



604/E2

**Simple colorimetric assay for determination of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity of plant extracts**

S C D Fernando and S S S B D P Soysa\*

*Department of Biochemistry & Molecular Biology, Faculty of Medicine, University of Colombo, Colombo 08*

The classical method to determine hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity of plant extracts is evaluated by measuring the disappearance of H<sub>2</sub>O<sub>2</sub> at a wavelength of 230 nm. Since this method suffers from the interference of phenolics having strong absorption in the UV region, an attempt was made to develop a simple and rapid colorimetric assay where plant extracts are introduced to a H<sub>2</sub>O<sub>2</sub>, 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<sub>2</sub>O<sub>2</sub> scavenged by the plant material.

Optimum conditions for the above enzyme catalyzed reaction were determined and half maximal effective concentration required to scavenge 50% of H<sub>2</sub>O<sub>2</sub> in the system (EC<sub>50</sub> value) was calculated for several plant extracts and standard antioxidants i.e.; L-ascorbic acid, gallic acid and tannic acid.

The optimum conditions for this assay was found to be 30 minute reaction time for completion of reaction, 37 °C, pH value of 7, enzyme concentration of 0.1 U/ml and H<sub>2</sub>O<sub>2</sub> concentration of 0.7 mM. Quinoneimine formed in the reaction between H<sub>2</sub>O<sub>2</sub>, phenol and 4-aminoantipyrine catalyzed by HRP decreased in a dose dependant manner when exposed to plant extracts and standard antioxidants. The current method for analysis of H<sub>2</sub>O<sub>2</sub> scavenging activity resulted in coefficient of variance (CV%) of less than 3.0 % for EC<sub>50</sub> values for all the plant extracts as well as for the standard antioxidants studied. The correlation coefficient values (R<sup>2</sup>) obtained for all dose response curves were > 0.95. The method developed is convenient and very precise and can be applied to assess the H<sub>2</sub>O<sub>2</sub> scavenging ability of standard antioxidants and natural antioxidants present in plant extracts.

Keywords: Colorimetry, hydrogen peroxide, plant extracts, scavenging