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Fabrication of an Eficient Antioxidant Capacity Assay Using Peroxidase-mimicking Trivalent DNAzyme

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So far, several methods, including DPPH, FRAP and TEAC have been suggested for considering the antioxidant capacity, each with disadvantages, including the need for expensive tools, low sensitivity, and complexity. One of the most accurate methods is the TEAC method, due to the use of protein enzymes, which possesses disadvantages such as activity in the limited temperature range, and instability against hydrolysates and hard storage conditions. Therefore, antioxidant capacity measurements were performed using the peroxidase-like trivalent deoxyribozyme. The results showed that trivalent deoxyribozyme has more catalytic activity than monomeric deoxyribozyme. Also, kinetic parameters such as k_{cat}, K_m, and V_{max} were calculated in the presence of H_2O_2, which were equal to 4.32 (min^{-1}), 8.744 (μM), and 0.864 (μM min^{-1}), respectively. The results of calculating RAC for the extracts of *Dacrocephalum* and *Black cardamom* plants were estimated to be 28.59 and 11.79, respectively. Also, the limit of detection (LOD) found for trolox, ferulic acid, and caffeic acid was about o.27, 0.14 and 0.28 (μM), respectively, by UV-Vis spectroscopy. The LOD values were 5.0, 2.0 and 2.5 (μM) by the naked eye for the mentioned antioxidants, respectively. These results indicated that antioxidant capacity measurements using the peroxidase-like trivalent deoxyribozyme have advantages such as cheapness, simplicity, observation with naked eye, stability, and high sensitivity to the other methods.

Keywords: Antioxidant capacity, TEAC, Peroxidase-mimicking, Trivalent deoxyribozyme

INTRODUCTION

The human body is continuously affected by oxidative stress, due to disturbing the balance between antioxidants (AOX) and free radicals [1-3]. In recent years, the reactions of free radicals have attracted a lot of attention because of their interference in the degradation of food polymers and the oxidative damage of DNA, proteins, and lipids [4]. The produced free radicals led to the development of diseases such as cancer and cardiovascular diseases, neurological, renal and liver disorders, and autoimmune disease [5-7]. Antioxidants, present in many nutrients, minerals, foods, in fresh or orange fruits, vitamins, and the extracts of some plants, are compounds that can prevent and delay the production of pro-oxidants and radicals, neutralize free radicals and as a result, prevent diseases [8]. Epidemiological studies have shown that people with a diet containing natural AOXs are less at risk for cardiovascular disease and cancers [9]. Therefore, considering the importance of AOXs in counteracting with free radicals, it is essential to measure antioxidant capacity, which may mean the ability to trap free radicals, oxidation inhibitory capacity, or the potential for prevention of disease [10].

Some methods have been developed to measure AOX capacity [11]. The most important of them are 1) DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical inhibitor, 2) FRAP (ferric reducing-antioxidant power) assay, 3) TEAC (trolox equivalent antioxidant capacity) methods [12]. The first method is based on the reducing DPPH by antioxidants in the absence of other free radicals in the environment,

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which results in changing color in an environment that the intensity of the color is measurable with the spectroscopy [1,13]. In the FRAP method, an oxidation/reduction reaction is used, which is accompanied by a change in color [14]. The TEAC method is the measurement of antioxidant capacity by deactivation of ABTS radicals. In the presence of AOX, the amount of $ABTS^{\bullet+}$ radical is reduced and converted to ABTS, and the amount of color reduction will be measured, and the intensity of color decreases [15]. In this assay, trolox was used as a standard. The advantages of the latter method are simple, low cost, higher stability of ABTS radical against pH, and concise response time [16, 17]. However, the TEAC method has some drawbacks such as low stability of peroxidase enzyme. Therefore, alternatives such as DNA enzyme can be used because of its simple structure, facile synthesis, high stability, low cost, and great heat resistance [18-20].

One of the best DNA enzymes is the peroxidase-mimicking DNAzyme (PDZ) [21]. PDZs are compounds with quadruplex DNA and hemin cofactor catalyzing the H_2O_2-mediated oxidation reaction, converting ABTS to ABTS radical, and changing the color of the reaction mixture; just like what occurs in the TEAC method [22]. There are various of PDZ compounds for the detection of various biomolecules and metal ions. So far, a limited studies have been conducted on the use of deoxyribozymes to measure antioxidant capacity [23,24]. The catalytic power of deoxyribozymes is important to improve their performance. The EPR studies showed that the heme proteins and free hemin can react with organic peroxides to split the O-O bond and produce mixtures of organic free radicals. The PS2.M-hemin complex evidently reacted with H_2O_2 to yield an oxidant talented of oxidizing substrates (such as ABTS) in a catalytic reaction. There are some ways to increase the PDZ efficiency, including modifications (imidazole group, dCCC, *etc*.), enzyme immobilization, the presence of some ions such as magnesium and copper, number of catalytic units and the addition of some nucleotide (ATP, GTP, *etc*.) [25-28]. One of the effective ways to increase the activity is using the multivalent DNAzyme consisting of the monomeric DNAzyme and nucleotide linkers. Comparison of the multivalent DNAzyme and the monomer DNAzyme in 2015 showed that the catalytic activity of a multivalent DNAzyme is higher. Also, several studies support the idea that ATP can play a key role in increasing the enzyme's efficiency [24, 29]. In this study, a trivalent deoxyribozyme was used for the antioxidant capacity measurement of standard antioxidant, and herbal extracts in the presence of ATP.

EXPERIMENTAL

Materials and Methods

All materials used in 40KT buffer (MES (2-(N-morpholino) ethanesulfonic acid buffer), Tris-HCl, KCl, Triton X-100), standard antioxidants (trolox, caffeic acid, tannic acid, Chlorogenic acid, L-cysteine, ascorbic acid, quercetin and ferulic acid), deoxyribozyme (ATP, hemin, ABTS) and other solutions (H_2O_2, DMSO, ethanol) were purchased from Sigma-Aldrich. Monomer Catg4 DNAzyme and trivalent deoxyribozyme sequence (5´-TGGGTAGGGCGGGTTGGGAAA-6T-TGGGTAGGGCGGGTTGGGAAA-6T-TGGGTAGGGCGGGTTGGGAAA-3´) were synthesized [29] and purified by Bioneer Company (South Korea). Molar extinction coefficients were detected by using UV-Vis absorption spectroscopy (A and E lab, UV 9000).

Preparation of 40KT Buffer

Materials required to prepare 40KT buffer (pH 6.2) include 50 mM Tris-HCl, 40 mM potassium chloride, 0.05% Triton x-100 and 100 mM MES [21,27].

Preparation of antioxidant solutions and herbal extracts

To evaluate the antioxidant capacity, various concentrations of the standard antioxidants and herbal extracts were prepared. To create the antioxidant stock solutions, the compounds were dissolved in a suitable solvent; trolox (50 μM), tannic acid (10 μM), and ferulic acid (20 μM) in dimethylsulfoxide (DMSO), L-cysteine (50 μM) and ascorbic acid (50 μM) in distilled water and caffeic acid (40 μM), chlorogenic acid (20 μM), quercetin (15 μM) in ethanol. The stock solutions were used for this experiment daily and fresh.

For preparation of herbal extracts (*Seminora*

Suffruticosa, Dracocephalum polychaetum Bornm, Black cardamom, Artemisia sibri and Ducrosia Anthifoli), at first, the required amount of the plant was weighed, and then dissolved in 10 ml of distilled water and stayed for 10 min on a magnetic stirrer and finally centrifuged at 10,000 rpm for 5 min. Lastly, supernatant materials were used to measure antioxidant capacity.

Preparation of Trivalent Deoxyribozyme

The DNA stock solution (500 µM) was placed at 95 °C for 5 min. It was then cooled to the temperature of 25 °C with a one-degree-per-minute process. After reaching the room temperature, it was incubated for 20 min. In the next step, 10 mM 40KT buffer was added to the DNA solution and placed in room temperature for 1 h to form four-strand DNA. Then, ATP (0.5, 1.5 and 1.5 mM) was added to the solution. At the last step, hemin (12.5 µM) was added to the DNA solution (1:1) and incubated at room temperature for 1 h [22].

Investigation of Deoxyribozyme Concentration

Different concentrations of deoxyribozyme (0.0-0.4 µM) (10 µl) were used in the assay volume to obtain the maximum activity of deoxyribozyme. The peroxidase activity of various concentrations of deoxyribozyme was measured in the presence of 0.61 mM ABTS (170 µl), 10 mM 40KT buffer with pH 6.2 (60 µl), and 0.072 mM hydrogen peroxide (10 µl) over 360 s. Absorbance considered in the 418 nm wavelength.

Investigation of Different Concentrations of ABTS and Hydrogen Peroxide

Different concentrations of ABTS (0.0-0.612 mM) and H_2O_2 (0-0.72 mM) were prepared in an assay volume to obtain the optimal concentration in the presence of 0.4 µM trivalent deoxyribozyme, and 10 mM 40KT buffer with pH 6.2, and the results were evaluated by using UV-Vis spectroscopy for 360 s. Also, the Michaelis-Menten and Line weaver-Burk curves were plotted for various concentrations of ABTS and H_2O_2 at 418 nm, and the kinetic parameters of V_{max} and *K*m were also determined. Furthermore, to plot the Michaelis-Menten curve, the absorbance obtained for different concentrations of ABTS and H_2O_2 in assay reactions was converted to velocity (µM min^{-1}) using the following equation:

$$A = \varepsilon CL$$

where A is the absorbance, C represents the substrate concentration, and L represents the length of the cuvette, and ε is molar extinction coefficient (ε_{ABTS} = 36000 M^{-1} cm^{-1}) [30,31]. Samples without any ABTS and hydrogen peroxide were used as control samples.

Measuring the Antioxidant Capacity of the Standard Antioxidant and Herbal Extracts

The absorbance-antioxidant concentration plot was investigated using the method proposed by Jia *et al.* in 2012, with some modification [24]. The antioxidant capacity of various concentrations of individual antioxidant solutions or plant extracts in the presence of 0.4 µM deoxyribozyme (prepared in the presence of 1.5 mM ATP), 0.61 mM ABTS, 0.72 mM hydrogen peroxide, and 10 mM 40KT buffer (pH 6.2) was investigated. After 5 min of incubation, the absorbance of the mixture was measured at 418 nm and subsequently the absorbance-antioxidant concentration plot was fabricated. Similarly, the color of the reaction mixtures was recorded by a digital camera. All determinations were carried out in triplicate.

Calculation of LOD and RAC

Limit of detection (LOD). One of the parameters in describing the system performance is LOD. The lowest level of analyte that can be detected by the biosensor is the lowest detection limit, which is calculated according to the following equation [32]:

$$LOD = (3\delta)/Slope$$

Relative antioxidant capacity (RAC). RAC, an important parameter used to compare the antioxidant capacity, is calculated from the following equation. The higher amount of RAC means a higher antioxidant capacity [24]:

$$RAC = S_{anti}/S_{trolox}$$

RESULTS

The Activity of Trivalent Deoxyribozyme

The results of the active factors in deoxyribozyme activity showed that the maximum adsorption of ABTS radical occurs only in the presence of deoxyribozyme, hydrogen peroxide, and ABTS; while there was no significant absorption in the absence of trivalent deoxyribozyme and hydrogen peroxide (Fig. 1). According to Fig. 1, the trivalent deoxyribozyme exhibited about 1.6 fold activity (at 418 nm) more than monovalent deoxyribozyme at the same concentration.

Activity Investigation of the Laboratory Conditions

To improve the peroxidase mimicking activity, different concentrations of trivalent deoxyribozyme, ABTS and hydrogen peroxide were investigated.

Investigation of the deoxyribozyme concentration. As shown in Fig. 2, the most peroxidation activity of the trivalent deoxyribozyme was observed at a concentration of 0.4 μM, and in this concentration, the curve was linearly absorbed in 418 nm. Also, according to Fig. S1, the maximum absorption of ABTS radical was plotted at different concentrations of the trivalent deoxyribozyme. Consequently, 0.4 μM deoxyribozyme was selected as the optimal concentration for enzyme activity in the presence of hydrogen peroxide and ABTS.

Investigation of the ABTS and H_2O_2 concentrations. As seen in Fig. 3, the most activity of deoxyribozyme was obtained in the presence of 0.61 mM for ABTS (Fig. 3a) and 0.72 mM for H_2O_2 concentration (Fig. 3b). As a result, this concentration was selected as the optimal concentration. Also, the Michaelis-Menten and Line weaver-Burk curves for ABTS (Fig. S2a) and H_2O_2 (Fig. S2b) were plotted at 418 nm, and the *K*m and Vmax parameters were obtained (Table 1).

Comparison of Trivalent and Monovalent Deoxyribozyme Peroxidation Activities in the Presence of ATP

To study the effect of ATP on the enhancement of enzyme activity, at first, monomer and trivalent deoxyribozyme were prepared in the presence of different concentrations of ATP (0.5, 1.5 and 4.5 mM). Then, the activity of trivalent and monomer deoxyribozyme (0.4 μM) was tested in the presence of 0.61 mM ABTS and 0.72 mM hydrogen peroxide (Figs. 4a-d). As shown in Fig. 4c, the peroxidase activity of trivalent and monovalent deoxyribozyme in the presence of 1.5 mM ATP was higher than the peroxidase activity in the presence of 0.5 and 4.5 mM ATP. Also, the catalytic activity of the trivalent deoxyribozyme in the presence of the same concentration of ATP is higher than monomer deoxyribozyme. It should be noted that the peroxidase activity in the presence of a trivalent deoxyribozyme with 1.5 mM ATP has higher stability than that in other experiments. So, it reaches the maximum after 60 s and remains in this mode for up to 650 s, but in other experiments, the color stability is reduced with increasing time (Fig. 4c).

Preparation of the ABTS Radical

As shown in Fig. 5, ABTS did not show any signal of absorption, whereas $ABTS^{\bullet+}$ showed two absorption peaks at 734 and 414 nm. Furthermore, after the addition of antioxidants (Trolox and Caffeic acid), a decrease in the color of $ABTS^{\bullet+}$ was obtained. Results showed that $ABTS^{\bullet+}$ could be scavenged by antioxidant, along with the decolorization of the blue-green color and the reduction of the absorption signal [23,24]. Observing antioxidant capacity at 414 nm results in the greatest change in the signal intensity and the lowest detection limits.

Measurement of Antioxidant Capacity of the Standard Antioxidant

Antioxidant capacity of each standard antioxidants was evaluated in the presence of 0.4 μM deoxyribozyme, 0.61 mM ABTS, 0.72 mM hydrogen peroxide, 1.5 mM ATP and 10 mM 40KT buffer (pH 6.2). As shown in Fig. 6, the dose-response and the linear range were obtained for each one. Also, various parameters, such as RAC and LOD were used for the performance of the system. RAC is an important parameter used to compare the antioxidant capacity of numerous antioxidants. RAC shows the antioxidant capacity of a specific antioxidant relative to a standard antioxidant. In Table 2, the values of the RAC for each antioxidant are given. LOD is the least amount of the analyte that the system can detect. Different values of the

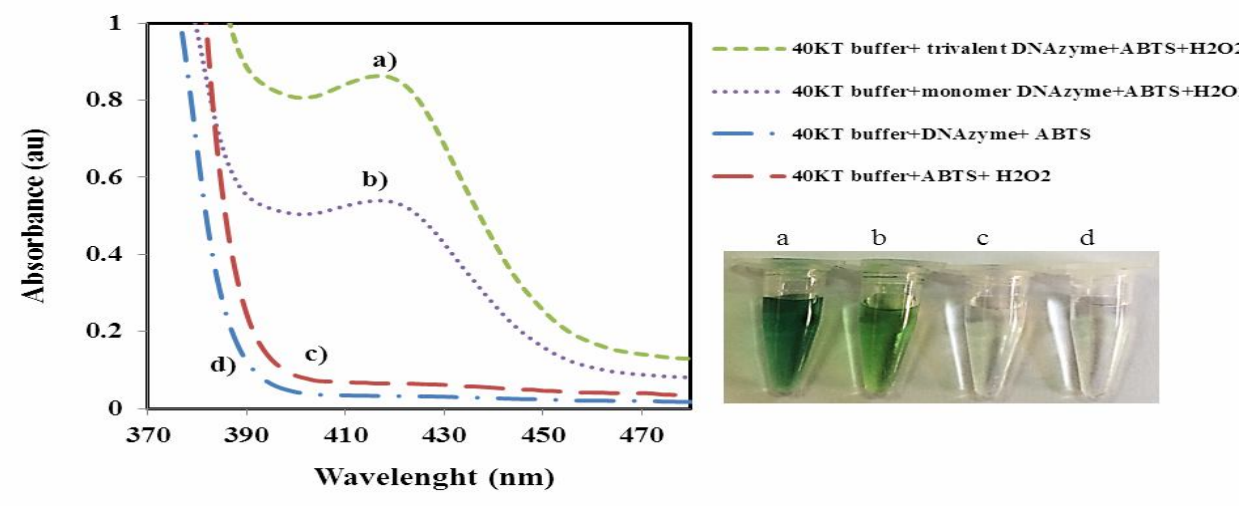

Fig. 1. Investigating the radical absorption spectrum of ABTS in 10 mM 40KT buffer, pH6.2: a) in the presence of 0.61 mM ABTS, 0.4 µM trivalent deoxyribozyme, and 0.72 mM H_2O_2; b) in the presence of 0.61 mM ABTS, 0.4 µM monomer deoxyribozyme and 0.72 mM H_2O_2 c) in the absence of H_2O_2, and d) in the absence of trivalent deoxyribozyme.

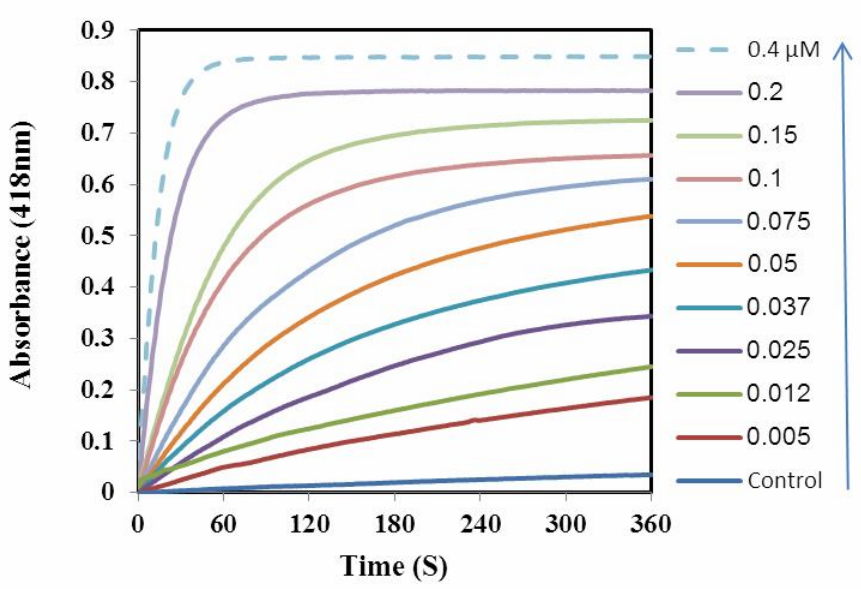

Fig. 2. Optimization of deoxyribozyme concentration in the oxidation reaction of ABTS in the presence of 0.72 mM H_2O_2, 0.61 mM ABTS and 10 mM 40 KT buffer, pH 6.2 in 418 nm for 6 min.

LOD are also in Table 2. LODs are 5.0, 2.0 and 2.5 (µM) by the naked eye for trolox, and ferulic acid and caffeic acid antioxidants, respectively.

Measurement of the Antioxidant Capacity of Herbal Extract

Similar to the standard antioxidants, the antioxidant

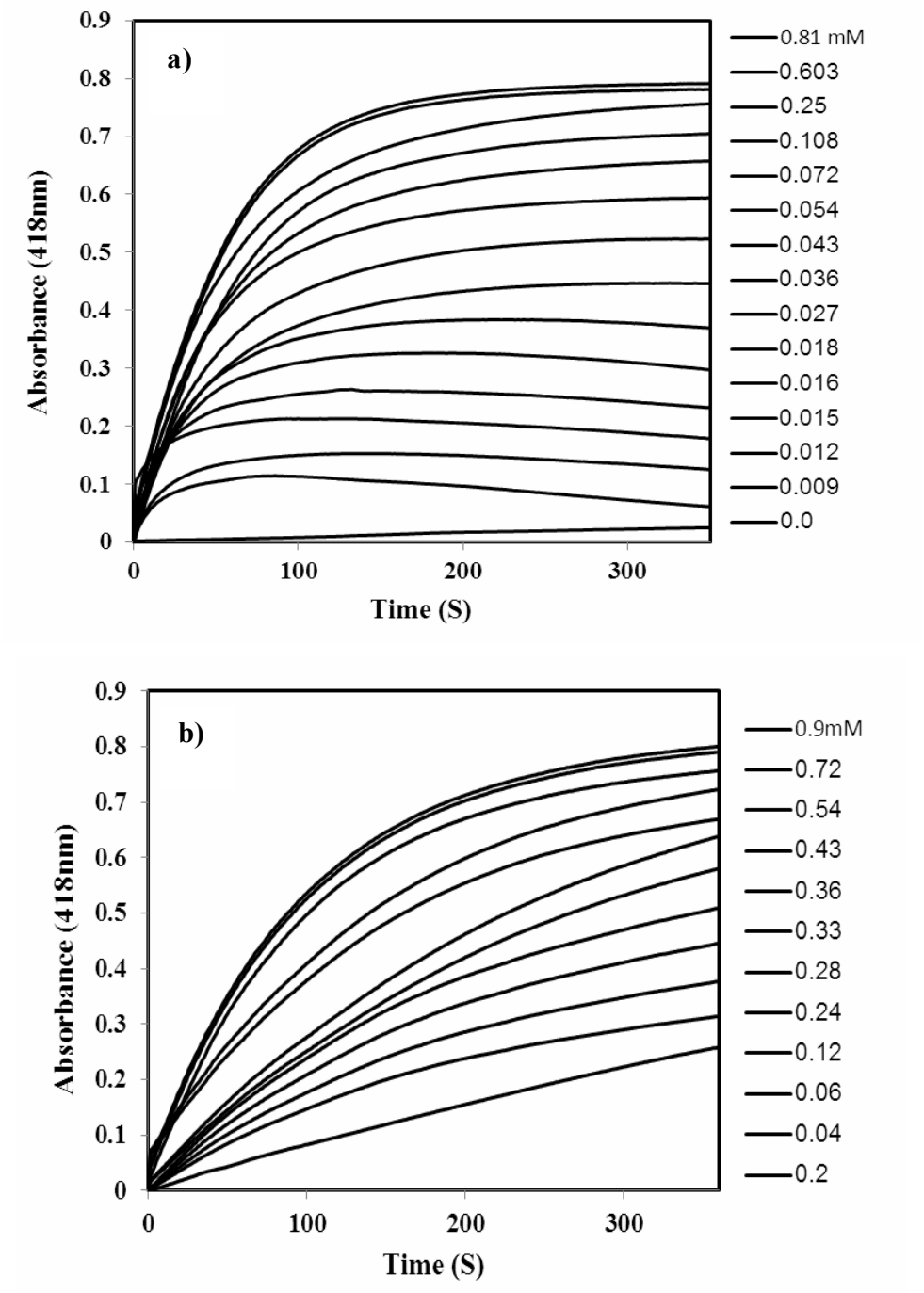

Fig. 3. Investigation of peroxidase activity of trivalent deoxyribozyme at different concentrations of ABTS and H_2O_2. a) Optimization of ABTS concentration in the presence of 0.72 mM H_2O_2, 0.4 µM deoxyribozyme, and 10 mM 40 KT buffer, pH 6.2 in 418 nm for 6 min. b) Optimization of H_2O_2 concentration in the presence of 0.14 mM ABTS, 0.4 µM deoxyribozyme, and 10 mM 40KT buffer, pH 6.2 in 418 nm for 6 min.

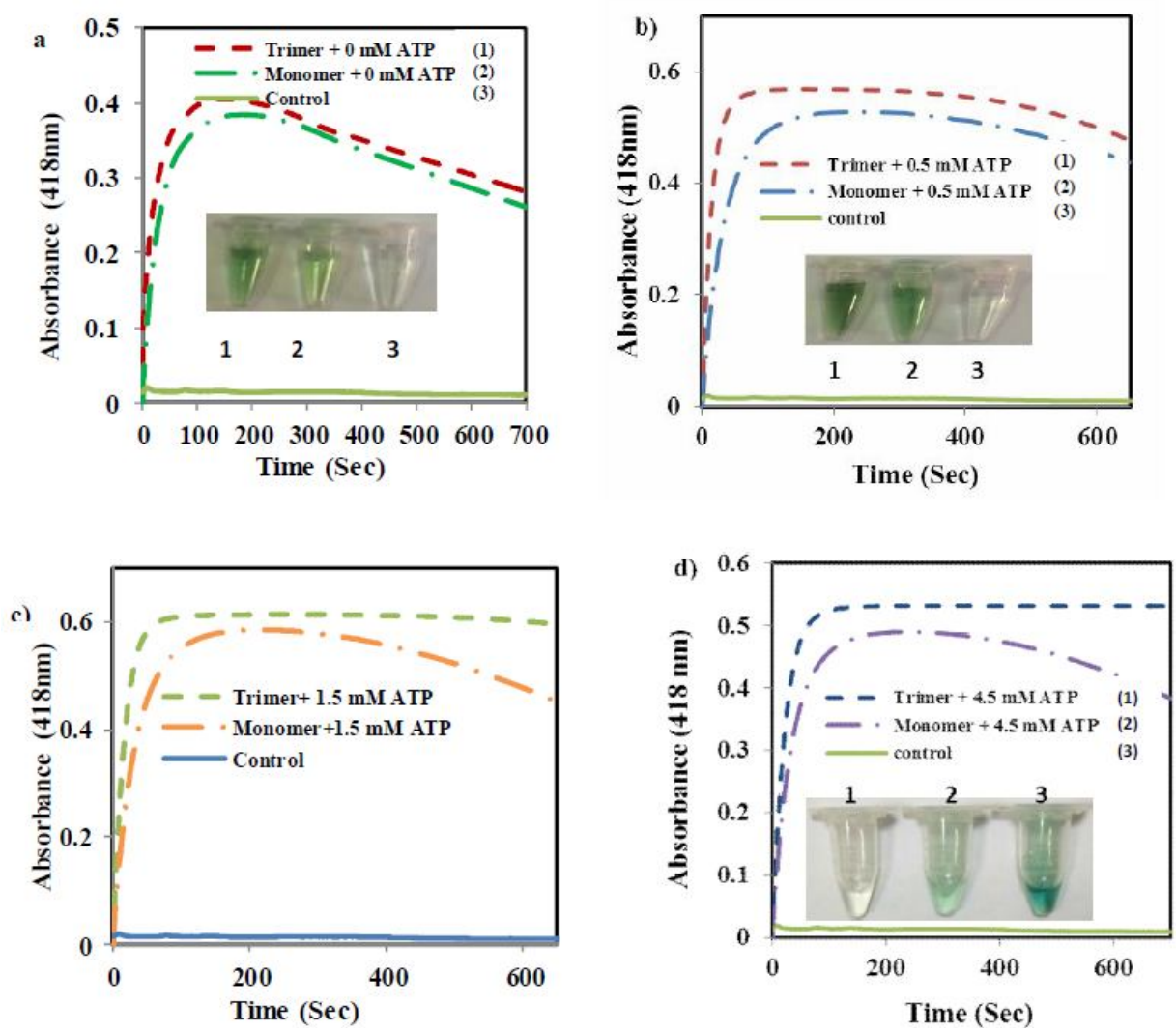

Fig. 4. Comparison of peroxidation activity of 0.4 µM deoxyribozyme in the presence of 10 mM 40KT buffer, pH 6.2, 0.61 mM ABTS and 0.72 mM hydrogen peroxide a) monomer and trivalent deoxiribozyme in the absence of ATP; b) monomer and trivalent deoxiribozyme in the presence of 0.5 mM ATP; c) monomer and trivalent deoxiribozyme in the presence of 1.5 mM ATP; d) monomer and trivalent deoxiribozyme in the presence of 4.5 mM ATP.

capacity of the herbal extracts was also measured in the presence of 10 mM 40KT buffer (pH 6.2), 0.4 µM deoxyribozyme, 0.61 mM ABTS, 0.72 mM hydrogen peroxide, and 1.5 mM ATP. As shown in Fig. 7, the dose-response and the linear range were obtained for each. According to Table 2, LOD and RAC for each extract were also calculated.

DISCUSSION

Because of the importance of free radicals in the pathogenesis of several diseases and human health, it is necessary to use antioxidants preventing the destructive effects of free radicals by inhibiting, trapping and neutralizing [10]. Therefore, measuring antioxidant capacity

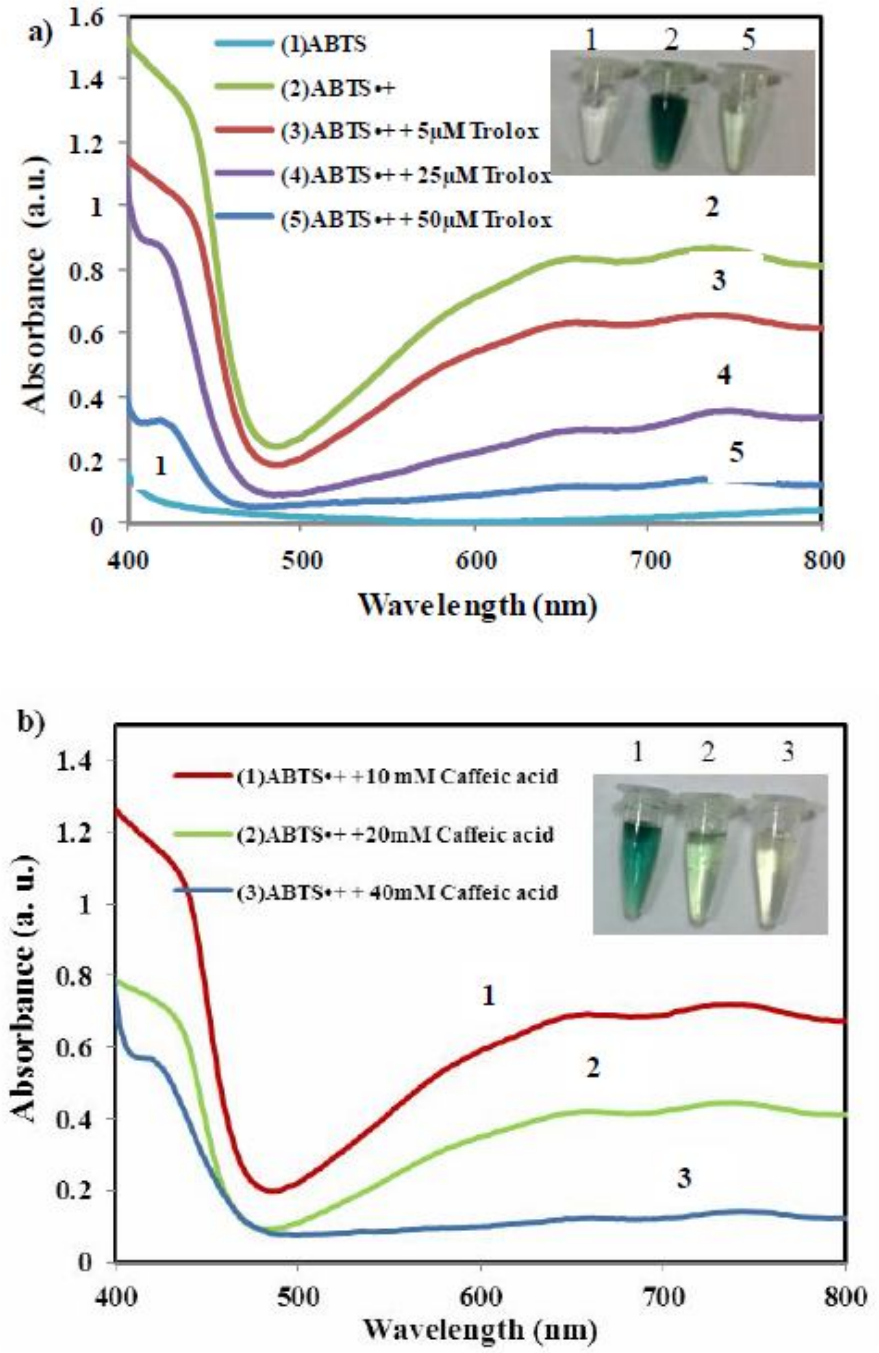

Fig. 5. UV-Vis spectra of ABTS, $ABTS^{\bullet+}$ and $ABTS^{\bullet+}$ with different concentrations of a) Trolox (5, 25, 50 μM), and b) Caffeic acid (10, 20, 40 μM). The inserts show the photographed images of the reaction tubes.

is critical to evaluate the antioxidant function. In this study, the reduction of green color of ABTS radical was used for the antioxidant capacity assay based on the TEAC method. Because of the disadvantages of the peroxidase mimicking enzymes such as temperature sensitivity, hydrolysis, hard storage conditions, and purification, which is commonly used trivalent deoxyribozyme as the appropriate replacement, was used for ABTS cation production [33].

The trivalent deoxyribozyme consists of three units of CatG4 quadruplex/hemin. In this study, the absorbance was in maximum after 5 min of incubation. The same results were also reported by Nikzad and Karami, 2018.

According to Yang *et al.'s* studies, the number of deoxyribozyme units seems to play an important role in the increase of enzyme activity. In other word, multivalent deoxyribozyme is more active than monomer deoxyribozyme, so, trivalent deoxyribozyme was used to measure the antioxidant capacity for increasing the system

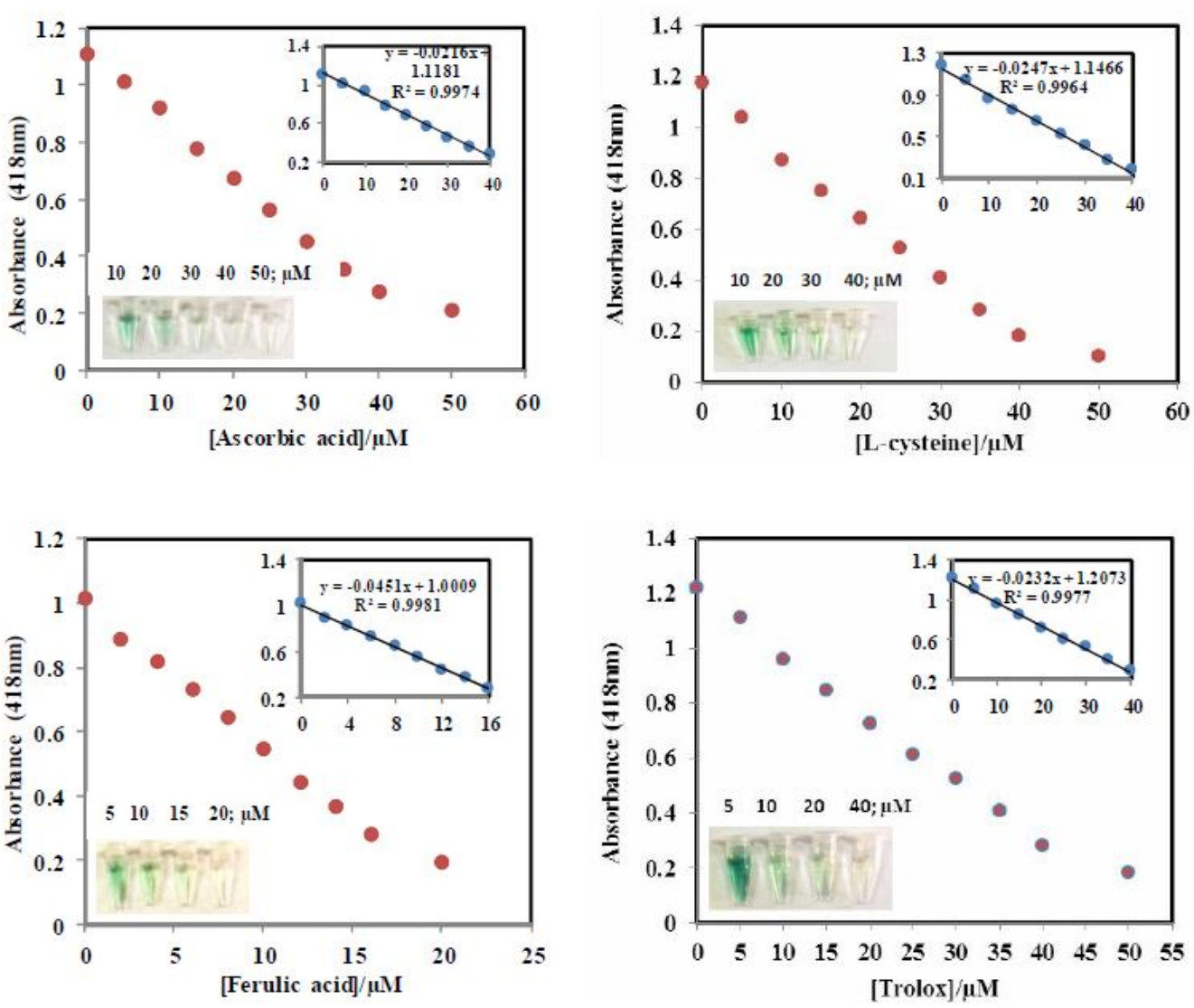

Fig. 6. Standard antioxidant absorption signal of the ABTS radical in the presence of 0.72 mM H_2O_2, 0.61 mM ABTS, 0.4 µM trivalent deoxyribozyme and 10 mM 40KT buffer, pH6.2. All experiments were repeated three times.

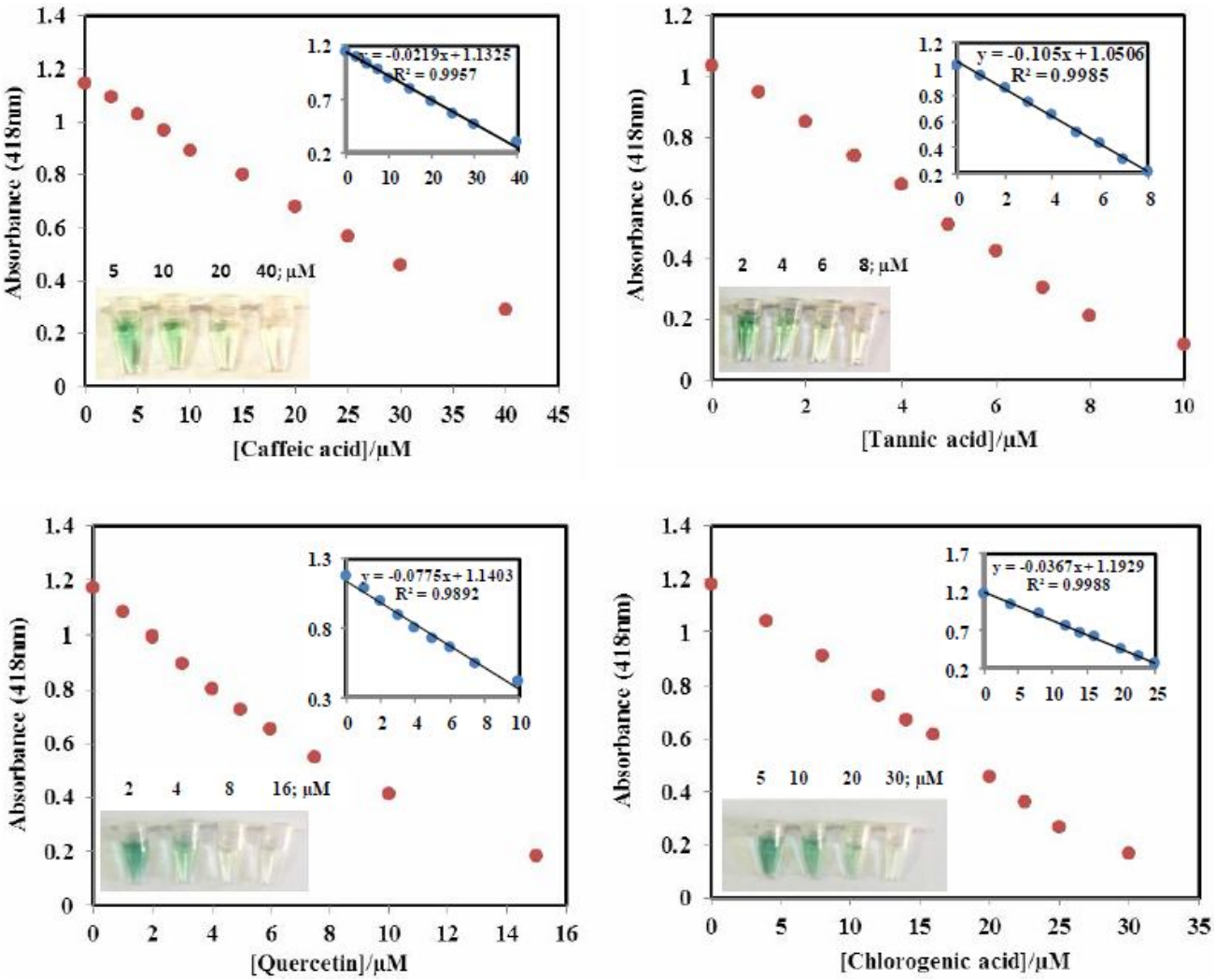

Fig. 6. Continued.

efficiency [29]. Due to the lack of optimization in the prior studies to improve the enzyme efficiency, in this study, we investigated the effects of various concentrations of ABTS and H_2O_2 on the activity of trivalent deoxyribozyme. According to the results of the optimization of ABTS and hydrogen peroxide, the optimal concentrations obtained were 0.61 and 0.72 mM, respectively. In 2012, Jia *et al.* obtained the optimal concentrations of ABTS and hydrogen peroxide at 0.6 and 1.5 mM, respectively [24]. In another report, Yang *et al.* in 2015, showed that the optimal values of ABTS and hydrogen peroxide were 0.5 and 2 mM, respectively [29]. Therefore, the designed system showed the highest activity at the same concentrations of ABTS reported by Jia *et al.* [24].

Another parameter evaluated in this study was ATP which was used for two reasons. The first is to contribute to the stability of ABTS cation, and the second to increase the catalytic activity of the enzyme [24,25,29]. ATP acts as a stabilizing agent both for the radical catalysis produced by the catalyst and for the protection of catalytic components, including the hemin damage caused by hydrogen peroxide [34]. Yang *et al.* showed that four-strand deoxyribozyme is deactivated after the reaction for a certain period, and this deactivation was attributed to the degradation of hemin by

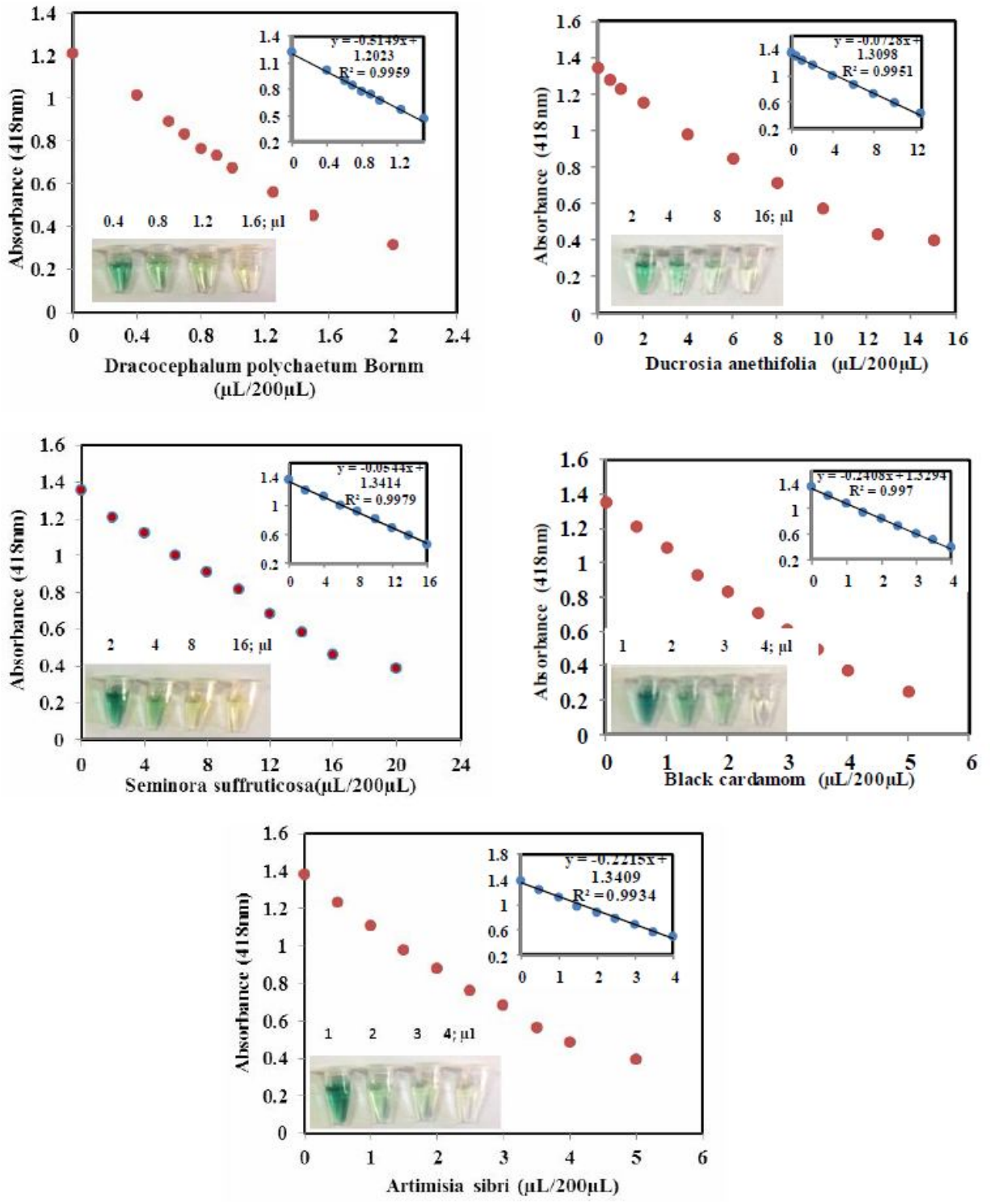

Fig. 7. Herbal extracts absorption signal of the ABTS radical in the presence of 0.72 mM H_2O_2, 0.61 mM ABTS, 0.4 µM trivalent deoxyribozyme and 10 mM 40KT buffer, pH 6.2. All experiments were repeated three times.

hydrogen peroxide. Jia and colleagues also found that the catalytic activity of the four-strand deoxyribozyme is protected in the presence of ATP for a long time [24,35]. According to the results shown in Fig. 4c, after more than 10 min, the color produced by trivalent deoxyribozyme is stable in the presence of 1.5 mM ATP (about 1.3 fold higher than monovalent deoxyribozyme), whereas the activity of monovalent deoxyribozyme begins to decrease after about 5 min of reaction time.

Therefore, due to the importance of the presence of ATP in the development of reaction and catalytic activity enhancement, the ATP concentration was investigated. Compared to previous reports, the concentration of ATP was obtained 1.5 mM, which is more cost-effective. For example, the optimal ATP concentration, reported by Jia *et al.*, was 2 mM. Yang *et al.,* in 2015, found that the high concentrations of ATP resulted in the loss of multivalent deoxyribozyme properties, because high levels of ATP cause aggression of hemin and consequently reduce the effectiveness of the hydrogen peroxide catalyst [24,29]. Also, the results showed that the catalytic activity of trivalent deoxiribozyme in the presence of ATP is higher than that of single-unit deoxyribozyme (Fig. 4).

Due to the use of trivalent deoxyribozyme as an enzyme, calculation of kinetic parameters, including V_{max} and K_m, is necessary to better understand the enzyme's behavior. V_{max} represents the maximum rate of the enzyme or the rate at which the active sites of all enzyme units are saturated with the substrate. K_m is a measure of the enzyme tendency to the substrate [36]. In this study, the kinetic parameters of V_{max} and K_m were obtained 0.857 μM min^{-1} and 9.41 μM, respectively (Table 1). The less K_m value indicates that the enzyme is active at lower concentrations of the substrate as obtained in this study.

After optimizing the factors affecting the activity of the enzyme, such as the optimal concentration of ABTS, H_2O_2, and deoxyribozyme, the parameters involved in the antioxidant capacity assay were calculated. Initially, the dose-response of the system was investigated. According to our results, the system can detect a higher concentration range of antioxidants. The results showed that the wide response range, in this system varies from 2 to 10 units in comparison with prior studies. In 2010, Wang *et al.* evaluated the detection range of caffeic acid at a concentration of 0.0-10 μM while they could not detect the higher concentrations [23]. In a similar study, Jia *et al*. in 2012, detected caffeic acid in the range of 0.0-30 μM [24]; while, the system designed in this study has the potential to measure the antioxidant capacity of caffeic acid in the range of 0.0-40 μM, indicating the response of the system to higher antioxidant doses. Also, the dose-response obtained for ascorbic acid, chlorogenic acid, ferulic acid, trolox, L-cysteine, and quercetin (25, 15, 12, 20, 25 and 9 units, respectively) were higher than those reported in other studies.

In the next step, the RAC parameter was evaluated to compare the antioxidant capacity. RAC is an important parameter used to compare the antioxidant capacity of various antioxidants. RAC indicates the antioxidant capacity of a specific antioxidant relative to a standard antioxidant [24]. While the RAC parameter for chlorogenic acid, reported by Jia *et al.,* was about 2.12, this parameter has been 1.75 in the present study, , indicating a significant increase in the antioxidant capacity of chlorogenic acid (Table 2). The results of this project indicate that RAC values for *Dacrocephalum* and *Black cardamom* plants were calculated to be 28.59 and 11.79, respectively. In 2012, Jia *et al.* obtained RAC for *Peach* and *Lemon juice*, 1.02 and 2.61, respectively [24].

The limit of detection, LOD, was the last parameter calculated in this study. The LOD is the lowest amount of analyte that can be detected by the system [32]. According to the results observed, and compared with other reports, this system was more sensitive and capable to detect a lower limit of the desired analyte. For example, in 2012, Jia *et al.* calculated the LOD for trolox, caffeic acid, and ferulic acid about 0.88, 0.70 and 0.42 μM, respectively [24], while in the present study, the LOD was detected for these antioxidants about 0.27 μM (about 3.5 times less), 0.28 μM (2.5 times less), and 0.14 μM (3 times less), respectively (Table 2).

CONCLUSIONS

Antioxidants are important factors in the elimination of free radicals, which are a critical part of health preventing pathogens. So, it is very noteworthy to know how diverse antioxidants are to the extent that they can neutralize free

Table 1. Kinetic Parameters of Trivalent Deoxyribozyme in the Presence of Different Concentration of ABTS and H_2O_2

Substrate	V_{max} ($\mu M\ min^{-1}$)	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($\mu M^{-1}\ min^{-1}$)
ABTS	3.295	355.0	8.23	0.023
H_2O_2	0.857	9.41	2.14	0.227

Table 2. RAC, LOD, Linear Range and Regression Equation Parameters

Compound/Sample	Regression equation	Linear range (μM)	LOD[a] (μM)	LOD (μM) (Naked eyes)	RAC[b]	Reported RAC
Trolox	-0.0232x + 1.2073	0-40	0.27	5.0	1.0 ± 0.02	1.0
Ferulic acid	-0.0451x + 1.0009	0-16	0.14	2.0	2.04 ± 0.01	2.10
Caffeic acid	-0.0219x + 1.1325	0-40	0.28	2.5	1.23 ± 0.06	1.26
Quercetin	-0.0775x + 1.1403	0-10	0.15	1.0	3.93 ± 0.04	4.45
Chlorogenic acid	-0.0367x + 1.1929	0-30	0.30	4.0	2.02 ± 0.02	1.75
Ascorbic acid	-0.0216x + 1.1181	0-35	0.40	5.0	1.10 ± 0.08	1.01
L-cysteine	-0.0247x + 1.1466	0-40	0.33	5.0	1.01 ± 0.05	1.16
Tannic acid	-0.105x + 1.0506	0-8	0.13	1.0	5.49 ± 0.10	-
Seminora suffroticosa	-0.0544x + 1.3414	0-16	-	-	2.82 ± 0.03	-
Dacrocephalum	-0.5149x + 1.2023	0-1.5	-	-	28.59 ± 0.06	-
Black cardamom	-0.2408x + 1.3294	0-4	-	-	11.79 ± 0.02	-
Artemisia sibri	-0.2215x + 1.3409	0-4	-	-	11.80 ± 0.07	-
Docrosia antipholia	-0.0728x + 1.3098	0-12	-	-	4.22 ± 0.04	-

[a]The lowest level of analyte that the biosensor system is capable of detecting is the lowest detection limit. [b]The higher amount of RAC means a higher antioxidant capacity and calculated as, RAC = Santi/Strolox.

radicals, and are known as antioxidant capacity. Therefore, the antioxidant capacity measurements have been performed using different methods such as DPPH, FRAP and TEAC, though, most of these methods are not desirable due to disadvantages such as high costs, complexity, and the use of protein enzymes. In this project, TEAC-based studies were performed on trivalent deoxyribozyme to measure the antioxidant capacity. Given the data and the results obtained, it can be concluded that the system designed in this study presents many advantages including simplicity,

sensitivity, cost-effectiveness, and stability against environmental factors.

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