Journal of Chemical Health Risks 2(4): 29-36, 2012
ISSN:2251-6719

Kinetic Study of Free Radicals Scavenging by Saffron Petal Extracts

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Abstract: Saffron petal is the main by-product of saffron processing which is produced in large amounts, annually. The objectives of this study were to study the antioxidant activity and free radical-scavenging effects of saffron petal extracts. The ability of saffron petal to act as an antioxidant using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical method was investigated by applying the Uv–Vis spectrometry. The Uv–Vis spectra of reaction mixtures in acetonitrile revealed that saffron petal has a considerable effect on scavenging free radical. Kinetic studies were conducted by measuring the disappearance of DPPH in acetonitrile over the wavelength range of 515-522 nm under pseudo-first-order conditions at 37^{o}C. Furthermore, the pseudo first order rate constants were determined.

Keywords: DPPH, antioxidant, saffron petal, kinetic study

INTRODUCTION

The free-radical mediated oxidation by the molecular oxygen is among the most important chemical reactions. Moreover many important industrial processes in petrochemical industry are based on the controlled oxidation of hydrocarbons. Oxidation is the main cause of deterioration of foodstuffs, oils and polymers [1].

Reactive oxygen species (ROS) including super oxide anion radical, hydroxyl radical, and hydrogen peroxide, which are formed and degraded by all aerobic organisms, can cause oxidative damage of all major groups of biomolecules (DNA, protein, lipids and small cellular molecules), which in turn leads to cardiovascular and neurodegenerative diseases [2]. The antioxidant defense systems including enzymes (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymes defenses (glutathione, vitamins C and E) play an important role in scavenging oxidants and in preventing cell injury [3,4]. In recent years, there has been a growing interest in finding alternative natural-based antioxidants. Plant material and their products are rich sources of a variety of biologically active compounds possessing antioxidant and radical scavenging activities. According to the literature, foods containing phytochemicals such as phenolic compounds have potential protective effects against many diseases [5] Therefore, consumption of a variety of antioxidant effects may reduce the risk of serious health disorders caused by ROS. Accordingly, much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation, or to provide a protection against the damage of free radicals [6-8].

In previous study, we reported the antioxidant activity of Zinc, Vitamin C and mixture of them [9].

Among the natural plants, saffron (*Crocus sativus*) is one of the most expensive spices of the world. Iran, with an annual production of about 170 tones of saffron, approximately 94% of the world production, is the main producer of this crop [10]. Spain, India, and Greece, respectively, hold lower ranks as minor producers. Saffron is usually used for cooking as a seasoning and coloring agent. It also has some other applications for medicinal and dye purposes [11]. Apart from stigmas, which have economical value, the other two parts of saffron flower (Fig 1) are usually used as animal feed. There are 2170 flowers in each kilo gram of harvested fresh saffron flower.

Saffron is also considered as a tonic for heart and nervous system [12] In addition, it is used in chemical pain tests as well as an acute and/or a chronic anti-inflammatory [13]. Clinical trials have demonstrated that saffron may be of therapeutic benefit in the treatment of mild to moderate depression [14, 15],

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acting as an anticonvulsant remedy. Experiments with mice using maximal electroshock seizure (MES) and pentylenetetrazole (PTZ) tests have demonstrated that the aqueous and ethanolic extracts of saffron capabilities as anticonvulsant [16] in preventing perkinsonism [17] and it has antiatherosclerosis, mutagenic or antimutagenic[18-20] and antigenotoxic agents [21-25]. Saffron petal was cheap and abundant; therefore, in this paper the effect of extract of saffron petal on scavenging of free radicals has been studied. The results of this study show that saffron petal has a perceptible effect as an antioxidant.

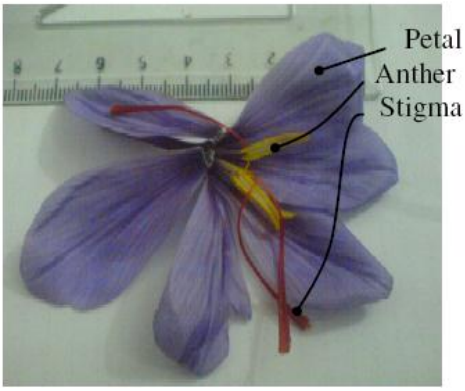


Fig1. Saffron flower and its three main parts.

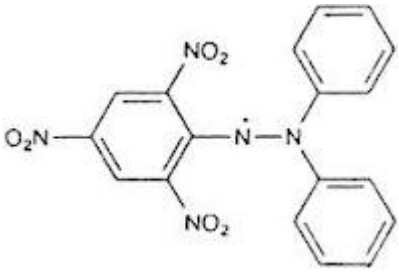


Fig2. Chemical structure of DPPH

MATERIALS AND METHODS

2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from a commercial Supplier (Sigma). Other chemicals and solvents used were of the highest analytical reagent grade.

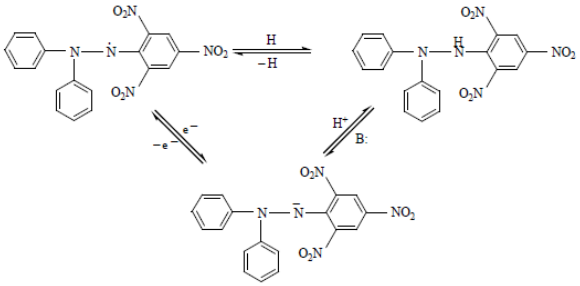
Perpetration of Alcoholic Extract from Saffron Petal

Five gram portions of saffron petal powder were added to a mixture of 85 mL of ethanol (95%) and 15 mL HCl (1.5 M). The mixture was stirred at a speed of 1000 rpm at room temperature for 30 min and then it was filtered.

UV–Vis assay for DPPH

The DPPH radical acts as a scavenger for other odd-electron species (Scheme 1)

Scheme1**.** The H-transfer reactions for DPPH radical



The H-transfer reactions from an antioxidant to DPPH were monitored using a UV–Vis spectrophotometer [26] (Perkin Elmer Lambda 25). The temperature in the cell was kept constant at 37 ^{o}C by means of a circulator. In a typical procedure, 2mL of a freshly prepared 2×10^{-4} M solution of DPPH in acetonitrile, was placed in the spectrophotometer cell. Then 0.01–0.05 mL of a freshly prepared alcoholic extracts of saffron petal was added as an antioxidant. All the spectra were recorded over two-minute intervals.

The second-order rate constant (k_2) was determined with the anti-radical compound [Antioxidant] in large excess as compared with the radical compound [DPPH], forcing the reaction to behave as first order in DPPH:

$$-\frac{d[DPPH]}{dt} = k_1[DPPH] \quad (2)$$

Where

$$k_1 = k_2[Antioxidant] \quad (3)$$

[Antioxidant] is assumed to remain constant throughout the reaction and can

be modified to obtain different k_1 values. Therefore, DPPH was depleted from the medium under pseudo-first-order conditions following the equation [27, 28]:

$$[DPPH] = [DPPH]_0 . e^{k_1 t} \quad (4)$$

Where [DPPH] is the radical concentration at any time (t), $[DPPH]_0$ is the radical concentration at time zero, and k_1 is the pseudo-first-order rate constant. This constant (k_1) is linearly dependent on the concentration of the antioxidant. The rate constants were determined by plotting $\ln(A_t-A_\infty)/(A_0-A_\infty)$ versus time as shown in Fig 3 and the calculated rate constants have been listed in Table 1.

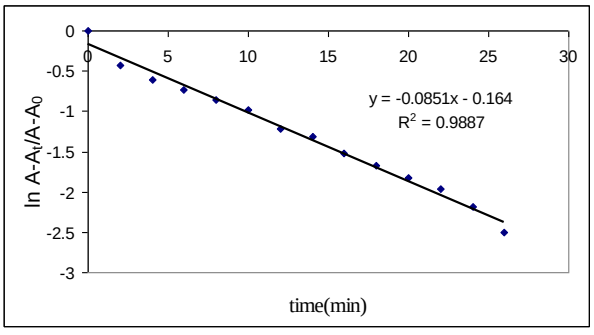
Kinetic studies were conducted by measuring the disappearance of DPPH in acetonitrile at the wavelength range 515-522 nm [29, 30] under pseudo-first-order conditions at 37 ^oC. The pseudo first order rate constants were determined by plotting $\ln(A_t-A_\infty)/(A_0-A_\infty)$ versus time as shown in Fig 3.

RESULTS AND DISCUSSION

DPPH is a stable free radical having maximum absorption at 517 nm [31] that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. In addition, DPPH is often used as a proper substrate to evaluate the antioxidant capacity of an antioxidant (via the unpaired electron which is delocalized over N and O heteroatoms. We have studied the ability of the antioxidants of extract of saffron petal to neutralize the free radicals such as DPPH radicals, an H atom is donated in the presence of saffron petal extract. Free radical scavenging hence the reduction in DPPH radical was determined by the decrease in its absorbance at 517 nm through the absorption spectra in the range of 400-1000 nm at 37 ^oC (Fig 4).

To study the kinetics of the reaction between DPPH and saffron petal extracts, the temperature was fixed at 37 ºC, and DPPH concentration was kept constant (from A to D in Fig 4); the amount of saffron petal extracts was varied from 0.01 mL to 0.05 mL. The disappearing of DPPH was monitored at ƛ max = 517 nm. The results show that the rate of disappearing of DPPH radical is increased by increasing in saffron petal extracts. The rate constants, which were determined by plotting $\ln(A_t-A_\infty)/(A_0-A_\infty)$ versus time as shown in Fig 3, are presented in Table 1. In this study, the rate constants of the first H atom abstraction by DPPH (k_1), were obtained (8.51×10^{-2}, 1.37×10^{-1}, 2.21×10^{-1} and 3.37×10^{-1} s^{-1}) in the presence of the saffron petal extract, under pseudo-first-order conditions at 37 ^oC.

A



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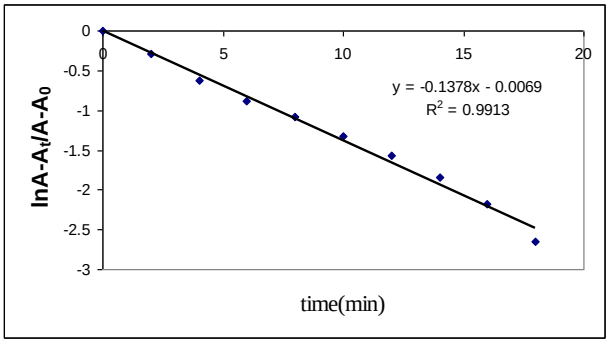

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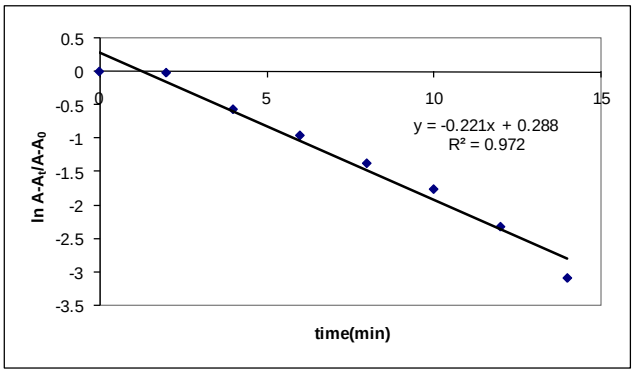

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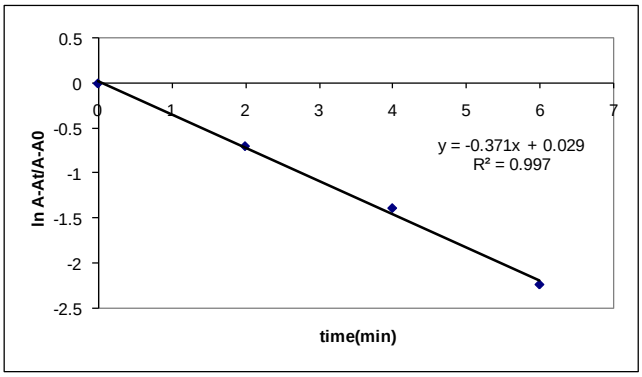
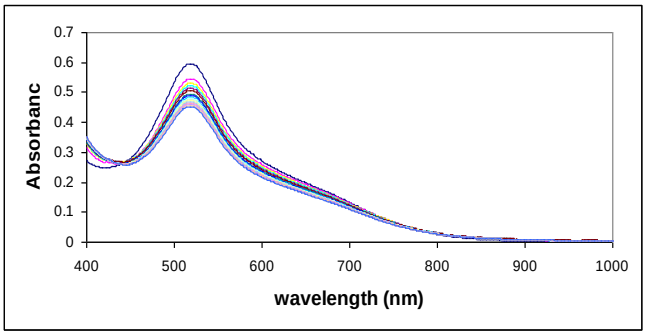


Fig 3. Plot of $\ln(A_t-A_\infty)/(A_0-A_\infty)$ versus time for determination of rate constants **A)** 0.01 mL **B)** 0.02 mL, **C)** 0.03 mL and **D)** 0.05 mL of extracts from saffron petal.

A



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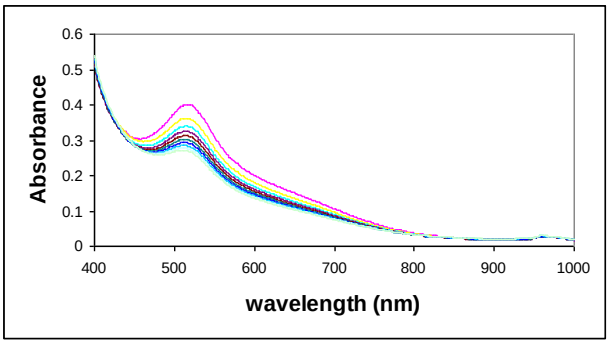

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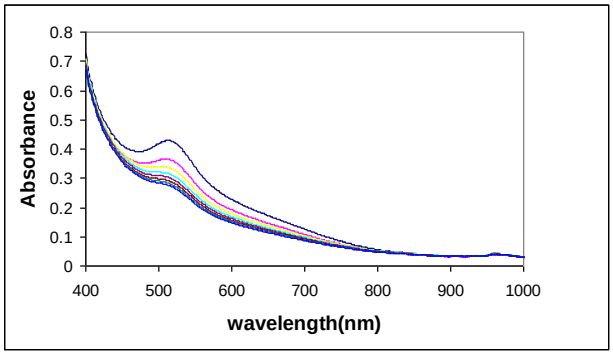

D

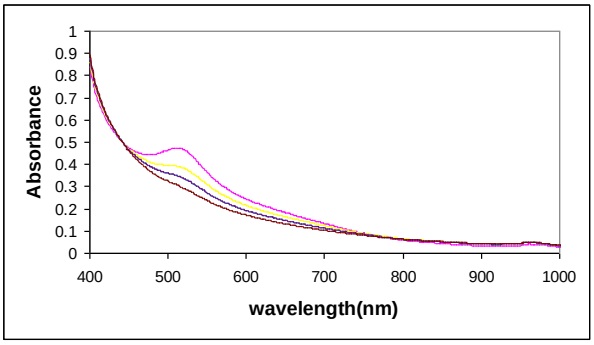

Fig 4.UV-Vis spectra of DPPH in the presence of **A)** 0.01 mL **B)** 0.02 mL, **C)** 0.03 mL, **D)** 0.05 mL extracts from saffron petal.

Table1. Calculated rate constants in the presence of different amount of extracts from saffron petal.

Extracts of saffron petal (mL)	0.01	0.02	0.03	0.05
Rate constant (1/s)	8.51×10^{-2}	1.37×10^{-1}	2.21×10^{-1}	3.37×10^{-1}

CONCLUSION

Many plants are consumed as vegetables, food complements or in medicinal

purposes. Saffron is widely used in the herbal and traditional medicinal preparations, especially among the Iranian food products. Many studies are required prior to its further utilization or commercialization. The World Health Organization (WHO) has estimated that about 80% of the world's inhabitants rely on traditional medicines for their primary health care needs, and most of these therapies involve the use of plant extracts or their active components. The present study elaborated the antioxidant effect of saffron. The results show that the extract of saffron petal has an appreciable effect on free radical scavenging.

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