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ANTIRADICAL ACTIVITY OF BIOACTIVE SUBSTANCES

***Zafarjon M. Ernazarov**, PhD*
Associate Professor, Department of Biology
Kokand State University, Uzbekistan
E-mail: zafarbek1985@gmail.com. ORCID: 0009-0009-5844-3852
***Shoirakhon R. Toshmatova**, PhD*
Associate Professor, Department of Biology
Kokand State University, Uzbekistan
e-mail: shoiraxon@gmail.com ORCID: 0009-0009-1535-0701
***Khilola E. Ernazarova**, PhD*
Lecturer, Department of English Language and Literature
Kokand State University, Uzbekistan
e-mail: hilolachik@gmail.com ORCID: 0009-0005-4634-9122

Abstract: This study investigates the antioxidant and antiradical activities of bioactive compounds—dihydroquercetin (DHQ), isoquinoline alkaloids, and their synthetic conjugate DKV-11. Oxidative stress, caused by free radicals, contributes to various diseases, including cardiovascular disorders, cancer, and neurodegenerative conditions. Natural antioxidants, such as flavonoids and alkaloids, play a crucial role in neutralizing free radicals and protecting cells from oxidative damage. The research evaluates the radical scavenging activity of DHQ, isoquinoline alkaloids, and DKV-11 using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. Results demonstrate that DKV-11 exhibits the highest antiradical activity (IC50 = 3.5 μM), significantly surpassing DHQ (IC50 = 45.3 μM). The findings highlight the potential of DKV-11 as a potent antioxidant agent for pharmacological applications, supporting further research into its therapeutic benefits.

Keywords: dihydroquercetin (DHQ), isoquinoline alkaloids, DKV-11 conjugate, antiradical activity, antioxidant properties, DPPH assay, oxidative stress, free radical scavenging, pharmacological potential.

Introduction

Currently, oxidative stress occurring in cells is recognized as one of the key factors negatively affecting human health. Under oxidative stress conditions, free radicals disrupt normal cellular functions, potentially leading to the development of various diseases, including cardiovascular disorders, cancer, neurodegenerative diseases, and inflammatory conditions. Therefore, the study and evaluation of the efficacy of antioxidant agents remain one of the most pressing scientific issues today.

The "Strategy of Actions for Further Development of the Republic of Uzbekistan" outlines key objectives, including "stimulating scientific research and innovation, as well as establishing effective mechanisms for implementing scientific and innovative achievements into practice" [2]. In particular, state programs aimed at improving the healthcare system and developing the national pharmaceutical industry emphasize the importance of studying natural bioactive compounds and expanding their therapeutic potential. Pharmacological research on natural antioxidants can significantly enhance human health and contribute to advancements in medicine.

Literature Review.

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In recent years, flavonoids have been actively studied as therapeutic agents against various diseases due to their free radical-binding properties [4]. Flavonoids and many other polyphenolic compounds possess free radical-scavenging capabilities (chain-breaking antioxidants), exhibiting high reactivity as hydrogen or electron donors [3]. Many compounds with antioxidant activity can reduce free radical formation and neutralize them. Dihydroquercetin and isoquinoline alkaloids are natural compounds, each demonstrating unique biological activity. Dihydroquercetin, a flavonoid renowned for its potent antioxidant properties, protects cells from oxidative stress by neutralizing free radicals. Isoquinoline alkaloids exhibit broad pharmacological effects, including anti-inflammatory, antiviral, antibacterial, and other biological activities [1].

The conjugate of these two compounds - DKV-11 - has been synthesized. This novel compound combines dihydroquercetin with 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline alkaloid, and its pharmacological properties are particularly noteworthy. Studies have shown that DKV-11 exerts positive inotropic effects on cardiac muscles and vasorelaxant properties. Research indicates that DKV-11 significantly increases the contractile force of papillary muscles and relaxes contractions induced by high KCl and phenylephrine in aortic rings. These effects are primarily mediated through β-adrenoreceptors and L-type calcium channels [5].

Furthermore, numerous studies have documented the broad-spectrum biological activities of isoquinoline alkaloids, including antitumor, antidiabetic, antibacterial, antifungal, antiparasitic, and neuroprotective properties, further enhancing their pharmacological significance [1].

The above findings highlight the importance of investigating the antiradical activity of the DKV-11 conjugate. Since its components - dihydroquercetin and isoquinoline alkaloids - individually exhibit strong antioxidant and other biological activities, a deeper study of DKV-11's antiradical properties and its pharmacological potential represents a crucial research priority.

Research Objective

The aim of this study is to investigate the antiradical activity of dihydroquercetin (DHQ), isoquinoline alkaloids, and their synthetic conjugate DKV-11.

Materials and methods.

The antiradical activity of bioactive substances was determined by a standard method, based on the kinetics of measuring the optical density of an alcoholic solution of 1,1–diphenyl–2–picrylhydrazyl (DPPH). The free radical concentration was 0.1 mM. The DPPH/polyphenol ratio was 1:10. The optical density of the alcoholic solution of DPPH was measured using a UV–755 spectrophotometer with an optical path length of 1 cm and a volume of 3 ml [6].

Results and their analysis.

The antiradical activity of DHQ and DKV-11 conjugates was determined by a standard method, based on the kinetics of measuring the optical density of an alcoholic solution of 1,1–diphenyl–2–picrylhydrazyl (DPPH). The free radical concentration was 0.1 mM. DHQ at concentrations of 20, 40 and 80 μM and DKV-11 conjugate at concentrations of 2, 4 and 7 μM were added to the DPPH solution in a cuvette. The change in the optical density of the DPPH alcoholic solution was measured using a UV–755 spectrophotometer, with an optical path length of 1 cm and a volume of 3 ml [6].

Despite the study of the free radical-binding properties of many flavonoids, the antiradical activities of biologically active compounds isolated from plants have not yet been determined.

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Based on this, the antiradical activity of the bioactive substances presented in this experiment was studied in relation to the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl).
Antioxidants can have different mechanisms of action, and it is recommended to study their activity using different methods. In this work, the antiradical activity of the studied substances was evaluated in relation to the free radical DPPH. When the studied substances were added to the alcoholic solution of DPPH, the free radical molecules were converted to a non-radical form, and the intensely purple solution of DPPH became colorless. The kinetics of the change in the optical density of the DPPH solution upon addition of the studied samples is shown.
Analyzing the results obtained, it can be concluded that when the tested DHQ and DKV-11 conjugates were added to the DPPH solution, a decrease in the optical density of the DPPH solution was observed, which indicates their antiradical activity. Among the bioactive substances, the highest antiradical activity was observed in the DKV-11 conjugate (Table 1).

Table 1

IC_{50} (50% Inhibitory Concentration) Values and the Time (t_{50}) Required to Reduce DPPH Concentration by 50% Upon Reaction with Bioactive Substances

Samples	IC_{50}, mkM	t_{50} sec
DHQ	45,3	75±7
DKV 11	3,5	250±11

To quantitatively evaluate the antiradical activity of bioactive substances, two indicators were used: t_{50}, which represents the time required to reduce the initial concentration of stable radicals by 50% upon reaction with the tested compound, and IC_{50}, the concentration of the substance needed to decrease the level of free radicals by 50%.
Accordingly, among the studied samples, the DHQ and DKV-11 conjugate demonstrated the highest antiradical activity. Their IC_{50} values were 45.3 ± 4.5 μM and 3.5 ± 0.09 μM, respectively. In the samples F-7, F-18, and F-19, no ability to reduce DPPH free radicals was observed.

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