Title: The in vitro antioxidant activities of *Gladiolus illyricus* Wilhelm Daniel Joseph Koch (W.D.J.Koch) plant

Research question: Is there DPPH radical scavenging effect at varying concentrations in the methanol extract (800 µg/mL, 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL) of The flowering aerial parts of the *Gladiolus illyricus* Wilhelm Daniel Joseph Koch (W.D.J.Koch) plant?

Biology Extended Essay

Word count: 4011

Index:

Introduction

Our body generates hydroxyl radicals (free radicals) under several conditions. These radicals are very unstable and, therefore, very reactive. After attack of radicals on membranes and lipoproteins, lipid peroxidation starts and may lead to the development of vascular lesions. Free radicals are known to be mostly associated with oxidative stress[1] [2]. Oxidative stress occurs when there is an excess of reactive oxygen species (ROS) produced by cellular mitochondrion. Under normal conditions, when the metabolism of a living organism is healthy, antioxidants and free radicals are in balance. However, when this balance shifts in favour of free radicals, susceptibility to oxidative stress-related diseases may be observed. As free radicals increase, endogenous antioxidants may become insufficient, necessitating the intake of exogenous antioxidants. For this purpose, natural resources are often used. Certain groups of molecules found in plants demonstrate antioxidant activity by affecting various metabolic pathways[3]. Flavonoids with known antioxidant activity prevent Low Density Lipoprotein oxidation in vitro and restrict cellular damage. In 1997, Alberico L. Catapano demonstrated that, although the biochemical mechanisms were not clearly outlined, flavonoid consumption in humans reduces the risk of cardiovascular diseases[4] . As shown by Alberico L. Catapano, 1997, *Gladiolus illyricus* W.D.J.Koch is rich in secondary metabolites, particularly flavonoids.

There are three major classes of plant chemicals: terpenoids, phenolic metabolites, and alkaloids. Phenolic compounds include phenolic acids , polyphenols, and flavonoids. There are many techniques to recover antioxidants from plants. Extraction yield and antioxidant activity not only depend on the extraction method but also on the solvent used for extraction. The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a solvent. Polar solvents are frequently used for recovering polyphenols from plant matrices. Methanol has been used as the most efficient solvent for the extraction of low molecular weight polyphenols. For this reason, methanol was used during the preparation of the extract[5].

Quercetin:

Quercetin is a natural flavonoid, abundantly found in vegetables and fruits, with potential therapeutic effects for the prevention and treatment of various diseases. This comparison was made based on the information that quercetin is one of the most prominent antioxidants in the market.

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[1] Gulcin, I.; Mshvildadze, V.; Gepdiremen, A.; Elias, R. Screening of antioxidant and antiradical activity of monodesmosides and crude extract from *Leontice smirnowii* Tuber. *Phytomedicine* **2006**, *13*, 343–351

[2] Altay, A.; Tohma, H.; Durmaz, L.; Taslimi, P.; Korkmaz, M.; Gulcin, I.; Koksal, E. Preliminary phytochemical analysis and evaluation of in vitro antioxidant, antiproliferative, antidiabetic and anticholinergics effects of endemic Gypsophila taxa from Turkey. *J. Food Biochem.* **2019**, *43*, e12908.

[3] Arıtuluk ZC, Çankaya İİT, Özkan AMG. Antioxidant activity, total phenolic and flavonoid contents of some Tanacetum L. (Asteraceae) taxa growing in Turkey. FABAD Journal of Pharmaceutical Sciences. 2016; 41:17-25.

[4] Alberico L. Catapano, PhD, Antioxidant Effect of Flavonoids, Angiology, Volume 48, Issue 1 (January 1997)

[5] Do, Quy Diem, et al. "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica." *Journal of food and drug analysis* 22.3 (2014): 296-302.

In order to determine if *Gladiolus illyricus* W.D.J.Koch had oxidant-scavenging capabilities quercetin was used as a reference compound[6].

Free Radicals and Oxidative Stress

Oxidation processes are essential for the survival of cells. Organisms that undergo aerobic cellular respiration metabolize glucose for energy requirements, while this metabolism also leads to the production of free radicals that can cause cellular damage[7]. Free radicals are known to be mostly associated with oxidative stress[8] [9]. Cells metabolize oxygen, creating potentially harmful reactive ROS. Oxidative stress occurs when there is an excess of ROS produced by cellular mitochondrion. "Free radicals, which are known to cause many degenerative diseases such as carcinogenesis, acute inflammation, high blood pressure, diabetes, preeclampsia, acute renal failure, atherosclerosis, Alzheimer's disease and Parkinson's disorders, mutagenesis, aging, and cardiovascular disorders, are produced in biological systems"[10] [11]. Under normal conditions, the rate and amplitude of oxidant formation are balanced by the rate at which they are removed[12]. However, disruption of the balance between antioxidants and pro-oxidants causes oxidative stress[13].

DPPH radical scavenging:

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) removing assay is the most popular and commonly used method among many techniques for measuring antioxidant activity. This method involves using a stable free radical, DPPH, to similarly determine antioxidant activity. "When a DPPH solution is mixed with a solution of a substance capable of donating a hydrogen atom, this violet colour disappears, resulting in the reduced form of the DPPH radical (DPPH-H)"[14]. The formation of hydrazine (DPPH-H) induces the disappearance of the visible band as the colour of the solution changes from violet to pale yellow because of radical reduction by hydrogen atom transfer from antioxidants, which are H donors. The colour intensity of this reaction, known as the "DPPH test" in the literature, can be easily recorded by

[6] Nutraceuticals (Second Edition), Efficacy, Safety and Toxicity, 2021, Pages 749-755
[7] Gulcin, I. Antioxidants and antioxidant methods-An updated overview. Arch. Toxicol. 2020, 94, 651–715.
[8] Gulcin, I.; Mshvildadze, V.; Gepdiremen, A.; Elias, R. Screening of antioxidant and antiradical activity of monodesmosides and crude extract from *Leontice smirnowii* Tuber. *Phytomedicine* **2006**, *13*, 343–351
[9] Altay, A.; Tohma, H.; Durmaz, L.; Taslimi, P.; Korkmaz, M.; Gulcin, I.; Koksal, E. Preliminary phytochemical analysis and evaluation of in vitro antioxidant, antiproliferative, antidiabetic and anticholinergics effects of endemic Gypsophila taxa from Turkey. *J. Food Biochem.* **2019**, *43*, e12908.
[10] Kedare, S.B.; Sing, R.P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* **2011**, *48*, 412–422
[11] Cetinkaya, Y.; Gocer, H.; Menzek, A.; Gulcin, I. Synthesis and antioxidant properties of (3,4-dihydroxyphenyl) (2,3,4-trihydroxyphenyl)methanone and its derivatives. *Arch. Pharm.* **2012**, *345*, 323–334.
[12] Tohma, H.; Altay, A.; Koksal, E.; Gören, A.C.; Gulcin, I. Measurement of anticancer, antidiabetic and anticholinergic properties of sumac (*Rhus coriaria*)-Analysis of its phenolic compounds by LC-MS/MS. *J. Food Meas. Charac.* **2019**, *13*, 1607–1619.
[13] Rodrigo, R. *Oxidative Stress and Antioxidants: Their Role in Human Diseases*; Nova: New York, NY, USA, 2009; pp. 9–10.
[14] Yapıcı, I.; Altay, A.; Ozturk Sarikaya, S.B.; Korkmaz, M.; Atila, A.; Gulcin, I.; Koksal, E. In vitro antioxidant and cytotoxic activities of extracts of endemic *Tanacetum erzincanense* together with phenolic content by LC-ESI-QTOF-MS. *Chem. Biodivers.* **2021**, *18*, e2000812.

UV-vis spectroscopy. “This method is widely used to evaluate the antioxidant capacity of pure antioxidant molecules, especially herbal extracts or phenolic compounds”[15].

Gladiolus illyricus:

Gladiolus illyricus W.D.J.Koch (Figure 1) is a member of the Iridaceae (Iris) family. It grows naturally in countries bordering the Mediterranean. In scientific literature, there are studies investigating the antioxidant, antifungal, and anticancer effects of the Gladiolus genus. The phytochemical content of the plant includes flavonoids, alkaloids, and terpenes. The group responsible for antioxidant activity is thought to be the flavonoid group (Iridaceae (Iris family)).

Figure 1: *Gladiolus illyricus* W.D.J.Koch

Link for figure 1:

https://identify.plantnet.org/tr/k-world-flora/species/Gladiolus%20illyricus%20W.D.J.Koch/data

Phytochemical Content:
Phytochemical studies on the *Gladiolus* genus have revealed the presence of flavonoids, terpenoids, and alkaloids. Flavonoids are known for their antioxidants, diuretic, anti-inflammatory, antiviral, and antimicrobial properties.

Methodology

Aim of study:

During my biotechnology internship, I read a scientific journal where I observed research on the therapeutic properties of plants. Inspired by this, I decided to conduct research on the *Gladiolus illyricus* W.D.J.Koch plant, which is native to my country. I managed to procure the plant extract from a nearby university and conducted the experiment in the laboratory of the same university. The main reason for using the aerial parts of the plant is their richness in flavonoids. Free radical scavengers are the compounds that remove the undesired free radicals generated because of impaired or disrupted mitochondrial respiratory reaction. In this experiment, I aimed to determine the presence of the radical scavenging effect of the plant *Gladiolus illyricus* W.D.J.Koch. The research aimed to identify whether the extract derived from this plant exhibits a more effective radical scavenging property compared to

[15] Xie, J.; Schaich, K.M. Re-evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. *J. Agric. Food Chem.* **2014**, *62*, 4251–4260.

quercetin since it possessed well known antioxidative properties. I wanted to examine the antioxidative properties of the *Gladiolus illyricus* W.D.J.Koch plant.

Hypothesis:

H_0: As the concentration of *Gladiolus illyricus* Wilhelm Daniel Joseph Koch plant extract increases, its radical scavenging activity does not increase.

H_1: As the concentration of *Gladiolus illyricus* Wilhelm Daniel Joseph Koch plant extract increases, its radical scavenging activity also increases.

An imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage, is termed 'oxidative stress'. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. Antioxidant defence involves several strategies, both enzymatic and non-enzymatic. Damage caused by oxidative changes in lipids, proteins, and DNA leads to tissue damage. [16]. Biological systems produce free radicals during their metabolism, which can lead to degenerative diseases [17] [18]. Radical scavenging agents terminate the radical chain reaction of peroxide radicals in the environment and enhance food quality and shelf life [19]. In order to demonstrate the radical scavenging capabilities of *Gladiolus illyricus* W.D.J.Koch I have opted to use the DPPH method. This method (DPPH) is a fundamental, standard, most popular, and widely used spectrophotometric test for determining antioxidant activity [20] . This method also the most popular and putative assay used for the determination of antioxidant activity. These tests commonly used because they are sensitive, simple, fast, and reproducible.

Free radicals produced by biological systems can cause acute inflammation, high blood pressure, pregnancy-induced hypertension, acute renal failure, atherosclerosis, Alzheimer's disease, and Parkinson's disease. Recent extensive scientific research has classified reactive species and free radicals into three main categories: reactive nitrogen species, reactive oxygen species (ROS), and reactive sulphur species. An antioxidant is defined as a substance that can significantly delay or completely prevent the oxidation of substrate molecules, even at low concentrations. "Many studies have suggested that flavonoids exhibit many biological activities. However, most interest has been devoted to their antioxidant activity, which is due to their ability to reduce free radical formation and to scavenge free radicals"[21].

In recent years, flavonoids as potent free radical scavengers have attracted tremendous interest as possible therapeutics against free radical mediated diseases. "Various structure activity relationship studies of flavonoids have pointed to the importance of the number and location of the phenolic OH

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[3] Artunc, T.; Menzek, A.; Taslimi, P.; Gulcin, I.; Kazaz, C.; Sahin, E. Synthesis and antioxidant activities of phenol derivatives from 1,6-bis(dimethoxyphenyl)hexane-1,6-dione. *Bioorg. Chem.* **2020**, *100*, 103884.

[17] Kedare, S.B.; Sing, R.P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* **2011**, *48*, 412–422

[18] Cetinkaya, Y.; Gocer, H.; Menzek, A.; Gulcin, I. Synthesis and antioxidant properties of (3,4-dihydroxyphenyl) (2,3,4-trihydroxyphenyl)methanone and its derivatives. *Arch. Pharm.* **2012**, *345*, 323–334.

[19] Gulcin, I. Antioxidant activity of food constituents: An overview. *Arch. Toxicol.* **2012**, *86*, 345–391.

[20] Gulcin, I. Antioxidants and antioxidant methods-An updated overview. *Arch. Toxicol.* **2020**, *94*, 651–715.

[21] Miller, A.L., 1996. Antioxidant flavonoids: structure, function and clinical usage. Alt. Med. Rev. 1, 103–111.

groups present, for effective radical scavenging activity"[22]. The mechanism and structural requirements necessary for the clearly defined radical scavenging effects of flavonoids have been established in previous studies. Therefore, based on the antioxidant and radical scavenging effects of flavonoids described in detail the radical scavenging activity of the flavonoid-containing plant *Gladiolus illyricus* W.D.J.Koch was investigated using the DPPH method. The radical scavenging activity of this plant was compared with quercetin, which is known to have a high radical scavenging effect.

The hypothesis is that as the concentration of Gladiolus illyricus W.D.J.Koch plant extract increases, the flavonoid content will also increase, and consequently, the radical scavenging activity will improve.

Method Development and Planning

Variables

Independent Variable: The diluted concentrations of the plant extract. There is a specific ratio between these variables. Each dilution is half of the previous concentration. (800 µg/mL - 400 µg/mL-200 µg/mL-100 µg/mL-50 µg/mL-25 µg/mL)

Dependent Variable: The absorbance values at different concentrations (measurable results are % inhibition values)

[22] van Acker, S.A., de Groot, M.J., van den Berg, D.J., Tromp, M.N., Donne´-Op den Kelder, G., van der Vijgh, W.J., Bast, A., 1996. A quantum chemical explanation of the antioxidant activity of flavonoids. Chem. Res. Toxicol. 9, 1305–1312.

Controlled variables:

Table 1: Explanation of controlled variables

Name of variables	**Possible effects on the variable**	**Method for control**
Species of plant	Plants in the Gladiolus genus are very similar to each other, so the correct species should be used. If a different plant that looks similar is used, the desired result cannot be achieved	A stereomicroscope is a type of microscope that provides three-dimensional images and allows for the identification of plant structures without entering the cellular structure The morphological characteristics of the plant are examined using a stereomicroscope
Incubation duration	Leaving the well plate for a long time may negatively affect the absorbance of the extract and DPPH solution	The well plate was left for 30 minutes
Evaporation	Damage to the extract may occur due to temperature.	A rotary evaporator set to low pressure was used. (20 mbar ±1-2 and 40 ˚C±0.05)
Pressure	Performing it under low pressure enables evaporation at low temperatures, therefore the plant may be negatively affected by the temperature.	A rotary evaporator set to low pressure was used. (20 mbar ±1-2),
Light	The absorbance to be measured may change because of the light on the solution content.	The solution in the well plate was covered with aluminium foil during the incubation period.
Volume and Concentration of DPPH	Not keeping the DPPH concentration constant may negatively affect radical scavenging activity.	50 µL of 1 mM DPPH were added to each well by using micropipette.

Table 2: Risk Assessment

Hazard	**Precaution**
Ethical	The work has been carried out in a laboratory environment without causing any harm to living organisms or the environment. The plant used for extraction has lost its vitality (dried up), and as little plant material as possible was judiciously used.
Environmental	The chemicals and materials used have been disposed of according to the Ministry of Health of the Republic of Turkey's medical waste management regulations.
Safety	There were chemicals used in the experiment. Therefore, during the experiment and cleaning process of the pots a laboratory coat, safety goggles, and latex gloves are required.

Table 3: Laboratory materials and other substances used

Measuring device	Materials	Chemicals
(1) Measuring cylinder (250 mL ±0.5) (2) Flask (250 mL±0.2) (3) Rotavapor (4) Glass funnel (100 mL±1) (5) Spectrophotometer (Thermo Scientific Orion AquaMate 8000, USA) (±0.05) (6) Weighing device (50 gr ±0.0001) (7) Micropipette (150 µL±0.15) (8) Falcon tube (15 mL±0.5) (9) Falcon tube (50 mL±0.5) (10) Glass beaker (11) Glass Erlenmeyer flask (12) Ultrasonic bath (13) Eppendorf tube 3 pieces	(1) Above-ground parts of *Gladiolus illyricus* W.D.J.Koch (2) Filter paper (25x5mm) 35 pieces (3) Metal spatula (4) 96-well plate (5) Micropipette tip 51 pieces	(1) Methanol %100 (500 µL) (2) DPPH (3500 µL) (3) Ethanol 5ml (4) Quercetin 200 µg/mL

Identification of the Plant:
The morphological characteristics of the plant are examined using a stereomicroscope. The plant's family, genus, and species are identified sequentially by comparing them with the descriptions in the *Flora of Turkey* book.[23]

Extraction:
Extraction is the process of soaking the utilized part of the plant in a suitable solvent, boiling it with the solvent, or pouring boiled and cooled solvent over the utilized part of the plant to transfer the chemical content into the solvent for the purpose of conducting phytochemical studies. Based on previous studies, methanol was used as the solvent. Powdered material (800 mg), obtained from the flowering aerial parts of the plant and was ground in a grinder. 50 mL of 100% methanol was added to this powder and left to stand for 12 hours. Afterward, the sample was filtered into an Erlenmeyer flask using filter paper and a funnel. The sample was then treated again by adding another 50 mL of methanol, left to stand for 12 hours, and filtered again into the Erlenmeyer flask to obtain the extract. Afterwards the filtrate is transferred to a flask and evaporated to dryness under low pressure (20 mbar) using a rotary evaporator. A rotary evaporator is used for the evaporation of liquids at low temperatures (40 ˚C) and for the removal of solvents. Additionally, the device is designed to operate under low pressure. The extract obtained was weighed, and it was found to be 80 mg.

[23] Davis, Peter. *Flora of Turkey*. Edinburgh University Press, 1986

A stock solution was prepared using 80 mg of extract and 100 ml of ethanol. This stock solution was then appropriately diluted to create an extract with a concentration of 800 µg/mL, 400 µg/mL,200 µg/mL,100 µg/mL,50 µg/mL,25 µg/mL. A 1 mM DPPH solution was prepared using 2 mg of DPPH and 5 ml of ethanol. During the examination period, the flask was wrapped in aluminium foil to protect it from light. Using a micropipette, 150 µL of each concentration of extract was transferred into three adjacent wells on a plate. Additionally, 50 µL of the 1 mM DPPH solution was added to each well using a micropipette. The plate was incubated at room temperature (20°C) in the dark for 30 minutes, after which the absorbance was measured at 517 nm using a spectrophotometer. Ethanol was used as the control group, and quercetin was used as a standard to compare antioxidant activity.

Analysis

Table 4: Absorbance values of extract

Absorbance values of extract			
Concentration of extract(µg/mL) (±0.05)	Absorbance 1	Absorbance 2	Absorbance 3
800	0.497	0.453	0.455
400	0.814	0.799	0.853
200	1.006	1.036	1.026
100	1.192	1.178	1.191
50	1.262	1.237	1.285
25	1.294	1.302	1.302

After the plate was incubated at room temperature in a dark environment for 30 minutes, the values read at 517 nm on the spectrophotometer were recorded as absorbance. Absorbance is a value that measures how much light is absorbed by a substance and is generally calculated using the following formula:

Formula 1:

$$A = -\log_{10}\frac{I}{I_0} = -\log T$$

A is absorbance,

I: the amount of light passing through the sample,

I_0: the initial amount of light entering the sample,

As absorbance increases, the substance absorbs more light. Absorbance provides a measurement as a ratio of light intensity. A spectrophotometer measures the absorbance value and automatically calculates it, providing a numerical value.

The radical scavenging capacity was calculated as a percentage of inhibition using the following formula:

Formula 2:

Formula for determining %inhibition:

$$\%\text{Inhibition} = [(\text{AControl} - \text{ASample}) / \text{AControl}] \text{ x } 100$$

In the formula, *AControl* represents the absorbance of the control, and *ASample* represents the absorbance of the tested sample.

Table 5- %Inhibition values of the extract:

Inhibition values of the extract			
Concentration of extract(µg/mL) (±0.05)	% Inhibition 1	% Inhibition 2	% Inhibition 3
800	62.051	65.411	62.258
400	37.846	38.992	34.868
200	23.186	20.895	21.659
100	8.984	10.053	9.137
50	3.639	0.548	1.883
25	1.196	0.585	0.585

Table 6: The mean and standard deviation of the % inhibition of the extract at different concentrations

Mean and Standard Deviation of extract at different concentrations			
Concentration of extract (µg/mL) (±0.05)	N	Mean	Std. Deviation
800	3	63240.00	1882.988
400	3	37235.33	2128.739
200	3	21913.33	1166.484
100	3	9391.33	578.104
50	3	2023.33	1550.271
25	3	788.67	352.761
Total	18	22432.00	22808.307

Table 7: One-Way ANOVA

ANOVA					
	Sum of Squares	df	Mean Square	F	p value
Between Groups	8819121290.667	5	1763824258.133	860.413	<,001
Within Groups	24599683.333	12	2049973.611		
Total	8843720974.000	17			

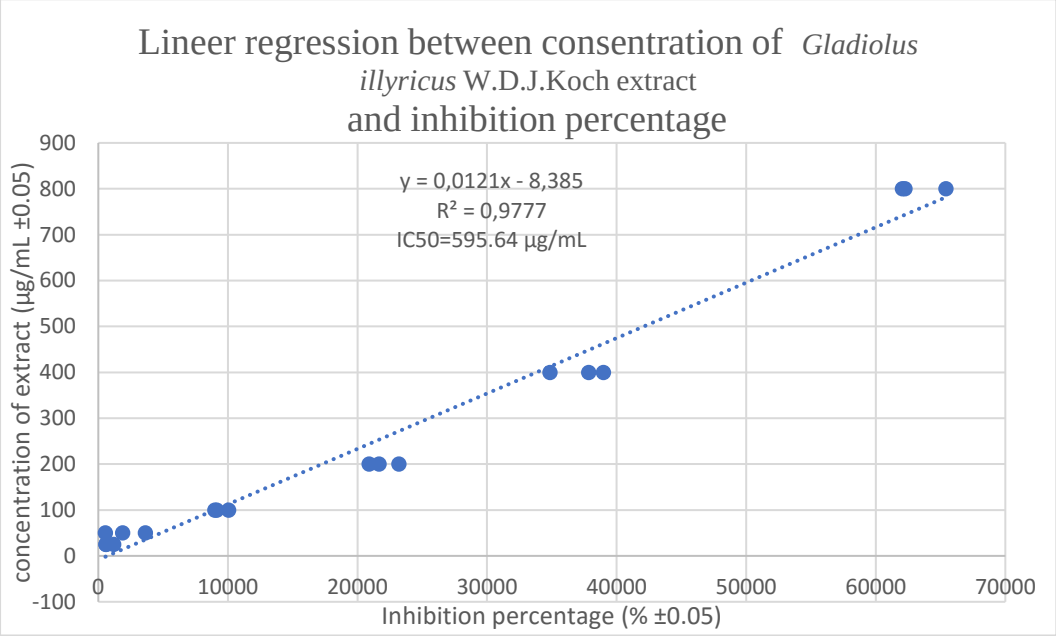
In the table above, a One-Way ANOVA test was conducted, and a statistically significant difference was found between the groups ($p < 0.001$).

Table 8: Multiple Comparisons of different concentrations of extract (Tukey test)

Multiple Comparisons					
Dependent Variable; Tukey HSD					
Concentration of extract (µg/mL) (±0.05)				95% Confidence Interval	
		Mean Difference	Sig.	Lower Bound	Upper Bound
25	50	-1234.667%	0,889	-5161.37%	2692.04%
	100	-8602.667%*	<,001	-12529.37%	-4675.96%
	200	-21124.667%*	<,001	-25051.37%	-17197.96%
	400	-36446.667%*	<,001	-40373.37%	-32519.96%
	800	-62451.333%*	<,001	-66378.04%	-58524.63%
50	25	1234.667%	0,889	-2692.04%	5161.37%
	100	-7368.000%*	<,001	-11294.71%	-3441.29%
	200	-19890.000%*	<,001	-23816.71%	-15963.29%
	400	-35212.000%*	<,001	-39138.71%	-31285.29%
	800	-61216.667%*	<,001	-65143.37%	-57289.96%
100	25	8602.667%*	<,001	4675.96%	12529.37%
	50	7368.000%*	<,001	3441,29%	11294.71%
	200	-12522.000%*	<,001	-16448.71%	-8595.29%
	400	-27844.000%*	<,001	-31770.71%	-23917.29%
	800	-53848.667%*	<,001	-57775.37%	-49921.96%
200	25	21124.667%*	<,001	17197.96%	25051.37%
	50	19890.000%*	<,001	15963.29%	23816.71%
	100	12522.000%*	<,001	8595.29%	16448.71%
	400	-15322.000%*	<,001	-19248.71%	-11395.29%
	800	-41326.667%*	<,001	-45253.37%	-37399.96%
400	25	36446.667%*	<,001	32519.96%	40373.37%
	50	35212.000%*	<,001	31285.29%	39138.71%
	100	27844.000%*	<,001	23917.29%	31770.71%
	200	15322.000%*	<,001	11395.29%	19248.71%
	800	-26004.667%*	<,001	-29931.37%	-22077.96%
800.	25	62451.333%*	<,001	58524.63%	66378.04%
	50	61216.667%*	<,001	57289.96%	65143.37%
	100	53848.667%*	<,001	49921.96%	57775.37%
	200	41326.667%*	<,001	37399.96%	45253.37%
	400	26004.667%*	<,001	22077.96%	29931.37%

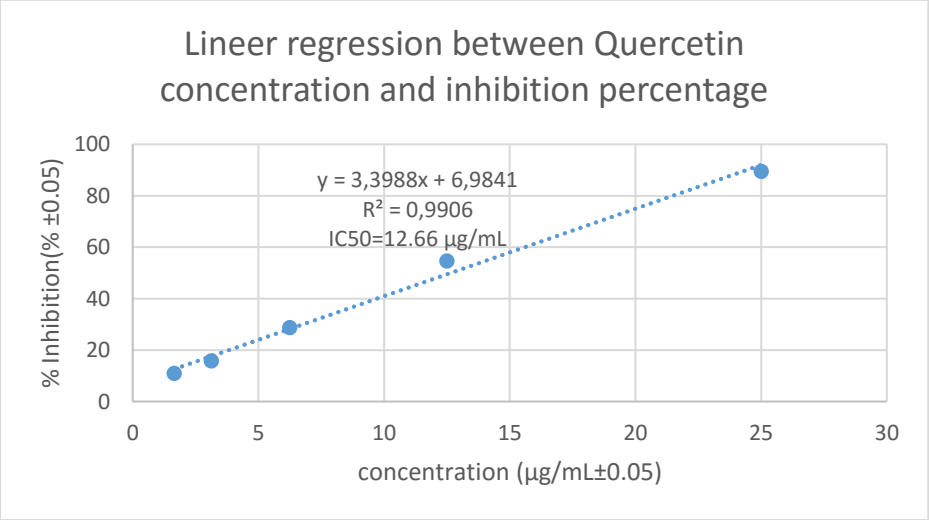
*. The mean difference is significant at the 0.05 level.

Graph 1: A graph showing the DPPH radical scavenging capacity of the plant extract at increasing concentrations:



According to the above linear regression graph, there is a positive relationship between concentration and % inhibition.

Graph 2: The DPPH radical scavenging capacity of quercetin at increasing concentrations (200–1.65 µg/mL)



Initially, a graph showing the DPPH radical scavenging capacity of quercetin at increasing concentrations (200–1.65 µg/mL) was created. Using the equation derived from this graph, the concentration of quercetin required to achieve 50% inhibition of the DPPH radical (IC50) was determined to be 12.66 µg/mL. The graph for quercetin is shown above.

Conclusion:

The aim of the study is to demonstrate the antioxidant activity of the extract obtained from *Gladiolus illyricus* W.D.J.Koch and to pave the way for future research that could evaluate the extract as a dietary

supplement or pharmaceutical. In this study, the antioxidant activity of the extract obtained from *Gladiolus illyricus* W.D.J.Koch plant was investigated by examining its radical scavenging effects at varying concentrations using the DPPH method. The absorbance values obtained using the spectrophotometer were used to calculate the inhibition values using the formula 1. The mean and standard deviation for different concentrations were calculated using Microsoft Excel (Table 6). A One-Way ANOVA test was conducted, and a statistically significant difference was found between the groups (p<,001). If the one-way ANOVA result provides a statistically significant outcome, the null hypothesis (H_0) is rejected, and the alternative hypothesis (H_0) is accepted. Subsequently, a Tukey test (Table 8) was performed, and no significant difference was observed between the % inhibition values measured at the 25 µg/mL and 50 µg/mL concentrations. However, for all other concentrations (800 µg/mL, 400 µg/mL,200 µg/mL,100 µg/mL), it was observed that as the concentration increased, the percentage inhibition values also increased, which was statistically significant. According to the linear regression graph (Graph 1), there is a positive relationship between concentration and % inhibition. As the concentration increases, the % inhibition value of extract also increases, indicating that the radical scavenging effect of the extract increases. Additionally, the R^2 (0.9777) value indicates that the explanatory power of this model is quite high.

The antioxidant activity of *Gladiolus illyricus* W.D.J.Koch plant extract was compared with quercetin via IC50 values and linear regression graphs, whose antioxidant activity has been demonstrated in previous studies. IC50 refers to the concentration of a compound required to inhibit a specific biological process or enzyme activity by half. The concentrations of the extract causing 50% inhibition (IC50 values) were calculated from the %inhibition graphs. When comparing the IC50 values of the extract (595.64 µg/mL) from the aerial parts of *Gladiolus illyricus* W.D.J.Koch with the reference compound quercetin (12.66 µg/mL)(Graph 2), it was observed that the RSA of the extract was significantly weaker (depending on the concentration value). when IC50 values are compared, it was concluded that higher concentrations of *Gladiolus illyricus* W.D.J.Koch extract would be required to reach the effect of quercetin. Based on this, it can be concluded that the *Gladiolus illyricus* W.D.J.Koch plant extract exhibits weaker antioxidant activity than quercetin. In the study by Başgedik[24], when the results of the DPPH radical scavenging capacity of the ethanol extract of the aerial parts of the plant were compared with those obtained in our study for the methanol extract of the aerial parts, the activity observed in our study was found to be significantly higher. This difference can be attributed to factors such as the polarity and quality of the solvent and the experimental conditions. In a study conducted using a similar method with another member of the *Gladiolus* species, *Gladiolus Segetum* methanolic extract showed the highest phenolic and flavonoid concentrations and strong antioxidant activity.[25]

As a result the methanol extract of *Gladiolus illyricus* W.D.J.Koch plant has radical scavenging activity, but it is weaker compared to quercetin. To achieve antioxidant activity comparable to that of quercetin, the concentration of *Gladiolus illyricus* W.D.J.Koch plant extract should be increased.

The approach used was the conclusion reached upon the thorough examination of similar assays conducted upon the family of Iridaceae. The final result was that DPPH antioxidant activity at varying concentrations in the methanol extract of the flowering aerial parts of the Gladiolus Illyricus

[24] Başgedik B. Investigation of the biological activities of *Iris germanica*, *Iris albicans*, *Gladiolus illyricus*, *Romulea ramiflora* [Master's Thesis]. Muğla: Muğla Sıtkı Koçman University; 2013.

[25] Marref, Salah Eddine, Naima Benkiki, and Mohamed Akram Melakhessou. "In vitro antioxidant activity, total phenolics and flavonoids contents of Gladiolus segetum Extracts." *Research Journal of Pharmacy and Technology* 11.11 (2018): 5017-5023.

W.D.J.Koch plant is present however the effect when compared to the antioxidative properties of quercetin was lower.

Evaluation:

During this assay objective and globally accepted ways of measurement were used and the conclusion reached was based around numerical values and provides a non-biased and objective result.

Based on the conducted studies, it is evident that relying on a single method for determining the antioxidant activity of the plant may not yield sufficient results. In this study, the DPPH method was used. Therefore, it is necessary to analyse antioxidant activity using various determination methods. Additionally, the use of plants in treatment should be carried out under the supervision of a specialist. This study has shown that as the *Gladiolus illyricus* W.D.J.Koch plant concentration increases, its radical scavenging activity also increases. Considering that a minimum concentration of 595.64 µg/mL is required to observe the antioxidant activity of the plant, it should also be noted that such a high concentration could potentially have toxic effects on living organisms. However, the potential toxic effects at these increased concentrations should be further studied, the difference between the effective dose and the toxic dose should be determined.

The solvent used during the extraction should not alter the chemical structure of the active substance. Methanol has been used in our study and is generally found to be more efficient in extracting lower molecular weight polyphenols. This, however, is one of the weaknesses of the study. However, repeating the experiment with different solvents is necessary to identify the most suitable solvent.

Although no phytochemical studies have been conducted specifically on *Gladiolus illyricus* W.D.J.Koch, phytochemical studies on different species of the same genus have revealed the presence of flavonoids, terpenoids, and alkaloids in the *Gladiolus* genus.[26] This study is one of the rare studies conducted with this *Gladiolus illyricus* W.D.J.Koch plant extract.

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Appendix 1: The document obtained from the authorized unit of the Hacettepe University where the study was conducted, proving that the study was carried out by me.

Appendix 1:

REPUBLIC OF TURKEY
HACETTEPE UNIVERSITY
Faculty of Pharmacy
Department of Pharmaceutical Botany

16.09.2024

Number :
Subject :

Arda Çağan Karaca, a 12th-grade student at Ted Ankara College Foundation High School, applied to the Department of Pharmaceutical Botany at Hacettepe University, Faculty of Pharmacy, for his project planned by his high school biology teacher for an extended assay within the scope of the IB program. He was accepted to our Department laboratory to work on his project and conducted his experiments between September 2-13, 2024.

The title of the extended essay was "*In vitro* antioxidant activities of the plant *Gladiolus illyricus* Wilhelm Daniel Joseph Koch (W.D.J. Koch)".

He participated in all stages of his research by conducting his study under the supervision of research assistants.

If you have any questions, please do not hesitate to contact us.

Best regards,

Prof. Dr. İ. İrem Tatlı Çankaya
Head of Department of Pharmaceutical Botany

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