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Screening of fruit pulp extract of Pithecellobium dulce (Manila tamarind) for antioxidant activity: An in vitro Study

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ABSTRACT:
In this study, the antioxidant property of fruit pulp extracts of Pithecellobium dulce was evaluated by using different model systems. The antioxidant potential was evaluated by determining the activity of superoxide, hydroxyl, hydrogen peroxide (H2O2), and DPPH assay using in vitro models. The HAPD showed the significant antioxidant capacity due to electron donating or hydrogen donating capacity. These antioxidant activities could be due to the presence of antioxidant phytochemicals such as flavonoids, phenols, terpenoids, and saponins among others. Therefore, the therapeutic potential of this plant could be due to the antioxidant properties of fruit pulp Pithecellobium dulce.

Keywords: Pithecellobium dulce, Superoxide radical, DPPH radical

I. INTRODUCTION:

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction. The Free radicals (e.g., superoxide, nitric oxide, and hydroxyl radicals) and other reactive species (e.g., hydrogen peroxide, peroxynitrite, and hypochlorous acid) are produced in the body, primarily as a result of aerobic metabolism and different processes such as glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Furthermore, natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. This activity is mainly due to the presence of phenolic compounds such as flavonoids, phenols, flavonols, and proanthocyanidins (Rice-Evans et al., 1995).

Synthetic and natural food antioxidants are used routinely in foods and medicine, especially those containing oils and fats to protect the food against oxidation. There are several synthetic phenolic antioxidants, butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) being prominent examples (Papas AM, 1999). Because of increased risk factors with the use of synthetic antioxidants to various deadly diseases of human, there has been a global trend toward the use of natural substance present in medicinal plants and dietary plants as therapeutic antioxidants. It has been reported that there is an inverse relationship between the dietary intake of antioxidant-rich food and medicinal plants and incidence of human diseases (Brown JE et al., 1998).

Attempts have been made to study the antioxidant potential of a wide variety of vegetables like potato, spinach, tomatoes, and legumes (Furuta S et al., 1997), fruits (berries, cherries, citrus, prunes, and olives) (Wang H et al., 1996) and Green and black teas have been extensively studied (Lin JK et al., 1998). Ascorbic acid is a standard antioxidant. It is used therapeutically in the treatment of wound healing, immunomodulator, hypertension, common cold and detoxification of organs and another metabolic process.

Hence, the present study focused on hydroalcoholic extracts of Pithecellobium dulce for antioxidant potential and which were compared with standard ascorbic acid. The in vitro antioxidant activity of selected plant extracts used in the present study was evaluated by

- Superoxide radical scavenging activity
- Hydroxyl radical scavenging activity
- Hydrogen peroxide scavenging activity
- DPPH radical scavenging activity

II. MATERIALS AND METHODS:

Preparation of plant materials: The selected extracts of Pithecellobium dulce were obtained from the local market. The fruit pulps are separated and cut into small pieces and shade dried. The fine powder was obtained after grinding at low speed and sieved to get a fine powder. The required quantity of pulp powers are measured and taken in motor and pestle, a creamy constituency was

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obtained upon triturating with a few drops of ethanol and makeup with water.

Superoxide radical scavenging activity

The assay was based on the capacity of the aqueous extracts in inhibiting the formation of formazan by scavenging the superoxide radicals generated in a riboflavin-light- NBT system 18 (McCord Martinez A C et al., 2001). The reaction mixture contained 58 mM phosphate buffer, pH 7.6, 20µM riboflavin, 6mM EDTA, and 50µM NBT, final volume was made up to 3 ml, and should be added in that sequence. Reaction was started by illuminating 40 volts. The reaction mixture with different concentrations of HAPD was left for 15 minutes. Immediately after illumination, the absorbance was measured at 560 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes with reaction mixtures, were kept in the dark and served as blanks. The percentage inhibition of superoxide anion generation was calculated by using the following formula:

$$\text{Percentage Inhibition} = \frac{\text{Average control O.D} - \text{Test sample O.D}}{\text{Average Control}} \times 100$$

Where A0 was the absorbance of the control, and A1 was the absorbance of the aqueous extract/standard.

Hydroxyl radical scavenging activity

Scavenging activity of hydroxyl radical was measured by the method of Halliwell et al., 1985. Hydroxyl radicals were generated by a Fenton reaction (Fe3+-ascorbate-EDTA-H2O2 system), and the scavenging capacity of the extracts and standard towards the hydroxyl radicals was measured by using deoxyribose degradation method. The reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations of the test sample in a final volume of 1 ml. The mixture was incubated for 1 h at 37 °C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium didecyl sulphate (0.2ml). The mixture was then heated (20 min at 90 °C) to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All experiments were performed in triplicates. The percentage of inhibition was expressed, according to the following equation:

$$\% \text{ Inhibition} = \frac{AO - A1 \times 100}{AO}$$

Where A0 was the absorbance of the control, and A1 was the absorbance of the aqueous extract/standard.

Free radical scavenging activity (DPPH•)

The free radical scavenging activity of HAPD was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH•) method of Blois (1958). 0.2mM solution of DPPH• in methanol was prepared and 100µl of this solution was added to various concentrations of HAPD at the concentrations of 50, 100, 150, 200 and 250µg/ml. After 30 minutes, absorbance was measured at 517nm. Butylated hydroxytoluene (BHT) was used as the reference material. All the tests were performed in triplicate and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

$$\text{Percentage of inhibition} = \frac{\text{Abs(cntrl)-Abs(test)} \times 100}{\text{Abs(cntrl)}}$$

The scavenging reaction between DPPH• and an antioxidant, H-A can be written as

$$\underset{\text{(Purple)}}{(\text{DPPH}\bullet) + (\text{H-A})} \longrightarrow \underset{\text{(Yellow)}}{\text{DPPH-H} + (\text{A}\bullet)}$$

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical scavenging activity was determined by the method of Ruch et al. (1984). Hydrogen peroxide (H_2O_2) is a biologically important oxidant because of its ability to generate the hydroxyl radical which is extremely potent. The ability of the hydroxyl radical to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems. It has very short half-life, however, restricts its diffusion capability and its potency. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of extracts in phosphate buffer were added to a H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing phosphate buffer without H_2O_2. The percentage of

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H_2O_2 scavenging of HAPD and Ascorbic acid as standard compound was calculated as
H_2O_2 radical scavenging activity (%) = [{Ao – A1/Ao}] ×100.
Where, Ao is the absorbance of the H_2O_2, A1 is the absorbance of the presence of the extract in H_2O_2 solution.

III. RESULTS AND DISCUSSION:

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants (Cheeseman KH., 1993). The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids (Young IS., 2001). Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets.

Oxidative stress was defined as the lack of balance between the occurrence of reactive oxygen/nitrogen species and the organism's capacity to counteract their action by the anti-oxidative protection systems. Increased oxidative stress has been proposed to be one of the major causes of the hyperglycemia-induced rigger of diabetic complications. High blood sugar causes the generation of free radicals that leads to oxidative stress in several tissues.

Antioxidants act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, synergists, and metal-chelating agents. Both enzymatic and nonenzymatic antioxidants exist in the intracellular and extracellular environments to detoxify ROS. Free radicals, either environmentally or internally produced, can be neutralized by antioxidants. Plants are rich in antioxidants; so much attention has been directed towards the development of ethnomedicine as they contain phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids, and many more phytochemicals responsible for different pharmacological activities. Herbal products are very well adapted by mankind due to fewer or no side effects. Various herbal products are used abundantly as antioxidant (Kumaran A and Karunakaran R J, 2007).

Several phytochemicals possessing polyphenolic structures are advocated as neutraceutical food supplements for better health care in recent years (Jose JK and Kuttan R, 1995), and most of them are claimed to possess antioxidant activity. Ayurveda and the naturopathic system of medicine (indigenous to India) clearly states the use of medicinal plants for treating various disorders. However, the system fails to provide clinical/ pre-clinical evidence, the failure to address the facts make the system not so popular due to lack of scientific justification. The claimed usefulness of herbs in several disorders might be due to their antioxidant activity.

Ascorbic acid or "vitamin C" is a monosaccharide antioxidant found in both animals and plants. As it cannot be synthesized in humans and must be obtained from the diet, it is a vitamin (Smirnoff N., 2001). Most other animals can produce this compound in their bodies and do not require it in their diets. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is a reducing agent and can reduce and thereby neutralize ROS such as hydrogen peroxide. In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants (Shigeoka S., 2002). The antioxidant potential of the hydroalcoholic extract of Pithecellobium dulce was investigated in comparison with the known antioxidant ascorbic acid (AA) following in vitro studies.

The superoxide radical (O_2^-)-the scavenging activity of the extracts, as measured by the riboflavin- NBT-light system in vitro. The superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species (Halliwell&Gutteridge et al., 1985). Many polyphenols present in our diet are effective xanthine oxidase inhibitors and/or superoxide radical scavengers. The activity of HAPD was found to be more among the selected plant extracts in scavenging superoxide radicals and extracts were

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capable of scavenging of superoxide radical in a concentrate dependent manner (Table 1).

Table 1: Effect of HAPD and ascorbic acid on superoxide radical scavenging activity

Concentrations(µg)	AA	HAPD
10	23.63±0.32	13.20±0.33
20	32.68±0.31	22.45±0.39
40	43.10±0.29	32.67±0.21
60	54.70±0.35	47.84±0.53
80	71.68±0.50	62.22±0.63
100	93.26±0.58	77.86±0.58
IC50	40.66	62.03
R^2	0.99	0.989

Each value represents the mean ± SEM (n = 3).

Hydroxyl radicals are capable to trap hydrogen atoms from cell membranes and producing a peroxidic reaction of lipids (Kitada et al., 1979) produced during immune action. The macrophages and microglia are mostly generating this compound. The destructive action of hydroxyl radicals has been implicated in several neurological autoimmune diseases. The hydroxyl radical can damage some macromolecules like carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation), and amino acids (Reiter RJ et al., 1995). The evaluation of hydroxyl radicals scavenging activity has been done by the Fenton reaction mechanism. The selected pulp extracts were found to have hydroxyl radical scavenging activity in a concentration-dependent manner (Table 2). Hydrogen peroxide is involved in the formation of hydroxyl radicals. The selected Pulp extract HAPD was found to have hydrogen peroxide free radical scavenging activity in a concentration-dependent manner (Table 3).

Table 2: Effect of HAPD and ascorbic acid on hydroxyl radical scavenging activity

Concentrations(µg)	AA	HAPD
10	25.07±1.07	25.95±0.23
20	33.63±0.69	33.55±0.63
40	47.20±0.38	43.54±0.67
60	54.83±0.11	54.24±0.34
80	67.50±0.77	67.05±0.23
100	84.19±0.79	77.89±0.58
IC50	48.39	51.08
R^2	0.986	0.998

Each value represents the mean ± SEM (n = 3).

Table 3: Effect of HAPD and ascorbic acid on hydrogen peroxide scavenging activity

Concentrations(µg)	AA	HAPD
1	27.50±0.46	18.68±0.48
2	42.54±0.39	25.97±0.52
4	55.66±0.75	38.70±0.41
6	74.85±0.26	53.87±0.20
8	83.03±0.38	65.60±0.34
10	93.01±0.19	78.66±0.63
IC50	3.37	5.63
R^2	0.970	0.99

Each value represents the mean ± SEM (n = 3).

Table 4: Effect of HAPD and ascorbic acid on DPPH radical scavenging activity

Concentrations(µg)	AA	HAPD
50	30.00±0.65	34.44±0.61
100	38.81±0.44	41.70±0.58
150	51.75±0.62	51.37±0.36
200	63.94±0.39	58.49±0.54
300	85.93±0.18	71.76±0.59
IC 50	147.36	150.62
R^2	0.99	0.97

Each value represents the mean ± SEM (n = 3).

DPPH is an exogenous free radical. The DPPH radical scavenging activity is extensively used in the evaluation of the antioxidant activity of natural substances (Feng Shi et al., 2010). The degree of the discoloration indicates the scavenging potential of the antioxidant action due to its hydrogen-donating ability (Barreira JCM et al., 2008). The selected HAPD was found to have DPPH free radical scavenging activity in a concentration-dependent manner (Table 4). The Pithecellobium dulce seeds were studied for antioxidant potential by using different in vitro assays such as inhibition of DPPH, nitric oxide, hydroxyl, superoxide anions, and lipid peroxidation (Dnyaneshwar MN., 2012). The free radical scavenging activity of HAPD extracts might be due to the presence of active phytochemicals such as Alkaloids, Flavanoids, Glycosides, Saponins, Fatty acids, Steroids, Tannins, and Terpenoids. Seven saponins named Pithedulosides A-G were isolated from the seeds of Pithecellobium dulce.

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