the highest concentrations of these essential amino acids in the tincture including leucine/isoleucine (78.00 ±1.15 mg.100g^{-1}), histidine (14.00 ±1.44 mg.100g^{-1}), lysine (49.33 ±2.02 mg.100g^{-1}), methionine (18.66 ±2.90 mg.100g^{-1}), cystine (29.00 ±0.57 mg.100g^{-1}), phenylalanine (24.16 ±1.87 mg.100g^{-1}) and threonine (32.50 ±1.22 mg.100g^{-1}) respectively (Figure 4). Being a source of flavonoids, tannins, saponins, and essential amino acids, the *E. purpurea* and *R. carthamoides* based tincture might be a useful source of secondary metabolites for the prevention and management of various diseases and thus warrant further detailed studies.

The resultant tincture has a pleasant agreeable taste coupled with acceptable herbal flavor which are important organoleptic properties for any product (Table 4). Disagreeable taste in flavor might be a reason for the non-compliance of several medicinal preparations. Further, the tincture has pleasant homogeneous and transparent preparations without sediments and adulterants with a light brown color which aid in increasing individuals' acceptability of the preparations for oral use. These organoleptic, physicochemical indicators of the tincture correspond to the requirements of the State Pharmacopoeia of the Republic of Kazakhstan **(The State Pharmacopeia, 2008)**.

CONCLUSION

In the current study, both plants exhibited low heavy metals contents which were within the WHO specified range for human use. Dried plant powder materials and resultant tincture exhibited considerably high contents of flavonoids, saponins, tannins, and essential amino acids. These metabolites own numerous pharmacological properties, thus both plants and their resultant tincture might be useful in the prevention and treatment of several diseases. Ideal organoleptic properties of the tincture yet another important aspect of the tincture for use in the general population. The outcomes of the current study will provide a scientific base for more systematic studies. Further detailed studies are required to signify the potential use of the selected plants in various disorders.

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Contact address:
Aitbek Kakimov, Shakarim University of Semey, Biotechnology and Standartisation Department, 20A Glinki Street, Semey city, 071412 Kazakhstan,
E-mail: bibi.53@mail.ru
ORCID: https://orcid.org/0000-0002-9607-1684

Alibek Muratbayev, Shakarim University of Semey, Food Engineering Department, 20A Glinki Street, Semey city, 071412 Kazakhstan,
E-mail: great_mister@mail.ru
ORCID: https://orcid.org/0000-0003-4765-8099

Klara Zharykbasova, Kazakh Humanitarian Law Innovative University, Department of Applied Biology, 11 Mangilik El Street, Semey city, 071400, Kazakhstan,
E-mail: klara_zharykbasova@mail.ru
ORCID: https://orcid.org/0000-0003-4800-5073

Samat Amanzholov, Kazakh University of Technology and Business, Department of Technology and Standartisation, 37A Mukhamedhanov Street, Nur-Sultan, 010000, Kazakhstan,
E-mail: samat_amanzholov@mail.ru
ORCID: https://orcid.org/0000-0003-4370-9629

Gulmira Mirasheva, Shakarim University of Semey, Biotechnology and Standartisation Department, 20A Glinki Street, Semey city, 071412 Kazakhstan,
Email: gulmira_mir@mail.ru
ORCID: https://orcid.org/0000-0002-4043-5020

Samat Kassymov, Shakarim University of Semey, Semey city, Food Technology Department, 20A Glinki Street, Semey city, 071412 Kazakhstan,
E-mail: samat-kasymov@mail.ru
ORCID: https://orcid.org/0000-0003-4370-9629

Asiya Utegenova, Shakarim University of Semey, Food Technology Department, 20A Glinki Street, Semey city, 071412 Kazakhstan,
E-mail: asia_aksu@mail.ru
ORCID: https://orcid.org/0000-0001-5133-5255

Madina Jumazhanova, Shakarim University of Semey, Biotechnology and Standartisation Department, 20A Glinki Street, Semey city, 071412 Kazakhstan,
E-mail: madina.omarova.89@mail.ru
ORCID: https://orcid.org/0000-0003-0396-8431

*Mohammad Ali Shariati, K. G. Razumovsky Moscow State University of technologies and management (the First Cossack University), Moscow, Russian Federation, Tel: +79048111166,
E-mail: shariatymohammadali@gmail.com
ORCID: https://orcid.org/0000-0001-9376-5771

*Corresponding author