STUDY OF THE ANTIRADICAL ACTIVITY OF THE POLYPHENOLS PLANTAGIN AND GLABTAN

Gayibov U.G.,
Rakhmonova G.G.,
Abdulladjhanova N.G.,
Rakhimov R.N.,
Makhmudov R.R.
Institute of Bioorganic chemistry, Academy of Sciences Republic of Uzbekistan
raxmonova-1987@mail.ru

Introduction. Natural compounds are an inexhaustible source of drugs with various therapeutic effects. The study of the molecular mechanisms of the pathogenesis of a huge number of plant, animal and human diseases has shown that all of them are to some extent associated with the activation or suppression of free radical processes. Therefore, the search and study of regulators of such processes based on natural and synthetic raw materials remains relevant.

All plant compounds in relation to animal organisms, to one degree or another, have an extremely wide spectrum of biological activity, due to the diversity of their chemical structure, and are currently in the center of scientific attention. In connection with the above, the search for antioxidants and the study of their inhibitory effect on the processes of free radical oxidation, uncontrolled lipid peroxidation, seems to be quite timely and in demand. Previously, polyphenolic compounds were isolated from the leaves of *Plantago major L*. and *Rhus glabra*, which we conventionally named Plantagin and Glabtan, respectively. Plantagin consists mainly of flavonoids and their glycosides. Glabtan consists of hydrolysable tannins.

Aim. Study of the antiradical activity of Plantagin and Glabtan with respect to the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydrogen peroxide (H_2O_2).

Materials and methods. DPPH method. The determination of the antiradical activity (ARA) of the studied samples was carried out according to the fixation of the change in the optical density of the free radical DPPH. To assess the antiradical activity, in this work we used the method of spectrophotometric measurement of the kinetics of the reduction of molecules of the stable radical DPPH by antioxidants. The test compounds were dissolved in water at a concentration of 1 mg/mL.

H_2O_2 method. To determine the ARA of the compounds with respect to hydrogen peroxide, a technique based on the Rouch method was used. Samples were added separately to each tube containing 2 ml of 20 mM phosphate buffer, 1 ml of 43 mM hydrogen peroxide solution, and 1 ml of distilled water. Absorbances of phosphate buffer solution and hydrogen peroxide solution without phosphate buffer were used as controls. After incubation at room temperature for 10 minutes, the optical density of the samples was measured at 230 nm with respect to a phosphate buffer control solution. The percentage absorption of hydrogen peroxide was calculated using the following formula:

$$H_2O_2\% = \frac{Ac-Ae}{Ac} \cdot 100$$

where: Ac - absorbance of the hydrogen peroxide solution without phosphate buffer;
Ae - absorbance of the samples.

Results and discussion. Since antioxidants can have different mechanisms of action, it is advisable to study their activity using various methods. In this work, the ARA of the compounds was evaluated by two methods: 1) against to the free radical DPPH and 2) against to hydrogen peroxide.

When the compounds under study are added to an alcoholic solution of DPPH, free-radical molecules transform into a non-radical form, while the intensely violet solution of DPPH becomes colorless. On figure 1 shows the kinetics of changes in the optical density of a DPPH solution upon addition of the samples we studied.

To compare the ARA of the studied samples, the concentration for each compound was chosen 5, 10, 25, 50 and 100 µl, respectively, from the prepared solution with a concentration of 1 mm of the substance. Analyzing the obtained results, we can conclude that when the studied polyphenols are added to the alcoholic solution of DPPH, a sharp decrease in the optical density of the DPPH solution is observed, which indicates the antiradical ability of the Plantagin and Glabtan compounds under study.

Figure 1 shows the results of the kinetics of the optical density of an alcoholic solution of DPPH with the addition of Glabtan and Plantagin in a volume of 50 µl (the ratio of DPPH:polyphenol corresponds to 2:1).

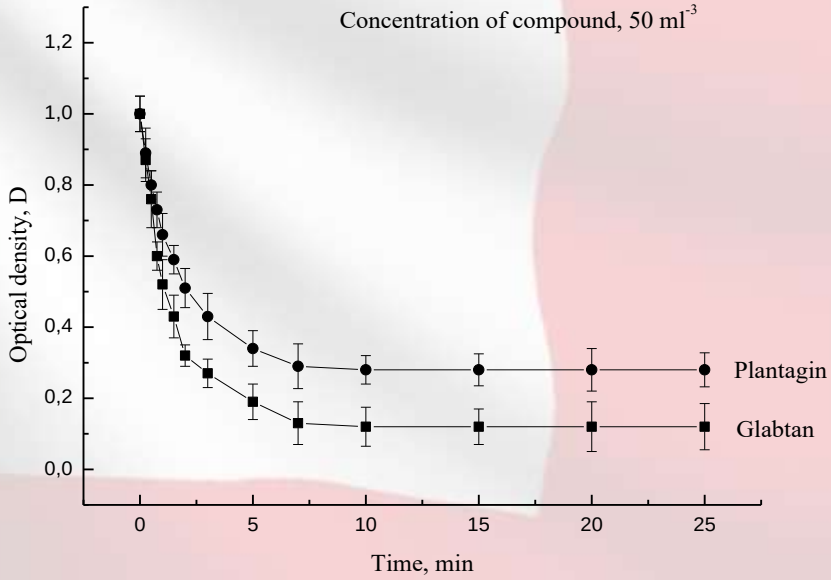

Fig.1. The change in the optical density of the alcoholic solution of DPPH in relation to the control when adding the test compounds depending on time. The solid line is based on non-linear regression. The concentration of DPPH is 0,1 mM. The measurements were carried out at 20°C immediately after the addition of the test preparations. The concentration of the studied compounds is 50 µl from the previously prepared 1 mM solution.

It follows from the experimental data that the studied compounds have a high ability to quench the DPPH free radical. To quantify the antiradical activity, we used the stable radical DPPH, as well as the parameter t_{50}, the time required for the studied preparations to reduce the initial concentration of the radical by 50%. In the DPPH reaction, t_{50} at 17°C is 105 sec for Glabtan and for Plantagin 158 sec. (Table 1).

Table 1

Reaction rate constant values, 50% inhibitory concentration (IC_{50}) and time required to reduce the DPPH concentration by 50% (t_{50}) when reacting with the studied polyphenols

K 10^{-3}, s^{-1}		IC_{50}, µl		t_{50}, sec at 50 µl of substance	
Glabtan	Plantagin	Glabtan	Plantagin	Glabtan	Plantagin
1,79	1,34	22	28	105	158

Further, the ARA of Glabtan and Plantagin with respect to hydrogen peroxide was studied. The results obtained showed the presence of an inhibitory effect in relation to this form of ROS.

The ARA of Glabtan and Plantagin with respect to hydrogen peroxide was 76±4,3 and 64±2,6, respectively, when the test samples were added to the incubation medium in a volume of 150 µl from a previously prepared 1 mM solution. This behavior is apparently due to the presence of several hydroxyl groups with mobile

hydrogen in the molecules of the compounds under study. Moreover, the antioxidant activity of Glabtan, according to the literature data, significantly exceeds the activity of rutin.

An analysis of the experimental results obtained during the study showed that the Glabtan compound has a higher ARA compared to Plantagin in relation to free radicals (DPPH and H_2O_2), for which the reaction constant with DPPH is the highest, the value of which can serve as the antiradical efficiency of the compound.

Conclusions. Polyphenols have a mobile hydrogen atom and therefore react with free radicals as well as free radical oxidation catalysts. The results obtained indicate that the molecules of the studied compounds contain mobile hydrogen atoms, which exhibit high antiradical activity. The studied compounds exhibit high antiradical activity towards DPPH and H_2O_2 free radicals in *in vitro* model systems. At the same time, a more pronounced activity was observed in Glabtan compared to Plantagin, realized as the interception of free radicals and reactive oxygen species. The results obtained in this work allow the creation of drugs with antioxidant activity based on these compounds.