# A REVIEW ON THE ANTIDIABETIC POTENTIAL OF SAFFRON IN THE TREATMENT OF TYPE 2 DIABETES MELLITUS AND ITS RESEARCH METHODOLOGIES

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## ÖZET

Bu çalışma Tip 2 Diyabet tedavisine yönelik Safran bitkisinin antidiyabetik potansiyeli ve araştırma metodolojileri üzerine bir derlemedir. Bu proje çalışmasının deneysel tasarımında, safran bitkisinden krosin elde edilmesi amaçlanmıştır. Krosin, yüksek antioksidan ve antidiyabetik özellikleri sayesinde potansiyel bir doğal ilaç etkeni olarak görülmektedir. Ancak, bu doğal etkeni elde etmede krosinin en yüksek verimini elde etmek için, doğru ekstraksiyon tekniklerini, fenolik içeriği, antioksidan ve antidiyabetik analizleri seçerek optimizasyonu sağlamak çok önemlidir. Bu projede, safran olarak da bilinen *Crocus sativus* bitkisinden krosin elde etmek için uygun koşullar altında ekstraksiyon tekniklerinin uygulanması amaçlanmıştır. Ayrıca, krosinin yüksek saflığını elde etmek için HPLC, toplam fenolik içeriğini belirlemek için TPC analizi, antioksidan kapasitesini belirlemek için ORAC ve DPPH analizleri ve antidiyabetik potansiyeli belirlemek için α-Amilaz İnhibisyon Testi ve α-Glukosidaz İnhibisyon Testi tekniklerini uygulamak üzere bir deneysel tasarım oluşturulmuştur. İstenilen sonuçlar elde edildiğinde, krosinin doğal bir pazar ilacı haline getirilmesi için deneysel çalışmalar başlatılacaktır.

**Anahtar Kelimeler:** Safran, Antidiyabetik, Doğal İlaç, Krosin, İlaç.

## ABSTRACT

This study is a review of the antidiabetic potential of the Saffron plant in the treatment of Type 2 Diabetes and research methodologies. The experimental design of this project study aimed to obtain crocin from the saffron plant. Crocin is considered a potential natural drug agent thanks to its high antioxidant and antidiabetic properties. However, in order to obtain the highest yield of crocin in obtaining this natural agent, it is very important to ensure optimization by selecting the right extraction techniques, phenolic content, antioxidant and antidiabetic analyses. In this project, it was aimed to apply extraction techniques under appropriate conditions to obtain crocin from the *Crocus sativus* plant, also known as saffron. In addition, an experimental design was created to apply HPLC to obtain high purity of crocin, TPC analysis to determine total phenolic content, ORAC and DPPH analyses to determine antioxidant capacity, and α-Amylase

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Inhibition Test and α-Glucosidase Inhibition Test techniques to determine antidiabetic potential. Once the desired results are achieved, experimental studies will be initiated to develop crocin into a natural market drug.
**Keywords:** Saffron, Antidiabetic, Natural Agent, Crocin, Medicine.

## 1. INTRODUCTION

*Crocus sativus,* popularly known as saffron, is a plant species belonging to the *Iridaceae* family [1]. This plant species is used for many different purposes in countries such as India and Iran, including as a spice to add extra flavor to dishes and as a medicinal plant for the treatment of diseases. Saffron is one of the plants that is best adapted to arid environments in its class [2]. While this plant can be planted at altitudes up to 2000 m above sea level, it can also grow on hillsides and in barren, arid places. However, carefully collecting and drying the stigma of this plant by hand is very important to obtain saffron efficiently [1]. The fact that it is so difficult to obtain saffron is one of the main reasons why saffron is expensive. Saffron, also known *as Crocus sativus*, has a yellow and orange color [3]. This is due to the carotenoids crocin and crocetin, which are components of saffron [1, 4]. In addition to crocin and crocetin, the stigma of saffron contains components such as vitamins B1 and B2, minerals, zeaxanthin, anthocyanin, lycopene, alpha- and beta-carotene The presence of secondary metabolites such as rutin, luteolin, hesperidin, quercetin, anthocyanin, and tannins in the leaves of saffron shows that saffron is one of the most important plants with various bioactive components used for therapeutic purposes [5]. Among these bioactive compounds, the four most important active components of the *Crocus sativus* plant, or saffron as it is commonly known, are crocin, croetin, picrocrocin and safranal. According to the literature, the most important reason for the intense color of saffron is the crocin and croetin components [4]. The compound crocin is a diester, a form of the diglycoside of crocetin, and its chemical formula is $C_{44}H_{64}O_{24}$ [6]. This compound is water-soluble. Due to its strong antioxidant properties, crocin protects cells from chronic stress [7]. It reduces the cellular damage caused by harmful complications such as retinopathy and nephropathy, which are associated with chronic diseases like Type 2 Diabetes mellitus (T2DM) [8]. In addition, it helps regulate blood glucose levels by increasing insulin sensitivity in cells [9]. In addition to all these bioactive properties, crocin also has anti-inflammatory and anticancer properties [7]. Picrocrocin is the main component that gives saffron its bitter taste and aroma. This ingredient has antimicrobial and antioxidant properties [4]. Especially when consumed, saffron provides the most important contribution to the therapeutic role in stomach and intestinal health. Another main ingredient is safranal [10]. Safranal is a volatile carboxaldehyde compound formed by the deglucosylation of picrocrocin [4]. This component, unlike other bioactive components, creates therapeutic effects on the central nervous system [5]. It is beneficial for psychological disorders such as depression by exhibiting antidepressant and sedative effects on the nervous system [11]. In addition, it slows down the progression of the disease by protecting brain cells in neurodegenerative diseases such as Parkinson's and Alzheimer's [5, 11].

As can be seen, crocin, one of the most important active ingredients of saffron, has very significant and therapeutic effects. This study aims to examine the relationship between crocin and T2DM. T2DM is a chronic disease characterized by high blood sugar as a result of the beta cells in the pancreas not producing enough insulin hormone or the cells not being able to use insulin properly. Within the scope of this study, it is aimed to reveal the antioxidant and anti-inflammatory properties, as well as the antidiabetic potential, of crocin bioactive substances—popularly known as saffron and extracted from the *Crocus sativus* plant—on the treatment of

T2DM. Depending on this aim, once the desired antidiabetic potential is obtained from the bioactive substance crocin, turning it into a natural drug product as an alternative to chemical drugs on the market will be the next goal of this study.

## 2. MATERIALS AND METHODS

Within the scope of this study, saffron stigmas obtained from companies, organic extraction solutions such as 80% ethanol distilled water, UV-Vis spectrophotometer device, HPLC (High Performance Liquid Chromatography) device, pure crocin standard, and additional glass materials such as Erlenmeyer flasks and beakers used in the laboratory, weighing device, TPC (Total Phenolic Content), DPPH (2,2-diphenyl-1-picrylhydrazyl), ORAC (oxygen radical absorbent capacity) assay, α-Amylase Inhibition Assay, and α-Glucosidase Inhibition Assay are used [12, 13].

*Harvesting and Preparation of Saffron*

Under normal circumstances, the stigma parts of the saffron plant, known as *Crocus sativus*, are collected by hand. Since the stigma is the most important region where the desired bioactive compounds are found, it is necessary to be very careful. Collected stigmas should be dried in a way that they do not receive direct sunlight. Then, the dried stigmas are ground with the help of a mortar or a grinder. Increasing the size and surface area of the particles enhances the extraction efficiency. Since harvesting and preparation could not be carried out within the scope of this study, saffron stigmas were provided [14, 15].

*Preparation of Extraction Solution*

The ground saffron stigmas are weighed on a precision scale to the amount of 2 grams. Then, 100 mL of 80% ethanol-distilled water solution is prepared. The saffron stigma in powder form is kept in this solution in a dark environment for 3 hours, and a 2% saffron solution is prepared [13, 16].

*Filtration and Lyophilization Process*

The saffron solution is filtered using a beaker, funnel, and filter paper. Following this process, the sample is freeze-dried in the lyophilization device. The extracted sample is stored at a temperature of -20°C and in a place away from light[14, 16].

*Preparation of Solution for Analysis*

The extract residues obtained as a result of all stages are weighed with a weighing device. They are then dissolved in normal saline and brought to a final concentration of 8.6%. The reason for using normal saline is that this medium has a salt concentration similar to body fluids due to the components it contains. In addition, it ensures the stability of crocin by helping it to dissolve better [13, 16].

*Sample Preparation*

The saffron solution is filtered again. Here, filter paper is selected according to the desired pore size.

*Purification of Crocin*

Within the scope of this study, HPLC analysis, one of the most well-known and best-yielding methods for the purification of crocin, is performed. A reverse phase-C18 column was

chosen for the HPLC analysis. The mobile phase flow is 1 mL/min. An 80:20 acetonitrile-water mixture, which is used to separate many bioactive substances, is used as the mobile phase. The previously prepared saffron extract solution sample is injected into the device in a volume of 10 µL with the help of a syringe. In the HPLC analysis, the wavelength was set to 440 nm. Then the analysis is started. In order to increase the purity of crocin, it is subjected to HPLC analysis again. The pure crocin fractions obtained are then analyzed with a UV-Vis spectrophotometer to obtain data. The pure crocin obtained is stored at -20°C by applying the lyophilization stage for later use [14].

*Total Phenolic Content (TPC) Analysis*

The Folin–Ciocalteu method was used in TPC analysis. A hydroalkaline solution at a v/v ratio of 50:50 was added to 125 mg of dry saffron stigma extract. The Folin–Ciocalteu reagent, diluted 10 times with deionized water, was mixed with 10% w/v sodium bicarbonate. This solution was incubated for 15 minutes at a temperature of 45°C. To measure the samples, the absorbance of the UV–vis spectrophotometer device was set to 765 nm. To calculate the measurements, the standard calibration curve was prepared with gallic acid at concentrations of 0, 0.05, 0.1, 0.15, 0.2, and 0.25 mg·mL−1. Then, the total phenolic content was calculated as in Equation (1) in mg Gallic Acid Equivalent (GAE)/g extract unit. In Equation (2), C represents the gallic acid concentration in the calibration curve (mg/mL), V represents the volume of the extract (mL), and m represents the mass of the dry stigma extract (g) [14].

$$\text{TPC (mg GAE/g extract)} = \frac{C\ x\ V}{m} \quad (1)$$

*ORAC (Oxygen Radical Absorbance Capacity) Assay*

The oxygen radical absorbent capacity (ORAC) method was used to determine the antioxidant activity of crocin obtained from saffron. The principle of this method is based on measuring the fluorescence loss due to peroxyl radical formation resulting from the degradation of AAPH (2,2′-azobis-2-methyl-propanimidamide dihydrochloride). The Trolox analogue was chosen as a positive control that inhibits fluorescence degradation relative to degradation. AAPH was preferred as the peroxyl radical generator. Phosphate buffer (pH 7.0) and 12.5 µM Trolox were used as blank and standard. Each diluted sample was plated with 50 µL of buffer and Trolox. A 12 nM fluorescent solution was added to all wells of the microplates. The microplate containing the sample, buffer, Trolox, and fluorescent solution was incubated at 37°C for 30 minutes. Then, a 100 mM AAPH solution was added to all wells, and fluorescence measurements were made at 540 nm excitation and 575 nm emission wavelengths, repeated three times. The calculation of ORAC values was made in µmol Trolox/µg sample as in Equation (2). Here, K represents the sample dilution factor and S represents the area under the fluorescence decay curve of Trolox or blank [14].

$$\text{ORAC value } \left(\mu\frac{\text{mol}}{\mu\text{g}}\right) = \frac{K(S\ sample - S\ blank)}{(S\ Trolox - Sblank)} \quad (2)$$

*DPPH (2,2-difenil-1-pikrilhidrazil) assay*

At this stage, a 0.06 mmol/L DPPH solution was first prepared using methanol, and this solution was incubated for 1 hour at 4°C in a light-free environment. This prepared solution was added to the previously prepared extracts at a rate of 3.5 mL and added to the samples created with new concentrations of 0.062, 0.125, 0.187, and 0.250. The solutions were mixed with a vortex to become homogeneous. Then, it was subjected to inhibition for 30 minutes in a dark

environment. Measurement was made at 517 nm using UV-Vis. The percentage of antioxidant activity (AA%) was calculated using Equation (3). The abbreviation $Abs_c$ represents the absorbance of the control solution containing DPPH and $Abs_s$ represents the absorbance of the sample solution containing DPPH. [3].

$$AA\% = \frac{Abs_c - Abs_s}{Abs_c}\ x\ 100 \quad (3)$$

*α-Amylase Inhibition Assay*

In this testing phase, the samples were reacted with starch solution and α-amylase enzyme. At this stage, 250 µl of α-amylase containing 240 U/ml, 0.02 M phosphate buffer solution (PBS), pH 6.9, 0.006 M NaCl, and 250 µl of extract were mixed. This solution was incubated at 37°C for 10 minutes. Then, 250 µl of 1% (w/v) soluble starch was added. Incubation was carried out again for 30 minutes at 37°C. After incubation, 50 µl (DNS) color reagent was added. It was heated in a boiling water bath for 10 minutes for the reaction to occur. The mixture was then cooled to room temperature and diluted with PBS. As a result of all these steps, absorbance measurement was made at 540 nm [17].

*α-Glucosidase Inhibition Assay*

Firstly, α-glucosidase enzyme and substrate p-Nitrophenyl-α-D-glucopyranoside were dissolved in PBS medium. Previously prepared samples and α-glucosidase enzyme were pre-incubated for 10 minutes at 37°C. After the incubation period, the substrate was added to the reaction mixture and another incubation step took place. Then, the reaction was stopped by adding 1 ml $Na_2CO_3$. This phase consisted of three repetitions. Absorbance measurement was performed at 405 nm and the inhibition percentage (%) was calculated using Equation (4). In this equation, the symbol AC represents control, $AC_b$ control blank, AS sample, and $AS_b$ sample blank [17].

$$Inhibition\ (\%) = \frac{(AC - ACb) - (AS - ASb)}{AC - ACb} x100 \quad (4)$$

*Statistical Analysis*

To determine whether these experimental results were statistically significant, the P value was checked using programs such as Design Expert and Microsoft Excel. As a result, it has been proven that the study is significant, and following all this methodology, the project work can be continued to convert crocin obtained from saffron into the final drug product [17].

*Further Studies for Conversion to the Final Product*

At the end of this methodology, when the antidiabetic potential of crocin obtained from saffron yields the desired result, it is necessary to optimize the extraction process in pilot production to convert it into the final product [18]. After optimization is achieved, further optimization must be made in the parameters required to scale up. Then, the preclinical study phase should be started. At this stage, studies such as toxicity and dosage should be carried out on animal models such as rats [19]. In addition, pharmacokinetic and pharmacodynamic studies of crocin, as well as ADME and bioavailability analyses, consisting of Absorption, Distribution, Metabolism, and Excretion, should be performed [20]. After all these stages are completed, the appropriate drug formulation needs to be developed. Clinical studies must be conducted before this drug can be marketed. These studies include Phase 1, Phase 2, and Phase 3 trials. Post-marketing Phase 4 studies are ongoing [19]. After everything is completed, final drug production

is completed with stages such as licensing and marketing for the drug to be used completely in society.

## 3. RESULTS AND DISCUSSION

Since only one experimental design was created within the scope of this study, only the methodology section was determined. However, when the literature studies are examined, there are many results from methods such as extraction steps for saffron and crocin, HPLC analysis, determination of antioxidant capacity with DPPH, and determination of antidiabetic potential according to the methods used. In this project methodology draft, an attempt has been made to create a methodology that is expected to yield optimum results.

In this project strategy, the extraction phase was first carried out to obtain crocin from saffron. Extraction efficiency is affected by factors such as the harvest time of the plant, harvest method, particle size, extraction method and duration, polarity and purity of the solvent used, solid-to-liquid ratios, pressure, and temperature [14, 21]. It is especially important to collect a plant with a sensitive stigma, such as saffron, without damaging the components in certain parts. Another factor that increases extraction efficiency is particle size and surface area [12]. Grinding the dry stigma of saffron to reduce its particle size increases the extraction efficiency [12, 13]. Providing incubation in a dark environment during the extraction process ensures that the crocin is extracted without degradation, while a light-free environment prevents the degradation of light-sensitive components [15].

While the filtration process removes undesirable compounds from the solution, lyophilization ensures stable storage of the crocin and drying of the compound without chemical degradation [21]. Storage temperature also ensures the preservation of the activity of the bioactive components and properties of crocin.

HPLC analysis is performed to purify crocin. HPLC is an analysis technique used to separate and analyze bioactive substances. Through this analysis, the purity of crocin is increased by quantitatively analyzing its quantity [17]. Parameters such as the C18 column, mobile phase, and wavelength selection chosen in HPLC are used to analyze the crocin sample for optimum purity and concentration [12]. This stage is a crucial step. The analysis results obtained here will be used to examine the antioxidant and antidiabetic potential of the natural agent crocin [22].

TPC is a method used to analyze the amount of phenolic compounds. Here, the antioxidant potential is determined by quantifying the phenolic compounds in the saffron extract [12, 21]. This stage enables comparative analysis by comparing the phenolic content results of other herbal extracts [21]. On the other hand, the ORAC assay quantitatively measures the antioxidant capacity of this compound by measuring the capacity of crocin to neutralize free radicals. With this analysis, a comparative analysis is made, and the antioxidant capacity results of other plant extracts are analyzed ([14]. DPPH is another antioxidant capacity measurement technique. Here, the antioxidant properties of crocin are determined by measuring its free radical scavenging potential. When the antioxidant capacity of crocin is proven through this analysis, it can be said that crocin is effective in preventing cellular damage caused by free radicals [3].

α-Amylase Inhibition Assay and α-Glucosidase Inhibition Assay are analysis techniques used to determine the antidiabetic potential. α-Amylase Inhibition Assay measures the inhibitory

effect of crocin on this enzyme. This enzyme inhibition helps maintain blood sugar at low levels by slowing the conversion of carbohydrates to glucose. Therefore, it is one of the main assays used to determine antidiabetic potential [17]. Another method is α-Glucosidase Inhibition Assay [8, 17]. Here, it measures the inhibition of crocin on the α-Glucosidase enzyme. The reason for choosing this methodology is that it is generally used in the literature and therefore has scientific validity [8]. The results of this project design can be compared with the literature studies conducted using these methods.

As a result, when studies in the literature are examined, TPC values of 25 to 30 (mg GAE/g of distilled water) are obtained for crocin obtained from saffron [21]. In HPLC analysis, the total amount of crocin component is 50 to 67 mg/g [22]. ORAC values are around 51 [14]. DPPH values are expected to be between 55 and 60 percent. IC50 (µg/mL) rates vary between 0.29 and 3.14 in different methodologies. In the literature, antidiabetic potential results are obtained in different inhibition tests according to different methodologies [17]. IC50 rates of α-Amylase Inhibition results range from 334.40 to 720.05 µg/ml. α-Glucosidase inhibition IC50 rates vary between 75.25 and 115.41 µg/ml [17]. The most important parameter to consider here is that the lowest IC50 rate shows both antioxidant and antidiabetic potential [8]. According to these data taken from the literature, the literature-based value ranges of the methodologies and expected results specified in Table 1 have been tabulated for better understanding. The expected results within the scope of this study are expected to be within this data and result range in the literature.

Table 1. Expected Ranges of Analysis Results Based on Literature for Evaluating the Antioxidant and Antidiabetic Potential of Crocin Natural Agent

| **Analysis Type** | **Expected Range Based on Literature** | **References** |
|---|---|---|
| TPC | 25 - 30 mg GAE/g | [21] |
| HPLC | 50 - 67 mg/g | [22] |
| ORAC Value | 51 µmol Trolox/µg | [14] |
| DPPH Antioxidant Activity | 55% - 60% | [14] |
| α-Amylase Inhibition | 334.40 - 720.05 µg/mL | [17] |
| α-Glucosidase Inhibition | 75.25 - 115.41 µg/mL | [17] |

## 4. CONCLUSION AND COMMENTS

The most basic bioactive component of the *Crocus sativus* plant, popularly known as saffron, is crocin. Crocin is the most important active ingredient that gives the saffron plant its basic color and scent. In addition, this bioactive substance has many properties such as antioxidant and antidiabetic effects. These properties make crocin obtained from saffron very popular for use in many industries such as food and health. Chemical drugs harm the human body when used for a long time in the treatment of many chronic diseases, so alternative drugs of natural origin are needed. For these reasons, crocin is an important drug candidate. However, as with every plant extract, it is very important that crocin is obtained in the most efficient way

with the most accurate techniques and that its bioactive properties are revealed by correct analysis. This project design aims to prove that crocin is a natural component with drug potential by determining the crocin extraction method that will provide high efficiency from saffron, separation techniques, and the determination of its antioxidant and antidiabetic potential with accurate methods. When the desired results are achieved, another goal is to continue the project with other drug production stages in order to turn it into a natural drug product.

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