O‘ZBEKISTON RESPUBLIKASI

OLIY TA’LIM, FAN VA INNOVATSIYALAR VAZIRLIGI

FARG‘ONA DAVLAT UNIVERSITETI

FarDU. ILMIY XABARLAR

1995-yildan nashr etiladi
Yilda 6 marta chiqadi

2-2024

НАУЧНЫЙ ВЕСТНИК. ФерГУ

Издаётся с 1995 года
Выходит 6 раз в год

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FarDU. Ilmiy xabarlar – Scientific journal of the Fergana State University

Volume 30 Issue 2, 2024-yil DOI: 10.56292/SJFSU/vol30_iss2/a118

UO'K: 547.962.9:547.917:615.4

O'SIMLIKLARDAN OLINGAN EKSTRAKTLAR ANTIRADIKALLIK FAOLLIGINI ANIQLASH

ОПРЕДЕЛЕНИЕ АНТИРАДИКАЛЬНОЙ АКТИВНОСТИ РАСТИТЕЛЬНЫХ ЭКСТРАКТОВ

DETERMINATION OF ANTIRADICAL ACTIVITY OF PLANT EXTRACTS

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Annotatsiya

*Ushbu maqolada 2,2-difenil-1-pikrilhidrazil (DPPH) erkin radikaliga qarshi qarag'ay gulchanglari va sharqiy biota o'simliklari ekstraktini antiradikal faolligini (ARA) o'rganib chiqildi. O'rganilgan moddalar orqali DPPHni kamaytirish reaktsiyasining miqdoriy xarakteristikalari aniqlandi. Ushbu jarayonda ARF ni baholash uchun biz antioksidantlar tomonidan barqaror radikal 2,2-difenil-1-pikrilgidrazil (**DPPG**) molekulalarini kamaytirish kinetikasini spektrofotometrik o'lchash usulidan foydalandik. O'rganilgan birikmalar erkin radikallarni o'chirish uchun yuqori qobiliyatga ega ekanligi aniqlandi.*

Аннотация

В данной стате изучена антирадикалная активност (АРА) пыльцы сосны и экстрактов растений биоты восточной в отношении свободного радикала 2,2-дифенил-1-пикрилгидразила (ДФПГ). Определены количественные характеристики реакции восстановления ДФПГ исследуемыми веществами. Для оценки АРА в этом процессе мы использовали спектрофотометрическое измерение кинетики восстановления молекул стабилного радикала 2,2-дифенил-1-пикрилгидразила (ДФПГ) антиоксидантами. Установлено, что изученные соединения обладают высокой способностью устранять свободные радикалы.

Abstract

This article examines the antiradical activity (ARA) of pine pollen and plant extracts from eastern biota in relation to the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Quantitative characteristics of the reaction of DPPH reduction by the studied substances were determined. To evaluate ARF in this process, we used spectrophotometric measurements of the reduction kinetics of stable radical molecules 2,2-diphenyl-1-picrylhydrazyl (DPPG) by antioxidants. It was found that the studied compounds have a high ability to remove free radicals.

Kalit so'zlar: *ekstrakt, antioksidant faollik, kislorodning faol shakli, radikal, adrenalin, spektrofotometr, oziq-ovqat qo'shilmalari.*

Ключевые слова*: экстракт, антиоксидантная активност, активная форма кислорода, радикал, адреналин, спектрофотометр, пищевые добавки.*

Key words*: extract, antioxidant activity, reactive oxygen species, radical, adrenaline, spectrophotometer, food additives.*

INTRODUCTION

The high reactivity of radicals and their presence in the cells of the body are inextricably linked with vital processes and play an important role in the pro-oxidant-antioxidant balance in the body. Oxidative damage is caused by endogenous and exogenous factors that generate reactive oxygen species. Internal sources of oxidants include the mitochondrial electron transport chain and nitric oxide synthase reaction, as well as non-mitochondrial reactions [1].

LITERATURE ANALYSIS AND METHODOLOGY

The human body has an antioxidant defense system that works through primary antioxidant enzymes and secondary antioxidant vitamin systems [2]. When the human body is exposed to

extreme factors such as radiation, poisons, many harmful molecules are produced, and in this case the body requires more antioxidants [3]. Because free radical molecules lack one or more electrons, they aggressively attack healthy molecules and cause a chain reaction. Free radicals usually accumulate in cell membranes and begin to corrode them, causing the cells of our body to gradually disintegrate [4].

Exogenous sources are environmental influences. Excessive production of free radicals damages the natural antioxidant defenses of cells and leads to deterioration in the body's functionality [6]. Elevated levels of free radicals are considered to be a cause of general aging and degenerative diseases of aging. One of the most important strategies for reducing oxidative stress is the consumption of exogenous antioxidants rich in beneficial compounds, usually from plant sources [5,6]. Antioxidants stop rapidly increasing oxidation processes, create inactive particles and remove them from the body [7]. Aromatic plant extracts are known to be promising substitutes for synthetic antioxidants [8,9]. Free radicals are the cause of many diseases in humans and animals. The body's antioxidant system helps neutralize these free radicals. Antioxidants are also widely used in modern practice. Antioxidants are able to neutralize the activity of free radicals, thereby protecting cells from oxidation [10]. A number of methods are used to assess antioxidant and antiradical activity. [11]. The most common are volumetric, photometric, chemiluminescent, fluorescent, electrochemical and biological marker methods [11].

Spices, various oils, teas, seeds, grains, cocoa beans, fruits and vegetables are used as natural antioxidants. Compounds with high antioxidant activity from natural compounds, including proven ascorbic acid, tocopherols, carotenoids, as well as flavonoids (quercetin, kaempferol, myricitin), catechins (carnosol, rosmanol, rosamiridifenol) or various individual antioxidants such as polyphenols and phenolic acids.

In this work, X1 (1+1), X2 (1+2), X3 (2+1) pine pollen and oriental biota, extract of antiradical activity (ARF); (pine pollen and eastern biota) against the stable free radical **DPPG** (2,2-diphenyl-1-picrylhydrazyl).

The materials were obtained based on 3 different samples:

1. No.1 X1 Extract; (1+1) (pine pollen and eastern biota)
2. No.1 X2 Extract; (1+2),
3. No.1 X3 Extract t; (2+1),

Method DPPG. In this study, we used spectrophotometric measurement of the reduction kinetics of stable radical molecules 2,2-diphenyl-1-picrylhydrazyl (DPPG) by antioxidants to assess ARF. The method is based on the interaction of antioxidants with the stable chromogenic radical 2,2-diphenyl-1-picrylhydrazyl (**DPPG**). A standard solution of **DPPG** ($5x10^{-4}$ M) in ethanol, acidified with acetic acid, was diluted with ethanol in a ratio of 1:10 to obtain a working solution. The resulting solution must have an optical density of no higher than 0,9 at 517 nm. 50 mkl of extracts of the studied plants were added to 5 ml of the DPPG working solution, mixed, and the kinetics of decrease in the optical density of the solution was recorded for 30 minutes at a wavelength of 517 nm. The DPPG working solution was used as a control sample..

Antiradical activity is determined by the following formula:

$$\% \text{ merge} = \frac{Akontr - Ax\,Akontr}{Akontr} x100\%$$

Here Ax is the optical density of the test solution, the numerator is the optical density of the test sample.

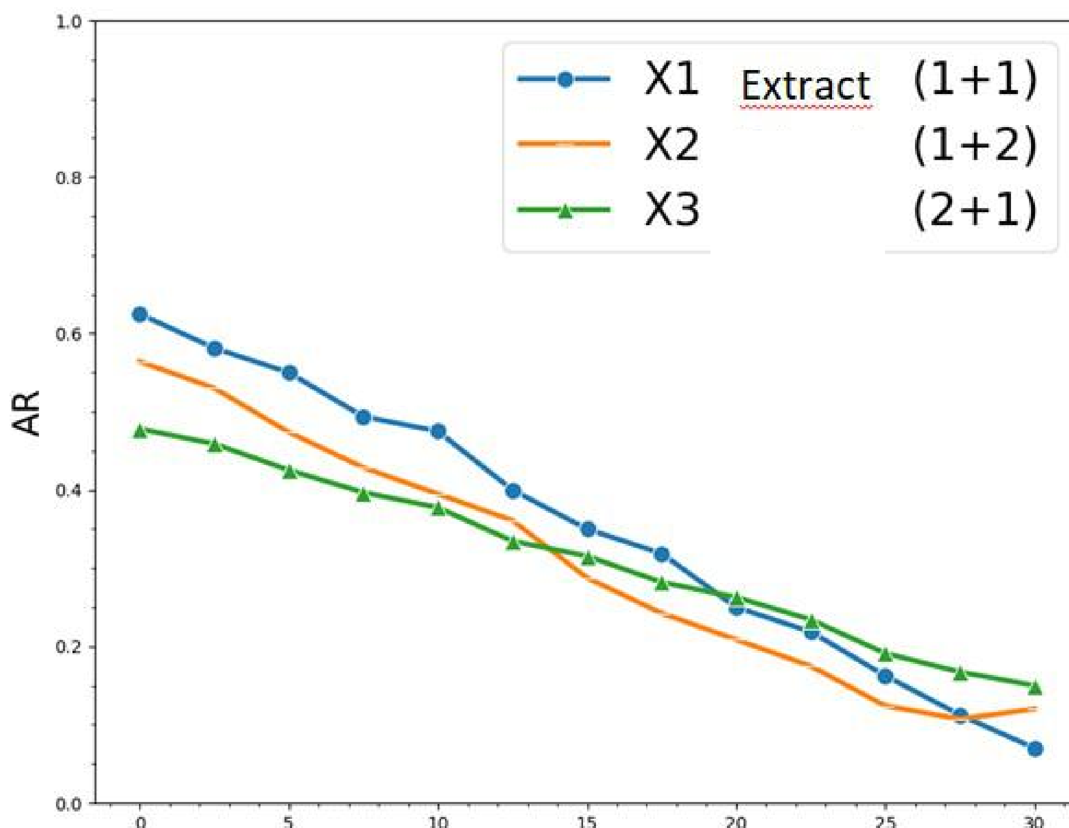
RESULT AND DISCUSSION

Antioxidants may have different mechanisms of action, and it is recommended to study their activity using different methods. In this study, ARF extracts were evaluated against DFPG free radicals. When the test plant compounds are added to an aqueous solution of DFPG, the free radical molecules are converted to non-radical forms, and the intense purple color of the DFPG solution is discolored. The kinetics of changes in the optical density of a DFPG solution upon addition of the studied samples is shown.

To compare the ARF of the tested samples, a concentration of 50 mkl of each extract was taken from the provided solution. Since samples 1–3 showed very high ARF, we diluted them with the appropriate solvent (DMSO) at a ratio of 1:100.

Analyzing the results obtained, we can conclude that when DFPG is added to the solution obtained from the studied preparations No. 1-3, a sharp decrease in the optical density of the DFPG solution is observed, which indicates their high ARF. ARF for 1 sample was assessed after 100-fold dilution, indicating a clear antiradical ability of plant extracts.

Plant name	Optical density, D	Time, minute	Concentration
No.1	0.1	30	0,07
No.1	0.2		0,11
No.1	0.3	25	0,16
No.1	0.4		0,22
No.1	0.5	20	0,25
No.1	0.6		0,32
No.1	0.7	15	0,35
No.1	0.8		0,4
No.1	0.9	10	0,47
No.1	1.0		0,49
No.1	1.1	5	0,55
No.1	1.2		0,58
No.1	1.3	0	0,62
No.2	0.1	30	0,12
No.2	0.2		0,11
No.2	0.3	25	0,12
No.2	0.4		0,17
No.2	0.5	20	0,21
No.2	0.6		0,24
No.2	0.7	15	0,29
No.2	0.8		0,36
No.2	0.9	10	0,39
No.2	1.0		0,43
No.2	1.1	5	0,47
No.2	1.2		0,53
No.2	1.3	0	0,56
No.3	0.1	30	0,15
No.3	0.2		0,17
No.3	0.3	25	0,19
No.3	0.4		0,23
No.3	0.5	20	0,26
No.3	0.6		0,28
No.3	0.7	15	0,32
No.3	0.8		0,33
No.3	0.9	10	0,38
No.3	1.0		0,40
No.3	1.1	5	0,43
No.3	1.2		0,46
No.3	1.3	0	0,48



Based on nonlinear regression. The concentration of DFPG is 0,1 mM. Measurements were carried out at 20°C immediately after adding the tested extracts. The concentration of the studied extracts is 50 mkl in a pre-supplied solution. Samples X1 (1+1), X2 (1+2), X3 (2+1) were diluted 100 times with appropriate solvents (water).

Change in the optical density of an aqueous solution of DFPG with the addition of extracts of the tested plants over time compared to the control.

According to experimental data, extracts No. 1 have the greatest ability to remove free radicals from the body. To quantify the antiradical activity, the stable radical 2,2-diphenyl-1-picrylhydrazyl (DFPG) was used, as well as t_{50} indicators - the time required for the studied drugs to reduce the initial concentration of the radical with 50% inhibitory activity.

In the reaction of DFPG with extracts at 20 °C, t_{50} for sample No. 1 is 9.9 ± 0.69, for sample No. 1 – 47.67 ± 3.08 (diluted 100 times). For sample No. 2 7.1 ± 0.57, for sample No. 2 42.65 ± 2.15 (diluted 100 times). For sample No. 3 6.9 ± 0.34, for sample No. 3 39.85 ± 1.38 (diluted 100 times).

(Table 1).

50% (IC_{50}) inhibitory concentration value and the time required to reduce the concentration of DFPG by 50% (t_{50}) when reacting with the tested extracts.

Table 1.

No. Plant extracts	IC_{50}, mkl	t_{50}, sec 50 mkl of substance
No.1	9.8±0.69	12.1±0.45
No.1 (diluted)	47.67±3.08	55.49±2.97
No.2	7.1±0.57	10.8±0.67
No.2 (diluted)	42.65±2.15	51.17±2.01

No.3	6.9±0.34	47.4±0.75
No.3 (diluted)	39.85±1.38	47.13±1.86

CONCLUSION

Analysis of the experimental results of the extract showed that free radical No.1 had the highest ARF compared to DFPG. Thus, the antiradical activity of plant extracts was studied. The aqueous extract X1 (1+1), X2 (1+2), X3 (2+1) found in water showed the greatest antiradical activity. There is sufficient information in the literature about the antiradical activity of medicinal plant extracts, and their maximum effect was found in extracts containing the largest amount of polyphenols and flavonoids. Thus, further work and creation of the ARF mechanism requires a detailed study of the qualitative and quantitative composition of the extracts regarding the composition of the components (polyphenols, flavonoids, tannins, alkaloids, etc.). Currently, we can safely talk about the feasibility of widespread use in the creation of food additives from pine pollen and extracts of oriental biota in folk medicine, as well as the production of medicines in the form of drugs approved by modern medicine against shortness of breath, asthma, prostatitis, prostate adenoma diseases and reduced immunity (since pine pollen is a natural antibiotic).

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