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Editorial Open Acces

Antioxidants Slow Down the Damage of Cells

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Editorial Note

Antioxidants have ability to neutralize excess oxidant production including enzymes that convert oxidants into less harmful or harmless species and small molecules that serve as oxidant sinks or scavengers. The carcinogenic adverse effects of synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) has led to a growing interest towards natural antioxidants of plant origin in recent years. Fruits, vegetables, grains and medicinal plants are known to contain number of phenolic compounds with strong antioxidant activity. These compounds are found to be well correlated with antioxidant potential. The antioxidant activities were determined by in vitro assays to compare their antioxidant effects. These include inhibition of DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox equivalent antioxidant capacity (TEAC) using ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) as an oxidant and FRAP (Ferric reducing antioxidant power). Total phenolic and flavonoid contents were also determined.

The capacity to scavenge the "stable" free radical DPPH by n-butanolic and ethyl acetate fraction was measured according to Hanato et al (1998) which is based on the reduction of methanolic solution of the coloured free radical of 1, 1- diphenyl-2-picryl hydrazyl (DPPH). A methanol DPPH solution (0.1 mM, 1 ml) was mixed with serial dilutions (10, 20, 40, 60, 80µg/ml) of the n-butanolic fraction and ethyl acetate fraction and incubated for 30 min at room temperature (250C). For each concentration the assay was run in triplicate and the absorbance was read at 517 nm using microplate reader (Powerwave XS, Biotek, USA). Ascorbic acid (Loba chiemie, India) was used as standard to compare with fractions. IC50 (the antiradical dose required to cause a 50% inhibition) for ascorbic acid, n-butanolic and ethyl acetate fraction was determined. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = (ADPPH – A test) / ADPPH X 100

The nitric oxide scavenging activity was determined according to method reported by Sreejayan (1997). The method is based on inhibition of nitric oxide (NO) radicals generated from sodium nitroprusside solution at physiological pH. Sodium nitroprosside (1ml of 10mM) was mixed with 1ml fractions of different concentrations (150- 300µg/ml) in phosphate buffer (pH 7.4). The mixture was incubated at 250C for 150 min. To 1 ml of incubated solution, 1ml of Griess reagent (α- naphthyl-ethylenediamine dihydrochloride 0.1% in water and sulfanilamide 5% in H3PO4) was added. The same reaction mixture without fractions but equivalent amount of distilled water was served as control. Absorbance was measured at 546 nm using microplate reader (Powerwave XS, Biotek, USA) and percentage inhibition was calculated using following formula:

% Inhibition = (Control – fraction) / Control X 100

The total antioxidant capacity of fractions was evaluated by the method of Prieto et al (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH (9). 0.1 ml of fraction (100-800 µg/ml) was mixed with 1 ml of the reagent solution (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid) and sample was incubated at 950C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm using an UV/Vis spectrophotometer (Beckman DU-530). The values are presented as the means of triplicate analysis. The antioxidant capacity was expressed as ascorbic acid equivalent by using the standard ascorbic acid graph.

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