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Original Article

The Determination of Antioxidant Activity of Ethanol Extracts of *Gynostemma Pentaphyllum*

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Abstract

Inflammatory reactions of active radicals play a primary role in many vital circumstances, and if they are not neutralized and persist in the body, many diseases will occur. Antioxidants are the very critical resources available in the body to eliminate these free radicals. Medicinal herbs have been used extensively in the treatment of diseases for centuries. Gynostemma Pentaphyllum is grown in many different locations of Europe and Asia is an adaptogenic plant that is rich in antioxidants. During this study, the antioxidant effects of Gynostemma Pentaphyllum extract are evaluated using two different methods of adrenaline autooxidation and Chemiluminescence, both patented in Russia. Our results show that the organic solvent is more suitable for the extraction of these antioxidants with the ratio of 70% ethanolic extract being yield in more extraction. This preliminary work might open a new door for future studies on the antioxidant effects of this herb grown in Russia for the treatment of inflammations and other diseases with such pathology.

Keywords: *Gynostemma Pentaphyllum, antioxidant, Chemiluminescence, adrenaline autooxidation, adaptogen, ROS.*

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1. Introduction

Antioxidants play an important role in maintaining our health, a large number of free radicals are created in our body daily, and antioxidants play an important role in neutralizing them and preventing a wide range

of diseases[1, 2]. Oxidative stress is one of the initiators of inflammation in the body, many internal and external factors can produce free radicals in the body, the body always tries to maintain balance and neutralize the effect of these free radicals because many chronic diseases such as diabetes, cancer, arthritis, etc. begin like this. Antioxidant compounds can well prevent the damage caused by these free radicals by trapping them, and plants are one of the rich sources of antioxidant compounds that can play an important role [3].

Due to the special geographical location of Russia and its location between Europe and Asia, as well as the types of soil that exists in this country, different types of plants grow in it, including plants with medicinal properties. Russian Far East is very important region of Russia regarding to distribution of medicinal and aromatic plants[4]. *Gynostemma pentaphyllum Makino* (family Cucurbitaceae) is a perennial creeping herb growing wild in the mountain regions of Vietnam, China, and some other Asian countries. it is endemic to the Far East (Kunashir Island)[5, 6]. *G. pentaphyllum* consumption is used to treat hematuria, edema, pain of the pharynx, heat and edema of the neck, and tumors and trauma[7]. It has been used in China for many years as a beverage and food additives, according to the traditional Chinese medicine, the taste and nature of *G. pentaphyllum* are slightly bitter, neutral, and warm[8]. This plant is also one of the adaptogenic plants, these plants have compounds that maintain the body's homeostasis in stressful conditions and make the body adapt to those conditions. Adaptogens exert their effects in a variety of ways, including the production of cortisol, increase the effectiveness of adrenal gland secretion and etc[9].

However, this plant perfectly adapts to the climatic conditions of the Urals: it is cultivated in the botanical garden and on the collection site of the department of pharmacognosy with a course of botany and the basics of phytotherapy at Bashkir State Medical University (Ufa, Russian Federation). In this regard, *G. pentaphyllum* is of great interest as a potential new type of medicinal plant material, since G. *pentaphyllum* is not consider as the natural flora of Russia and as recently been grown by Republic of Bashkortostan, an investigation on the possible altered phytochemical characteristics of this herb in the new environment is required and justified, the aim of this study was to investigate the antioxidant effects of this plant.

1. Materials and Methods

2.1. Materials

Ethanol 40 % (ECOlab Company, Russia), Ethanol 70 % (ECOlab Company, Russia), Adrenalin (Ellara Company, Russia), Ascorbic acid (Askoprom Company, Russia), Phosphate buffer (Bio-optica Milano, Italy), Solution of luminol (Asfarma Company, Russia), Potassium hydroxide (Unid, Korea), Sodium citrate Component-Reagent Company, Russia), Iron sulfate (Energy-chemical Company, Russia), Sodium carbonate (Chemical Union, Russia).

2.1. Plant Materials and Preparation of the Extracts

The object of the study was the *Gynostemma pentaphyllum* (Thunb.) Makino herb introduced on the territory of the Republic of Bashkortostan. aerial parts of plant was collected during the growing summer season 2019 and dried naturally by an air-shadow method. 40% and 70% methanol extracts from the medicinal material were prepared using methods of the State Pharmacopoeia of the 14th edition [10]. For water extract (infusion) preparation, the raw materials (in a ratio of 1:10) were placed in a perforated infuse glass, and then in a ceramic infuse container previously heated in a boiling water bath. The infusion time in the water bath was 15 minutes, then at room temperature – 45 minutes (infusion mode). The ethanol extracts were extracted in a ratio of 1:10 in a boiling water bath using a reflux condenser for 30 minutes and then alcohol was removed, antioxidant activity was studied using two *in vitro* methods[11].

2.2. Evaluating Antioxidant Effect

2.2.1. Standard Method for Evaluating AOQ

The *in vitro* method for determining antioxidant activity is based on the adrenaline ability to form a product with maximum absorption at 347 nm during the autooxidation in an alkaline medium at room temperature. The appearance of this oxidation product is ahead of time in the formation of adrenochrome (absorption maximum of 480 nm).

0.1 ml of a 0.1% prepared solution of adrenaline hydrochloride solution was added to 2 ml of a carbonate solution (pH = 12), and after 3, 5, and 8 minutes the optical density was determined at a wavelength of 347 nm in a 10 mm thick cuvette using a Shimadzu UV-spectrophotometer 1800 (OD_1). Then, 0.01 ml of the test extract and 0.1 ml of a 0.1% prepared solution of adrenaline hydrochloride were added to 2 ml of the carbonate solution, mixed, and after 3, 5, and 8 minutes the optical density was also determined at a wavelength of 347 nm in a 10-cell cell mm (OD_2). As a control sample, we used a buffered solution of the extract, without adrenaline solution. The 0.5% ascorbic acid solution was a comparison solution.

The indicator of antioxidant activity was calculated by the formula:

$$AOA = (OD_1\text{-}OD_2) *100 / OD_1$$

When calculating the antioxidant activity, it was taken into account that the extracts have their color. They absorb a certain wavelength in the visible region of the spectrum. AOA value of more than 10% indicates the antioxidant activity of the studied objects[11].

2.2.2. Chemiluminescence Method

It is the method of establishing antioxidant activity *in vitro*. The ability to reduce the chemiluminescence intensity in systems simulating the processes of production of reactive oxygen species (ROS) and lipid peroxidation (LPO) was determined. A 0.5% ascorbic acid solution was used as a control. The first model system, where ROS were generated, consisted of 10 ml of phosphate

buffer, a solution of luminol (10 mm) and sodium citrate (50 mm). The pH of the system was adjusted to 7.5 by titration with 0.1 N potassium hydroxide solution. To activate the reactions, 1 ml of iron sulfate solution (50 mM) was added. Fe^{2+} salts due to the production of free radicals, resulting in the formation of chemiluminescence, enhanced in the presence of luminol. Registration of the glow lasted for 5 minutes with continuous mixing.

As a second model, lipoprotein complexes from the chicken yolk, similar to blood lipids, were prepared to evaluate the effect of extracts on lipid peroxidation. The chicken yolk was combined with phosphate buffer in a ratio of 1:5, homogenized and diluted 20 times. A sample volume of the lipoprotein complex was 10 ml. Chemiluminescence was activated by adding 1 ml of a 50 mM Fe^{2+} solution to the system, which triggered the oxidation of unsaturated acids that make up the lipids. The chemiluminescence of model systems was characterized by spontaneous emission, a sharp flash, and an unhurried flash developing after that. All studies were carried out in 3 replicates[12].

2. Results and Discussion

The antioxidant effects of the adrenaline autooxidation method occur at different times of 3, 5, and 8 minutes and the results are as follows (Table1)

As shown in the table above, except for the water extract, in other cases the extracts have an antioxidant effect similar to ascorbic acid or even better. The best result was observed in 70% ethanolic extract after 3 minutes, which was able to neutralize about 50% of active radicals in the environment. According to the obtained results, the changes in intensity in 70% ethanol extract were examined, and again it was observed that the maximum rate of changes is related to the period time of 3 minutes (Figure 1).

According to studies, most of the compounds in *G. Pentaphyllum* that are biochemically active need are: flavonoids in terms of rutin, procyanidins in terms of cyanidin chloride, catechins, Triterpene saponins and tannins [13].

The antioxidant properties of the extracts were measured using ROS and AFC models and through chemiluminescence and the results are as follows. As can be seen in the table and figures, the antioxidant effects are dose-response and by increasing the dose, antioxidant effects are raised, but among the extracts, 70% ethanol extract has more antioxidant effects than other extracts.

In the study of Dunja Samec et al., *G. pentaphyllum* was studied with two origins from Germany and Malaysia. During this study, aqueous and methanolic extracts were taken and then the antioxidant effects were investigated and the results were similar to the results of our experiments. Thus, organic extracts have better antioxidant effects than aqueous extracts, and in this article it is stated that antioxidant effects can be due to the phenolic content of the plant[14]. In another study, ethanolic extracts of the plant and

flavonoids isolated from it were examined and 9 flavonoids with different antioxidant effects were isolated, which is the routine composition, the largest quantity of active component showed high activity[15]. Also, a lot of research has been done on the polysaccharide compounds in this plant. In the study of Bo Li et al., 3 models of these polysaccharides were isolated and it was found that they have strong antioxidant effects [16].

According to various studies, the antioxidant effects of this plant have been preserved due to its growth in different geographical areas where the plant is grown. This plant contains several groups of biochemical compounds to which its antioxidant effects can be attributed, and the presence of all of them in this plant can cause a strong antioxidant effect, which is evident in Fig. 2, with increasing dose (more statistical information about the experiment performed on the plant is shown in Table 2. The use of organic solvents in extraction has also shown positive impact on the occurrence of antioxidant effects.

4. Conclusion

During this study, the antioxidant effects of aqueous and ethanolic extracts of *G. pentaphyllum* were evaluated in two different in-vitro methods and it was found that 70% ethanolic extract has better effects, due to the adaptogenic and antioxidant effects of this plant and diversity of biochemically active compounds that exists in this plant, it is promising that with more research and purification of different fractions of it, active fraction is identified that can use in the treatment of diseases especially those in which active radicals and the inflammatory system play an important role.

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Tables

Table 1. Determination of the antioxidant activity of adrenaline solutions with *G. pentaphyllum* extracts.

No	Extracts	Time, Percentage		
		3	5	8
1	Infusion (Water extract)	21,1±0,63	17,1±0,51	14,4±0,43
2	40% ethanol extract	37,8±1,13	33,6±1,0	29,3±0,88
3	70% ethanol extract	50,5±1,50	45,4±1,36	47,3±1,42
4	0.5% ascorbic acid solution (Control)	41±1,23	35±1,05	33±0,99

Table 2.The antioxidant effect of *G. pentaphyllum* extracts measuring by ROS and LPO models through chemiluminescence.(* p<0.5 is significant)

Systems	C, g/ml	ROS model		LPO model	
		Light sum ± SD	% reduction control	Light sum ± SD	% reduction control
Control	–	66,49	100	73,2	100
Solution of ascorbic acid (comparison solution) (1)	0,01	28,58±0,86	57*	27,25±0,82	62,7*
	0,1	6,12±0,18	90,7*	2,59±0,08	96,4*
	1	1,13±0,03	96*	1,83±0,06	97,5*
40% water-ethanol extract (2)	0,01	52,71±1,58	20,7*	55,57±1,67	24,1*
	0,1	40,96±1,23	38,4*	16,03±0,48	78,1*
	1	17,51±0,53	73,6*	8,15±0,25	88,8*
Infusion (3)	0,01	52,63±1,58	20,8*	57,55±1,73	21,4*
	0,1	29,27±0,88	55,9*	35,56±1,07	51,4*
	1	19,77±0,59	70,3*	24,18±0,73	66,9*
70% Water-ethanol extract (2)	0,01	50,52±1,52	24*	44,67±1,34	39*
	0,1	19,51±0,59	70,6*	10,01±0,30	86,3*
	1	8,89±0,27	**86,6***	2,95±0,09	**95,9***

Figures:

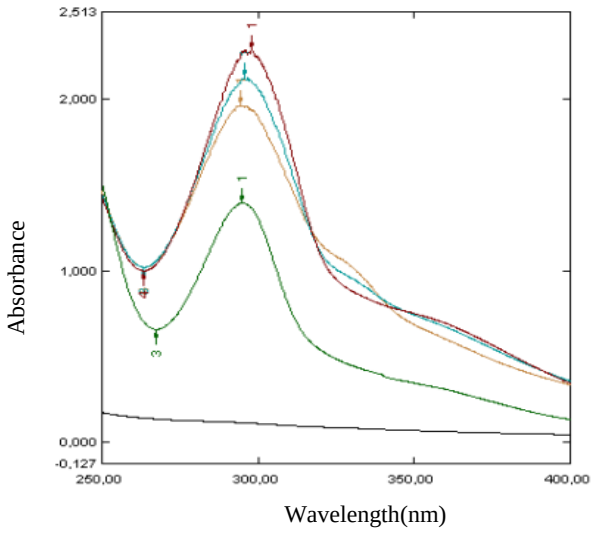

Figure 1. Intensity changes of the 70% ethanol extract during the autooxidation of adrenaline versus time (black line: buffer, green: 0.9 min, brown: 8 min, blue: 5 min, red: 3 min).

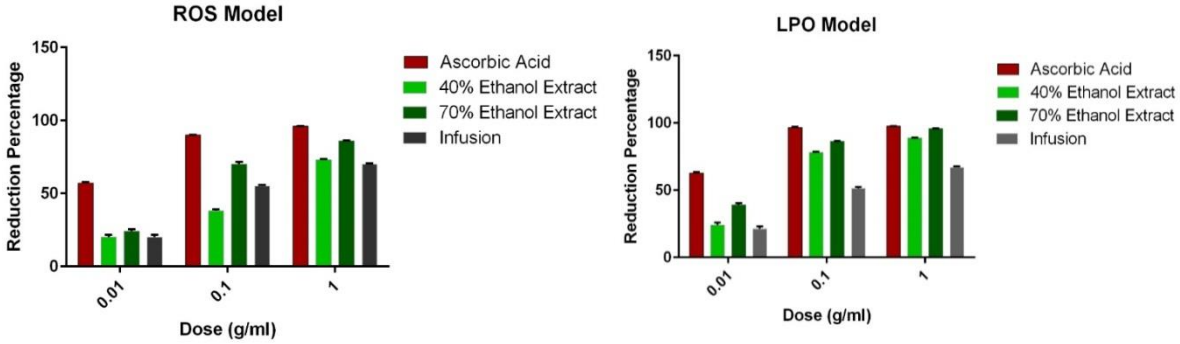

Figure 2. The antioxidant effect of G. pentaphyllum extracts measuring by (A): ROS model and (B): LPO model through chemiluminescence.