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## Free Radical Scavenging Activity of Folklore: *Pithecellobium dulce* Benth. Leaves

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### Abstract

In the present study, the aqueous and alcoholic extract of *Pithecellobium dulce* leaves were evaluated for radical scavenging activity using reducing power assay method. Aqueous extract showed potent free radical scavenging activity, than alcohol extract. The observed activity could be due to higher phenolic content in the extracts (0.2171 & 0.2042 mg/g in aqueous and alcohol extract respectively). HPTLC fingerprint profile of the ethanol and aqueous extracts were developed which would serve as reference standard for quality control of these extracts.

### Introduction

*Pithecellobium dulce* Benth. (Leguminosae)<sup>1</sup> is a small to medium sized, evergreen, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. It is known as 'Vilayati babul' in Hindi and 'Kodukkapuli' in Tamil. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also useful in dermatitis and eye inflammation. The leaves have been reported to possess astringent, emollient, abortifacient and antidiabetic properties. The presence of steroids, saponins, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported in the seeds.<sup>2-5</sup> The bark contains 37% of tannins of catechol type. Quercetin, kaempferol, dulcitol and afezilin have been reported from the leaves.<sup>6</sup> <sup>7</sup> Roots have been reported to possess estrogenic activity.<sup>8</sup> Studies on alkylated resins from seed oil have been reported recently.<sup>9</sup>

**Table 1.** Ethanomedical uses of *P. dulce*.

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Name of the	Ethanomedical Uses in Mexico
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## Part

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Leaves      Astringent, emollient, abortifacient, dysentery, anticonvulsant, antiulcer and antidiabetic. The leaves can be applied as plasters for pain and venereal sores. Decoctions of leaves are also used for indigestion, abortifacient, intestinal disorder and used in ear ache, leprosy, tooth ache and larvicide

It is evident that the plant has great potentials in treating a number of ailments where the free radicals have been reported to be the major factors contributing to the disorders.<sup>10</sup> In continuation of our work on evaluation of ethano pharmacological properties<sup>11-13</sup> of *Pithecellobium dulce*, the present investigation was aimed to evaluate the in vitro antioxidant activity of ethanolic and aqueous leaf extract of *P. dulce* by reducing power assay method based on the medicinal values in folk medicine of Mexico ( Table 1).

## Materials and Methods

### Plant material

Fresh leaves of *Pithecellobium dulce* were collected from Sembulam Village at Kancheepuram District, T.N. in the month of January 2005. The plant was identified by local people of that village and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai. A herbarium specimen (APCP-3/ 2005) of the plant was preserved in the Department of Pharmacognosy of our institute for further reference. All the reagents used were of analytical grade obtained from S.D. Fine chemicals Ltd., Mumbai and Qualigens Fine Chemicals, Mumbai.

### Preparation of Aqueous and Alcoholic Extracts

The fresh leaves of *P. dulce* were washed with water, air-dried at room temperature and then reduced to coarse powder. The powdered mass of leaf was defatted with petroleum ether (60-80<sup>0</sup> c) followed by extraction with alcohol (95% v/v) and then water for about 18 hr by using Soxhlet apparatus. The extracts were filtered and the filtrates were concentrated under reduced pressure using rotary evaporator to obtain the extracts as solid residues. Extractive value (%w/w) of alcohol and aqueous extracts were 17.93 and 18.58 respectively.

### Preliminary Phytochemical Screening

The freshly prepared extracts were chemically tested for the presence of different constituents using standard methods.<sup>14</sup>

### Reducing Power Assay Method

Reducing power of 70% ethanolic extract of *P. dulce* was carried out as per Oyaizu.<sup>15</sup> Different doses of *P. dulce* were prepared and 1ml of each solution was mixed with Phosphate buffer (2.5 ml, 0.2M, pH 6.60) and potassium ferricyanide 9 (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. To this mixture, 2.5 ml of 10% trichloro acetic acid (TCA) was added and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and FeCl<sub>3</sub> (0.5 ml, 0.1%) was added and the absorbance was measured at 700

nm. Increased absorbance of the reaction mixture indicated increased reducing power. The experiment was performed in triplicate. The percentage scavenging was calculated by using the formula,  $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$ , where  $A_{\text{control}}$  = Absorbance of blank and  $A_{\text{sample}}$  = Absorbance with different dilutions of drug. The results are shown in Table-2.

**Table 2.** Reducing power activity of *P. dulce* leaf extracts.

<b>Treatment</b>	<b>Absorbance*</b>	<b>Percentage inhibition</b>
Control	0.1173	-----
Aqueous extract ( 5 µg)	0.1644	40.23
Aqueous extract ( 10 µg)	0.1824	55.58
Aqueous extract ( 25 µg )	0.1903	62.31
Aqueous extract (50 µg )	0.2294	95.65
Aqueous extract ( 100 µg )	0.3105	165.13
Ethanollic extract ( 5µg)	0.150	27.11
Ethanollic extract (10 µg)	0.183	55.08
Ethanollic extract (25µg)	0.195	65.25
Ethanollic extract (50µg)	0.210	77.96
Ethanollic extract (100µg)	0.224	89.98

\*Each value is an average of three determinations.

### Estimation of Phenolic Content

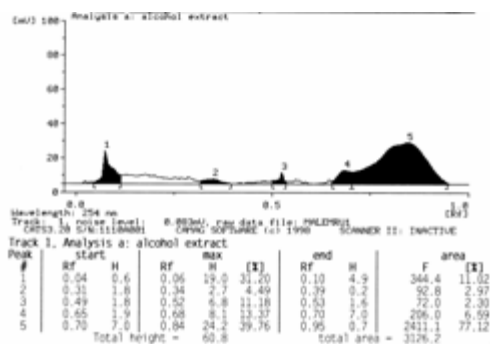
Clear aqueous and alcoholic leaf extracts of *P. dulce* were prepared and each 1.0 ml of the test solution was withdrawn in 10 ml volumetric flask separately. The blue colored complex was developed in the similar manner as in calibration curve studies, replacing the tannic acid with extracts and absorbance for aliquots of each extract was noted at 700 nm. The corresponding concentrations of total phenol against respective absorbances were determined as tannic acid using the calibration curve. All determinations were performed in triplicate. Total phenolic content of *P. dulce* was calculated in terms of tannic acid equivalent (TAE) by the following formula,  $C = c.v / m$ , Where C is total content of phenolic compounds in mg/ g of plant extract, c is the con of polyphenol established from the calibration curve in µg/ ml, v is the volume of extract in ml and m is the weight of pure plant extract in g.<sup>16</sup> The results are shown in Table 3.

**Table 3.** Amount of phenolic compounds in *p.dulce* benth leaves.

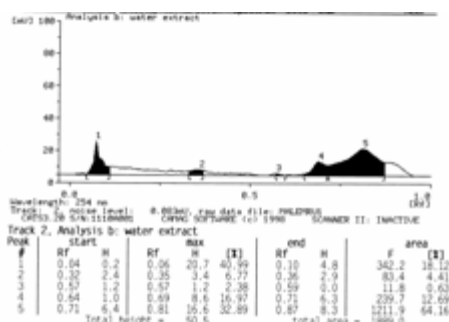
<b>Month</b>	<b>Absorbance ( at 700 nm)</b>		<b>Concentration (mg/ml)</b>		<b>Total amount (mg/g)</b>	
	<b>Aqueous extract</b>	<b>Alcoholic extract</b>	<b>Aqueous extract</b>	<b>Alcoholic extract</b>	<b>Aqueous extract</b>	<b>Alcoholic extract</b>

### HPTLC Analysis

HPTLC fingerprinting was performed on 20x 20 cm aluminum sheets precoated with silica gel 60 F<sub>254</sub> Merck plates of 0.2 mm thickness. CAMAG HPTLC linear thin trough (20x20 cm) was saturated with the developing solvent, for 2 hrs at 20-250°C. The sample 2 or 5 ml was applied in a 5 mm width band through LINOMAT IV in a space of 5 mm. Detection was carried at scanning wavelength 254 nm using Camag scanner II equipped with CATS 3.20 version software. <sup>17</sup>



**Fig 1.** HPTLC Finger printing of alcohol extract of *Pithecellobium dulce* Benth.



**Fig. 2.** HPTLC Finger printing of water extract of *Pithecellobium dulce* Benth

### Results and Discussion

Preliminary phytochemical investigation showed the presence of phenolics including flavonoids as a major class of compounds. It was observed that absorbance of the test sample was increased with increase in concentration of test. So, *P. dulce* showed concentration dependant reducing capacity. Aqueous extract showed potent free radical scavenging activity, than ethanol extract. The aqueous extract has the highest phenolic content (0.2171 mg/g), followed by water extract (0.2042mg/g). HPTLC fingerprint profile of the ethanol and aqueous extract were developed in n-butanol: water: acetic acid (9.0: 0.5:0.5) at 254 nm and shown in Figs.1 and 2.

Reducing power of *P. dulce* was determined based on the ability of antioxidant to form coloured complex with potassium ferricyanide, TCA and FeCl<sub>3</sub>. The polyphenolic content of ethanolic and aqueous leaf extract of *P. dulce* was estimated by Folin- Denis method. The method is based on the oxidation of the molecules containing a phenolic

hydroxyl group. The tannin and tannin-like compounds reduce phospho tungsto molybdic acid in alkaline solution to produce a highly colored blue solution. The intensity of which is proportional to the amount of polyphenolic compounds and can be estimated against standard tannic acid solution at wavelength of 700 nm. The total phenolic compounds of two successive extracts were expressed as tannic acid equivalent in mg/ g of extracts. Phenolic compounds were reported<sup>18</sup> to be potent free radical terminators and thus, the results are further supported by the varying amounts of total phenolic content in different fractions of leaves. HPTLC fingerprint profile of the ethanol and aqueous extracts were developed which would serve as reference standard for quality control of these extracts. Further work is, therefore, under progress to identify and isolate the anti oxidative constituents and to establish the activity in animal models.

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