RESEARCH PAPER

Phytochemical Profiling and Platelet-Augmentation Activity of *Pithecellobium dulce* Fruit

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Abstract

Pithecellobium dulce (Roxb.) Benth. (Fabaceae), commonly known as Manila tamarind, is traditionally used in India as food and folk medicine. Its fruit pulp is rich in nutrients and bioactive compounds, yet its therapeutic potential remains underexplored.

Fruit pulp extracts prepared using solvents of varying polarity were subjected to qualitative phytochemical screening. Reported pharmacological activities were reviewed, including antioxidant, antidiabetic, anti-inflammatory, and antimicrobial effects.

Screening indicated the presence of flavonoids, phenolics, tannins, saponins, alkaloids, terpenoids, steroids, and glycosides, along with vitamin C and minerals. The pulp exhibited notable antioxidant activity, attributed mainly to its phenolic and flavonoid content. Antimicrobial activity was linked to tannins and saponins, while antidiabetic and anti-inflammatory effects were associated with polyphenols and flavonoids. Hepatoprotective, anthelmintic, and antiulcer activities have also been reported in preclinical studies.

P. dulce fruit pulp is a promising source of bioactive phytoconstituents with multiple pharmacological benefits, supporting its traditional use. Further isolation of active compounds, mechanistic studies, toxicity assessment, and clinical validation are warranted for nutraceutical and therapeutic applications.

Keywords: Pithecellobium dulce, Manila tamarind, fruit pulp, phytochemical screening, flavonoids, antioxidant, Platelet augmentation etc.

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Introduction

Thrombocytopenia, a platelet count below 150,000/µL, is a common complication of dengue, chemotherapy-induced myelosuppression, immune thrombocytopenia, and other viral and hematological disorders. Dengue alone causes an estimated 390 million infections annually, mainly in tropical and subtropical regions (1,2). In dengue, platelet counts fall during the critical phase, and severe thrombocytopenia raises the risk of hemorrhage (3). Management is largely supportive, and platelet transfusion carries risks such as alloimmunization, transfusion reactions, and limited availability. This has driven interest in safe, accessible agents that support platelet recovery.

Medicinal plants have long been a source of therapeutic leads (4). *Carica papaya* leaf extract is the most widely studied for platelet augmentation, with clinical and experimental reports of increased platelet counts in dengue-associated thrombocytopenia (5,6). This has encouraged investigation of other traditionally used plants.

Pithecellobium dulce (Roxb.) Benth. (Fabaceae), commonly known as Manila tamarind, is a fast-growing tree native to tropical America and widely naturalized in India and Southeast Asia. Its sweet-sour fleshy aril (fruit pulp) is eaten fresh and used in traditional medicine. Various plant parts show antioxidant, antimicrobial, anti-inflammatory, and gastroprotective activities, linked to phenolics, flavonoids, tannins, saponins, and related metabolites (7,8). Because flavonoids and polyphenols may influence hematopoiesis and megakaryocyte activity and protect marrow and blood cells from oxidative damage, the fruit pulp is a plausible platelet-enhancing candidate. Evidence for this specific effect, however, remains limited.

Phytochemical screening is the first step in linking traditional use to pharmacological action. It identifies the classes of secondary metabolites present and guides later fractionation, standardization, and mechanistic work (9–10). Pairing this profile with in vivo assessment of platelet augmentation in experimentally induced thrombocytopenia allows the observed activity to be considered alongside the likely responsible phytoconstituents.

Despite the nutritional and ethnomedicinal importance of *P. dulce* fruit pulp, studies combining its phytochemical profile with platelet-augmenting potential are scarce. This study therefore aims to (i) carry out preliminary phytochemical screening of the fruit pulp extract and (ii) evaluate its platelet augmentation activity, to provide a scientific basis for its use as supportive therapy in thrombocytopenic conditions.

The antioxidant potential of the hydroalcoholic fruit extract of *Pithecellobium dulce* (HEPD) was evaluated using several in vitro free radical models. Phytochemical screening showed tannins, saponins, flavonoids, terpenoids, cardiac glycosides, alkaloids, and phenols. The extract was tested for DPPH, nitric oxide, superoxide, hydroxyl radical, and lipid peroxide scavenging, as well as reducing power, total antioxidant capacity, and total phenolic content (11)

Materials and Method Plant material

Plant Part Selection: fruits were chosen for study due to their reported pharmacological relevance. (12). Collection of plant material: Fresh fruits of *Pithecellobium dulce* were collected from local area of Sakur, roadside plantation in Sangamner, Maharashtra, India, during the flowering and fruiting season

(Dec -April, 2023-24).

Collection Time: Collection was carried out in the early morning to avoid moisture loss and enzymatic degradation of active constituents.

Drying of plant materials: Cleaned and dried at room temperature in shade and away from direct sunlight. The dried coarsely powdered in grinder. Large difference in particle size of crude drug result in long extraction time as the course particle increase the extraction time and fine powdered material was sieved through 60-120 mesh to remove fines and large particles and the powder was subjected for further study.

Figure 1 Collection, Cleaning, Drying, and Powdering Process of *Pithecellobium dulce* Fruits

The stepwise preparation of *Pithecellobium dulce* fruit samples for phytochemical analysis is shown in Figure The fruits were first collected and rinsed with tap water followed by distilled water to remove dirt and other impurities. They were then dried in the shade to protect heat-sensitive bioactive constituents and mechanically ground into a fine powder. This powder was used for maceration in the experiments that followed.

Preparation of Herbal Extracts The hydroalcoholic fruit extract of Pithecellobium dulce was prepared by macerating about 500 g of air-dried ground homogenous powder of the fruits in 70 per cent ethanol for a week. The extract was filtered with Whatman filter paper no. 1, and the filtrate was allowed to evaporate by rotary evaporator. (13).

Phytochemical Investigation

The preliminary phytochemical analysis of the extracts was performed using hydroalcoholic extracts utilizing established techniques to identify the primary components (14)

Table 1. **Phytochemical analysis**

Sr. NO	Phytochemical	Test	Reagents	Positive indication
1	Tannins	Dissolve extract in warm water and filter. Add 5% ferric chloride drops in 90% alcohol to the filtrate	5% ferric chloride in 90% alcohol	Bluish-black or greenish-black precipitate
2	Alkaloids	Stir 0.4 g of extract with 8ml of 1% HCL, warm and filter. To 2 ml of filter, add (a) Mayers reagent or (b) Dragendroffs reagent.	1% HCL Mayers reagent or	Yellow PPT
3	Flavonoids (Ferric Chloride Test)	Boil 0.5g of extract with ml of water then filter. Add 10% ferric Chloride solution to 2ml of filtrate	10% ferric Chloride solution	Yellow, orange, or red colour
4	Saponins	Boil 1g of extract with 5ml of water and filter. Add 3ml of water to the filtrate and shake vigorously for 5 minutes	water	Frothing (foam formation)
5	Terpenoids	Dissolve the extract in chloroform and add a few drops of Conc. Sulfuric acid	Chloroform, Conc. Sulfuric acid.	Red, pink, or purple colour
6	Phenols	Dissolve 500mg of extract in 5 ml of water. Add a few drops of neutral 5% ferric chloride solution	5% Ferric chloride (neutral ferric Chloride solution)	Dark green colour

Preliminary screening (Table 1) showed that *P. dulce* fruit extract contains phenolic, flavonoid, tannins, alkaloids, saponins and terpenoids content may contribute to its platelet-augmenting activity.

Methodology for Antioxidant Activity Antioxidant activity of extracts

(Radical scavenging activity by DPPH assay method)
Anti-oxidant activity of *Pithecellobium dulce* fruit extract was investigated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method.

Principle-
DPPH (1,1-diphenyl-2-picrylhydrazyl) is considered as a stable radical because of the Para magnetism conferred by its odd electron (delocalization of the spare electron on the molecule as a whole). The solution appears as a deep violet color and shows strong absorption at 520 nm. DPPH can accept the electron or hydrogen to become a stable diamagnetic molecule and is pale violet. If sample mixed with DPPH solution gives rise to pale violet color, it suggests that it has antioxidant effect by mechanism of free radical scavenging activity. The % scavenging activity is calculated by following formula (15)

% scavenging Activity = $\frac{(A \text{ control} - A \text{ sample}) \times 100}{A \text{ control}}$

Where,
$A_{control}$ = Absorbance of blank DPPH solution. A_{sample}= Absorbance of sample solution.

Procedure:

a) Preparation of standard solution:
Stock solution (1mg/ml) was prepared by dissolving 100 mg of ascorbic acid in 100ml of distilled water. Aliquotes 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml were withdrawn from stock solution and diluted to 10 ml with distilled water to get final concentration of 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml respectively.

b) Preparation of sample solution:
100 mg of extract was accurately weighed and diluted up to 100 ml with methanol. The solution was sonicated for 10 mins. Aliquots 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml were withdrawn from stock solution and each diluted to 10.0 ml with distilled water to get final concentration of 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml respectively.

c) Preparation of Reagents:
2,2-Diphenyl-1-picrylhydrazyl (DPPH) Solution (200 μM**):**
13.31mg of DPPH was dissolved in 5 ml of methanol and transferred to a 25 ml volumetric flask and volume was made up to the mark with methanol. 10 ml of the stock solution was pipetted out and further diluted to 100 ml in a volumetric flask to get 0.135 mM solution which was used for the estimation.

d) Procedure:
The assay was performed in 96-well plates. The *Pithecellobium dulce* fruit extract, containing 100 μL of DPPH and 100 μL of the test sample, was incubated at 37^0 C for 30 min. The absorbance was measured at 515 nm. Ascorbic acid was used as a positive control. (16,17,18)

Platelet augmentation activity:
Animals: Albino Wistar rats (150-250g) of either sex were used for the study. They were housed under standard conditions of temperature and light and fed with standard diet and water ad libitum. Experimental protocol was approved by Institutional Animal Ethics Committee (19)
Thrombocytopenia was induced in rats with cyclophosphamide, and the animals were randomly allocated into four groups (n = 6 per group): The rats were divided into four groups of six animals each:

- **Group I (normal control):** received normal saline (10 mL/kg, p.o.) for 15 days.
- **Group II (toxicant control):** received cyclophosphamide (50 mg/kg, s.c.) for three consecutive days.
- **Group III (low dose):** received *P. dulce* fruit extract (400 mg/kg, p.o.) for 15 days, along with cyclophosphamide (50 mg/kg, s.c.) during the first three days.
- **Group IV (high dose):** received Standard Caripill (114mg/kg, p.o.) for 15 days, along with cyclophosphamide (50 mg/kg, s.c.) during the first three days. Under light ether anesthesia, blood samples were collected from the retro-orbital plexus on days 1, 3, 7, and 11 of the study. Platelet counts were measured with a Coulter Act-Diff automated cell counter. On day 15, the blood clotting time was determined using the capillary method. (20,21)

Figure 2 Study Design

Results and Discussion:

Extraction
The fruit of *Pithecellobium dulce* was extracted using ethanol: water (8:2). The *Pithecellobium dulce* fruit yielded slightly sticky brown extract.

Figure 3 Extraction Figure 4 PD Fruit Extract

Table 2 Extraction

Sample	Extraction Method	Solvent used	Extraction value (%w/w)
Pithecellobium dulce fruit	Maceration	Ethanol +Water	17.733 % w/w

Evaluation of Extract

1. Phytochemical investigation

Figure 5 Phytochemical Tests
Preliminary qualitative tests were used to screen the aqueous and ethanolic extracts of *Pithecellobium dulce* fruit for tannins, alkaloids, flavonoids, saponins, terpenoids, and phenols. The ethanolic extract showed a stronger presence of phenols, flavonoids, and terpenoids than the aqueous extract.

Table 3 Phytochemical qualitative analysis

SI No.	Test	Hydroethanolic extract
1	Tannins	+
2	Alkaloids	+
3	Flavonoids	++
4	Saponins	+
5	Terpenoids	++

6	Phenols	+++

Note: High +, Highest +++, Note present –

Antioxidant activity of extracts

The DPPH assay method was used to determine the antioxidant activity of fruit extracts of *Pithecellobium dulce* using ascorbic acid as a standard. The reading for control was found to be 0.421. Various concentrations of ascorbic acid, fruit extracts were prepared and similar treatment was given.

Table 4 Antioxidant activity of ascorbic acid and PD Fruit extract

Concentration (µg/ml)	Absorbance (Mean ± S.D, n=3)	
	Ascorbic acid	Fruit
0.5	0.115±0.07	0.219±0.21
1	0.100±0.11	0.105±0.18
2	0.096±0.09	0.098±0.12
3	0.095±0.14	0.090±0.09
4	0.089±0.06	0.087±0.13
5	0.087±0.11	0.082±0.07

Table 5 % Radical scavenging activity of PD Fruit extract

Concentration (µg/ml)	% radical scavenging activity	
	Ascorbic acid	PD Fruit
0.5	81.71±0.10	65.18±0.09
1	84.1±0.08	83.36±0.11
2	84.73±0.09	84.42±0.08
3	84.89±0.04	85.69±0.06
4	85.85±0.12	86.58±0.13
5	86.1±0.07	86.96±0.05

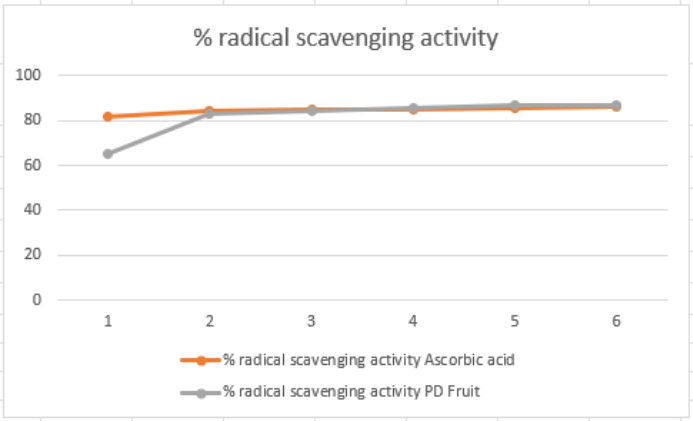


Figure 6 Antioxidant activity of PD Fruit extract in comparison to Ascorbic acid

Figure 7 Antioxidant activity

The antioxidant activity of PD fruit extract was found to be comparable to standard ascorbic acid.

Evaluation of platelet augmentation activity by cyclophosphamide induced thrombocytopenia model

Figure 8 Oral Dosing of animal

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igure 9 Subcutaneous Dosing of animal

The extract was studied for platelet augmentation activity using cyclophosphamide induced haematological cytopenia model at 400mg/kg dose level.

Evaluation Parameters:

Bleeding time & Clotting time

Groups	Bleeding time (min) (Mean ± SD, n=5)	Clotting time (min) (Mean ± SD, n=5)
Vehicle Control	2.214±1.29	2.013±0.29
CYP	4.625±0.41	4.6175±1.29
PDFE extract 400mg/kg	2.212±0.45	2.27±0.14
Standard Caripill 114mg/kg	1.15±0.07	1.34±0.14

- The bleeding and clotting time of Inducer group was found more then other test group and standard group, signifying a hampered platelet function.
- The test and standard groups were found to show bleeding time and clotting time comparable to vehicle control presenting the normal functioning of the platelets in these groups

Effect on platelet-

The test and standard groups showed less reduction in platelet count compared to cyclophosphamide treated group on day 7. Cyclophosphamide group showed significant reduction in platelet count ($p<0.05$) when compared to vehicle control.

The test and standard groups showed no significant ($p>0.05$) reduction in platelet count on day 7, when compared to

cyclophosphamide group.

On day 11, significant ($p<0.05$) improvement in platelet count was observed in test and standard groups in compared to cyclophosphamide presenting their platelet augmentation effect.

On day 15, the animal of cyclophosphamide group showed platelet counts comparable to vehicle control group, showing recovery.

The rats of PDFE (400mg/kg) and standard formulation treated group showed significant increase ($p=0.004$) in platelet count, presenting their platelet augmentation effect.

The prevention of decrease in platelet count and increase in platelet count on day 11 and a certain the platelet booster effect of PDFE extract(400mg/kg), beneficial in the management of platelet count.

Conclusion

The hydroalcoholic extract of *Pithecellobium dulce* fruit pulp contained phenols, flavonoids, terpenoids, tannins, alkaloids, and saponins, with phenols the most abundant. It showed strong DPPH radical scavenging, comparable to ascorbic acid. In cyclophosphamide-induced thrombocytopenic rats, the extract at 400 mg/kg limited the fall in platelet count and improved it significantly by day 11. It also restored bleeding and clotting times to near-normal values, similar to the standard Caripill. These findings support the traditional use of *P. dulce* fruit pulp and suggest it may serve as a supportive platelet-augmenting agent. Further work is needed to isolate the active constituents, clarify the mechanism, and confirm safety and efficacy at multiple doses.

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