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Studying the Properties of Biologically Active Substances Isolated from Some Plants

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Abstract This article explores the identification, isolation, and biological activities of active compounds derived from various plant species. The focus is on understanding how these biologically active substances such as alkaloids, flavonoids, terpenoids, and phenolic compounds can impact human health and disease prevention. The study examines both traditional uses and modern scientific validations of these plant-derived substances in the treatment of ailments, including cancer, infections, and inflammation. Additionally, the article discusses the methodologies used in the extraction and characterization of these compounds, as well as the potential for developing new pharmacological agents from plants. The findings contribute to the growing field of natural products research and underscore the importance of plants as a source of novel therapeutic agents.

Keywords Antioxidant activity, Anti-radical activity, Polyphenolic compounds, Flavonoids

1. Introduction

The antioxidant status of organism is one of the universal indicators characterizing the state of human health. Almost all pathological processes in the body, in particular coronary heart disease, atherosclerosis, heart valve pathology and other cardiovascular diseases, are accompanied the development of oxidative stress and the formation of free radicals [1]. Reactive oxygen species (ROS) induce various free radical oxidative reactions in cells, the targets of which are cell membrane lipids, nucleic acids, proteins, enzymes, DNA molecules, resulting in a wide range of pathogenetic effects may develop. The following ROS are of greatest biological significance: singlet oxygen, radical anion superoxide (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), peroxyl radical (R-COO), nitric oxide (NO), peroxynitrite (ONOO) [2,3]. Since free radical oxidation is a chain of branched reactions initiated by various types of ROS, in the process of which various products of degradation of molecules with their own activity, there is no universal method for assessing the antioxidant activity (AOA) of biologically active substances. The results obtained using only one test can be interpreted with great caution in relation to biological objects. Therefore, in vitro assessment of AOA is currently performed using several test systems [4,5].

Objective of the work: To determine the antioxidant and antiradical properties of polyphenols, terpenoids, tannins, and flavonoid compounds obtained from certain plants.

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2. Materials and Methods of Research

To determine the antioxidant and antiradical properties of selected biologically active substances, the solubility of the preparations is considered. The spectrophotometric method with the Folin-Ciocalteu reagent is used to determine the total amount of polyphenol-rich compounds. The sum of polyphenols is determined based on phosphor-molybdenum and phosphor-tungsten acids in an alkaline medium [6]. To determine flavonoids, 2 ml of the solution, 0.5 ml of a 33% acetic acid solution, and 95% ethanol are sufficient. The optical density is measured after 20 minutes at a wavelength of 410 nm [5]. When determining the amount of proanthocyanidins (PACs), 15 ml of concentrated hydrochloric acid and 10 ml of water are added to a 5 ml sample, and the mixture is boiled for 80 minutes. The optical density of the solution is measured at a wavelength of 545 nm [4]. The antioxidant activity in vitro is determined by various methods. The spectrophotometric method for determining the total antioxidant activity is based on the Fe^{+2} -induced peroxidation model. The optical density of the samples is measured at a wavelength of 532 nm [7]. Spectrophotometric determination of the inhibition of hydroxyl radicals (OH). Hydroxyl radicals are generated by the Fenton reaction (Fe2+ – EDTA- H_2O_2 system). The antiradical activity against hydroxyl radicals is measured using the deoxyribose method. The optical density of the samples is measured at a wavelength of 520 nm [8]. Spectrophotometric determination of the

inhibition of the superoxide radical in an alkaline solution of dimethyl sulfoxide (DMSO). The optical density of the samples is measured at a wavelength of 560 nm [9]. Spectrophotometric method for measuring NO-inhibitory activity. This method is based on measuring the concentration of nitric oxide (NO), formed by the reaction of sodium nitroprusside with the Griess reagent [9]. The optical density of the resulting solution is measured at a wavelength of 540 nm. The DPPH spectrophotometric method [10] is based on the interaction of antioxidants with the stable chromogenic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The optical density of the resulting solution is measured at a wavelength of 517 nm. As a control sample, a working DPPH solution was used. The ABTS spectrophotometric method [8] generates the ABTS+ radical cation by incubating a mixture (1:1) containing 7 mM of diammonium salt of 2,2´-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (Sigma) and 2.45 mM of sodium persulfate at a temperature of 37℃ for 12-16 hours. The optical density of the samples is measured at a wavelength of 734 nm. Determination of Fe+2-chelating activity [9]. The optical density of the resulting solution is measured at a wavelength of 510 nm. Spectrophotometric method based on the study of the kinetics of the inhibition reaction of adrenaline autoxidation [10]. All experiments were conducted using the Cary-60 Agilent Technology spectrophotometer. Statistical processing of the experimental results was carried out using Origin 8.6 USA software.

3. Results and Discussion

The antioxidant activity (AOA) of plant samples was conducted by comparing the total amount of polyphenolic compounds with the concentration of flavonoids and PACs under the influence of biologically active substances. (Table 1).

Table 1. The amount of polyphenols (mg/ml) in 70% aqueous and ethanolic solutions of certain plants (n=5)

Preparations	Content of polyphenolic compounds		
	In 70% water-ethanol extract, mg/ml		
	Total content of polyphenolic compounds	Flavonoids	PACs
Quercetin	153,87±1,25	17,63±0,85	57,42±0,63
Euphorbin-1	184,21±1,80	12,93±0,75**	72,07±0,31
Euphorbin-2	261,53±2,04*	19,12±0,58	71,69±0,48*
Euphorbin-3	217,18±1,74	12,09±0,62*	66,46±0,95
Gossitan	139,18±1,86	14,52±0,97	52,01±0,52
Sumakh	189,83±1,97**	16,78±0,69	61,02±0,42
Glabra	206,15±2,70	15,03±0,57**	67,85±0,94
Bex	201,21±2,11	16,95±0,86	64,26±0,71
Karelinia caspia	189,81±2,53*	14,79±0,74*	59,24±0,63*

(application: *p=0.05, **p=0.01)

The data presented in Table 1 show that the content of phenolic compounds varied widely depending on the growth phase of the plants. In particular, the total content of phenolic compounds in the studied extracts, relative to the extract from raw material collected at the beginning of the vegetation period, increased 1.46 times during the budding phase and 1.19 times during the flowering phase, and decreased 0.9 times during the fruiting phase.

A similar pattern of change in concentration was observed for flavonoids, though with a larger amplitude of fluctuations. Their content, relative to the extract from raw material at the beginning of the vegetation period, increased 4.7 times during the budding phase and decreased to 4.13 and 2.89 times during the flowering and fruiting phases, respectively. As for the content of proanthocyanidins (PACs), during the budding and flowering phases, relative to the beginning of vegetation, there was a slight decrease - 0.98 and 0.92 times respectively - and an increase of 1.66 times during the fruiting phase.

Considering that plant phenolic bioactive compounds are multifunctional antioxidants capable of neutralizing ROS, binding metal ions involved in the ROS-generating Fenton reaction, and neutralizing free radicals formed in intermediate stages of free radical oxidation, the evaluation of the antioxidant and antiradical potential of plant raw material should be based on the use of a complex of methods reflecting various aspects of their activity.

In this study, methods were used to assess the effectiveness of natural and synthetic antioxidants in terms of inhibiting model reactions of free radical oxidation of biomolecules, particularly fatty acids, nucleic acids, proteins, etc. (direct methods), and reactions of reducing ROS activity and inactivating free radicals (indirect methods) [13-15].

The direct method used to determine AOA involved the Fe+2-induced peroxidation of arachidonic acid. It was established that all studied extracts showed similar levels of inhibition of free radical oxidation (about 70%) with no significant differences in different phases of development (Table 2).

4. Assessment of Antiradical Activity

The evaluation of direct antiradical activity was carried out using two methods, differing in the radicals they inactivate. The first method is based on the interaction of antioxidants with the stable chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). This method was proposed by M.S. Blois [10,16] and shows a correlation with the results obtained from direct methods [7,17]. The research results (Table 2) confirmed the presence of significant antiradical activity in all studied extracts. The obtained values were similar in magnitude and ranged from 86% to 89%. The second method for assessing antiradical activity is based on the reaction of antioxidants with the cation-radical 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+). Results

obtained using this method, compared to the DPPH method, show a high correlation with the content of phenolic compounds and inhibitory activity concerning ROS. The results presented in Table 2 demonstrated greater variation in the expression of antiradical activity for different extracts: the highest activity was observed in extracts from raw material collected during the flowering phase (90.85 ± 2.07%), while the lowest activity was found during the fruiting phase (64.63 ± 2.36%).

Another method for determining antioxidant activity (AOA) is based on assessing the reduction of iron-radical activity. It is known that ions of metals with variable valence often act as initiators of the lipid peroxidation (LPO) process (Fenton reaction). Given this fact, an evaluation of Fe^{+2}-chelating activity and inhibition of the hydroxyl radical generated in the Fenton reaction was carried out. It was found that the extracts from the grass *Agropyron repens*, collected during the budding and flowering phases, exhibited the highest Fe-chelating activity (Table 2). This activity might be due to the presence of flavonoids and tannins in their composition, which, according to literature, are effective chelators of heavy metal ions [3,18]. Furthermore, such high chelating activity could also be related to the high content of polysaccharides present in the studied extracts.

As an integral method for assessing the effectiveness of plant raw material, one can consider the method based on evaluating the inhibitory activity of substances regarding the autoxidation process of adrenaline in an alkaline medium in vitro, thus preventing the formation of ROS [10,19]. According to the results obtained using this method, the highest activity was recorded in the extract from raw material collected during the budding phase (48.89 ± 1.13%), and the lowest activity was observed at the beginning of the vegetation period (23.75 ± 0.78%).

In general, the obtained results, using different evaluation methods, confirmed the presence of pronounced antioxidant and antiradical activity in the extracts of *Agropyron repens* grass.

5. Conclusions

Thus, the presented data indicate that the above-ground part of *Agropyron repens*, containing polyphenols extracted from the plants, can be considered as a promising raw material for the creation of phytopreparations with antioxidant and antiradical properties.

Table 2. Antioxidant and antiradical activity of polyphenolic compounds (%) (n=5)

Methods for Determining AOA	**AOA %**									
	Gliclazide	**Euphorbin-1**	**Euphorbin-2**	**Euphorbin-3**	**Quercetin**	**Getasan**	**Sunakh**	**Glabra**	**Bex**	**Karelinia caspia**
Method of Inhibition of Lipid Peroxidation	52,23 ± 0,98	69,01 ± 0,56	73,11 ± 0,91**	72,25 ± 0,35**	71,20 ± 1,02	64,15 ± 1,17*	68,49 ± 1,85	69,90 ± 2,08	63,14 ± 1,76*	62,18 ± 1,91*
Hydroxyl Radical Inhibition Method	47,89 ± 1,12	66,43 ± 0,89	65,56 ± 1,06*	77,60 ± 0,97	65,92 ± 1,21	68,75 ± 1,85	69,12 ± 1,88	70,56 ±2,01**	62,14 ± 1,73	64,35 ± 1,85
Superoxide Radical Inhibition Method	42,91 ± 0,87	59,93 ± 0,98*	74,31 ± 1,34**	97,01 ± 0,59	67,39 ± 1,13	63,45 ± 1,85	69,54 ± 2,15*	70,02 ± 2,07	68,25 ± 2,45*	78,63 ± 2,33**
Nitric Oxide Radical Inhibition Method	20,14 ± 0,72	28,44 ± 2,88	38,78 ± 2,01	32,29 ± 2,48*	28,87 ± 1,99	26,53 ±1,53	29,63 ± 1,67	31,01 ± 1,09	29,51 ±1,07**	27,59 ± 1,67
DPPH	67,48 ± 1,08	86,98 ± 2,43**	89,91 ± 2,13	82,41 ± 1,53*	92,46 ± 1,28*	82,69 ±1,13*	84,69 ±1,59**	86,57 ± 1,61	80,68 ± 1,12	83,69 ± 1,08
ABTS	61,77 ±1,85	77,42 ± 2,64	91,85 ± 2,07	86,77 ± 2,12	65,63 ± 2,36	72.63 ±1,95	78,54 ± 2,08	67,95 ± 2,45	69,12 ± 1,86	70,25 ±2,08
Fe^{2+} chelating activity	31,02 ± 1,14	38,49 ± 1,90**	61,19 ± 1,95	55,76 ± 1,73	41,53 ± 2,04	39,62 ±1,39**	49,57 ± 1,88	50,14 ±1,70**	41,68 ± 1,29	47.23 ± 1.42*
Autoxidation of adrenaline	18,25 ± 0,69	23,68 ± 0,78	48,82 ± 1,13	33,67 ± 0,57	37,01 ± 1,89	27,46 ±1,05	29,36 ± 1,15*	32,74 ±1,21**	28,96 ± 1,36	33,85 ± 1,67**

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