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Research Article

EVALUATION OF ANTIOXIDANT CAPACITIES OF SOME PHENOLIC ACIDS BY DPPH METHOD

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ABSTRACT

Antioxidants are the agents capable of slowing or preventing non-enzymatic oxidation. In oxidation, electrons are transferred from substance to oxidizing agent which leads to production of free radicals and initiates chain reaction. Phenolic acids and its derivatives like 3,4,5-trihydroxy benzoic acid, Propyl 3,4,5-trihydroxybenzoate, Octyl 3,4,5-trihydroxybenzoate, 2,4-dihydroxy benzoic acid are known to possess antioxidant capacity and are used in food products to scavenge reactive oxygen species. The present study was conducted to evaluate antioxidant capacity of some phenolic acids in vitro, as their ability to scavenge the 1, 1-diphenyl -2 picryl – hydrazyl (DPPH) radical and to correlate the number of free –OH groups, position of these groups on aromatic ring and its antioxidant capacity. It was found that phenolic acids having three free hydroxyl groups at position 3, 4, 5 on aromatic ring have maximum antioxidant capacity. Phenolic acids that have two free hydroxyl groups at ortho position to each other, on aromatic ring have optimum antioxidant capacity while, phenolic acids that have only one free hydroxyl group at position 2 or 4 have negligible antioxidant capacity.

Keywords: Antioxidant, Phenolic acid, free radical.

INTRODUCTION

Free radicals are molecules or molecular fragments containing a single unpaired electron, this unpaired electron gives considerable degree of chemical reactivity to the free radical.

Reactive free radicals may be reducing or oxidizing in character. Oxidizing free radicals may initiate cell injury by abstracting hydrogen atom from a polyunsaturated fatty acid (PUFA) to initiate the degradative process that is lipid peroxidation or add across unsaturated centers in molecule to give covalently bound adducts that may disturb biological function.[1]

These free radicals can originate endogenously from normal metabolic reactions or exogenously as components of tobacco smoke, air pollutants, through metabolism and certain solvents, drugs, pesticides or through exposure to radiation. These free radicals contribute to etiology of many chronic hearth problems as emphysema, cardiovascular cerebro-vascular and inflammatory disease cataract, cancer etc. DNA damage may occur when cells are exposed to oxidative stress. It may be due to activation of nucleases or direct reaction hydroxyl radicals with the DNA.

Defenses against free radical damage include tocopherol (vitamin E), Ascorbic acid (vitamin C),beta carotene, glutathione,uric acid, bilirubin and several metalloenzymes including glutathione peroxidase (selenium),catalase (Iron) and superoxide dismutase(copper, zinc, manganese) and proteins such as cerulloplasmin. The extent of tissue damage is the result of the imbalance between the free radicals generated and the antioxidant protective defense system.[2,3]

An antioxidant is a molecule capable of inhibiting or preventing the oxidation of other molecules. Antioxidants help to counter the detrimental effects of reactive oxygen species (ROS) and free radicals which play part in atherosclerosis, some forms of cancer and reperfusion injuries. They may be reducing agents such as thiols, ascorbic acid or poly phenols (Sies,1997) or poly peptides like

glutathione, vitamins such as vitamin C and vitamin E or enzymes such as catalase, peroxidases, superoxide dismutase that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids (Sies, 1997 and Vertuani et al 2004) [4,5].

Phenolic compounds are important components of the human diet due to their potential antioxidant activity[6]. Their capacity to diminish oxidative stress induced tissue damage resulted from chronic diseases[7].Gallic acid(3,4,5-trihydroxy benzoic acid) is a phenolic acid posses antioxidant property [8].The gallate esters of n-alkanols also act as potent antioxidant, propyl, octyl and dodecyl(lauryl) gallates are permitted addetives for antioxidation in foods [9] .

MATERIALS AND METHODS

Chemicals and reagents

3,4,5-trihydroxy benzoic acid(3,4,5 THBA), Propyl 3,4,5-trihydroxybenzoate(P 3,4,5 THBA), 3,4,5-trimethoxy benzoic acid(3,4,5 TMBA), 2,3-dihydroxy benzoic acid (2,3DHBA), 2,4-dihydroxy benzoic acid (2,4 DHBA), 2,5-dihydroxy benzoic acid (2,5DHBA), 2,6-dihydroxy benzoic acid (2,6 DHBA), 3,5-dihydroxy benzoic acid (3,5DHBA)), 4-hydroxy-3-methoxy benzoic acid (4H3MBA), 2-hydroxy benzoic acid (2HBA), 4-hydroxy benzoic acid (4HBA) (50 µg/ml each) as well as 1, 1-Diphenly-2-Picryl Hydrazyl (DPPH) were purchased from Sigma Aldrich. Methanol was purchased from Merck.

Analysis of Antioxidant Capacity

DPPH free radical scavenging activity of phenolic acids was determined using UV-Vis Spectrophotometer (Chemito).[11]

A 0.3 mM solution of DPPH was prepared in methanol. The initial absorbance of DPPH was measured at 517 nm. 1 ml of aliquots of 3,4,5-trihydroxy benzoic acid (25 µg/ml), Propyl 3,4,5-trihydroxy benzoate (25µg/ml) and 3,4,5-trimethoxy benzoic acid 2,3-dihydroxy benzoic acid, 2,4-dihydroxy benzoic acid, 2,5-dihydroxy benzoic acid, 2,6-dihydroxy benzoic acid, 3,5-dihydroxy benzoic acid, 4-hydroxy-3-methoxy benzoic acid, 2-hydroxy benzoic acid, 4-hydroxy benzoic acid (each 50 µg/ml) was added to 1 ml of 0.3 mM DPPH solution in methanol and 1 ml of methanol. The decrease in absorbance at 517 nm was measured after 20 minutes. The readings were taken in triplicates.

The antioxidant capacity based on DPPH free radical scavenging ability was expressed as % scavenging activity which was determined using formula

Percentage scavenging activity (%) = [(Acontrol – Asample) / Acontrol] x 100

Where, Acontrol = Absorbance of the control.
Asample **=** Absorbance of the sample.

RESULT AND DISCUSSION

The interest in the search for new antioxidants has grown over the past years, as reactive oxygen species (ROS) production and oxidative stress have been shown to be linked to ageing related diseases. Evaluation of antioxidant capacity of phenolic acid, correlation of number of-OH groups and bonding of these groups on aromatic ring of phenolic acid and antioxidant capacity could be a valuable tool for designing a pharmacophore for drugs for treatment of various diseases.

The antioxidant capacities of some hydroxy benzoic acids estimated by DPPH radical scavenging assay are described in table 1

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Brand-Williams et al 1995).

According to the current understanding of radical-scavenging processes of phenolic antioxidant (Ar- OH), H-atom donation is the dominant mechanism which involves two pathways that is one step H-atom transfer and stepwise electron transfer / proton transfer.

The first pathway does not involve charge separation, it is preferred in non-polar solvent and can be characterized by bond dissociation energy; whereas second is favored in polar media due to charge separation processes and can be characterized by ionization potential.

The factors which might have contributed to the difference in antioxidant capacities of these phenolic acids may be the number of free hydroxyl groups and the position of these groups on aromatic ring of phenolic acid.

In this set of phenolic acids it was found that, acid having three free hydroxyl groups at position 3, 4, 5 on aromatic ring have maximum antioxidant capacity, while blocking of hydroxyl groups at position 3, 4, 5 on aromatic ring of phenolic acid resulted in profound decrease in antioxidant capacity. Phenolic acid having two free hydroxyl groups at ortho position to each other on aromatic ring has more antioxidant capacity than the phenolic acid having two free hydroxyl groups at meta position to each other, while phenolic acid having one free hydroxyl group at position 2 or 4 has no antioxidant capacity.

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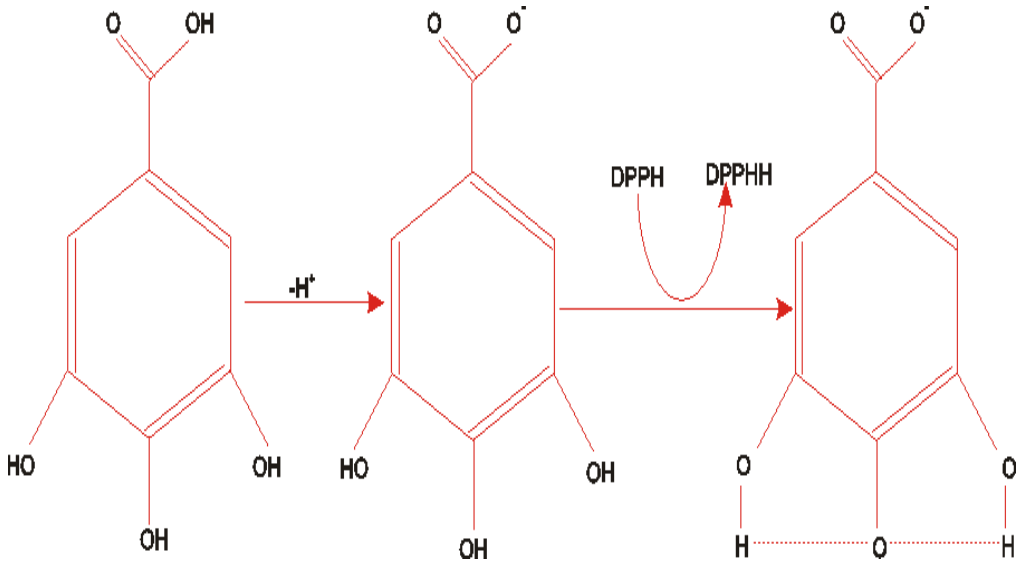
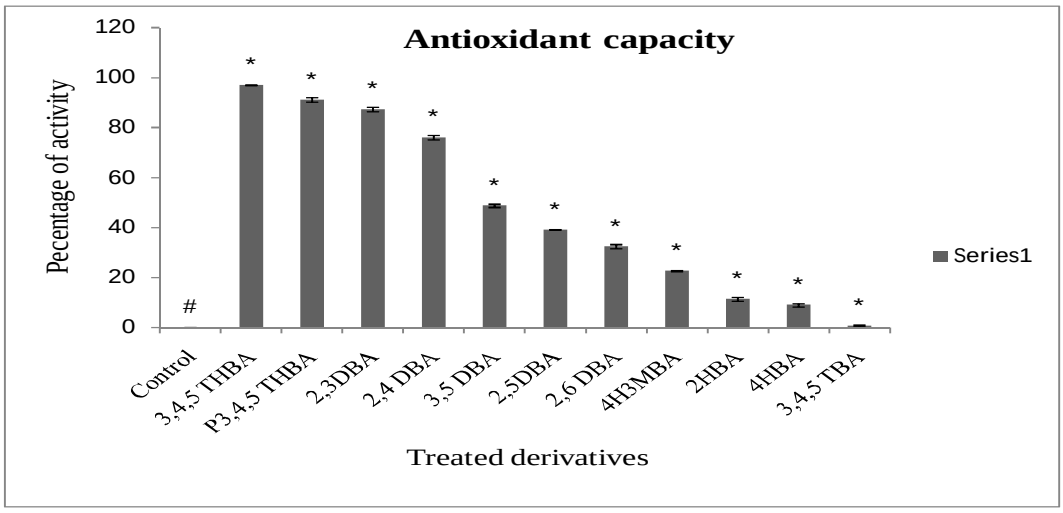


Fig. 1: DPPH-radical-scavenging mechanism of gallic acid



Graph: Antioxidant capacity of phenolic acids in methanol

Table 1: Antioxidant capacity of some phenolic acids by DPPH radical scavenging assay method

S. No.	Name	Free hydroxyl groups	Concentration (µg/ml)	Percentage Scavenging Activity (%)
1	Control	--	--	--
2	3,4,5- trihydroxy benzoic acid (3,4,5 THBA)	03	25	96.96 ± 0.070
3	Propyl 3,4,5-trihydroxy benzoate (P3,4,5 THBA)	03	25	91.12 ± 0.8570
4	2,3-dihydroxy benzoic acid(2,3DBA)	02	50	87.26 ± 0.874
5	2,4-dihydroxy benzoic acid(2,4 DBA)	02	50	75.99 ± 0.9646
6	3,5-dihydroxy benzoic acid(3,5 DBA)	02	50	48.71± 0.6180
7	2,5-dihydroxy benzoic acid (2,5DBA)	02	50	39.13 ± 0.1181
8	2,6-dihydroxy benzoic acid (2,6 DBA)	02	50	32.4 ± 0.8908
9	4-hydroxy 3-methoxy benzoic acid (4H3MBA)	01	50	22.68 ± 0.3485
10	2-hydroxy benzoic acid(2HBA)	01	50	11.35± 0.725
11	4-hydroxy benzoic acid(4HBA)	01	50	8.99 ± 0.7067
12	3,4,5-trimethoxy benzoic acid(3,4,5 TBA)	Nil	50	0.80 ± 0.1586

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