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Kinetic study of DPPH scavenging in the presence of mixture of Zinc and Vitamin C as an antioxidant

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Abstract: Reactions of free radicals and reactive oxygen species (ROS) with biological molecules in vivo play an important physiological role in many diseases. 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical and often used as a routine reagent to evaluate the antioxidant capacity of an antioxidant. This study was undertaken to investigate the free radical-scavenging and antioxidant activities of Zinc, Vitamin C and mixture of them. UV-Vis spectrophotometry method was used to evaluate the ability of Zinc, Vitamin C and mixture of them to scavenge DPPH radical. The kinetic parameters such as rate constant and activation energy in experimental conditions were calculated. The rate constants of the H atom abstraction by DPPH (k_1), in the presence of Zinc, Vitamin C and mixture of them were obtained (0.4209, 2.092 and 1.82 min^{-1}) respectively, under pseudo-first-order conditions at 25 ^oC.

Keywords: Antioxidant, DPPH, Kinetic, Free radical, UV-Vis spectrometry

INTRODUCTION

Free radicals are electrically charged molecules, they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Free radicals and reactive oxygen species (ROS) play a key role in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's, Parkinson's and even in the aging process [1-3]

ROS are entities containing one or more reactive oxygen atoms including hydroxyl radical ($OH^{\cdot}$), superoxide anion radical ($O_2^{\cdot -}$), and hydrogen peroxide (H_2O_2). Their formation is an unavoidable consequence of respiration in aerobic organisms. These species are very unstable and react rapidly with other substances in the body, leading to cell or tissue injury [4].

In cellular defense system, scavenging of free radicals is an important issue affiliated by utilization of both exogenous and endogenous antioxidants. Antioxidants are defined as substances that when present at low concentrations compared with those of an oxidizable substrate significantly delay or prevent oxidation of that substrate [5-7].

Small-molecule antioxidants can be present extra- and intracellularly. Antioxidants work by preventing the formation of new free radical species, by converting existing free radicals into less harmful molecules, and by preventing chain reactions. In the last decade, the antioxidants from

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food resources have attracted a great deal of attention related to their ROS scavenging capacity. In diets, metal binding proteins and non-enzymatic natural antioxidants such as α-tocopherol (Vitamin E), Vitamin C and quercetin can be consumed as food supplements [8,9]. Vitamin C is considered-as the most important water-soluble antioxidant in extracellular fluids. It is capable to neutralize ROS in the aqueous phase before lipid peroxidation is initiated.

Also the commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest [10-12]. Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases [13]. These effects have been attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids among others [14]. Basils (Ocimum spp., Lamiaceae) contain a wide range of essential oils rich in phenolic compounds [15] and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins [16].

In this study, we report the antioxidant activity of Zinc, Vitamin C and mixture of them [17].

The DPPH radical-scavenging capacity of individual selected Zinc, Vitamin C and mixture of them was determined with UV-Vis spectrometry method.

Previous studies have reported the influence of some variables (temperature, time.) on the antioxidant activity of Zinc, Vitamin C and mixture of them.

MATERIALS AND METHODS

Analytical grade of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Company, (St. Louis, MO, USA). Food supplements of Vitamin C and Zinc Sulfate were purchased from a pharmacy. Also analytical grade acetonitrile solvent (CH_3CN) was purchased from Merck Company.

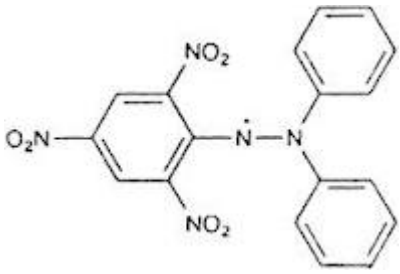

Fig 1. Chemical structure of DPPH

L-Ascorbic Acid

L-ascorbic acid ($C_6H_8O_6$) is the trivial name of Vitamin C. The chemical name of this compound according to IUPAC nomenclature is 2-oxo-L-threo-hexono-1, 4-lactone-2, 3-endiol (Fig 2). Ascorbic acid being a water soluble compound is easily absorbed but it is not stored in the body. The major metabolites of ascorbic acid in human are dehydroascorbic acid, 2, 3-diketogluconic acid and oxalic acid (Fig 3). The main route of elimination of ascorbic acid and its metabolites is through urine. It is excreted unchanged when high doses of ascorbic acid are consumed. Ascorbic acid is generally non-toxic but at high doses (2-6g/day) it can cause gastrointestinal disturbances or diarrhea [18, 19].

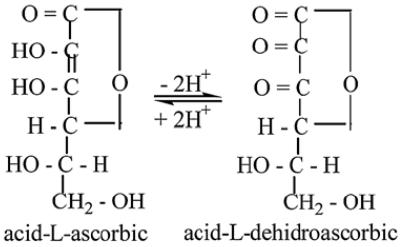

Fig 2. Vitamin C oxidation

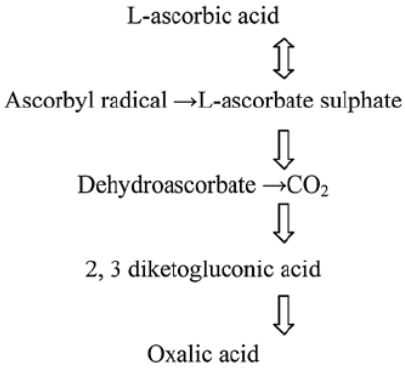

Fig 3. Catabolism of ascorbic aci

UV-Vis - assay for dpph

The scavenging of DPPH was monitored using UV-Vis spectrophotometer (Perkin-Elmer Lambda 25). The temperature in the cell was kept at 25 ^{o}C by using a circulator. In a typical procedure, to a 3mL portion of a freshly prepared solution of DPPH (2×10^{-5} M) in acetonitrile, placed in the spectrometer cell, 30-80 μL of a freshly prepared solution of the antioxidant (2×10^{-2} M) in the same solvent was added. All the spectra were recorded over 1 min intervals for the determination of rate constants.

Kinetic UV-Vis assay for DPPH

Second-order anti-radical kinetic determinations were obtained using DPPH and antioxidants of Zinc, Vitamin C and mixture of them.

$$-\frac{d[DPPH]}{dt} = k_2[DPPH]\ [Antioxidan\ t] \quad (1)$$

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], thus 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

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

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, and from the slope of these plots, k_2 is determined. Kinetic studies were conducted by measuring the disappearance of DPPH in acetonitrile at a wavelength of 515-522 nm under pseudo-first-order conditions at 25 ^{o}C. Determinations of k_1 were repeated ten time using different antioxidant concentrations for each sample.

RESULTS AND DISCUSSION

DPPH is a stable free radical having maximum absorption at 517 nm [20-22] that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. In addition, DPPH is often used as substrate to evaluate the antioxidant capacity of an antioxidant (the unpaired electron is delocalized over N atoms and over O atoms, respectively).

We have studied the ability of the antioxidants such as Zinc, Vitamin C and mixture of them to neutralize the free radicals like DPPH radicals.

The antioxidant activity of Zinc, Vitamin C and mixture of them monitored by following the decrease in DPPH absorbance at 515 nm[23-25]. The decrease in DPPH absorption in the presence of Zinc, Vitamin C and mixture of them was monitored by measuring absorption spectra in the range of 400-900 nm at 25 ^{o}C (Fig 4A, B and C). In this figure, spectra were recorded every 1 min for the determination of rate constants. The kinetic studies of DPPH scavenging in the presence of Zinc, Vitamin C and mixture of them were carried out by varying temperature between 25 to 40 ^{o}C.

The rate constants were determined by plotting $\ln(A_t-A_\infty)/(A_0-A_\infty)$ versus time as shown in Fig 5A, B and C and are presented in Table 1. In this plot, A_t is the absorbance at time t, A_∞ is the absorbance after reaction has gone to completion and A_0 is the initial absorbance.

In this study, the rate constants of the scavenging of DPPH (k_1), in the presence of Zinc, Vitamin C

and mixture of them were obtained (0.4209, 2.092 and 1.82 min^{-1}) respectively, under pseudo-first-order conditions at 25 ^{o}C. Table 1 and figure 5 show that the pseudo first order rate constant of scavenging DPPH in the presence of vitamin C is larger than pseudo first order rate constants in the presence of zinc and mixture of zinc- vitamin C. Vitamin C attracts free radicals because its LUMO, or lowest unoccupied molecular orbital, has a low energy. The free radical, if it is donating an electron, which is most often the case, must put the electron in the LUMO. When the electron is placed in the LUMO, the overall energy of the vitamin C molecule stays about the same, it changes from –681.008 au to –680.951 au. The energy of the LUMO of the neutral vitamin C is 3.82 eV while the HOMO (highest occupied molecular orbital, the same as the LUMO of the neutral vitamin C) of the vitamin C anion doublet (vitamin C with a negative charge and one unpaired electron) is –1.15 eV. In other words, adding an electron to vitamin C will hardly have any major effect to it. On the other hand in biochemical systems, the antioxidant properties of zinc have been clearly demonstrated and, for the most part, appear to be independent of zinc metalloenzyme activity. In general, the mechanism of antioxidation can be divided into acute and chronic effects. Chronic effects involve the exposure of an organism to zinc on a long-term basis, resulting in an induction in some other substance that is the ultimate antioxidant. On the other hand, chronic zinc deprivation generally results in increased sensitivity to some oxidative stress [26], although the biochemical basis of many of these effects is not clear. The acute effects are generally thought to involve two mechanisms: protection of protein sulfhydryls or reduction in the formation of $^{\cdot}OH$ from H_2O_2 through the antagonism of redox-active transition metals, such as iron and copper. In general the results show that antioxidant activity of vitamin C decrease in the presence of Zinc.

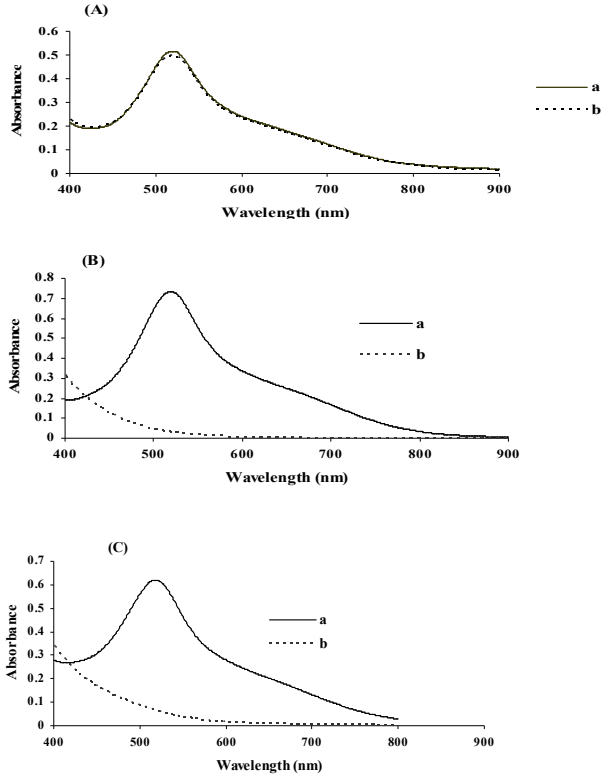

Fig 4. The spectra of DPPH in the presence of (A) Zinc a) at time zero b) t = 16 min (B) Vitamin C a) at time zero b) t = 4 min (C) Zinc and Vitamin C a) at time zero b) t = 3 min.

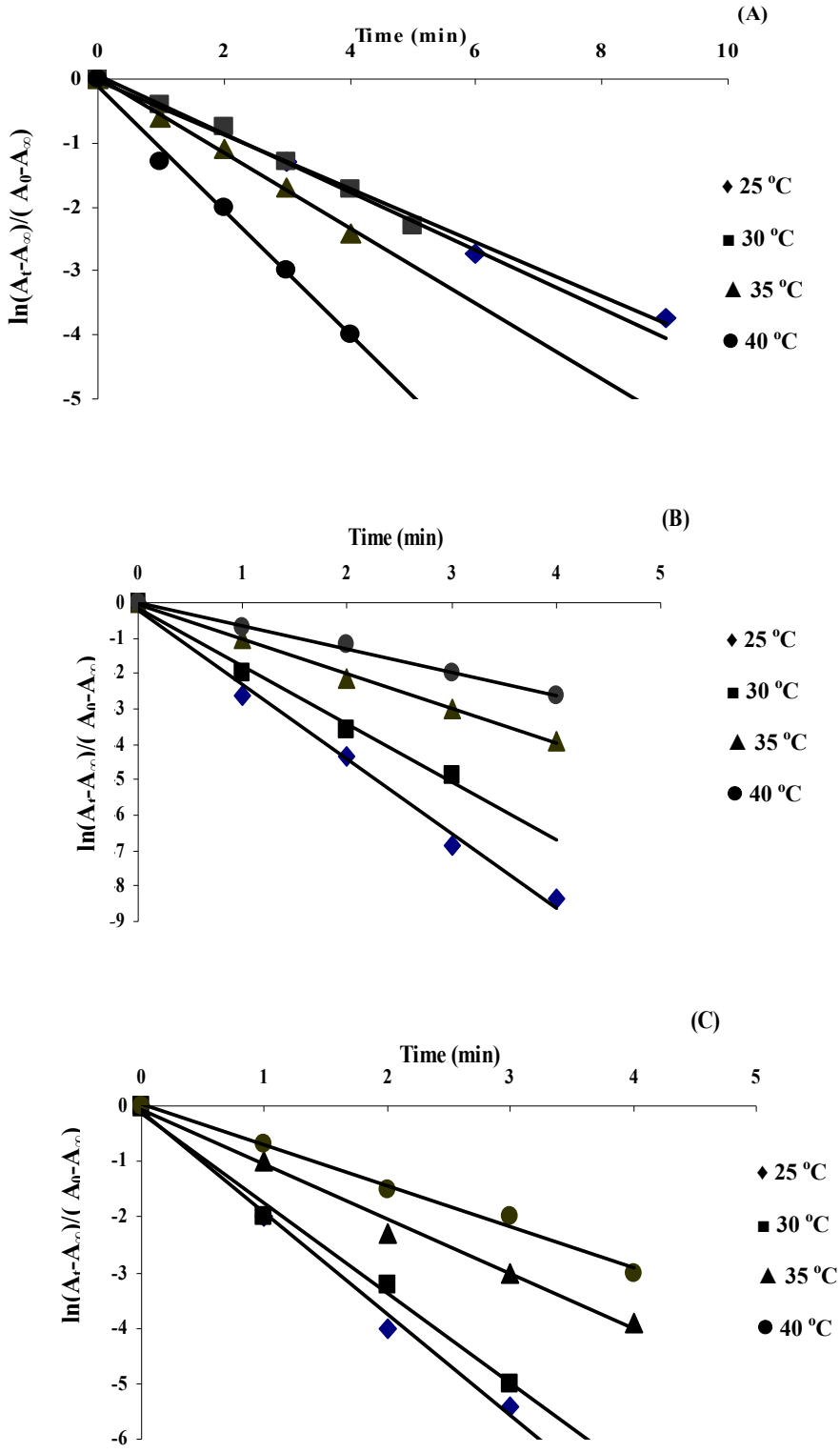

Fig 5. Plots of $\ln(A_t-A_\infty)/(A_0-A_\infty)$ versus time in the presence of (A) Zinc, (B) Vitamin C and (C) mixture of them at various temperatures

Table 1. Rate constants (k_1) for DPPH scavenging in the presence of: Zinc, Vitamin C and mixture of them in different temperatures.

T(°C)	$k_1(\text{min}^{-1})$		
	Vitamin C	Zinc	Mixture of Zinc and Vitamin C
25	2.092	0.4209	1.82
30	1.63	0.4577	1.623
35	0.982	0.59	0.98
40	0.65	0.97	0.73

The activation energy for DPPH scavenging in the presence of Zinc, Vitamin C and mixture of them was calculated from the angular coefficient of the plot of $\ln(k)$ versus 1/T as shown in Fig 6A, B and C. The values found were E_a = 43.14 kJ.mol^{-1} for Zinc, E_a = -59 kJ.mol^{-1} for Vitamin C and E_a = -45.56 kJ.mol^{-1} for mixture of them. Figure 6 B and 6 C show that these radical scavenging reactions have negative activation energies. In general activation energy for most of free radical reaction is negative.

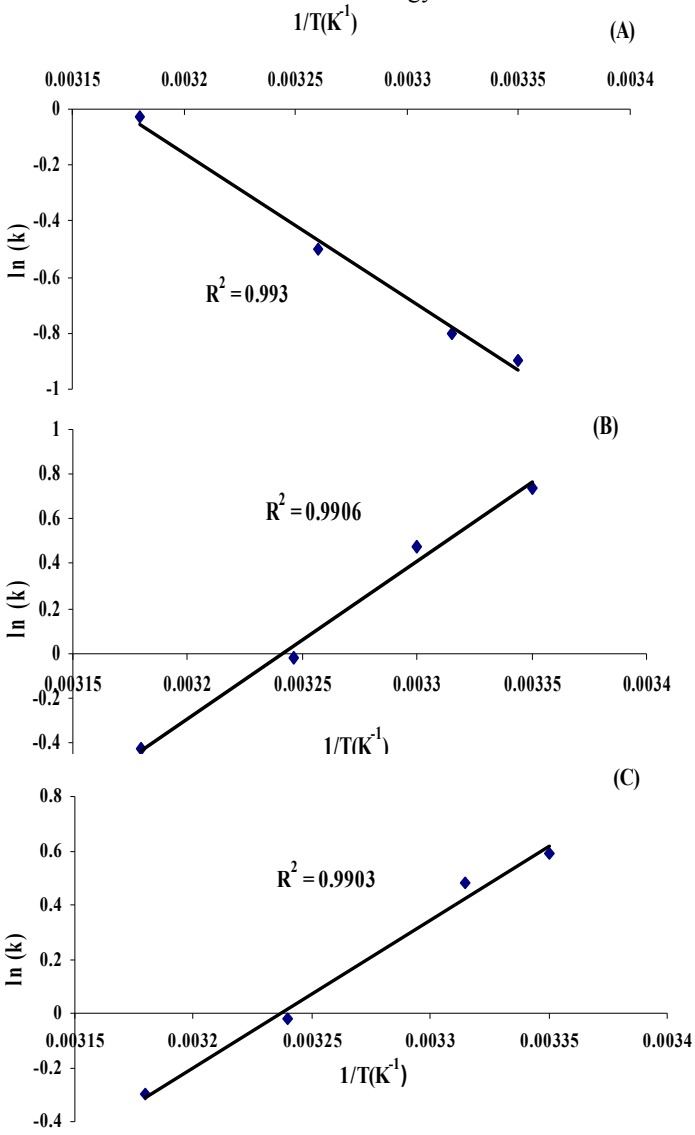

Fig 6. Plots of Arrhenius equation for DPPH scavenging in the presence of (A) Zinc, (B) Vitamin C and (C) mixture of them.

CONCLUSION

In This study free radical-scavenging and antioxidant activities of Zinc, Vitamin C and mixture of them was investigated. The kinetic parameters such as rate constant and activation energy in experimental conditions were calculated. The rate constants of the DPPH scavenging (k_1), in the presence of Zinc, Vitamin C and mixture of them were obtained (0.4209, 2.092 and 1.82 min^{-1}) respectively, under pseudo-first-order conditions at 25 ^oC. The results show that scavenging of DPPH in the presence of vitamin C is larger than zinc and mixture of zinc-vitamin C. Because the LUMO orbital of vitamin C has a low energy. In general, the antioxidant activity of vitamin C decreases in the presence of Zinc. Calculated activation energy show that DPPH scavenging reactions have negative activation energies in the presence of vitamin C and mixture of vitamin C-Zinc. In general activation energy for most of free radical reaction is negative.

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