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Phytochemical composition and antioxidant activity of three medicinal plants from southeastern Kazakhstan

Abstract. Main results on study of phytochemical composition and antioxidant activity of three plants growing in southeastern part of Kazakhstan, namely *A. schrenkiana*, *L. turkestanicus* and *C. tianschanica*. Plant material was collected on flowering stage in July 2020. This study was carried out to evaluate the phytochemical constituents and antioxidant potential in order to validate the medicinal potential of these herbs. The antioxidant activity of alcoholic extracts was evaluated using 2,2 diphenyl-l-picrylhydrazyl (DPPH) assays. The total polyphenol and flavonoid content were determined according respectively to Spanos-Wrolstad and Zhishen method. The essential oil composition of samples was analyzed by gas chromatography (GC) (Agilent 7890A) coupled by flame ionization detector and mass spectrometry (Agilent 5975C) using capillary column (HP Innowax Capillary; 60.0 m×0.25 mm×0.25 μm). Phenols in *A. schrenkiana* (2.83 mg OE/g), in L. turkestanicus (2.86 mg OE/g), in C. tianschanica (16.08 mg OE/g). Flavonoids in *C. tianschanica* (21.79 mg RE/g), in *L. turkestanicus* (4.29 mg RE/g), in *A. schrenkiana* (3.8 mg RE/g). *C. tianschanica* (IC_{50} = 0.78 μg.mL) had greater antioxidant activity than *A. schrenkiana* (IC_{50} = 3.7 μg.mL) and *L. turkestanicus* (IC_{50} = 3.38 μg.mL). The components of the essential oil of *A. schrenkiana* revealed 13 types of chemical elements, of which the indicators of camphor (44.79%) and 1.8-cineole (29.05%) were high.

Key words: *Artemisia schrenkiana* Ledeb*, Leonurus turkestanicus* V.I.Krecz*, Cerasus tianschanica* Pojark*,* antioxidant activity, phytochemical composition.

Introduction

More than 8,000 polyphenolic compounds have been identified in various plant species [1]. Polyphenolic compounds are natural antioxidants used for protection against oxidative damage to such important biological molecules like DNA, lipids, and proteins involved in numerous diseases, this means that they can stop the reaction of free radicals with other molecules in the body, preventing DNA damage, as well as long-term health effects [2]. In the scavenging of various free radicals, flavonoids are highly effective by their redox potentialas they can destroy free radicals. They help in DNA repair, as well as inhibit angiogenesis and tumor invasion. There has been an increasing interest in research on plant flavonoids since their pharmacological properties are directly linked with their antioxidant potential [3]. Like in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, a purple-colored solution of DPPH radical, which by accepting electron is converted to discolour DPPH. In fact, the degree of color change is related to the concentration and effectiveness of the antioxidants. The degree of discoloration with respect to the decrease in the absorbance of the reaction mixture indicates the free radical scavenging action [4].

Many species of *Artemis*ia is a valuable forage plant in Central Asia. It have a corresponding economic value as ornamental crops, medicinal and aromatic plants, many species of these plants are a source of essential oils used in medicine, cosmetics and pharmaceutical industry [5-6]. For example, new phytopreparations with antibiotic, antimicrobial, phytohormonal and antioxidant action were developed on the basis of santonin derivatives isolated from *A. cina* in the Institute of Phytochemistry of Medical University Karaganda (Kazakhstan) [7]. *A. schrenkiana* is used in folk medicine for allergies, skin diseases, respiratory diseases, as it contains many

biologically active substances, such as essential oils, saponins, tannins, phenolic compounds, vitamins, amino acids, organic acids, enzymes, absiogin and santonin [8].

Second plant *L. turkestanicus* is a perennial Woody rhizomatous plant belonging to the Lamiaceae family [9]. Plant species from the Lamiaceae family have been used in traditional herbal medicine for thousands of years. Traditional applications of plants from the Lamiaceae family are as a common tea, as an insect repellent, in flu control, and as an anti-inflammatory, sedative and analgesic agent. Tea and infusion from the aerial parts of *L. turkestanicus* is used in Uzbekistan to treat nervous disorders, hypertonia, hysteria, epilepsy, tachycardia, and gastrointestinal and female diseases and as soporific, anti-inflammatory, pathogenic, and laxative agents [10]. A previous phytochemical study of the aerial part of *L. turkestanicus* revealed the presence of the flavonoid genkwanin and iridoids 6-deoxy-8-acetylharpagide, 8-acetylharpagide, and harpagide [11].

The third plant, *C. tianschanica* is a tree-shrub plant belonging to the Rosaceae family. It grows in tropical and subtropical regions. It grows only in Central Asia (in the Pamirs and Tien Shan). In our country, it is distributed mainly in the Almaty region, where it can often be found in the foothills of the Takyr mountains [12]. It is used in folk medicine to treat skin rashes and can also be used for decorative purposes. It has been a favorite food of the local ethnic minorities in China since ancient times and was known as the "sacred xueyu fruit" [13]. It contains alkaloids (0.15%), tannins (10.4%), various sugars, malic acids and a very small amount of essential oil, fiber, vitamin C, carotenoids, and anthocyanins, each of which play an important role in cancer prevention. One of the most important groups of biologically active compounds in the composition are phenolic compounds. Their action on the human and animal body is diverse and underlies a number of vital functions and processes, in particular, metabolism, hematopoiesis, strengthening of vessel walls, etc. [14, 15].

Studied plants were long used in folk medicine to treat various diseases. However, despite their promising biological activities, these plants have been little studied in Kazakhstan. We aimed to focus on studying the total content of phenols and flavonoids and antioxidant activity of specimens from southeastern Kazakhstan. The composition of the essential oils of *A. schrenkiana* was determined as well.

Materials and methods

Objects of study. Three plants have been evaluated in this study, namely *A. schrenkiana, L. turkestanicus* and *C. tianschanica*. It is well known that the quantitative concentration of biologically active compounds changes depending on the stage of plant growth and development. For harvesting the plants, the best time for harvesting was determined. The three plants were gathered in August 2020. *C. tianschanica* plant was collected from the territory of Mount Sogeti of Almaty region (43° 27' 0" N, 78° 54' 0" E), *A. schrenkiana* plant was collected from the territory of Urdzhar District of eastern Kazakhstan region (47° 6' 13" N, 81° 33' 16" E), and *L. turkestanicus* was collected from the mountain Alma-Arasan gorge located in the south-western part of Almaty (43° 5′ 11″ N, 76° 54′ 25″ E).

The aerial parts were air dried in shade for ten days, then grinded to fine powder by using electric blender (Ruian Kangyuan Pharmaceutical Machinery limited company, China).

Preparation of the plant extracts. 20 mL methanol with 80% purity was added to the 2 g plant sample and extraction is made in the orbital shaker for 1 hour. The tube was centrifuged in 5,000 rpm during 5 minutes. Later, the liquid phase was collected in a test tube by filtering the solution. 5 mL methanol with 80% purity added to the residual part in the tube and the same process were recurred three times. After this, the extracts were taken to the 50 mL volumetric flask and it was diluted to the volume of the volumetric flask [16].

Determination of total phenolics. The total phenolic contents of the plant samples were determined as spectrophotometrically in accordance with the method of Spanos and Wrolstad [17]. For this goal, 100 μL were taken from the extracts and 900 μL deionized water, 4 mL Na_2CO_4 solution (75 g/L), 5 mL 0.2 N Folin Ciocalteau reactive was added. The mixture was stored at a dark place for 2 hours. The absorbance of the mixture was read in 765 nm wavelength in spectrophotometer device (Shimadzu UV-Vis 160A, Japan). At the end of the experiment, results were calculated as a gallic acid equivalent [18].

Determination of total flavonoids. The total flavonoid contents of the extracts were determined as spectrophotometrically according to the method of Zhishen. To achieve this goal, 1 mL was taken from the extracts and 4 mL deionized water and 0.3 mL $NaNO_2$ solution (5%) were added. After waiting 5 minutes, 0.60 mL $AlCl_3$ solution (10%) was added.

Then, 2 mL NaOH (4%) was added after 5 minutes. Total volume was completed with deionized water to 10 mL. The absorbance of the mixture was read in 510 nm.

Antioxidant activity. DPPH radical scavenging assay was performed by dilution with methanol after extraction. The 1mM DPPH radical solution was prepared depending on the number of samples; 600 µL of DPPH was added to each of the tubes. Different amounts (20, 40, 60, 80, 100 µl) of plant extract were added to the test tubes, the total volume of the tube was filled with up to 6 ml of methanol. All tubes were vortexed; the incubation procedure was made during 15 minutes in a dark place in the room temperature (25°C). 5,400 µl MeOH was added to the 600 µL DPPH for using as a replicate sample. Incubation of the copied sample was carried out within 15 minutes. The absorption values of the samples were read on a spectrophotometer with a wavelength of 517 nm at the end of the incubation procedure. The percentage inhibition values corresponding to the sample size were calculated according to the equation below.

$$\% \text{ Inhibition}=[(Abs_{DPPH}–Abs_{extract})/Abs_{DPPH}] \times 100$$

Certain inhibition values were shown on a graph showing the values of the sample volume, and a linear regression analysis was also performed, and curved equation was determined depending on the sample. The value of IC_{50} was calculated based on this equation. Obtaining the DPPH ($1/IC_{50}$) value showed a reverse value converted to mg for the new plant, which inhibits 50% of the 1 g DPPH radical [19].

Essential oil. The each dried samples were weighted as 20 g, the weighed sample was put into the Clevenger apparatus, deionized water was added as 200 mL, the hydrodistillation was realized as 2 hours. Essential oil content was calculated by using essential oil amount and weighed plant material [20].

Essential oil composition. The essential oil composition of plant samples was analysed by gas chromatography (Agilent 7890A, Agilent, USA) coupled by flame ionization detector and mass spectrometry (Agilent 5975C, Agilent, USA) using capillary column (HP Innowax Capillary, Agilent, USA; 60.0 m×0.25 mm×0.25 µm). Essential oils were diluted 1:50 ratio with hexane. Gas chromatography (Agilent 7890A)-mass detector (Agilent 5975C) (GC-MS/FID) analysis was carried out at split mode of 40:1. Injection volume and temperature were adjusted as 1µL and 250 °C, respectively. Helium was the carrier gas at a constant flow rate of 0.8 mL/min. The oven temperature was programmed as follows: 60 °C for 10 minutes, increased at 4 °C/minute to 220 °C, and held at 220 °C for 10 minutes. Mass spectrometry (MS) spectra was recorded between 35 to 450 amu and the ionization mode used was electronic impact at 70eV. The relative percentage of the components was calculated from GC-FID peak areas [21].

Results and discussion

The results of quantitative determination of the content of biologically active substances in the studied samples of medicinal plants are presented in the drawings. The data obtained show that the content of biologically active substances in plants varies widely. Thus, for example, the mass fraction of phenolic and flavonoids in plant raw materials varies considerably from ~ 0.5% in *A. schrenkiana* to ~ 8% in *C. tianschanica.*

In this study, the amount of total phenols varied in the three plants and ranged from 2.02 to 20.81 mg/g dry matter. The highest level of phenols was found in *C. tianschanica*. It was noted that the total amount of phenolic compounds in Rosaceae varieties, it is higher than in other families. Relatively low levels of phenols were found in *A. schrenkiana* (2.83 mg EHA/g), *C. tianschanica* (16.08 mg EHA/g) and *L. turkestanicus* (2.86 mg EHA/g dry weight) (Figure 1).

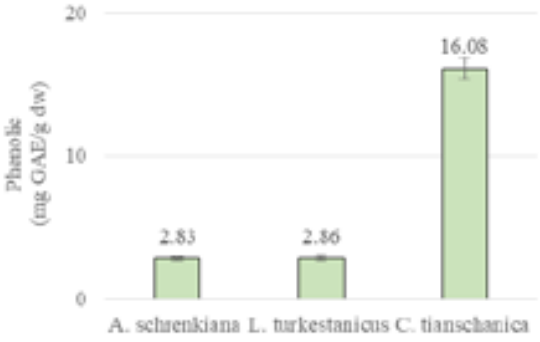

Figure 1 – Total phenol contents for the studied plants, mg GAE/g dry weight

Flavonoids are considered one of the most common groups of natural compounds found in plants. The Zhishen method, most commonly used for determining the total flavonoid content, showed relatively good reproducibility. *C. tianschanica* was distinguished by the highest content of flavonoids (21.79 mGRE/g), *A. schrenkiana* (3.80 mGRE/g) and *L. turkestanicus* (4.29 mGRE/g) (Figure 2).

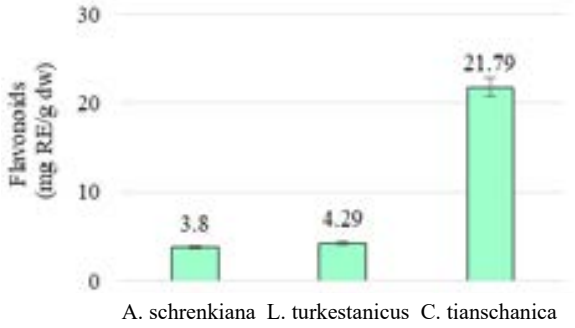

Figure 2 – Total flavonoid contents for the studied plants mg RE/g dry weight

Antioxidant activity. Among extracts tested for antioxidant activity by DPPH, crude methanol extracts of *C tianschanica*. IC_{50} 0.78 µg/mL, *A. schrenkiana* and *L. turkestanicus* showed antioxidant activity with (IC_{50}) values of 3.7 µg/mL and (3, 38 µg/ml) respectively. The results show that the antioxidant activity of the crude extract of *C. tianschanica* is relatively high compared to the other two plants (Figure 3).

A. schrenkiana is a valuable fodder and medicinal plant and contains a large amount of essential oils. The amount of essential oil collected from the *A. schrenkiana* plant was 0.15 ml and the composition of the essential oil in the plant *A. schrenkiana* is 0.37%. The main components of the essential oil: camphene – 3.65%, *1.8*-cineole – 29.05%, *g*-terpinene – 0.30%, p-cymene – 1.08%, α-thujone – 0.35%, camphor – 44.79%, bornyl acetate – 0.93%, terpinen-4-ol – 1.16%, borneol – 4.26%, carvone – 0.70%, β-oplopenone – 1.57%, spatulenol – 3.76% and others – 8.40%. These components are displayed on the histogram (Figure 4).

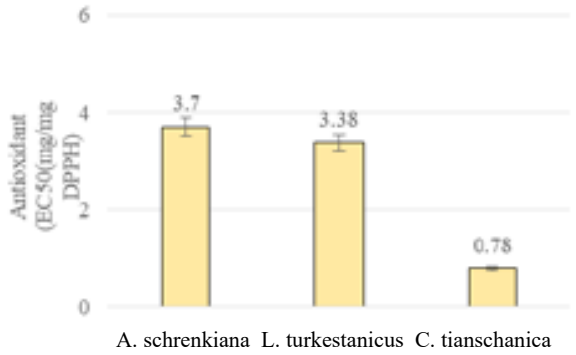

Figure 3 – The value of antioxidant activity (µg/ml DPPH)

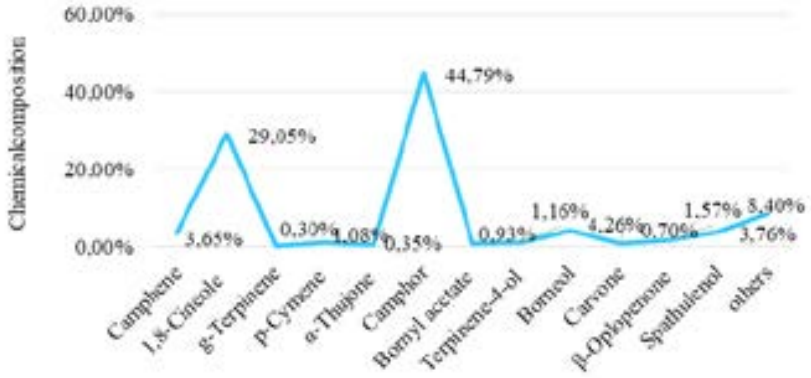

Figure 4 – Chemical composition of *A. schrenkiana* essential oil

We have previously studied nine different elements in *A. schrenkiana*, including macro and microelements lead, cadmium, zinc, copper, iron, manganese, sodium, and potassium [22]. A second study was on the effect of *A. schrenkiana* herbal extract on insulin, glucose, and homeostatic model for assessing insulin resistance (HOMA-IR) serum levels in diabetic rats and *A. schrenkiana* extract improved the alloxan-induced diabetic metabolic abnormality [23].

Phenols are valuable secondary plant metabolites found in plants and their products, which might potentially be associated with antioxidant, anti-inflammatory, anticancer,

hypoglycemic, hypocholesterolemic, antibacterial, antifungal, antiviral, and analgesic activities [24-26]. Interestingly in some cases the cytotoxic effect of many plant extracts might be related to their antioxidant activity. According to the US National Cancer Institute, the IC_{50} value should be <20 g/mL for plant extract to be considered as a potential cytotoxic agent, and <4 mcg/ml for isolated compounds [27]. Higher content of total phenols and flavonoids was observed in *C. tianschanica* (13.869 mg GAE/g and 21.79 mg RE/g). All three different plants analyzed have high antioxidant properties, however the highest antioxidant activity was observed in *A. schrenkiana*.

A plant growing in different places has different phytochemical components. For example, in Kazakhstan, *A. schrenkiana* had high levels of camphor (44.79%) and 1.8-cineole (29.05%). The reason for this phenomenon might be the favorable climate and fertile soils of southeastern Kazakhstan. They give plants unique healing properties. In particular, it is a common tea used an anti-inflammatory, sedative and analgesic for influenza, and due to 1,8-cineol, it can be used in therapeutic soaps, sprays or cosmetic flavors and other hygiene products, such as toothpaste, which have a bactericidal effect [28, 29].

Conclusion

It was found that despite the higher content of total phenols and flavonoids in *C. tianschanica* (13.869 mg GAE/g and 21.79 mg RE/g), higher antioxidant activity was observed in *A. schrenkiana*, which looks promising by the composition of its essential oil, in which prevailing compounds are camphor (44.79%) and 1.8-cineole (29.05%).

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