Pak. J. Bot., 55(5): 1857-1865, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-5(33)

STUDIES ON THE BIOLOGICAL ACTIVITY OF DIFFERENT POPULATIONS OF THE MEDICINAL PLANT *RHODIOLA ROSEA* L. (GOLDEN ROOT)

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Abstract

Rhodiola rosea species have a wide spectrum of therapeutic action and are actively used as a tonic, adaptogenic, antidepressant, anti-inflammatory, and for other treatments. We analyzed the phytochemical composition of essential oils of *R. rosea* from the high mountainous regions of Kazakhstan Altai (Eastern Kazakhstan) and Austria. The results showed high percentages of cinnamyl alcohol (31.28%), Cyclobutanecarboxylic acid, 3-methylbutyl ester (9.65%) (9.65%) in the roots of *R. rosea*. of Kazakhstan Altai and the content of cinnamyl alcohol (27.28%), Tyrosol in the root (19.39%) native to Hochkar, Austrian Alps. The antioxidant activity and cytotoxicity of species of different populations of *R. rosea* were also studied. The results revealed antioxidant activity of this species. Biologically active compounds of *R. rosea* have many pharmaceutical effects in various diseases. The formula of essential oils from the root of *R. rosea* were analyzed using GC-MS. The antioxidative activity was determined by FRAP method. The essential oil has shown a low antioxidative activity compared to butylhydroxyanisole(BHA)at concentrations of 0.25-1.0 mg ml-1, which confirms the antioxidative properties of this species. The results of cytotoxic activity against the larvae of *Artemia salina* has revealed that its essential oil shows lethal toxicity at all tested concentrations, proving the cytoxicity of golden root.

Keywords: *Rhodiola rosea*, Antioxidative activity, Biological activity, Cytotoxic activity.

Introduction

The arctic-alpine *R. rosea* (golden root, rose root; synonym *Sedum roseum* (L.) Scop., Family Crassulaceae), is a popular medicinal species distributed in Eurasia. It is known for its pharmacological characteristics as immunostimulant, anti-depressant and adaptogenic (Cunningham *et al*., 2020; Tao *et al*., 2020). This species is extremely variable and some of its geographical races have already been assigned to a number of independent species (Abramchun *et al*., 2014) (Fig. 1).

This medicinal plant has its origin in Asia and Europe and is traditionally used as adaptogen, antidepressant and anti-inflammatory agent. It is classified currently as a rare and endangered species and in many countries is listed as a protected plant in many areas of Eurasia; including the Czech Republic, Slovakia, Bosnia and Herzegovina, Bulgaria, Germany, Austria, the Russian Federation, Mongolia, and China (Kubentayev *et al.,* 2021).

The distribution is observed on a wide Eurasian mountain range (Anon., 2015). Depending on the habitat conditions, roseroot populations exhibit a large variation in phenotypic traits, such as the height of shoots, shape and size of leaves, number of flowers from the inflorerescenses and strength of root system (Wei-Ling *et al*., 2020).

Several centuries ago people have explained the relationship between the disease and cellular pathology and there are records of the medicinal use of *R. rosea* in the Tibetan pharmacopoeia in this connection (Marchev *et al*., 2016). Latest pharmaceutical studies have identified 140 compounds isolated from *Rhodiola* species, including flavones, coumarins, volatiles, anthraquinone, and organic constituents (Panossian *et al*., 2010).

Lately the importance of herbal medicines has increased significantly, leading to a marked increase in the number of studies on their wild populations (Ganzera *et al*., 2001). There is a long history of human use of plants as medicine and much data is found in various fields of biomedical knowledge (Ozturk & Hakeem, 2019ab; Ozturk *et al*., 2017, 2018, 2020, 2022; Olennikov *et al.,* 2020; Malik *et al*., 2021). In the traditional medicine *R. rosea* has been used to increase physical endurance, productivity, longevity, resistance to altitude sickness, and in the treatment of system disorders. In Chinese medical documents golden root has been used to increase the body's resistance to fatigue and to prolong human life (Saratikov *et al*., 1968).

The root system of this species is a branched rhizome with numerous roots. The size and weight of the rhizomes varies greatly depending on the habitat of plants and average weight ranges from 70 to 400g and maximum weight around 2.5–3.5 kg. The rhizomes annually grow from upper part and break down from lower side, their surface is smooth, with a golden shine. The smell is characteristic, slightly reminiscent of pink attar; taste is bitter. The medicinal raw material used are the rhizomes, which are harvested from the end of flowering period to the end of the plant's growing season (Evstatieva *et al*., 2010).

The plant has long been used as an adaptogen in traditional Chinese medicine and it is reported to have many pharmacological features (Tolonen *et al*., 2003). The pharmacological and medicinal properties of this plant depend on the species (Saratikov & Krasnov, 1987). The researchers have discovered other relevant beneficial compounds such as synthetic melanin. They have studied the effects of aqueous-alcoholic extract of *R. rosea* and its hydrolyzate on melanin synthesis which was found to suppress the melanin synthesis and tyrosinase activity in mouse melanoma cells (Kurkin *et al*., 1985). The extract also inhibited the expression of the melanocortin receptor gene as well as protein and inhibited the phosphorylation of the c-AMP response element binding protein (CREB), inhibited the activation of AKT and glycogen synthase-3 beta (GSK3b) kinase and expression of the microphthalmia-associated transcription factor (MITF), and tyrosinase-bound protein 1 (TRP-1) (Chiang *et al*., 2014).

It is important to note that, salidroside as the most potent compound of *R. rosea* can attenuate asthma or cerebral ischemia caused by excessive inflammatory responses by regulating auxiliary T-cell balance (Th1 / Th2) or polarization of macrophages; has regulatory effects on various immune cells and can exhibit excellent immune regeneration of effects in various diseases (Wang *et al*., 2014, 2018).

In the food industry the acetone extract of *R. rosea* has been observed to show antityrosinase activity, the aqueous and methanol extracts of *R. sacra*, a species closely related to *R. rosea* exhibits active oxygen uptake (Liu *et al*., 2018). The natural populations of *R. rosea* are facing a degradation in its natural reserves (Chen *et al*., 2009).

Lately its root extracts have been used globally as ingredients in beverages, food supplements, and commercial pharmaceuticals. Studies on the phytochemistry of *R. rosea* roots has revealed that they contain phenylpropanoids, phenylethanol derivatives (salidroside and tyrosol), flavanoids, monoterpenes, triterpenes, and phenolic acids (Ohsugi *et al*., 1999).

Salt stress has a significant impact on agricultural output, affecting seed germination and seedling growth. Under such circumstances, seed priming may be a feasible and practicable approach for achieving rapid, uniform emergence, vigorous seedlings, and higher crop yields (Hussain *et al*., 2023).

The aim of this work was to obtain biologically active substances from Austrian and Kazakhstani *Rhodiola* species so as to give an idea of how to get an effective herbal medicine for the human body (Johansen *et al*., 1940).

Collecting material for analysis: *R. rosea* rhizomes and roots were collected from Kazakhstan and Austrian Alps (Figs. 2-3), from the plants growing at different places and under varying climates.

1) The underground part of the raw material *R. rosea* was collected in the fruiting phase in August 2020 (Kazakhstan Altai, Ivanovsky ridge (50°18'36.9 "N, 83°44'44.7" E at an altitude of 2000-2100 v. above sea level). The raw materials were dried and crushed. Herbarium specimens of the plant were kept in the herbarium fund of the Astana Botanical Garden (NUR).The plants growing in Hochkar, Austrian Alps: 47°43'24.3"N 14°55'24.9"E., were harvested during fruiting period in August 2020.

The samples were dried and ground into fine powder. 1 g of selected plants was extracted with 97% ethanol (20 ml) in an ultrasonic bath for 30 minutes. After filtering the extracts, the filtrates were pooled and concentrated under vacuum at 30°C until dry.

Fig. 1. General view of the plant *R. rosea*).

Materials and Methods

GC-MS analysis of rapeseed extract: Analysis was performed on rapeseed extract obtained by subcritical CO_2 extraction. Chromatographic analysis conditions: sample volume 1.0 µl, sample inlet temperature 240°C, flow division 1:10. Separation was carried out using a WAXetr chromatographic capillary column with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 µm at a constant carrier gas (helium) speed of 1 ml / min. The chromatographic temperature was programmed from 40°C (exposure 0 min) to 260°C with a heating rate of 10°C / min (exposure 20 min). Detection was carried out in the SCAN m / z 34-850 mode. To control the gas chromatography system, register and process the results and data obtained, Agilent MSD ChemStation software (version 1701EA) was used. Data processing included determination of retention times, peak areas, and processing of spectral information obtained using a mass spectrometric detector (Fig. 4). For decoding the obtained mass spectra, the Wiley 7th edition and NIST'02 libraries were used (the total number of spectra in the libraries was more than 550 thousand) (Tables 1 and 2).

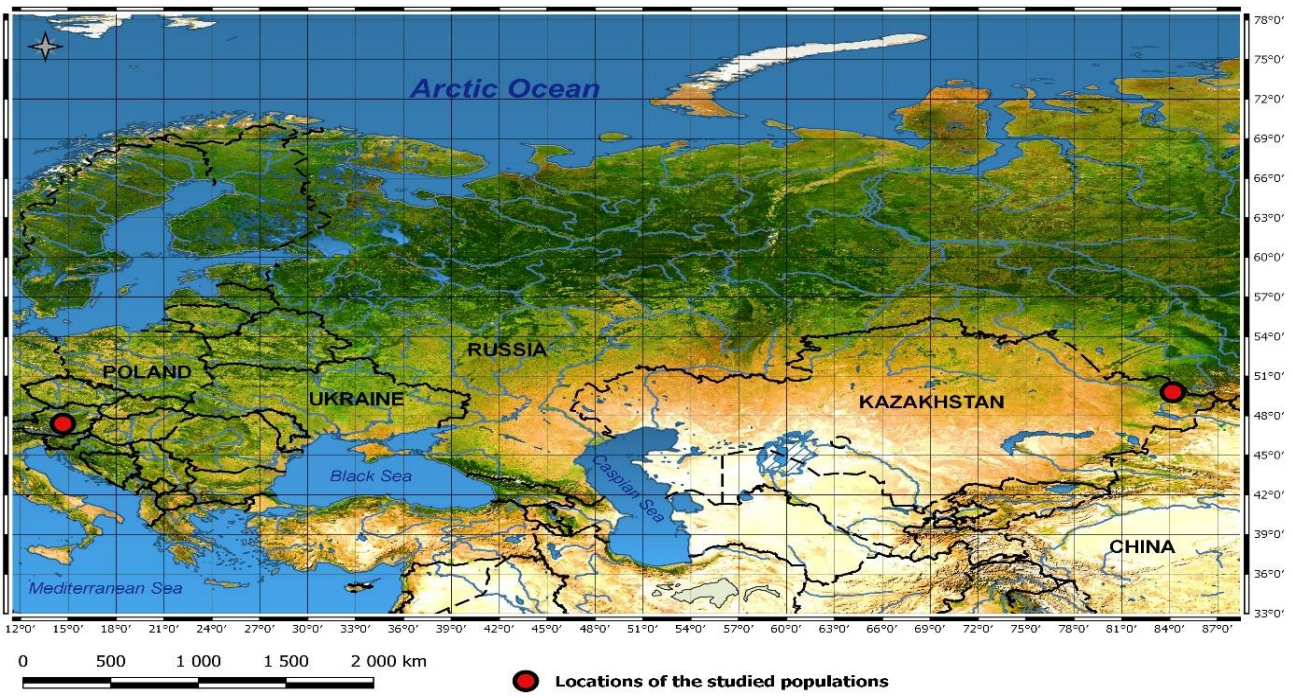


Fig. 2. Schematic map of the distribution of *R. rosea* in East Kazakhstan Alps

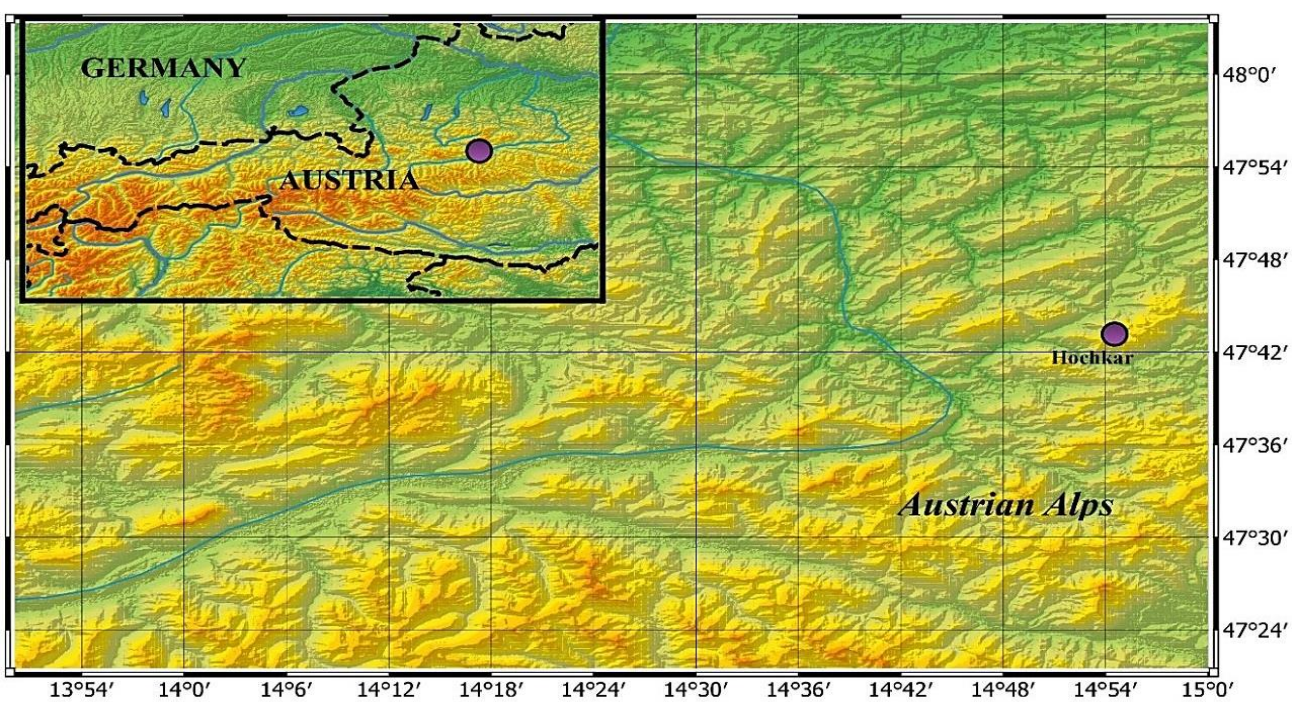


Fig. 3. Schematic map of the distribution of the *R. rosea* in Austrian Alps.

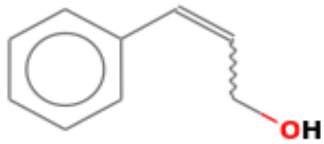
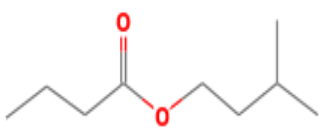
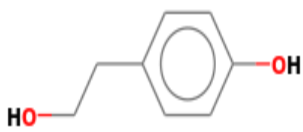
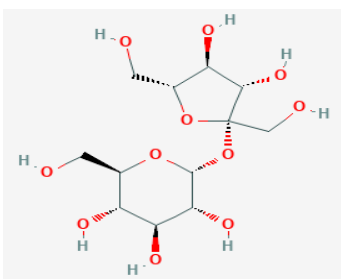
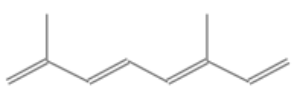
The method of determination of antioxidative activity: 0.25 ml 0.2 M phosphate buffer (pH=6,6) and 0.25 ml of 1% solution of potassium (III) hexacyanoferrate were added to 0.1 ml of the test substances in the concentration range of 0.25; 0.5; 0.75; 1.0 mg/ml. The reaction mixture was incubated within 20 minutes at 50°C, the reaction stopped by adding 0.25 ml of 10% trichloroacetic acid solution; mixture was centrifuged for 10 minutes (3000 rpm). The top layer of 0.5 ml was mixed with 0.5 ml of dist. water and 0.1 ml of 0.1% of $FeCl_3$. Measurement of optical density (OD) was performed at 700 nm. The antioxidative activity (AOA) of the samples was compared with the AOA of butylhydroxyanisole (BHA).

The method of determination of cytotoxic activity: A 55 ml separating funnel was filled with artificial seawater and 200 mg of *Artemia salina* eggs were added. These were kept for 3 days under a soft air delivery until the crustaceans hatched from their eggs. One side of the tube was covered with aluminum foil, and 5 minutes later, the larvae that had gathered on the bright side of the separating funnel were removed with a Pasteur pipette.

Table 1. Results of chromatographic analysis of ethanol extract from Kazakhstan samples.

No.	Holding time, min	Compound	Identification probability, %	Percentages, %
1.	10,89	1,6-Dimethylhepta-1,3,5-triene	89	1,23
2.	11,31	2,6-Dimethyl-1,3,5,7-octatetraene	90	1,33
3.	11,58	2-Cyclopenten-1-one, 2-hydroxy-	92	0,38
4.	12,92	Benzene, 2-propenyl-	85	0,23
5.	13,59	2,6-Octadienal, 3,7-dimethyl-	81	1,02
6.	14,18	Benzylalcohol	92	0,59
7.	14,39	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1α,2α,5α)-	72	0,55
8.	14,68	2-Hydroxy-gamma-butyrolactone	86	0,90
9.	14,82	2,6-Dimethyl-1,3,5,7-octatetraene	91	3,90
10.	15,44	Thymine	79	1,08
11.	15,52	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1α,3α,5α)]-	84	0,43
12.	15,88	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-	81	0,15
13.	16,53	cis-Verbenol	82	0,43
14.	16,89	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	90	1,04
15.	17,10	Pentanal	75	0,50
16.	17,90	Geraniol	93	1,19
17.	18,12	2,6-Octadienal, 3,7-dimethyl-	92	0,68
18.	18,39	Benzofuran, 2,3-dihydro-	86	0,92
19.	18,67	3-Phenylpropanol	76	0,92
20.	18,80	2,6-Octadienal, 3,7-dimethyl-	91	1,04
21.	19,68	p-Cymen-7-ol	85	0,89
22.	19,84	5-Hydroxymethylfurfural	81	1,07
23.	20,25	Cinnamaldehyde	93	1,78
24.	20,37	2-Methoxy-4-vinylphenol	84	0,66
25.	20,72	2-Propen-1-ol, 3-phenyl-	91	31,28
26.	20,98	Eugenol	88	0,42
27.	21,50	Isopulegol	74	0,38
28.	21,98	Pentanoicacid, 3-methylbutyl ester	66	0,52
29.	22,74	Cyclobutanecarboxylicacid, 3-methylbutyl ester	75	9,65
30.	23,64	Benzeneethanol, 4-hydroxy-	89	5,42
31.	24,02	Acetophenone, 4'-hydroxy-	88	0,40
32.	24,91	Sucrose	72	4,17
33.	25,88	D-Allose	88	1,15
34.	26,66	2,4,6-Cycloheptatrien-1-one, 2-hydroxy-4-(1-methylethyl)-	72	0,37
35.	35,64	Heneicosane	93	3,08
36.	38,97	Hexacosane	90	1,15
37.	40,29	1-Docosene	87	0,61
38.	41,95	1-Docosanol, acetate	94	2,72
39.	42,53	Hexadecanal	85	0,91
40.	42,87	Benzyl β-d-glucoside	77	1,36
41.	43,40	1-Eicosanol	89	1,40
42.	44,90	Tetracosylacetate	96	6,27
43.	44,99	Tetratetracontane	87	1,47
44.	45,47	Octadecanal	79	0,45
45.	47,64	Hexacosylacetate	92	3,28
46.	54,62	γ-Sitosterol	79	0,64
47.	44,90	Squalene	76	1,56

Table 2. Chemical composition of *R. rosea* root extract from Kazakhstan.

Compounds	Description	Chemical structure	Retention time, min	Percentage, %
2-Propen-1-ol, 3-phenyl	Cinnamyl alcohol has been shown to be a skin sensitizer		91	31,28
Cyclobutanecarboxylicacid, 3-methylbutyl ester	3-methylbutyl octanoate is a fatty acid ester obtained by the formal condensation of isoamyl with caprylic acid. It has a role as a metabolite. It derives from an isoamyl and an octanoic acid		75	9,65
Benzeneethanol, 4-hydroxy	It has a role as an anti-arrhythmia drug, an antioxidant, a cardiovascular drug, a protective agent and a fungal metabolite. It derives from a 2-phenylethanol.		89	5,42
Sucrose	It has a role as an osmolyte, a sweetening agent, a human metabolite, an algal metabolite, a Saccharomyces cerevisiae metabolite, an Escherichia coli metabolite and a mouse metabolite.		72	4,17
2,6-Dimethyl-1,3,5,7-octatetraene	It has a role as a metabolite.		91	3,90
Hexacosylacetate	It has a role as a pheromone, a plant metabolite and a volatile oil component.		92	3,28
1-Docosanol, acetate And Tetracosylacetate	It has a role as a pheromone, a plant metabolite and a volatile oil component.		94	2,72

20-40 larvae were placed in 990 ml of seawater in each of 24 microbeads. Dead larvae were counted under a microscope. 10 ml of dimethyl sulfoxide solution was added per 10 mg/ml sample. Actinomycin D or staurosporin was used as a reference drug. Only 10 ml of dimethyl sulfoxide were added for negative test control. Dead larvae were counted under a microscope after 24 hours of incubation and further incubation of the microbeds for 24 hours (to ensure immobility).

The death rate was determined by the following formula (1):

$$P = \frac{A - N - B}{Z} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

A – Number of the dead larvae after 24 hours;
N – Number of the dead larvae before the test;
B – Average amount of the dead larvae in the negative test control;
Z – Total amount of larvae.

The correlation analysis was done by Pearson in the R-studio program.

Results and Discussion

In the course of present study, we evaluated Kazakh and European *R. rosea* extracts and their active components. We found many useful active ingredients *R. rosea*.

As per our results on the composition of the *R. rosea* roots extract large quantities of chemical compounds were found: 2-Propen-1-ol, 3-phenyl- 31, 28%, Cyclobutanecarboxylicacid, 3-methylbutyl ester-9.65%, Tetracosylacetate - 6,27%, Benzeneethanol, 4-hydroxy-5.45%, Sucrose -4, 17%, 2, 6-Dimethyl-1, 3, 5, 7-octatetraene-3.90%, Hexacosylacetate – 3, 28, Heneicosane - 3, 08% and 1-Docosanol, acetate – 2,72 (Table 1).

During phytochemical investigations, 29 main compounds of various nature were isolated from the roots (Fig. 4 and Table 3).

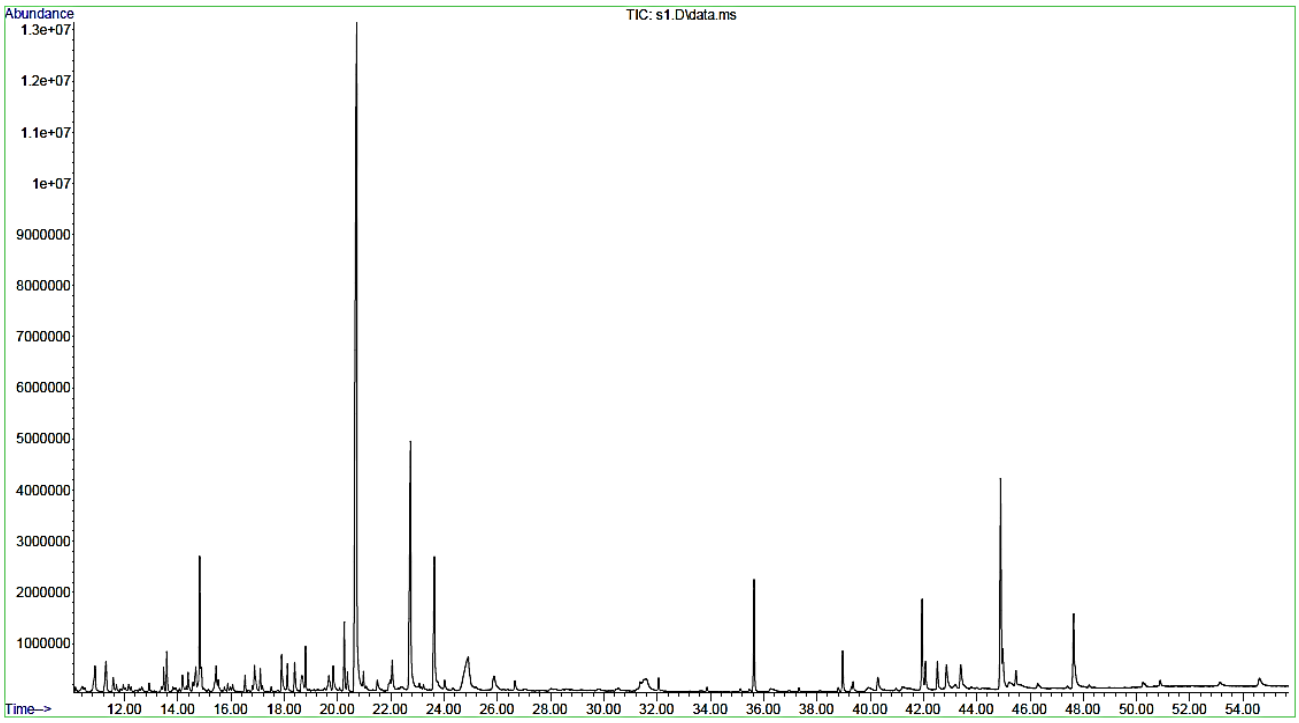


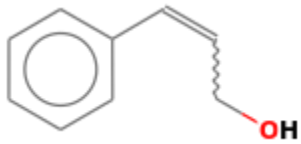
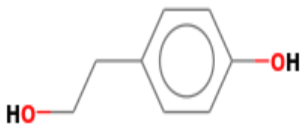
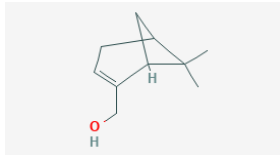
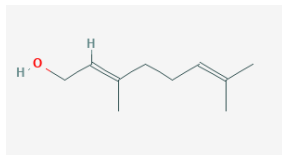
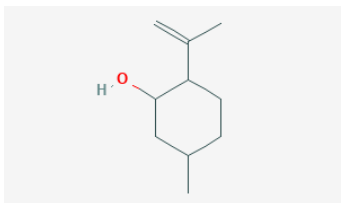
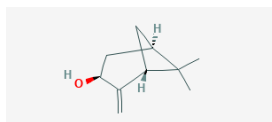
Fig. 4. Phytochemical investigations.

Table 3. Results of chromatographic analysis of ethanol extract from Austrian samples.

No.	Holding time, min	Compound	Identification probability, %	Percentages, %
1.	10,3	Propanoic acid, 2-hydroxy-, ethyl ester	91	0,44
2.	12,0	Ethyl glycolate	91	0,38
3.	12,7	Acetic acid	97	4,43
4.	13,0	Methyl pyruvate	86	3,99
8.	15,9	4-Cyclopentene-1,3-dione	80	0,49
9.	17,6	L-trans-Pinocarveol	92	2,73
10.	17,7	Furfuryl alcohol	91	0,63
11.	18,9	2(5H)-Furanone, 3-methyl-	87	0,60
12.	20,0	2-Cyclopenten-1-one, 2-hydroxy-	93	1,18
13.	20,7	Myrtenol	95	7,03
14.	21,9	Geraniol	95	6,39
15.	22,4	Benzyl alcohol	96	2,38
16.	23,1	Phenylethyl Alcohol	95	2,66
17.	25,8	3-Phenylpropanol	86	0,71
18.	26,5	Cyclopropyl carbinol	75	0,75
19.	27,1	1,3-Dioxol-2-one,4,5-dimethyl-	74	1,87
20.	28,1	2-Hydroxy-gamma-butyrolactone	87	1,70
21.	28,7	4-Vinylguaiacol	86	0,46
22.	30,2	Cinnamyl alcohol	92	27,28
23.	30,6	Glycerin	90	1,78
24.	30,8	Isopulegol	78	6,06
25.	31,0	4-Methoxyphenethyl alcohol	74	1,78
26.	32,5	Myrtenoic acid, butyl ester	71	0,53
27.	33,2	5-Methyl-1-heptene	75	3,47
28.	41,7	Tyrosol	88	19,39
29.	43,6	Squalene	73	0,89

Identification of probability in percentage, prevailing in the rhizomes was as follows: Cinnamyl alcohol – 27,28; Tyrosol – 19,39; Myrtenol – 7,03; Geraniol – 6,39; Isopulegol – 6,06; L-trans-Pinocarveol – 2,73

Table 4. Chemical composition of *R. Rosea* L. roots extract (Austria).

Compounds	Description	Chemical structure	Retention time, min	Percentage, %
Cinnamyl alcohol	Cinnamyl alcohol has been shown to be a skin sensitizer		92	27,28
Tyrosol	It has a role as an anti-arrhythmia drug, an antioxidant, a cardiovascular drug, a protective agent and a fungal metabolite. It derives from a 2-phenylethanol.		88	19,39
Myrtenol	Myrtenol is a bicyclic monoterpene with anti-inflammatory properties.		95	7,03
Geraniol	antitumor, anti-inflammatory, antioxidative, and antimicrobial activities, and hepatoprotective, cardioprotective, and neuroprotective effects.		95	6,39
Isopulegol	isopulegol presented depressant- and anxiolytic-like effects.		78	6,06
L-trans-Pinocarveol	It is a pinene monoterpene, a secondary alcohol and a carbocyclic compound.		92	2,73

GC analysis results revealed the identification of 46 and 29 (Table 1) individual compounds, at concentrations more than 0.20% at least in one of the studied extracts. The identified components represented 99,99% of the total extracts. 2-Propen-1-ol, 3-phenyl- (31.28%) was the most abundant in the Kazakhstan extract. Cyclobutanecarboxylic acid, 3-methylbutyl ester (9.65%) and Benzeneethanol, 4-hydroxy- (5.42%) were present in significant amounts too. Cinnamyl alcohol was the principal component of Austria (27.28%) and Kazakhstan (31.28%), followed by Cyclobutanecarboxylic acid, 3-methylbutyl ester (9.65%) in Kazakhstan or Tyrosol (19.39%) in Austria sample. It is notable that cinnamyl alcohol was present in a large concentration in Kazakhstan sample. Geraniol and Benzeneethanol, 4-hydroxy were identified as main rose like odor compounds.

Cytotoxic and antioxidative activity : Cytotoxic activity of essential oils of *R. rosea* plants was determined according to the normal method, three parallel experiments were carried out, in each of which 20-40 larvae were used (Suleimenov, 2009.).

Based on the results of our experiment, the *R. rosea* essential oil demonstrated the cytotoxicity at concentrations of 10, 5, and 1 mg/ml and the death rate of larvae was 96% (Table 5).

All *R. rosea* essential oils have low antioxidative activity compared to butylhydroxyanisole in all concentrations (Table 6). A chromatographic analysis had been carried out on *R. rosea* of another population growing in the Kazakh Altai, where first detected was squalene, it was first discovered in *R. rosea* by Zhumagul *et al*., (2019). It is also found in the European populations of *R. rosea*. The probability of the appearance of a new component of squalene is attributed to the evolution of species, genetic differences, geographical distribution and ecological growth conditions (Yang *et al*., 2016). Vitamins, collagen and hyaluronic acid are popular globaly among the useful substances, but squalene is one of the little-known, confirming the antioxidant activity of *R. rosea.*

There are various complex antioxidant protection systems in plants including enzymatic or non-enzymatic ingredients which make the reactive oxygen species, while antioxidant enzymes comprise of superoxide dismutase, ascorbate peroxidases, guaiacol peroxidase, glutathione reductase, catalase and dehydroascorbate reductase, amongst them, superoxide dismutase is of main interest as it changes the superoxide into H_2O_2 (Adil *et al*., 2023).

Table 5. Cytotoxic activity of *R. rosea* essential oil.

Name	Concentration, mg/ml	Number of larvae in the control		Number of larvae in the sampling			% Surviving larvae in control	% Surviving larvae in sampling	Death rate, A,%	The presence of neurotoxicity,%
		Surv.	Dead	Surv.	Dead	Par.				
Rh. rosea essential oil	10	22	1	0	26	0	96	0	96	0
	5	22	1	0	23	0	96	0	96	0
	1	22	1	0	26	0	96	0	96	0

Antioxidative activity was determined by FRAP-method (Ferric Reducing Antioxidant Powerassay) (Yang *et al*., 2022)

Table 6. Changes in OD solutions depending on the concentration of working solutions.

No.	Samples	Optical density value at concentration (mg/ml)			
		0,25	0,5	0,75	1,0
1.	Butylhydroxyanisole (BHA)	1,6339	1,6785	1,7822	1,8032
2.	*Rh. Rosea*	0,4180	0,4475	0,5000	0,5091

Conclusions

R. rosea is well known for its unique adaptogenic and antioxidant features, as well as a wide range of biological effects. It is used in traditional medicine to treat various diseases. It was found that *R. rosea* essential oil has cytotoxic and antioxidant effect. GS analysis of the biological activity and composition of extracts confirmed the activity of several known biologically active compounds. Several compounds were identified for the first time, with their previously unknown activity. The study showed a highest percentage of cinnamon alcohol as 31.28 % in the Kazakh Altai samples and cinnamyl alcohol 27.28 % in the European population. Squalene was discovered for the first time in this study. This study is one of the first to determine the cytotoxicity and antioxidant activity of Kazakh rhodiola essential oils. However, more detailed investigations are required to confirm the compounds responsible for the activity in the treatment of many diseases.

Acknowledgment

This study was supported by the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan (Program, IRN: BR10264557)

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(Received for publication 2 May 2022)