

## Research Article



## Evaluation of Antioxidant and Anticancer Activity of *Pithecellobium dulce* Fruits

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### ABSTRACT

The present study was designed to investigate the antioxidant and anticancer activities of *Pithecellobium dulce* fruits. The dried coarse powder of *P.dulce* fruits was exhaustively extracted with methanol and the resulting crude methanol extract was assayed for antioxidant and anticancer activities, since both are inter related. The antioxidant activity of *Pithecellobium dulce* fruits was determined by reductive potential in reducing ferric ions and anticancer activity was determined using breast cancer cell lines (MCF 7). The extract showed strong antioxidant and anticancer activities which suggest the use of these fruits for therapeutic purpose.

**Keywords:** *In vitro* anticancer, *Pithecellobium dulce*, MCF -7 cells, antioxidant.

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### INTRODUCTION

Cancer is one of the major health problems of global concern<sup>1</sup>. Cancer statistics project will have target in the year 2030, there will be 26 million new cases and 17 million deaths per year<sup>2</sup>. In worldwide, breast cancer is the second leading cause of death in women<sup>3</sup>. The increasing incidence of breast neoplasia reported over the last a few decades has led to development of new anticancer drugs, drugs combinations and chemotherapy strategies by methodical and scientific exploration of enormous pool of synthetic, biological and natural products<sup>4</sup>. In light of the continuing need for effective anticancer agents and the association of fruits and vegetables consumption with reduced cancer risk, edible plants are increasingly being considered as a sources of anticancer drugs<sup>5</sup>. There is a large amount of scientific evidence showing that fruits and vegetables lower the risk of cancer<sup>6</sup>. However, whether fruits, vegetables and antioxidant micronutrient consumption are associated with reduction in breast cancer incidence remains unresolved<sup>7</sup>. Experimental investigation demonstrated that many naturally occurring agents and plant extracts have shown antioxidant and anticancer potential in a variety of bioassay system and animal models having relevance to human disease<sup>8</sup>. Epidemiological studies suggested that antioxidant supplements might reduce the risk of breast cancer related mortality<sup>9</sup> and consuming rich in anthocyanin inhibit the growth of tumor cells.<sup>10, 11</sup>

*Pithecellobium dulce* (Roxb.) Benth belonging to the family Fabaceae. It is locally woody known as Jangal Jalebe and with English name as Manila Tamarind, is a small to medium sized evergreen, spiny wood legume up to 18 m height. It is native of a tree that originally from tropical America and also found throughout India and Pakistan. Pharmacological studies showed fruits of the plant possess antiulcer (Megala and Geetha, 2010)<sup>12</sup>, antidiabetic (Pradeepa *et al.*, 2013)<sup>13</sup>, hypoglycemia (Jayaraman and Arumugam 2015)<sup>14</sup>, hepatoprotective (Raju and Jagadeeshwar 2014).<sup>15</sup> The main objective of this study to find out the *in vitro* anticancer and antioxidant activity of *Pithecellobium dulce* fruit.

### MATERIALS AND METHODS

#### Collection and Preparation of the Selected Samples

The *Pithecellobium dulce* ripen fruits were collected from in and around Coimbatore district, Tamil Nadu, India. The fruits were cut in to pieces and air dried. The dried samples were pounded in to powder using mortar and pestle. The powder obtained was kept in 4°C in the refrigerator and used during the period of research.

#### Fruit Extract

The powered form (80g) of fruits was extracted using methanol (200ml) for 72 hr by using soxhlet apparatus. The extract was filtered through Whatman No. 1 filter paper and evaporated in a rotary vapour at 40°C to get it completely dried. The percentage yield of the powered methanol extract was 8.056 %. It was transferred to sterile screw caps and stored at - 20° C When needed the frozen dried extracts were dissolved in media.

#### Methodology for *In vitro* Assay

##### Cell Culture

The Human Breast cancer (MCF-7) cells were procured from the National Center for Cell Sciences (NCCS), Pune,



India. The cancer cells were maintained in Dulbecco's modified eagles medium (DMEM) supplemented with 2mM l-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/L Na<sub>2</sub>CO<sub>3</sub>, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100µg) were adjusted to 1ml/L. The cells were maintained at 37°C with 5% CO<sub>2</sub> in a humidified CO<sub>2</sub> incubator.

### Evaluation of Cytotoxicity

The inhibitory concentration (IC<sub>50</sub>) value was evaluated using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cancer cells were grown (1×10<sup>4</sup> cells/well) in a 96-well plate for 48 h in to 75% confluence. The medium was replaced with fresh medium containing serially diluted synthesized compounds and the cells were further incubated for 48 hr. The culture medium was removed and 100µl of the MTT [3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37 °C for 4 h. After removal of the supernatant, 50 µl of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multi well plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula.

$$\% \text{ of viability} = \frac{\text{OD value of experimental sample}}{\text{OD value of control}} \times 100$$

### Morphological Study of *In vitro* Anticancerous Activity

The MCF-7 cells that were grown on cover slips (1×10<sup>5</sup> cells/cover slip) incubated for 6-24hr by using different concentration, and they were fixed in an ethanol: acetic acid solution (3:1v/v). The cover slips were gently mounted on glass slides for the morphometric analysis. Three monolayers per experimental group were photo micro graphed. The morphological changes of the MCF-7 cells were analyzed using Nikon (Japan) bright field inverted light microscopy at 40x magnification.

**Standard Used:** Doxorubicin

### Statistics

All the in vitro experiments were done in triplicates. The statistical software SPSS version 17.0 was used for the analysis. *P* value < 0.01 was considered significant.

### Methodology for Antioxidant Activity

#### Diphenyl Picryl Hydrazyl (DPPH) Radical Scavenging Activity

The radical scavenging and antioxidant potential of the fruit extracts were determined by the ability of plant extracts to scavenge the stable free radical DPPH and convert it into Diphenyl picryl hydrazine. The degree of decolorization from purple to yellow color was measured

spectrophotometrically at 517 nm. 500µl of 0.3mM DPPH prepared in methanol was mixed with 2.5 ml of extracts. The reaction mixture was mixed well and left in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the fruit extract was calculated using this equation;

$$\text{DPPH Scavenging activity (\%)} = \frac{[(\text{Abs control} - \text{Abs sample})]}{(\text{Abs control})} \times 100$$

Where Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample (i.e. extract).

### Inhibition of Nitric Oxide Generation

The extent of nitric oxide generation was studied using Griess reagent method. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide which interacts with oxygen to produce nitrite ions determined by the use of Griess reagents. 300µl of 100 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (p<sup>H</sup> 7.2) was mixed with fruit extract .The mixture was incubated at 25°C. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 540 nm. The amount of nitric oxide radical was calculated following this equation

$$\% \text{ inhibition of NO} = \text{Abs control} / \text{Abs sample} \times 100$$

### Inhibition of Superoxide Generation

Superoxide generation was investigated on the basis of production of Nitroblue tetrazolium formazan of the superoxide ion by the fruit sample. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 560 nm. The amount of Superoxide radical was calculated following this equation

$$\% \text{ inhibition of SO} = \text{Abs control} / \text{Abs sample} \times 100.$$

### Statistical Analysis

All the parameters studied were analyzed statistically using Sigma Stat statistical package (Version 3.1). One way ANOVA with *P*<0.05 was considered significant and, one way ANOVA followed by post-hoc Fischer analysis was done to test the levels of statistical significance.

## RESULTS AND DISCUSSION

### Anticancer Activity of *Pithecellobium dulce* Fruits

*Pithecellobium dulce* methanol fruit extract was treated by using different concentrations viz., 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml against MCF human breast cancer cell lines. 27 % cell viability exhibited at 50 µg/ml, 33 % cell viability at 40 µg/ml, 49 % cell viability at 30 µg/ml, 70.2 % cell viability at 20 µg/ml, 95.5 % cell viability at 10 µg/ml (Table 1, Plate 1). The morphology of cultured breast cancer cell significantly changed upon the treatment with the fruit extract compared with control.



Effect of inhibition of cell growth showed significantly cytotoxic against MCF – 7 with an IC<sub>50</sub> of 24 µg/ml. The overall results indicate the promising baseline information for the potential uses of the methanol extract of *P.dulce* fruit has anticancer activity.

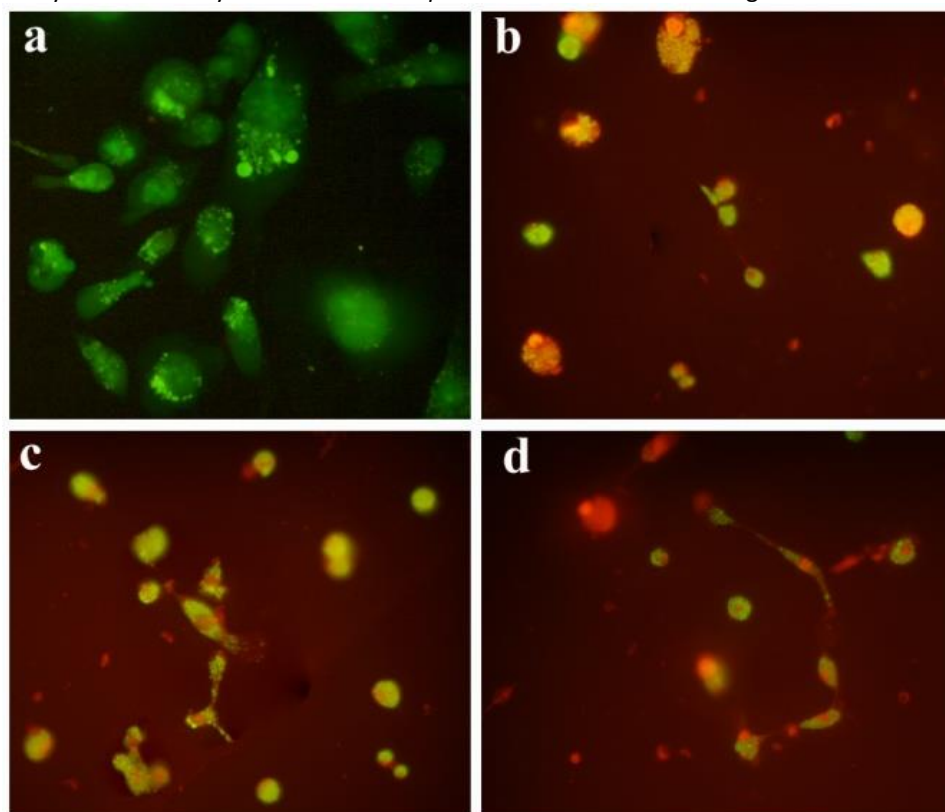
Anthocyanin rich extracts from berries and grapes exhibited proapoptotic effects in multiple cell types *in vitro*<sup>16,17</sup>. The anticancer activities of anthocyanins from many fruits were inhibit the initiation, promotion and

progression of several types of cancers<sup>18, 19</sup>. The anthocyanins induce apoptosis through both intrinsic (mitochondrial) and extrinsic (FAS) pathways<sup>20</sup>. Similar experiment conducted in *P.dulce* leaf (570 µg/ml) and flower (624 µg/ml)<sup>21</sup> but there is only meagre in fruits. Isolation of active anticancerous compounds to find out the detailed mode of action for the development of new drug. This will be useful in the treatment or prevention of cancer may be a boon for the society.

**Table 1:** *In vitro* Cytotoxicity of *Pithecellobium dulce* Fruits against MCF Human Breast Cancer Cell Line in MTT Assay

Conc (µg/ml)	% Cell Viability	Doxorubicin (Standard)	IC <sub>50</sub> (µg/ml)
10	95.5 ± 0.31	92	24
20	70.2 ± 0.11	55	
30	49 ± 0.41	27	
40	33 ± 0.21	13.5	
50	27 ± 0.33	9.0	

**Plate 1:** In Vitro Cytotoxic Efficacy of *Mukia maderaspatana* Fruit Extract on Michigan Cancer Foundation-7 Cell Line



a = Control (Untreated); b= 10µg/ml; c= 20µg/ml; d= 40µg/ml

#### Antioxidant Activity of *Pithecellobium dulce* Fruit Methanol Extract

##### DPPH Scavenging activity

DPPH is a stable free radical compound and has an absorbance in its oxidized form around 515 – 520 nm<sup>22, 23</sup>. DPPH assay is relatively rapid and efficient method to evaluate free radical scavenging activity. DPPH is able to accept an electron or hydrogen radical to form a stable

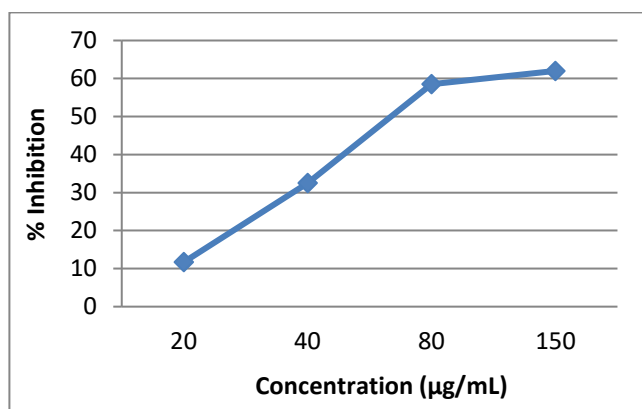
diamagnetic molecule. Changes in colour from purple to yellow indicate decrease in absorbance of DPPH radical. This demonstrates that the antioxidant found in a mixture solution interact with the free radical<sup>24</sup>. In the present study, percentage of inhibition (Table 2 and Chart 2) was measured to determine the antioxidant activity of the extracts which is able to inhibit free radicals. Four varying concentrations (20, 40, 80 and 150 µg/ml) of methanol extract of *Pithecellobium dulce* fruits demonstrated

different percentage of inhibition. The EC<sub>50</sub> values of the methanolic extract of *P.dulce* fruits were 60 µg/ml whereas gallic acid showed the value of 5.50 µg/ml which were significant enough to be considered for further research. Methanol has been known as potential polar solvent to extract phenolic compounds<sup>22, 25</sup>. The antioxidant activity measurement has revealed that methanol fruit extract of *P.dulce* has greater activity than gallic acid. Some reports have shown that methanol extract of medicinal plants possessed good pharmaceutical activity.

**Table 2:** Antiradical (DPPH) Activity of *P.dulce* Ripen Fruit Methanol Extract

Sample	Concentration (µg/mL)	(%) Inhibition	EC <sub>50</sub> (µg/ml)
<i>P.dulce</i>	20	11.7 ± 1.5	60
	40	32.5 ± 1.2	
	80	58.5 ± 1.5	
	150	62.0 ± 1.0	
Gallic acid			5.50

**Chart 2:** Antiradical Scavenging Activity of *Pithecellobium dulce* Fruit Methanol Extract



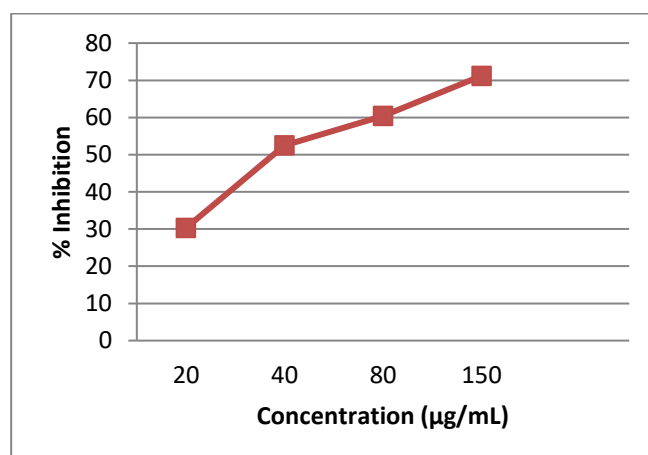
### Superoxide Anion Radical Scavenging Activity

Superoxide anions radicals play a critical role in the formation of reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide and singlet oxygen, which induce oxidative damage in lipids, protein and DNA<sup>26, 27</sup>. As the data of table shows different concentration of methanol extract of *P.dulce* fruits (20 – 150 µg/ml) exhibited different superoxide anion scavenging activity. With an increase in the concentration of methanol extract of *P.dulce* fruits, the activities become stronger. At the highest evaluated concentration (150 µg/ml), 74.5 % of superoxide anions radicals. The EC<sub>50</sub> value of methanol extract of *P.dulce* fruits for scavenging superoxide anion radical was 55 µg/ml, whereas the EC<sub>50</sub> value of ascorbic acid was 12.5 µg/ml (Table 3 and Chart 3). When compared to that of ascorbic acid, the superoxide radical scavenging activity of methanol extract of *P.dulce* fruits was highly significant. These results indicated that the tested extract had a noticeable effect on scavenging superoxide when compared with ascorbic acid which was used as positive control.

**Table 3:** Superoxide Anion Scavenging (SO) Activity of *P.dulce* Ripen Fruit Methanol Extract

Sample	Concentration (µg/mL)	(%) Inhibition	EC <sub>50</sub> (µg/ml)
<i>P.dulce</i>	20	23.3 ± 1.2	55
	40	38.5 ± 1.3	
	80	63.8 ± 1.6	
	150	74.5 ± 1.2	
Ascorbic acid			12.50

**Chart 3:** Superoxide Anion Scavenging (SO) of *Pithecellobium dulce* Fruit Methanol Extract



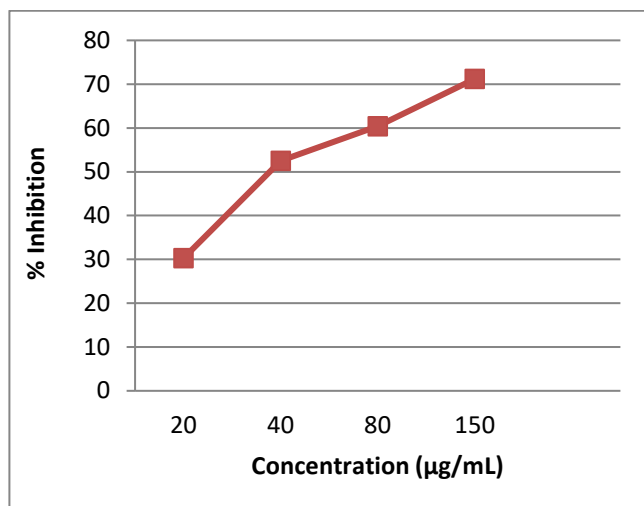
### Nitric oxide Scavenging Activity

Nitric oxide is also implicated in inflammation, cancer and other pathological conditions.<sup>28</sup> The plant products may have the property to counteract the effect of nitric oxide formation and in turn may be considerable interest in preventing the ill effects of excessive nitric oxide generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of nitric oxide that is detrimental to human health. The extract showed a moderate nitric oxide scavenging activity between 20 – 150 µg/ml in a dose dependent manner (EC<sub>50</sub> = 46 µg/ml). The percent inhibition was increased with an increasing concentration of the extract (Table 4 and Chart 4). Curcumin, the natural antioxidant was used as positive control for comparison.<sup>29</sup>

**Table 4:** Nitric Oxide Scavenging Activity of *P.dulce* Ripen Fruit Methanol Extract

Sample	Concentration (µg/mL)	(%) Inhibition	EC <sub>50</sub> (µg/ml)
<i>P.dulce</i>	20	27.3 ± 1.2	46
	40	45.5 ± 1.7	
	80	71.8 ± 1.5	
	150	88.5 ± 1.5	
Curcumin			20.8



**Chart 4:** Nitric Oxide Scavenging Activity of *P. dulce* Fruit Methanol Extract

### CONCLUSION

In the Present study, the fruit extract of *Pithecellobium dulce* showed invitro anticancer and antioxidant activity. The effect of inhibition of cell growth showed significant cytotoxic against MCF – 7 cell line with an inhibit cell growth by 50 % (IC<sub>50</sub>) of 24 µg/ml. *Pithecellobium dulce* fruit extract were screened for in vitro anticancer and antioxidant activity with view to suggest anticancer herbal drug for managing breast cancer.

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