Journal of Chemistry of Goods and Traditional Medicine

J Chem Good Trad Med, Volume 3, Issue 4, 2024 Traditional Medicine

STUDYING THE ANTIOXIDANT ACTIVITY OF ROSEHIP AND LICORICE PLANTS

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DOI: https://doi.org/10.55475/jcgtm/vol3.iss4.2024.355

Keywords:
Rosa canina L, Glycyrrhiza glabra L, the active form of oxygen, bronchitis, immunostimulant

Abstract

The antioxidant activity of aqueous and ethanolic extracts from the local medicinal plants Rosa canina L. and Glycyrrhiza glabra L. was experimentally studied using different quantitative ratios. A spectrophotometric method was employed to analyze the properties of adrenaline, which inhibits the autooxidation reaction, to determine the antioxidant activity of the extracts. The results indicated that the ethanolic extract from sample 3, prepared in a 75:25 ratio of Rosa canina L. fruits to Glycyrrhiza glabra L. roots, exhibited higher antioxidant activity than the other extracts. Given its high antioxidant capacity and rich chemical composition, recommendations were made for its use in strengthening the immune system, treating upper respiratory tract diseases, and aiding in sputum removal.

How to Cite

Askarov, I. R., Abdulloev, O. S., Otakhonov, K. K., Razzakov, Z. N., & Latipov, H. A. (2024). STUDYING THE ANTIOXIDANT ACTIVITY OF ROSEHIP AND LICORICE PLANTS. Journal of Chemistry of Goods and Traditional Medicine, 3(4), 320–340. https://doi.org/10.55475/jcgtm/vol3.iss4.2024.355

Rosa canina L. belongs to the Rosaceae family, which contains over 100 species widely distributed in Europe, Asia, Africa, the Middle East, and North America [2]. This thorny shrub, also known as rosehip, dog rose, or briar rose, is mightily resistant to hard weather conditions [3]. Its pseudo-fruits [4], rose hips, ripen in August–September [5] and gain a brick-red to deep-red colour [6]. Rose hips are a valuable resource from both medical and economic perspectives [3].

Typically, 13 species of rosehip are used in medicine, of which 3 species are most common in our sunny country. Rosehip (*Rosa canina* L), Fedchenko dog rose (Rosa fedtschenkoana), Begger rosehip (Akhargul, Rosa beggeriana Sehrenk) grow in gardens, fields, mountain slopes, and water bodies in the middle and upper parts of the mountains, in juniper groves, and in walnut groves in Tashkent, Andijan, Syrdarya, Jizzakh, Samarkand, Fergana, Namangan, Kashkadarya, and Surkhandarya regions [7]. Therefore, there is a complete reserve of the plant, and in medicine, its fruits are mainly used as a natural source of medicinal properties [8].

The licorice plant (Glycyrrhiza) belongs to the Fabaceae family, and currently, 36 species are known to science. Of these, 8 species are mainly found in Uzbekistan. Among these species, the medicinal properties of smooth licorice (*Glycyrrhiza glabra* L) are of particular interest. In Uzbekistan, licorice is found on the banks of the Syrdarya, Zarafshan, Chirchik, Angren, and Amu Darya rivers. To create a reserve of this medicinal plant and its widespread use in the pharmaceutical industry and folk medicine, plantations of G. Glabra have been established in our country on an area of 2,858 hectares, and 23 thousand tons of products were produced in 2018 alone. Since the underground part of the plant is rich in biologically active substances, its root is widely used in medicine [9].

To prevent and treat infectious diseases in humans, scientific research is being conducted to develop synthetic drugs and natural food additives containing chemical compounds with immunostimulating properties and to determine their chemical composition. In this regard, the process of reducing radicals formed as a result of biochemical processes characteristic of oxidation-reduction in the body, that is, antioxidant activity (AO%), plays an important role in strengthening the immune system.

Antioxidants are important micronutrients due to their ability to neutralize free radicals or their effects. Reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals, atomic oxygen, and hydrogen peroxide, are often formed as byproducts of biochemical reactions or as a result of exogenous factors [10]. These radicals have a wide range of pathological effects, causing DNA damage, carcinogenesis, and various diseases such as cardiovascular disease, aging, and neurodegenerative diseases [9]. One of the challenges facing modern medicine is the production and use of drugs with effective antioxidant activity. The study of the AO% of medicinal plants and the application of the results of the research into practice serve as a solution to this problem.

Research object and method. In our research, the AO% of the fruit of the rosehip and the root of the licorice were studied spectrophotometrically. The samples were evaluated by the method of inhibition of the autooxidation reaction of adrenaline in vitro, that is, by the ability to inhibit the autooxidation reaction of adrenaline and at the same time prevent the formation of reactive oxygen species (ROS) [11, 12, 13].

Experimental part. Rosehip (*Rosa canina* L) fruit and licorice (*Glycyrrhiza glabra* L) root were ground to 0.1-1 mm size and 3 different samples were prepared based on them. The quantitative ratio of plant parts in the samples is given in the table below.

Table 1. Proportions of plant parts in sample preparation.

Sample	Part of the plant, %	
	Rosa canina L fruit	*Glycyrrhiza glabra* L corn
Sample 1	25	75
Sample 2	50	50
Sample 3	75	25

Extracts of the samples were prepared in two different ways.

1. Preparation of aqueous extract. 1 g of plant sample was boiled in 25 ml of water for 10 minutes in a flask equipped with a reflux condenser. The obtained extract was passed through a 0.45 μm syringe filter and used for analysis.

2. Preparation of ethanolic extract. 1 g of plant sample was ultrasonically extracted in 25 ml of 96% ethanol for 20 minutes at 60 °C. The resulting extract was filtered through a 0.45 μm syringe filter and used for analysis.

For spectrophotometric analysis of the obtained plant extracts to determine the AO%, 3 ml of 0.2 M carbonate buffer (Na_2CO_3-$NaHCO_3$, pH=10.65) and 0.15 ml of 0.18% adrenaline tartrate solution were added, mixed rapidly, and the optical density D1 was determined at a wavelength of 347 nm every 30 seconds for 10 minutes in a K7000 (YOKE, China) spectrophotometer in a 10 mm thick cuvette. 45 μl and 90 μl of the tested plant extract, 3 ml of buffer solution and 0.15 ml of 0.18% adrenaline tartrate solution were taken, mixed as above, and the optical density at a wavelength of 347 nm was measured (D_2) [8].

Discussion of results. The kinetics of inhibition of adrenaline autooxidation by extracts prepared from *Rosa canina* L. fruit and *Glycyrrhiza glabra* L. root were studied by spectrophotometric measurement. The table below shows the time dependence of the optical density of the oxidized form of adrenaline with the addition of aqueous (Table 2) and ethanolic (Table 3) extracts.

Table 2. Optical densities of solutions containing adrenaline and aqueous extracts of samples were measured for 10 minutes.

Time, sec	Adrenaline (D_1)	Sample 1 (D_2)		Sample 2 (D_2)		Sample 3 (D_2)	
		45 mcL	90 mcL	45 mcL	90 mcL	45 mcL	90 mcL
0	0.029	0,044	0,04	0,055	0,057	0,061	0,072
30	0.062	0,066	0,063	0,076	0,078	0,078	0,089
60	0.097	0,086	0,085	0,096	0,102	0,095	0,107
90	0.13	0,106	0,106	0,116	0,121	0,111	0,125
120	0.163	0,126	0,128	0,135	0,139	0,128	0,145
150	0.195	0,145	0,149	0,154	0,157	0,143	0,163
180	0.226	0,164	0,169	0,174	0,177	0,16	0,181
210	0.256	0,182	0,189	0,192	0,198	0,175	0,199
240	0.285	0,201	0,208	0,211	0,215	0,19	0,216
270	0.312	0,219	0,228	0,229	0,232	0,205	0,232
300	0.337	0,237	0,246	0,246	0,248	0,219	0,247
330	0.361	0,254	0,264	0,262	0,266	0,233	0,263
360	0.383	0,271	0,281	0,278	0,282	0,247	0,28
390	0.403	0,288	0,297	0,293	0,298	0,26	0,295
420	0.422	0,304	0,313	0,308	0,315	0,273	0,309
450	0.439	0,318	0,328	0,322	0,33	0,287	0,324
480	0.455	0,333	0,342	0,336	0,345	0,299	0,337
510	0.469	0,347	0,357	0,349	0,356	0,311	0,35
540	0.482	0,361	0,37	0,361	0,369	0,322	0,363
570	0.494	0,374	0,383	0,374	0,383	0,334	0,375
600	0.504	0,386	0,396	0,387	0,395	0,345	0,387

In the table above, the optical densities of various concentrations of aqueous extracts of fenugreek fruit and licorice root with adrenaline were measured at different time intervals and the results were compared with the optical density of the adrenaline solution. The results obtained are presented in the form of a diagram in the figure below:

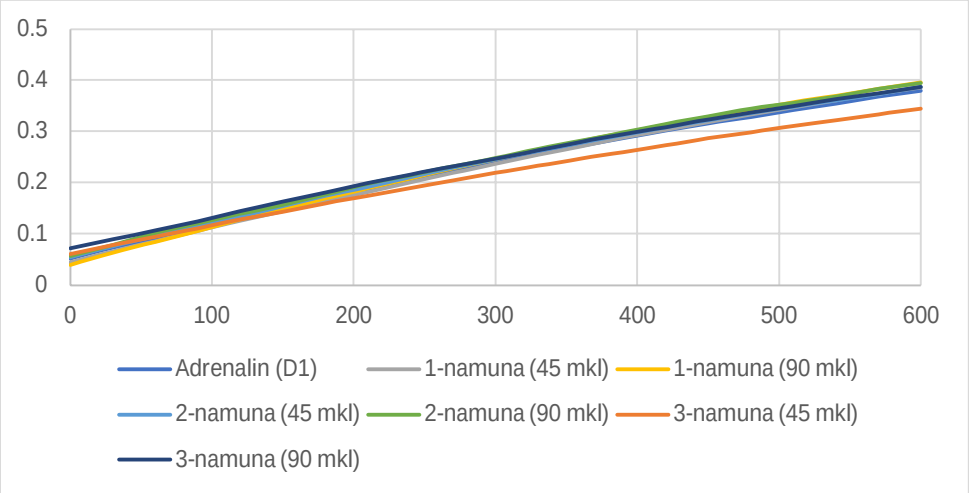


Figure 1. Diagram of optical density increase for adrenaline and adrenaline mixed with aqueous extracts of samples (λ = 347 nm).

In the next part of the study, we determined the optical density of alcoholic extracts from the fruit of the rosehip and the roots of licorice. The results are displayed in the table below:

Table 3. Optical densities of solutions containing adrenaline and ethanol extracts of samples were measured for 10 minutes.

Time, sec	Adrenaline (D_1)	Sample 1 (D_2)		Sample 2 (D_2)		Sample 3 (D_2)	
		45 mcL	90 mcL	45 mcL	90 mcL	45 mcL	90 mcL
0	0.029	0,048	0,047	0,049	0,039	0,054	0,041
30	0.062	0,076	0,084	0,078	0,074	0,082	0,072
60	0.097	0,104	0,12	0,104	0,108	0,108	0,102
90	0.13	0,131	0,156	0,133	0,142	0,134	0,133
120	0.163	0,159	0,191	0,16	0,174	0,16	0,162
150	0.195	0,186	0,225	0,188	0,207	0,185	0,19
180	0.226	0,213	0,257	0,215	0,238	0,21	0,218
210	0.256	0,239	0,287	0,239	0,268	0,234	0,244
240	0.285	0,264	0,317	0,264	0,297	0,257	0,269
270	0.312	0,287	0,346	0,288	0,325	0,279	0,294
300	0.337	0,311	0,372	0,31	0,351	0,3	0,316
330	0.361	0,333	0,397	0,331	0,374	0,319	0,337
360	0.383	0,354	0,421	0,351	0,397	0,338	0,356
390	0.403	0,373	0,443	0,371	0,426	0,356	0,376
420	0.422	0,391	0,463	0,389	0,439	0,372	0,394
450	0.439	0,408	0,483	0,409	0,458	0,388	0,409
480	0.455	0,425	0,5	0,424	0,474	0,403	0,423
510	0.469	0,441	0,516	0,438	0,489	0,416	0,437
540	0.482	0,457	0,531	0,451	0,504	0,429	0,45
570	0.494	0,47	0,543	0,463	0,516	0,441	0,462
600	0.504	0,481	0,554	0,475	0,531	0,452	0,474

In the table above, the optical densities of various concentrations of alcoholic extracts of fenugreek fruit and licorice root with adrenaline were measured at different time intervals and the results were compared with the optical density of the adrenaline solution. The results obtained are presented in the form of a diagram in the figure below:

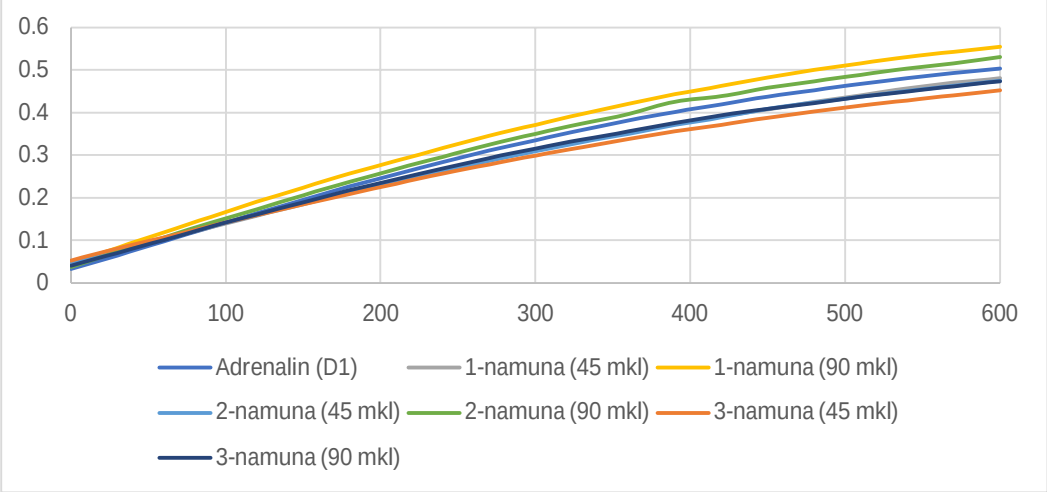


Figure 2. Diagram of optical density increase for adrenaline and adrenaline mixed with alcoholic extracts of samples (λ = 347 nm).

The antioxidant activity of the tested samples is expressed in percentage (AO%) of inhibition of adrenaline autooxidation and was calculated using the following formula and presented in Tables 4-5:

$$AO = \frac{(D_1 - D_2) \cdot 100}{D_1}$$

Here, D_1 is the optical density of the adrenaline tartrate solution added to buffer, and D_2 is the optical density of the sample extract and adrenaline tartrate added to buffer.

Table 4. Antioxidant activity values of aqueous extracts of samples.

Extract under investigation	AO, %					
	Sample 1 (45 mcL)	Sample 1 (90 mcL)	Sample 2 (45 mcL)	Sample 2 (90 mcL)	Sample 3 (45 mcL)	Sample 3 (90 mcL)
1-min	6,522	7,61	-4,35	-10,87	-3,26	-16,30
3- min	2,381	-0,60	-3,57	-5,36	4,76	-7,74
5- min	0,837	-2,93	-2,93	-3,77	8,37	-3,35
10- min	-1,579	-4,21	-1,84	-3,95	9,21	-1,84
Average	**2,04**	**-0,03**	**-3,17**	**-5,98**	**4,77**	**-7,31**

The following table shows the AO% of alcoholic extracts of *Rosa canina* L. fruits and *Glycyrrhiza glabra* L. roots:

Table 5. Antioxidant activity values of alcoholic extracts of samples.

Extract under investigation	AO, %					
	Sample 1 (45 mcL)	Sample 1 (90 mcL)	Sample 2 (45 mcL)	Sample 2 (90 mcL)	Sample 3 (45 mcL)	Sample 3 (90 mcL)
1- min	-5,051	-21,21	-5,05	-9,09	-9,09	-3,03
3- min	6,167	-13,22	5,29	-4,85	7,49	3,96
5- min	7,44	-10,71	7,74	-4,46	10,71	5,95
10- min	4,563	-9,92	5,75	-5,36	10,32	5,95

Average	**3,28**	**-13,77**	**3,43**	**-5,94**	**4,86**	**3,21**

Conclusion: The results indicate that the alcoholic extracts of the tested samples demonstrate higher antioxidant activity compared to the aqueous extracts. However, when the volume of the sample extract was doubled to 90 μL, a decrease in antioxidant activity was observed. This suggests that the optimal amount for assessing antioxidant activity is 45 μL of the extract, as it effectively inhibits the autooxidation of adrenaline. In conclusion, sample 3 exhibited the highest antioxidant activity among all the samples tested.

Considering that the alcoholic extract of the plants of the genus rosehip and licorice has high antioxidant activity is rich in chemical micro- and macroelements, and is widely used in folk medicine, it is advisable to develop a new food product based on them for the prevention and auxiliary treatment of upper respiratory tract diseases.

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