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ORIGINAL ARTICLE

Colorimetric determination of radical scavenging activity of antioxidants using Fe_3O_4 magnetic nanoparticles

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Abstract We report the first use of iron oxide magnetic nanoparticles (Fe_3O_4 MNPs) as a novel, alternative, simple and reliable agents for colorimetric measurement of radical scavenging activity of the antioxidants. In the presence of H_2O_2 and the peroxidase colorimetric substrate, Fe_3O_4 MNPs catalyzed the oxidation of colorless peroxidase substrate to form colorimetric products via the generation of hydroxyl radicals. After adding antioxidants, the catalytic activity of Fe_3O_4 MNPs was inhibited due to scavenging of hydroxyl radicals by the antioxidants, producing less colorimetric products resulting in the reduction of color intensity. Two model antioxidant standards including gallic acid (GA) and epigallocatechin gallate (EGCG) were successfully evaluated for their hydroxyl radical scavenging activity using the developed assay. The performance of the developed method was validated against traditional antioxidant assays for 9 tea samples. Using the Spearman rank correlation coefficient method, the antioxidant activity of tea samples obtained from the Fe_3O_4 MNP assay correlated well with the traditional assays at the 95% confidence level. Furthermore, the developed assay was successfully carried out on a paper-based device to provide for high throughput analysis with low amounts of reagents and sample consumption and low analysis cost for screening of radical scavenging activity of the antioxidants. The results from the analysis of antioxidant activity in tea samples obtained from the Fe_3O_4 MNP paper-based assay were not significantly different to those obtained from the developed Fe_3O_4 MNP spectrophotometric assay indicating that the developed assay was also applicable in a low-cost analysis platform.

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1. Introduction

Free radicals commonly produced in metabolism processes in the body are highly reactive towards other biological molecules and can cause the oxidative damage to the affected area resulting in many diseases such as cancer, dermatitis, heart attack, cardiovascular disease and Alzheimer's disease (Valko et al., 2007; Lobo et al., 2010). The reactivity of free radicals as well as reactive oxygen species (ROS) such as hydroxyl radicals (HO), superoxide anion (O_2^-) and hypochlorous acid (HOCl)) can be quenched or removed by antioxidants which are both naturally found in vegetables, tea and fruits as well as synthetic chemicals and nanoparticles (Pham-Huy et al., 2008; Hamid et al., 2010; Hu et al., 2020; Li et al., 2020; Li et al., 2020). Antioxidants have been recommended to consume to prevent the diseases and also been used as conventional additives in food products, cosmetics as well as dietary supplements. Therefore, suitable methods for determining the activity of antioxidants, especially their free radical scavenging activity, are strongly required. Several methods have been developed to determine antioxidant radical scavenging ability including electron spin resonance (Peng et al., 2009; Zang et al., 2017), electrochemical sensors (Prieto-Simón et al., 2008; Oliveira et al., 2016), and spectrophotometric assays (Sánchez-Moreno, 2002; Marinova and Batchvarov, 2011; Naguib, 1998; Naguib, 2000). Of these methods, spectrophotometric methods using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay (Kedare and Singh, 2011; Xie and Schaich, 2014), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay (Re et al., 1999; Thaipong et al., 2006) and oxygen radical absorbance capacity (ORAC) assay (Ou et al., 2002; Zulueta et al., 2009) have been the most widely used. However, they have some disadvantages. The DPPH and ABTS assays rely on the scavenging activity of antioxidants toward the synthetic free radicals, DPPH and $ABTS^+$ radicals, which are not commonly found in natural systems. The ORAC assay requires relatively complicated instruments such as fluorescence spectrophotometers for detection. Therefore, development of an alternative, novel, simple and reliable method to evaluate antioxidant radical scavenging activity is still necessary.

Iron oxide magnetic nanoparticles (Fe_3O_4 MNP) are well-known and widely used nanozymes (defined as nanomaterials that have enzyme-like characteristics) since they exhibit both magnetic and peroxidase-like properties to induce the production of hydroxyl radicals (HO) from H_2O_2 substrate (Zhang et al., 2008; Wang et al., 2010; Chen et al., 2012) and the generated HO Can further oxidize a chromogenic substrate to cause the color change (Gao et al., 2007). Fe_3O_4 MNPs, therefore, have been widely demonstrated as novel sensing platforms including H_2O_2 detection (Chang et al., 2009; Gao et al., 2011; Jiang et al., 2011), glucose detection (Wei and Wang, 2008; Yu et al., 2010; Dong et al., 2012), DNA detection (Park et al., 2011), aptasensors (Zhang et al., 2010; Zhang et al., 2011) as well as immunoassays (Gao et al., 2007; Gao et al., 2008; Kaittanis et al., 2009). The analysis in these applications were based on the direct catalytic oxidation of H_2O_2 (produced from the reaction of target analytes by the oxidase enzymes) by the Fe_3O_4 MNPs to generate HO that further trigger the color reaction of a chromogenic substrate. By monitoring the increased color, the amount of the analyte can be evaluated. However, to the best of our knowledge, there is no report for the application of Fe_3O_4 MNPs for antioxidant evaluation where the analysis is based on the quenching of HO by the antioxidants.

This work presents, for the first time, an alternative, novel, reliable, simple assay for the colorimetric evaluation of antioxidant radical scavenging activity using Fe_3O_4 MNPs. The analysis is based on the quenching ability of the antioxidants to the HO , a free radical found naturally, that was produced from the catalytic reaction of Fe_3O_4 MNPs and H_2O_2. In an absence of antioxidants, H_2O_2 is catalytically oxidized by Fe_3O_4 MNPs and produced HO to reactively oxidize the substrate such as 3,3′,5,5′-tetramethylbenzidine (TMB) or *o*-Phenylenediamine (OPD) to change the color from colorless to blue or yellow, respectively (Fig. 1A). In the presence of antioxidants, the HO are captured resulting in lower number of the species to react with the peroxidase substrate and hence, decreasing the color intensity (Fig. 1B). By recording the suppressed color, the analysis of oxidation resistance of antioxidants can be obtained. Here, gallic acid (GA) and epigallocatechin gallate (EGCG) were employed as model antioxidant standards for a proof of concept. Firstly, colorimetric detection was carried out using UV–Vis spectrophotometer for studying the developed antioxidant assay. The inhibition rate of antioxidant to the oxidative catalytic activity of Fe_3O_4 MNP was found to depend on the antioxidant concentration and antioxidant types. The method has high tolerance to the potential interferences normally found in tea samples. The performance of the developed method was validated against traditional antioxidant assays and the results showed that the antioxidant activity of tea samples obtained from the Fe_3O_4 MNP assay correlated well with the traditional assays at the 95% confidence level. Finally, the assay was further carried out on the paper-based device used as a detection platform where the colorimetric detection was performed by digital camera and imaging software to provide for simple, low-reagent consumption, low-cost and high-throughput analysis of antioxidant radical scavenging activity.

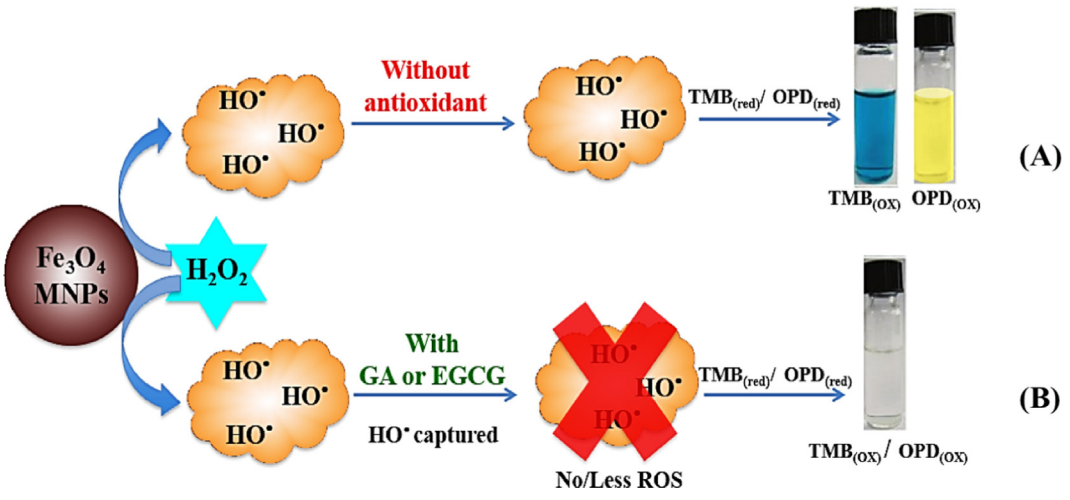

Fig. 1 The principle of the analysis of radical scavenging activity of antioxidants using Fe_3O_4 MNPs (A) without antioxidant (B) with antioxidants.

2. Materials and methods

2.1. Chemicals and instrumentations

Unless otherwise stated, all chemicals were purchased from Sigma Aldrich (Singapore). The 15–20 nm Fe_3O_4 MNPs water dispersion (20 wt%) was obtained from US Research Nanomaterials, Inc. (Houston, Texas, USA). Hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), glucose, galactose, fructose, surcrose and DL-tartaric acid were purchased from Merck (Germany). Oxalic acid was obtained from Carlo Erba Reagents (Bangkok, Thailand). Epigallocatechin gallate (EGCG), disodium L-(+)- tartrate dehydrate was purchased from TCI Chemicals (Japan). DL-Alanine was obtained from Himedia laboratories LLC (USA). Glycine was from Omni-Pur. Tea samples were purchased from local markets in Chon buri, Thailand. The UV–VIS spectrophotometer for colorimetric measurement was Analytik Jena 210 spectrophotometer. Xerox wax printer ColorQube 8870–13 (Malaysia) was employed for the wax printing process used to fabricate a paper-based device. Filter paper Whatman™ No.4 was purchased from GE Healthcare (China).

2.2. Antioxidant activity measurement using Fe_3O_4 MNPs and UV–Vis spectrophotometry

The general procedure for analysis of antioxidant activity using Fe_3O_4 MNPs with UV–Vis spectrophotometric detection is as follows. Two antioxidant standards including gallic acid and EGCG were employed to demonstrate the radical scavenging activity measurement obtained from the Fe_3O_4 MNP assay. The Fe_3O_4 MNP (400 μg/mL, 20 μL) suspension was mixed with TMB (20 mM, 20 μL) and acetate buffer pH 3.7 (200 mM, 1170 μL). 50 μL of a specific concentration of an antioxidant standard/sample (0–20 mM gallic acid, 0–500 μM EGCG) was then added to the mixture followed by 20 μL of 10 mM H_2O_2. The mixture was allowed to react in the dark for a desired time interval and the absorbance was measured by a UV–Vis spectrophotometer at 654 nm. A graph of the relative absorbance (A_t-A_0 where A_t and A_0 are absorbance at reaction time of 0 min and t min, respectively) versus the time (min) was used to evaluate the quenching kinetic of the antioxidant on the HO produced from catalytic reaction of Fe_3O_4 MNPs and H_2O_2. The two-point fixed time method was then employed to generate the antioxidant standard curve which was the plot of differences in the relative absorbance at two reaction times as a function of antioxidant concentrations. The linear range, reproducibility and limit of detection from the analysis of the two model antioxidant standards were then evaluated.

For assay optimization to evaluate suitable Fe_3O_4 MNP concentration and reaction time, the procedure was similar to the method described above excepted that no antioxidant standard was added to the assay.

2.3. Traditional antioxidant assays

2.3.1. DPPH assay

DPPH assay was performed according to the method described previously with some modifications (Blois, 1958). The 2 mM DPPH solution was prepared by dissolving DPPH in absolute ethanol and kept in the dark. Then, 10 μL of sample or GA standard (0–2 mM GA) was added. Next, 2 mM DPPH (100 μL) and methanol (3000 μL) were added. The mixture was incubated at ambient temperature for 30 min and the absorbance measured at 515 nm. The analysis was performed in three replicates. Finally, the antioxidant activity expressed as GAE of each sample was calculated.

2.3.2. FRAP assay

FRAP assay was performed in a similar manner to the method described previously (Benzie and Strain, 1996). The stock solutions including 300 mM acetate buffer pH 3, 10 mM of 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3{\cdot}6H_2O$ were prepared. FRAP reagent was freshly prepared by mixing solutions of acetate buffer with TPTZ and $FeCl_3{\cdot}6H_2O$ in a ratio of 10: 1: 1 by volume and then warming at 37 °C before use. Then, 50 μL of GA standard (0–4 mM) or sample was mixed with 150 μL of deionized water and 1500 μL of the FRAP reagent. The mixture was shaken and incubated at ambient temperature for 4 min. The absorbance was measured at 593 nm. Finally, the antioxidant activity of each samples expressed as GAE was calculated.

2.4. Paper-based Fe_3O_4 MNP antioxidant assay

The Fe_3O_4 MNP antioxidant assay was also performed on the paper-based detection platform to evaluate the possibility of the developed assay to be carried out in a low-cost platform. The paper-based device was designed using the Adobe Illustrator CS5 program to have a 5-mm diameter circular test zones. The device was fabricated using a wax-printing technique (Carrilho et al., 2009). For antioxidant activity analysis on the paper-based device, 2 μL of 400 μg/mL Fe_3O_4 NPs was deposited onto the device. After dried, 2 μL of 40 mM TMB and 3 μL of GA antioxidant standard (0–35 μM) or tea samples were added. Finally, 20 μL of 100 mM H_2O_2 in 200 mM acetate buffer pH 3.7 was added. The mixture was allowed to react in the dark for 30 min. The colorimetric detection on the paper-based device was performed using a light box and camera and the color intensity was measured according to the method described previously (Adkins et al., 2017). A graph of color intensity versus GA concentration was used to determine the linear range of the analysis. Antioxidant activity of the samples was presented in units of gallic acid equivalent (GAE).

2.5. Preparation of tea samples

Tea samples were obtained from local markets in Chon buri, Thailand. The extraction process was carried out according to the method described previously (Piyanan et al., 2018). A 2 g of commercial tea was heated in 200 mL deionized water at 80 °C for 5 min. The tea solutions were filtered using Whatman No.1 filter paper. The solution was allowed to cool to ambient temperature prior to analysis for antioxidant activity using the Fe_3O_4 MNP assay and the traditional antioxidant assays including DPPH and FRAP assays. The gallic acid was used as an antioxidant standard to construct the standard curve for all assays and the antioxidant activities of the sam-

ples were measured, expressed and compared as gallic acid equivalent (GAE, μmolGA/g samples) values.

3. Results and discussion

3.1. Assay optimization

In the Fe_3O_4 MNP-based assay developed here, the antioxidant activity was measured based on the ability of the antioxidant to quench the HO radicals generated from the catalytic reaction of Fe_3O_4 MNPs and H_2O_2. Therefore, the initial concentration of Fe_3O_4 MNPs played important role for assay sensitive-response analysis of antioxidant radical scavenging activity and was first investigated. A mixture of investigated concentration of Fe_3O_4 MNPs, substrate (TMB or OPD), acetate buffer pH 3.7 and H_2O_2 without the addition of antioxidants was performed to optimize the catalytic assay. Since the Fe_3O_4 MNP solution at high concentration has background color, the control which is a mixture of Fe_3O_4 MNPs solution at each concentration with all reagents excepted H_2O_2 was also measured and used for background subtraction. As shown in the spectra (Figure S1A and S1C, Supplementary Information), the absorbance at 654 and 450 nm as corresponded to the TMB and OPD substrates, respectively, increased as the concentration of Fe_3O_4 MNPs increased. This was a result of increasing peroxidase-like activity of the increasing concentration of Fe_3O_4 MNPs. However, the control signal increased when Fe_3O_4 MNPs concentration increased as the nanoparticles have their own background color. Therefore, when the background-subtracted absorbance as a function of Fe_3O_4 MNPs concentration was plotted, the signal increased as the Fe_3O_4 MNPs concentration increased in the range of 50–400 μg/mL (Figure S1B and S1D, Supplementary Information). The signal dropped when the nanoparticle concentration higher than 400 μg/mL since the background color was high. Therefore, the Fe_3O_4 MNPs concentration of 400 μg/mL was considered as the optimal value to allow for sensitive-response analysis of antioxidant activity and used for further experiments. These results demonstrated that it is possible to measure antioxidant activity that quench the Fe_3O_4 MNP catalytic activity in a dose–response manner.

Since the developed antioxidant assay is based on catalytic reaction, optimal reaction time and suitable kinetic method of analysis has been investigated. Assay reaction time was therefore studied in the range of 0–120 min using the similar mixture described for Fe_3O_4 MNP concentration optimization. The absorbance of the mixture recorded at 654 nm (Figure S2A, Supplementary Information) and 450 nm (Figure S2B, Supplementary Information) using TMB and OPD as substrates, respectively, increased linearly as the reaction time increased. These were the results of continuous catalytic oxidation of unlimited concentration of substrates (H_2O_2 and TMB/OPD) by Fe_3O_4 MNP. Therefore, to analyze the antioxidant radical scavenging activity using the developed method, the kinetic method of analysis was employed and carried out using two-point fixed time method (Pardue, 1977). The two fixed points of absorbance measurement are at 8 and 10 min reaction time for gallic acid and at 2 and 4 min reaction time for EGCG.

3.2. Analysis of antioxidant standards

Once the optimized conditions were obtained, model antioxidant standards including gallic acid (GA) and epigallocatechin gallate (EGCG) were analyzed and studied for their quenching ability on the HO generated from peroxidase-like catalytic activity of Fe_3O_4 MNPs to H_2O_2. GA is a natural occurring low molecular weight triphenolic compound found in gallnuts, grapes, hops, oak bark and tea which acts as a strong antioxidant and an efficient apoptosis of cancer cells and also helps to protect human cells against oxidative damage (Daneshfar et al., 2008). EGCG is a phenolic antioxidant found in a number of plants such as green and black tea which has been demonstrated to possess strong free radical scavenging activity (Wang et al., 2016). Without antioxidants, color development in the reaction between H_2O_2 and the colorimetric substrates such as TMB and OPD catalyzed by Fe_3O_4 MNPs appears as intense blue and yellow, respectively (Fig. 2A). On addition of either GA or EGCG, the color intensity decreased compared to the control demonstrating the quenching ability of the antioxidants to the assay. Even testing at lower concentration, EGCG (150 μM) was more efficient than GA (600 μM) in inhibiting the catalytic oxidation of TMB or OPD due to higher number of phenolic rings in its structure to scavenge the HO produced from the assay (Figure S3, Supplementary information).

The quenching kinetics of the two antioxidants on the HO Produced from the peroxidase-like activity of Fe_3O_4 MNPs were demonstrated by recording the relative absorbance of the colorimetric products over time. The slopes of the kinetic plot (relative absorbance vs time) decreased as the concentrations of the two model antioxidant standards increased (Fig. 2B and 2D). This result demonstrated that the HO generated from the catalytic activity of Fe_3O_4 MNPs toward the oxidation of H_2O_2 was quenched and found to depend on the concentration of antioxidant. The two-point fixed time method was then employed to construct the calibration curve of the two model antioxidant standards which was the plot of absorbance differences at two reaction times as a function of antioxidant concentrations given in Fig. 2C and 2E, respectively. The linear range of GA and EGCG were 0–1000 μM and 0–150 μM, respectively. The linear range of GA obtained by the developed method was similar to that obtained from ABTS, DPPH and free radical method reported previously which were 5–100 μM, 0–500 μM and 5–5000 μM, respectively (Sochor et al., 2010; Sirivibulkovit et al., 2018). The reproducibility was in the range 6.6–16.1 and 9.8–14.4 %RSD ($n = 5$) for GA and EGCG, respectively for all concentrations tested. The limit of detection (LOD), defined as the antioxidant concentration that causes a decrease in the signal three times the signal-to-noise ratio relative to the control, for GA and EGCG were 0.3 mM and 34.2 μM, respectively.

We further evaluated the mechanism of the two model antioxidant standards for the quenching ability on the HO produced from Fe_3O_4 MNPs peroxidase-like activity. Spectra of the antioxidant standards with or without the reagents including Fe_3O_4 MNPs and H_2O_2 were recorded every 3 min for 30 min. Spectra of only GA and EGCG showed good stability over time (Inset of Fig. 3A and 3C). When either GA or EGCG was mixed with H_2O_2, good stability was still observed from the spectra (Inset of Fig. 3B and 3D) indicating that there

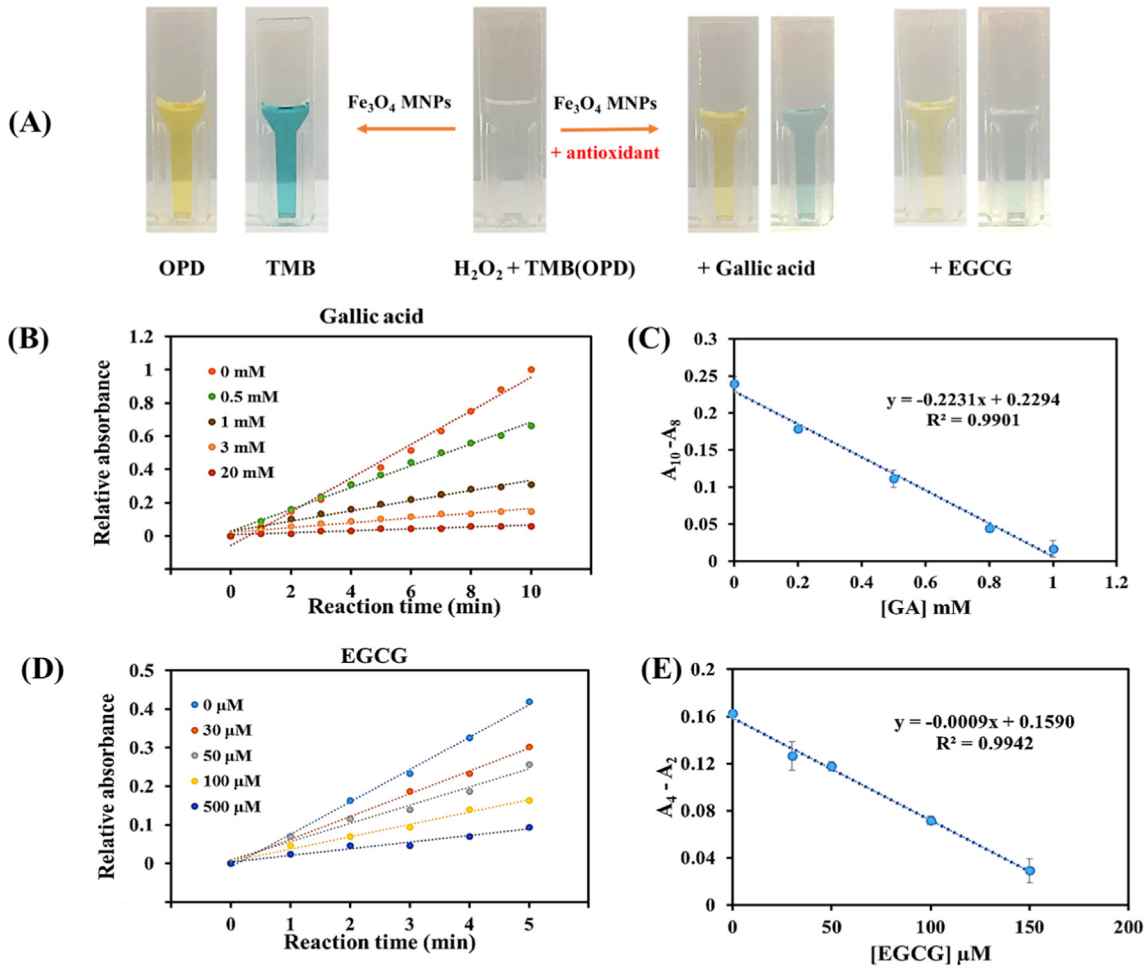

Fig. 2 The quenching effect of GA and EGCG on HO• radicals produced from the peroxidase-like activity of Fe_3O_4 MNPs to catalytically oxidize H_2O_2. (A) Pictures of representative colorimetric substrates after catalytic oxidation by Fe_3O_4 MNPs in the absence and presence of antioxidants; GA (0.6 mM), EGCG (150 µM). Concentration-dependent quenching effects of (B) GA, (D) EGCG on the catalytic oxidation of TMB and H_2O_2 by Fe_3O_4 MNPs. Calibration plots of differences in the relative absorbance at two reaction times as a function of antioxidant concentrations for (C) GA, (E) EGCG (n = 5).

was no reaction of GA or EGCG with H_2O_2. Furthermore, when each antioxidant standard was mixed with Fe_3O_4 MNPs, only negligible decrease of the absorbance over time of GA and EGCG was demonstrated indicating the weak electron between the two antioxidants and Fe_3O_4 MNPs (Fig. 3A and 3C). However, in the presence of both Fe_3O_4 MNPs and H_2O_2, the absorption spectra of both GA and EGCG obviously decreased over time (Fig. 3B and 3D) indicated that the GA and EGCG were oxidized by the HO products from the reaction between H_2O_2 and Fe_3O_4 MNPs in acidic condition (Zhang et al., 2008; Wang et al., 2010; Chen et al., 2012). These mechanism studies can explain the results from the inhibitory effect of GA and EGCG on the peroxidase-like activity through the scavenging activity of the antioxidants to the HO radicals generated by decomposition of H_2O_2 catalyzed by the Fe_3O_4 MNPs. Compared with natural horseradish peroxidase (HRP) that is commonly used for evaluation of antioxidant scavenging activity to the HO radicals, the Fe_3O_4 MNPs provide more robustness against denaturing and the cost of the materials is relatively lower.

3.3. Interference study

The effect of potential interferences was investigated using compounds frequently found in antioxidant-rich samples such as tea leaves and tea beverages including organic acids and bases (tartrate and tartaric acid), amino acids (glycine and alanine) and sugars (galactose, fructose, glucose and sucrose) (Balentine et al., 1997; Shi and Schlegel, 2012). Major component which is phenolic acid such as GA is found at approximately 30 and 50 % of the total dry matter of dried green tea leaves and beverages, respectively.[43] Minor components are organic acids (0.5%) and amino acids (4%).[43] Interfering compounds were considered to affect the developed assay if the difference of absorbance at reaction times of 8 and 10 min is significantly different from that of the control (blank), using pooled variance *t*-test at the $P = 0.05$ confidence level. The results showed that there was a high tolerance of the developed assay to sugar and amino acids but low tolerance to the organic acids at a concentration of 0.8 mM (Table S1, Supplementary Information). However, as has been

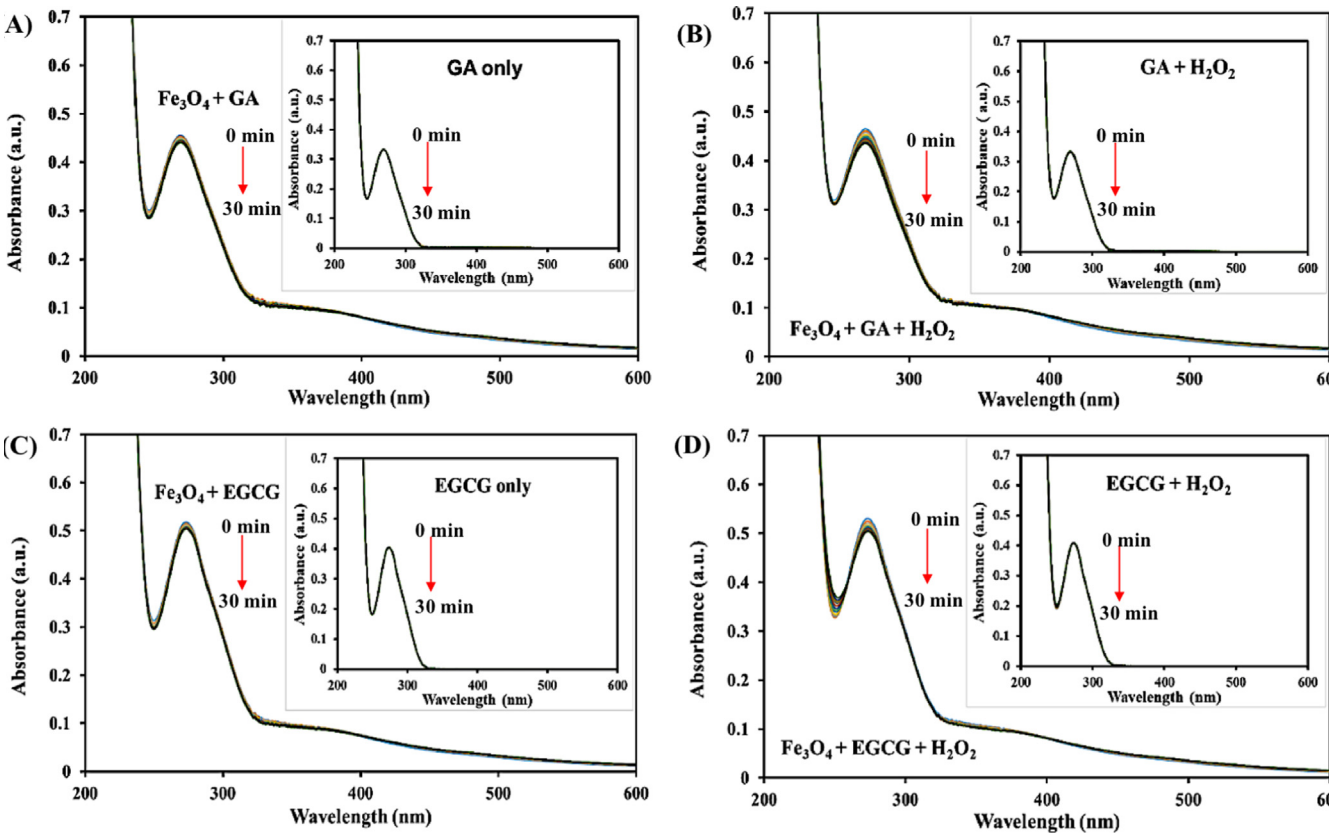

Fig. 3 The investigation of UV–VIS spectra over time for 1 mM GA (A, B) and 1 mM EGCG (C, D) catalyzed by Fe_3O_4 MNPs in the absence and presence of H_2O_2. Inset show the control experiment without the addition of Fe_3O_4 MNPs. Conditions: Fe_3O_4 MNPs 400 μg/mL 20 μL, TMB 20 mM 20 μL, H_2O_2 10 mM 20 μL, acetate buffer (pH 3.7) 200 mM 1170 μL. The spectra were recorded every 3 min for 30 min.

mentioned above, the organic acids are present in much lower amounts than the antioxidant compounds in tea samples. The results are shown in Fig. 4 where all of the investigated interfering compounds at the tolerance limit concentration did not cause the difference in absorbance at reaction time of 8 and 10 min to the control (expressed as %differences). Unlike GA antioxidant standards, the interfering compounds did not have a quenching effect to the HO radicals generated from the peroxidase-like activity of Fe_3O_4 MNPs.

3.4. Analysis of tea samples

The performance of the Fe_3O_4 MNP assay was validated against the traditional assays including DPPH and FRAP assays by analyzing the antioxidant activity of 9 tea samples. Gallic acid was used as an antioxidant standard since it is a major constituent in tea. The antioxidant activity was expressed as gallic acid equivalent (GAE, mol/g tea) (Shi and Schlegel, 2012). The Spearman rank correlation coefficient method was employed for comparing the GAE measured by the developed method and the traditional methods. The antioxidant activity of tea samples obtained from all methods are summarized in Table 1. The developed Fe_3O_4 MNP assay gave antioxidant activity in a similar sample ranking order to the conventional assays, indicating that they are well correlated at $P = 0.05$ level. When the developed assay was compared with DPPH and FRAP assays, $P < 0.01$ ($r_s = 0.883$) and $P < 0.001$ ($r_s = 0.950$) were obtained, respectively. These results indicated that the proposed method was well correlated for antioxidant activity measurement to the traditional assays and hence, it could be used as an alternative assay for antioxidant radical scavenging activity analysis of tea samples. The analysis of other types of samples as well as the method validation against other traditional antioxidant assays are currently being investigated.

3.5. Paper-based devices for antioxidant activity using the Fe_3O_4 MNP assay

To further demonstrate the applicability of the developed Fe_3O_4 MNP assay, the paper-based devices was employed for the measurement of antioxidant radical scavenging activity to allow for high throughput analysis, low amount of reagent and sample consumption and inexpensive analysis cost. Paper-based devices have gained much interest recently as an analytical platform in several areas such as clinical sciences, environmental monitoring as well as food safety (Hu et al., 2014; Busa et al., 2016; Meredith et al., 2016). They provide several advantages over traditional analysis system including simplicity, rapidity, low cost, portability low sample and reagent consumption and ease of disposal. The analysis of the GA antioxidant standard using the paper-based devices was demonstrated as a proof-of-concept. The results show that as the GA concentration increased, the color intensity of the

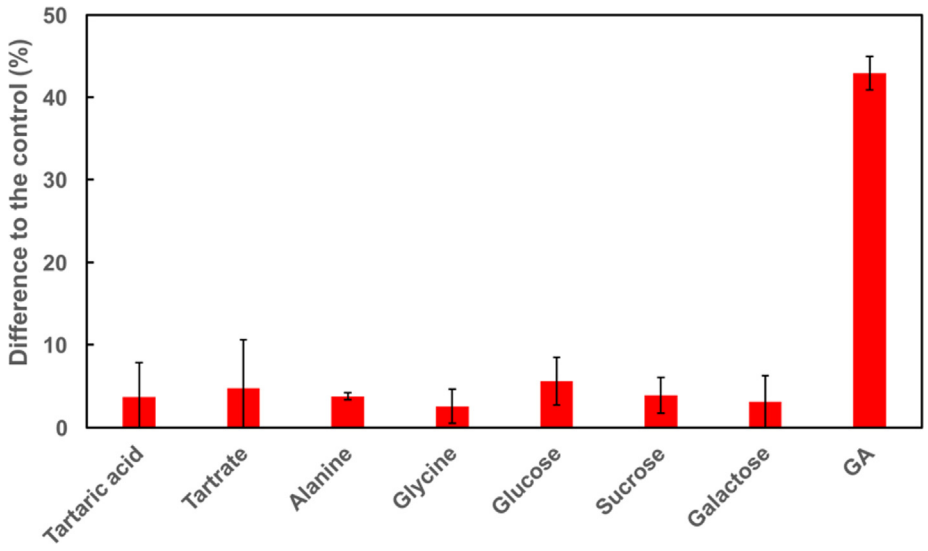

Fig. 4 Response of the Fe_3O_4 MNP assay to various compounds at the tolerance limit concentrations. The percentage difference to control is the absorbance differences at reaction time of 8 and 10 min from the interference analysis compared to those obtained from the control (DI water) analysis (n = 3).

Table 1 Antioxidant activity expressed as GAE and ranking order of 9 tea samples obtained from the Fe_3O_4 MNP assays and the traditional DPPH and FRAP antioxidant assays (n = 3).

Sample	Fe_3O_4 NPs	Rank	DPPH	Rank	FRAP	Rank
	mmol GA/ g sample		mmol GA/ g sample		μmol GA/ g sample	
Black tea 1	1.95 ± 0.54	1	0.16 ± 0.01	3	65.00 ± 3.30	2
Black tea 2	4.86 ± 0.38	4	0.17 ± 0.00	4	133.00 ± 4.15	4
Black tea 3	6.05 ± 0.44	7	0.31 ± 0.01	6	230.69 ± 0.12	7
Green tea 1	4.24 ± 0.46	2	0.10 ± 0.01	2	24.83 ± 2.56	3
Green tea 2	4.81 ± 0.41	3	0.01 ± 0.00	1	6.54 ± 0.38	1
Green tea 3	6.31 ± 0.61	8	0.33 ± 0.01	7	240.90 ± 3.54	8
Green tea 4	6.52 ± 0.50	9	0.33 ± 0.02	8	365.52 ± 5.36	9
Oolong tea 1	5.05 ± 0.08	5	0.20 ± 0.01	5	196.04 ± 0.70	5
Oolong tea 2	5.14 ± 0.52	6	0.38 ± 0.02	9	204.66 ± 5.61	6

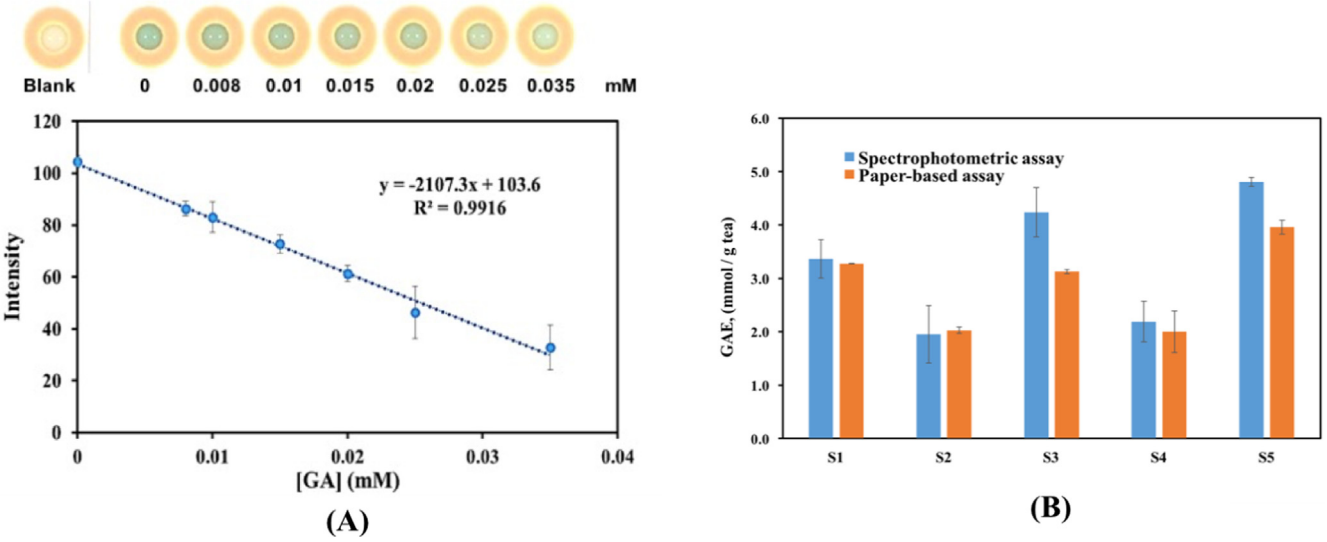

Fig. 5 (A) Analysis of the GA antioxidant standard using the paper-based Fe_3O_4 MNP assay (n = 3) (B) Antioxidant activity expressed as GAE in mmol gallic acid equivalent/g tea for the analysis of five tea samples using the paper-based Fe_3O_4 MNP assay and the spectrophotometric Fe_3O_4 MNP assay (n = 3).

oxidized TMB product decreased (Fig. 5A), indicating that the inhibitory effect of antioxidant on the peroxidase-like activity of Fe_3O_4 MNPs occurred. The linear range was found to be 0–35 μM with the limit of detection of 8 μM which are in similar range to those obtained from the analysis of GA using DPPH assay performed on the paper-based devices (Sirivibulkovit et al., 2018). The reproducibility of the analysis of GA in the linear concentration range was found to be 1.76–4.67 %RSD (n = 6). Using this assay, reducing amounts of total reagent and sample was obtained by factors of 60 times and 10 times, respectively when compared to the spectrophotometric assay. Moreover, high throughput analysis can be obtained where up to 40 samples can be analyzed at a time by capturing the pictures and analyzing their color intensity using imaging software.

The performance of the paper-based Fe_3O_4 MNP assay was further evaluated for the analysis of antioxidant activity in tea samples and the results compared with those obtained from the spectrophotometric Fe_3O_4 MNP assay (Fig. 5B). There was no significant difference in GAE obtained from the two methods based on a 95% confidence interval (two-tailed $P = 0.15$). The high degree of equivalence between the two methods demonstrates that the paper-based Fe_3O_4 MNP assay is appropriate as an alternative method for antioxidant activity in real world samples.

4. Conclusions

A Fe_3O_4 MNP assay has been developed for colorimetric detection of antioxidant radical scavenging activity. The analysis is based on the quenching ability of antioxidants to the HO radicals generated from peroxidase-like activity of Fe_3O_4 MNP to the oxidation of H_2O_2. The analytical performance has been investigated and the assay gave a low limit of detection for the antioxidant standard with a high tolerance to the potential interferences normally found in tea samples. From the analysis of tea samples, the developed assay gave antioxidant activities in a similar order to the traditional antioxidant assays such as DPPH and FRAP, indicating that it can be an alternative and simple method for antioxidant activity in real world samples. The assay can also be performed in paper-based devices to offer high throughput analysis with low reagent and sample consumption. Similar antioxidant activities from five tea sample analyses were obtained from both the paper-based Fe_3O_4 MNP method and the spectrophotometric Fe_3O_4 MNP assay.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103475.

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