Electronic Supplementary Material (ESI) for Analytical Methods.

Analytical Methods: Supplementary Information

Nanoceria-Based Reactive Species Scavenging Activity of Antioxidants Using N, N- Dimethyl-*p*-Phenylenediamine (DMPD) Probe

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In this study, CeONPs were synthesized using green chemistry to produce reactive species (RS) upon reaction with H_2O_2. These RS caused a coloration on the DMPD reagent, which was bleached by antioxidants. The initial and remaining amounts of antioxidants were measured with the CUPRAC method to validate the proposed assay. A series of experiments were performed to optimize the catalytical activity of CeONPs to generate RS. The results of these experiments used for optimizing reaction and assay parameters are shown in Table S1 and Figures S1-S6.

Table S1 Linear correlation equations of antioxidants using the CUPRAC assay, as absorbance (A) *versus* molar concentration (C)

Scavenger antioxidant	Linear correlation equation
Gallic acid	A = 35539 C + 0.1049
Quercetin	A = 75582 C + 0.0250
Trolox	A = 15287 C – 0.0043
Caffeic acid	A = 44013 C – 0.0190
Rutin	A = 40953 C – 0.0652
Catechin	A = 39649 C + 0.0409
Naringenin	-
Ferulic Acid	A = 18902 C + 0.005

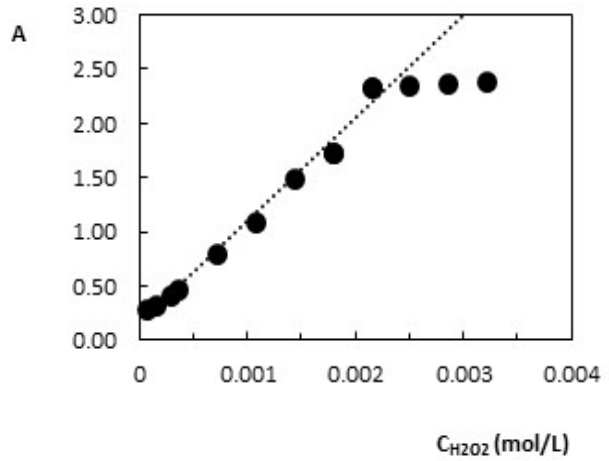

Fig. S1 Effect of hydrogen peroxide concn.: 0.25 mL of 2-90 mM H_2O_2 + 1 mL of 5.0×10^{-3} M DMPD reagent + 3.75 mL of deionized water + 2.0 mL of 2000 ppm nanoceria (10s vortex).

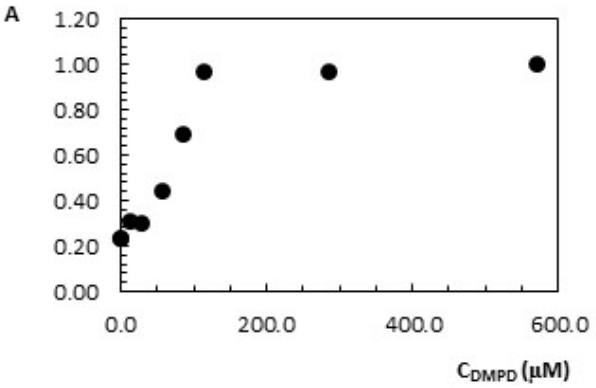

Fig. S2 Effect of DMPD concn.: 0.25 mL of 60 mM H_2O_2 + 1 mL of 10^{-4} – 10 mM DMPD solution + 3.75 mL deionized water + 2.0 mL of 2000 ppm nanoceria (10s vortex).

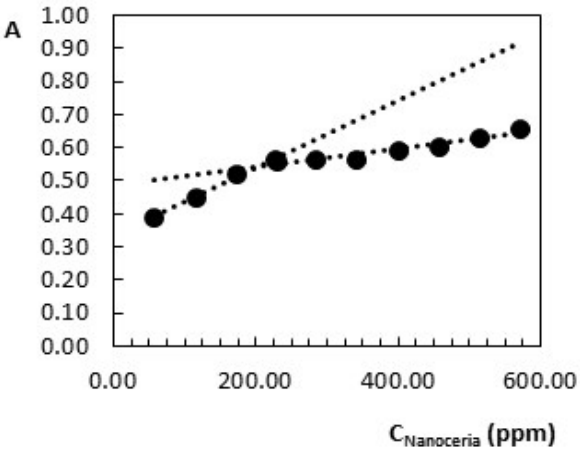

Fig. S3 Effect of nanoceria concn.: 0.25 mL of 60 mM H_2O_2 + 1 mL of 8.0×10^{-4} M DMPD + 3.75 mL deionized water + 2.0 mL of 200-2000 ppm nanoceria (10s vortex).

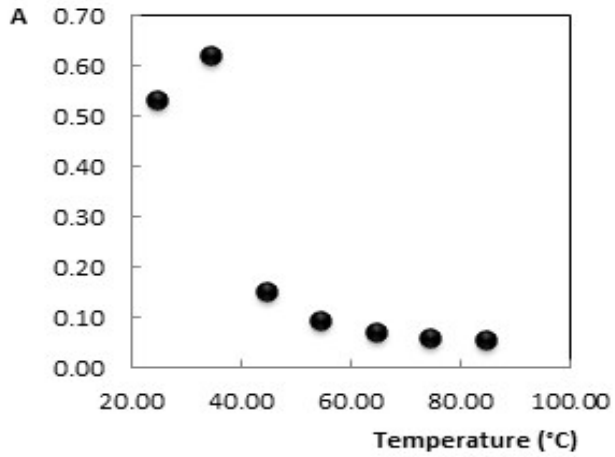

Fig. S4 Effect of temperature: 0.25 mL of 60 mM H_2O_2 + 1 mL of 8.0×10^{-4} M DMPD + 3.75 mL deionized water + 2.0 mL of 800 ppm nanoceria (10s vortex).

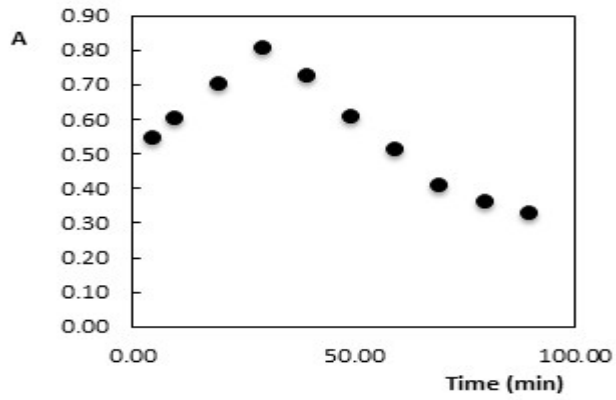

Fig. S5 Effect of incubation time: 0.25 mL of 60 mM H_2O_2 + 1 mL of 8.0×10^{-4} M DMPD + 3.75 mL deionized water + 2.0 mL of 800 ppm nanoceria (10 s vortex).

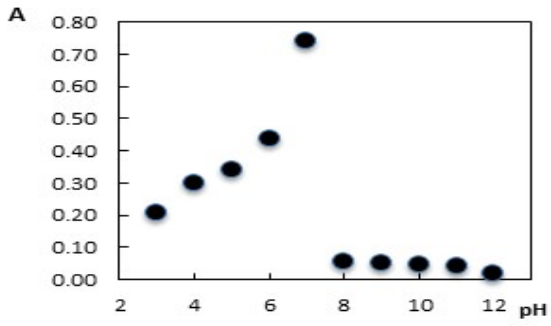

Fig. S6 Effect of pH: 0.25 mL of 60 mM H_2O_2 + 1 mL of 8.0×10^{-4} M DMPD + 3.75 mL buffer solution + 2.0 mL of 800 ppm nanoceria (10s vortex)

Certain parameters that affected the production of RS were optimized. These parameters included the concentration of H_2O_2, DMPD and nanoceria suspension, temperature, reaction time, pH, and the order of reagent addition (Supporting Information, Figures S1-S6).

These results showed that best values for the proposed colorimetric determination were as follows: 2.14 mM of H_2O_2, 1.14×10^{-4} M of DMPD, 228 ppm of nanoceria, room temperature, 30 min of reaction time and a pH value of 7 (Supporting Information, Figures S1-S6).

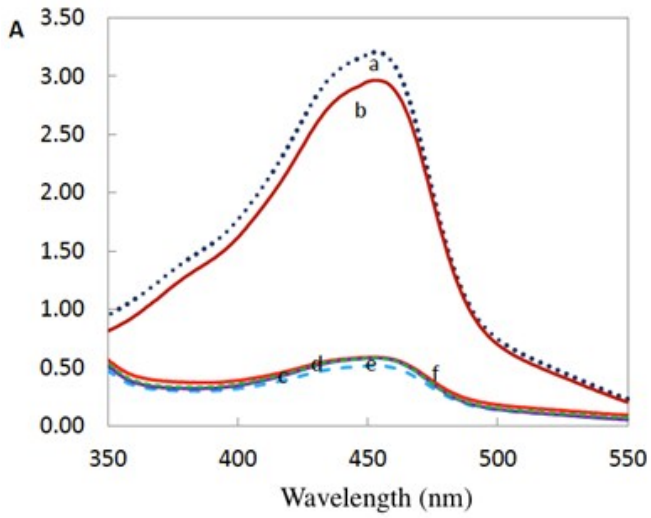

Fig. S7 The interrelated effects of caffeic acid, nanoceria, H_2O_2 and catalase in the CUPRAC method: **(a)** 0.05 mL of 20 mM H_2O_2 + 0.5 mL of 1.0×10^{-4} M caffeic acid **(b)** 0.05 mL of 20 mM H_2O_2 **(c)** 0.5 mL of 1.0×10^{-4} M caffeic acid **(d)** 0.5 mL of 1.0×10^{-4} M caffeic acid + 0.4 mL of 500 ppm nanoceria suspension **(e)** 0.5 mL of 1.0×10^{-4} M caffeic acid + 0.5 mL of 804 U mL^{-1} catalase **(f)** 0.05 mL of 20 mM H_2O_2 + 0.5 mL of 1.0×10^{-4} M caffeic acid + 0.5 mL of 804 U mL^{-1} catalase.

Figure S7-a almost gave an additive spectrum of H_2O_2 (Figure S7-b) and caffeic acid (Figure S7-c). Neither nanoceria (Figure S7-d) nor catalase (Figure S7-e) affected the CUPRAC spectrum of caffeic acid alone (Figure S7-c). When the excess of H_2O_2 was annihilated with catalase, the CUPRAC spectrum of caffeic acid constituent of the mixture (Figure S7-d) was identical to that of caffeic acid alone (Figure S7-c).