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Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H_2O_2) scavenging activity of plant extracts

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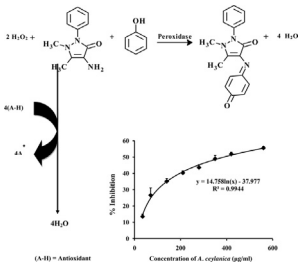
GRAPHICAL ABSTRACT



ABSTRACT

The classical method to determine hydrogen peroxide (H_2O_2) scavenging activity of plant extracts is evaluated by measuring the disappearance of H_2O_2 at a wavelength of 230 nm. Since this method suffers from the interference of phenolics having strong absorption in the UV region, a simple and rapid colorimetric assay was developed where plant extracts are introduced to H_2O_2, phenol and 4-aminoantipyrine reaction system in the presence of horseradish peroxidase (HRP). This reaction yields a quinoneimine chromogen which can be measured at 504 nm. Decrease in the colour intensity reflects the H_2O_2 scavenged by the plant material.

- Optimum conditions determined for this assay were 30 min reaction time, 37 °C, pH 7, enzyme concentration of 1 U/ml and H_2O_2 concentration of 0.7 mM. The limit of detection (LOD) and limit of quantitation (LOQ) were 136 μM and 411 μM, respectively.
- Half maximal effective concentration required to scavenge 50% of H_2O_2 in the system (EC_{50} value) calculated for several plant extracts and standard antioxidants resulted in coefficient of variance (CV%) of the EC_{50} values less than 3.0% and correlation coefficient values (R^2) > 0.95 for all dose response curves obtained.

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- This method is convenient and very precise which is suitable for the rapid quantification of H_2O_2 scavenging ability of standard antioxidants and natural antioxidants present in plant extracts.

ARTICLE INFO
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Method details

Background information

Hydrogen peroxide (H_2O_2) scavenging activity of natural antioxidants present in plant extracts has been determined widely [1–5] by measuring decrement of H_2O_2 in an incubation system containing H_2O_2 and the scavenger using the classical UV-method at 230 nm [6]. The main disadvantage of this method is the possible interference from secondary metabolites present in plants which absorb in UV region [7]. Therefore, a simple and rapid colorimetric assay was developed to determine H_2O_2 scavenging activity of plant extracts and standard antioxidants based on the reaction system where H_2O_2 rapidly reacts with phenol and 4-aminoantipyrine in the presence of horseradish peroxidase (HRP) to produce a pink coloured quinoneimine dye (Fig. 1) [8]. H_2O_2 scavengers will eventually result in decreased production of this particular chromophore. This method was applied to standard antioxidants ascorbic acid, gallic acid and tannic acid in addition to selected plant extracts to determine their hydrogen peroxide scavenging abilities.

Chemicals and equipment

The chemicals gallic acid, 4-aminoantipyrine and horse radish peroxidase (HRP) were purchased from Sigma Chemicals Co. (P.O. Box 14508, St. Louis, MO 63178, USA). L-Ascorbic acid and hydrogen peroxide were purchased from BDH Chemicals (BDH Chemicals Ltd Poole, England). Tannic acid was purchased from Riedel De Haen Ag, Wunstorfer Strasse 40, SEELZE1, D3016, Germany. Phenol was purchased from Fluka (Fluka chemie GmbH, CH-9471, Buchs, Switzerland). Plant extracts were freeze dried using LFT 600EC freeze dryer. SHIMADZU UV 1601 UV Visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used to measure the absorbance.

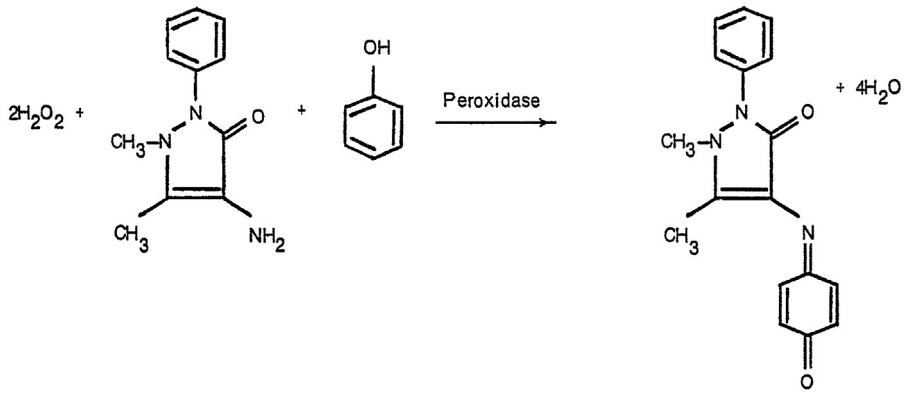

Fig. 1. The chemical reaction catalyzed by HRP [8].