ORIGINAL ARTICLE

# Flash Chromatography-Based Phytochemical Profiling and Pharmacological Evaluation of *Pithecellobium dulce* (Roxb.) Benth Leaves

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## Abstract

**Background:** *Pithecellobium dulce (P. dulce),* plant widely distributed in tropical regions, is traditionally recognized for its nutritional and therapeutic value. Although its antibacterial, anti-inflammatory, antidiabetic, hepatoprotective, antioxidant, and anticancer activities are well reported, its neuroprotective potential remains largely unexplored. **Objective:** This study aimed to evaluate the antioxidant and anti-Alzheimer activities of the 90% ethanol extract of *P. dulce* leaves (EEPD). **Methods:** Preliminary phytochemical screening was conducted to identify major bioactive constituents. Antioxidant activity was assessed using the DPPH free radical scavenging assay. EEPD was fractionated by flash chromatography guided by thin-layer chromatography (toluene: ethyl acetate, 9:1), and enriched fractions were characterized using LC-MS. Cytotoxicity and neuroprotective effects were evaluated in SH-SY5Y neuroblastoma cells, with memantine as the reference drug. **Results:** EEPD contained flavonoids, alkaloids, tannins, terpenoids, steroids, and phenolic compounds. It exhibited the strongest antioxidant activity with the lowest $IC_{50}$ value among tested extracts. Phenolic-rich fractions showed significant neuroprotective effects, enhancing SH-SY5Y cell viability and demonstrating notable anti-Alzheimer activity comparable to memantine. **Conclusion:** The study highlights the potent antioxidant and neuroprotective properties of *P. dulce* leaves, supporting their potential for developing plant-based therapeutics for neurodegenerative disorders.

**Key words:** Alzheimer's disease, flash chromatography, liquid chromatography-mass spectrometry, phenolic compounds, *Pithecellobium dulce*, SH-SY5Y cells

## INTRODUCTION

*Pithecellobium dulce*, widely distributed across Asia, Africa, the Pacific Islands, and Central and South America.[1,2] It is common in Tamil Nadu, Kerala, Andhra Pradesh, and numerous other Indian states, where it thrives in coastal belts, scrublands, and dry deciduous forests.[3,4] Its edible fruits are consumed fresh or processed into jams and jellies.[5]

The plant has been extensively explored for its pharmacological activities, such as antimicrobial, antihyperglycemic, anti-diabetic, hepatoprotective, antioxidant, and anticancer effects.[6-12] Although many of its therapeutic properties are documented, its neuroprotective potential remains comparatively underreported. Recent studies highlight the value of plant-derived phenolics and flavonoids in neurodegenerative disease management.[13-15]

Therefore, the present study goals to examine the neuroprotective efficacy of *P. dulce* by examining its effects on neuronal metabolic pathways using *in vitro* models. This work specifically evaluates the possible relevance of *P. dulce* extracts in mitigating neurodegenerative disorders (ND), such as Alzheimer's disease (AD).[16]

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## MATERIALS AND METHODS

### Phytochemical studies

#### *Collection and authentication of plant*

Leaves of *P. dulce* were sourced from the Pattukkottai region of Thanjavur District, Tamil Nadu. Botanical identification was confirmed by Dr. B. D. Sheeja, Associate Professor, Department of Botany, Government Arts College, Udhagamandalam. A specimen has been archived in the Pharmacognosy Laboratory at JSS College of Pharmacy, Ooty.

### Extraction procedure

The leaves were properly cleaned with tap water, shade-dried for a week at room temperature, and then ground into a powder. The dried material was sieved through a mesh no. 40 and 80 to obtain uniform particles, which were stored in airtight containers. Approximately 2 kg of powdered leaves was consecutively extracted using a Soxhlet apparatus with n-hexane, chloroform, ethyl acetate, and 90% ethanol.[17-19] Each extract was concentrated by rotary vacuum evaporator, weighed, and the percentage yield was calculated based on the dry leaf weight. The color and consistency of the extracts were documented. All solvents used were of analytical grade. The extracts were subsequently subjected to preliminary phytochemical screening and *in vitro* antioxidant and free radical scavenging assays.

### *In vitro* antioxidant and free radical scavenging studies

Different quantities of *P. dulce* extracts were combined with 1 mL of a 1 mM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution made in methanol. The mixtures were incubated in the dark at room temperature for 30 min, and absorbance was recorded at 517 nm. Percentage inhibition was measured relative to a control containing 1 mL of methanol added to 1 mL of DPPH solution.[20,21] Based on its robust phytochemical profile and antioxidant activity, the ethanol extract (EEPD) was selected for further isolation and characterization.[22]

### Thin layer chromatography (TLC)

TLC was performed based on the adsorption principle, where components migrate according to their affinity toward the stationary phase. Silica gel G (60–120 mesh) was mixed with water to form a slurry, spread uniformly (2 mm thickness) on cleansed glass plates, dried, and activated. The mobile phase (toluene: Ethyl acetate: Formic acid) in the ratio of 5:4:1was allowed to saturate, the chamber before development. EEPD was spotted 2 cm above the baseline, and plates were developed to three-fourths of their length. After drying, separated spots were visualized using suitable detection reagents or an iodine chamber, and Rf values were determined.[23]

### Flash chromatography

Flash chromatography (Biotage system) was performed using toluene (A) and ethyl acetate (B) in a 9:1 ratio using a SNAP 25 g cartridge. The instrument was primed with solvents, the EEPD extract was loaded, and elution was carried out at a 5 mL/min flow rate. As needed, fractions were gathered in 100 test tubes, concentrated, and recrystallized. Collected fractions were subjected to additional analytical analysis.[24]

### Liquid chromatography: Mass spectrometry (LC-MS)

Bioactive components of the fractions were identified and characterized using LC-MS analysis. This method combines mass spectrometry to determine molecular masses, elemental makeup, and structural details with liquid chromatography to separate compounds. Chemical components can be precisely profiled by detecting ionized molecules based on their mass-to-charge (m/z) ratios.[25]

### Pharmacological studies

When compared to all four extracts, the findings of the cytotoxicity investigation in SH-SY5Y cell lines demonstrated robust anti-AD action in EEPD as compared to memantine as a standard which was mentioned in Figure 3 and Table 1.

**Table 1:** *In vitro* cytotoxicity studies for AD

| S. No. | Sample description | IC50 µg/mL |
|---|---|---|
| 1. | Memantine | 64.59 |
| 2. | n-hexane extract of *P.dulce* | 183.16 |
| 3. | Chloroform extract of *P.dulce* | 194.72 |
| 4. | Ethyl acetate extract of *P.dulce* | 169.42 |
| 5. | Ethanol extract of *P.dulce* | 96.67 |

*P. dulce*: *Pithecellobium dulce*, AD: Alzheimer's disease

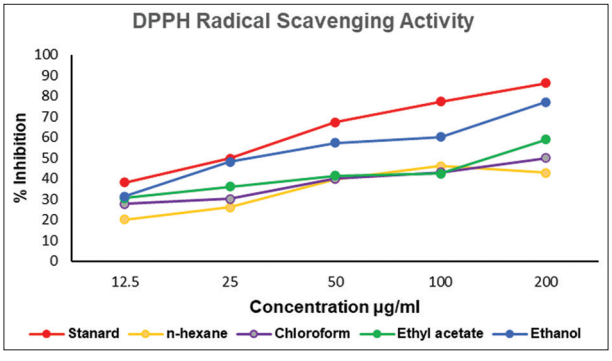


**Figure 1:** 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of *Pithecellobium dulce* leaves extract

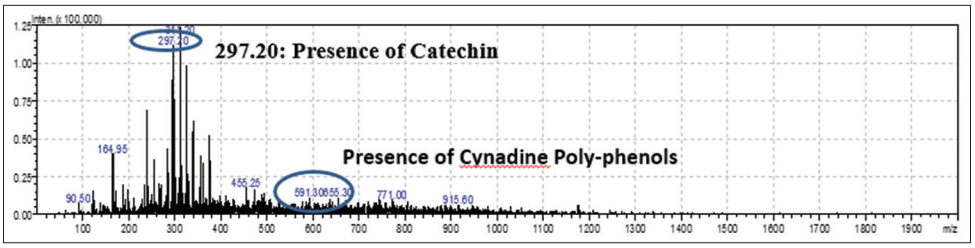


**Figure 2:** Liquid chromatography-mass spectrometry spectrum of fraction 15–21

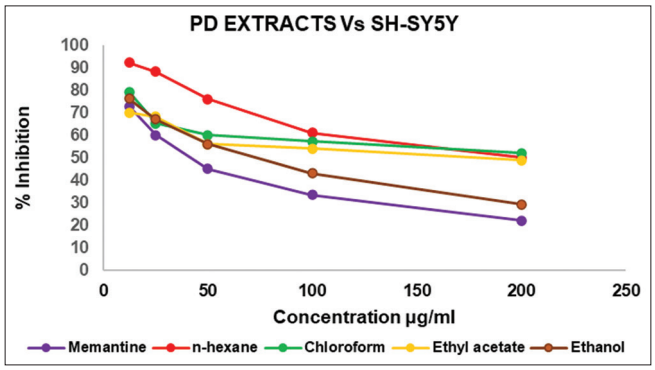


**Figure 3:** *In vitro* cytotoxicity studies for Alzheimer's disease

### *In vitro cytotoxicity studies for AD*

MTT assay was performed to determine the cytotoxicity level of cells for the *in vitro* cytotoxicity assessment.[26] The test system used the SH-SY5Y cell line, which is frequently utilized for performing *in vitro* for neurobiological research and neurodegenerative diseases, including Parkinson's and Alzheimer's. This cell line is useful for researching synaptic function, neurite outgrowth, and neuronal development. To examine the extract's possible anti-Alzheimer action, SH-SY5Y cells were treated with it at doses of such as 12.5, 25, 50, 100, and 200 µg/mL.

## RESULTS AND ANALYSIS

### Phytochemical studies

Flavonoids, alkaloids, tannins, terpenoids, steroids, and phenolic chemicals were all found in different extracts, according to preliminary screening.

### *In vitro* free radical scavenging activities

### *DPPH radical scavenging activity*

A lipophilic radical chain reaction brought on by lipid auto-oxidation is modeled by the DPPH radical. Figure 1 showed that EEPD demonstrated high dose-dependent DPPH radical scavenging capabilities among the several extracts used in the study. The percentage of scavenging activity or inhibition was calculated using the linear regression method. The $IC_{50}$ values for DPPH radical scavenging activity of standard ascorbic acid, n-hexane extract, chloroform extract, ethyl acetate extract, and ethanol extract were 18.08 µg/mL, 215.94 µg/mL, 182.53 µg/mL, 137.40 µg/mL, and 53.75 µg/mL, respectively.

On the basis of phytochemical evaluation and *in vitro* free radical scavenging activities, the ethanol extract of *P. dulce* leaves (EEPD) was selected for isolation.

### *TLC*

EEPD was treated to TLC on silica gel G which has demonstrated good resolution of solutes system such Toluene: Ethyl acetate (9:1). The matching detecting agent was used to identify the various spot developments in each system, and Rf values were computed.

### *Flash chromatography*

On the basis of phytochemical screening and TLC analysis, the isolation of active ingredients of EEPD was done by flash chromatography through the isocratic elution technique with the help of the solvent system Toluene: Ethyl acetate (9:1). The identical color-collected fractions 15–21 [Figure 2], 10–13, and, 26–33 produced by flash chromatography were subjected to LC-MS investigations for validating the phytoconstituents.

### *Spectral analysis: LC-MS of fractions*

Methanol-water (70:30) was used as the diluent for LC analysis, with a rate of flow of 0.5 mL/min. A Shimadzu LC-MS 8030 system (Japan) with an ESI interface running in scan mode was used for mass spectrometric finding. The probe was kept at room temperature, with a block temperature of 350°C and a DL temperature of 250°C. Nitrogen was employed as the nebulizing gas (3 L/min) and drying gas (15 L/min). Data acquisition and processing were carried out using Lab Solutions software. A total of 36 fractions were collected during purification and subsequently pooled into three major groups. LC-MS profiling of these pooled fractions revealed a high concentration of phenolic constituents.

### Pharmacological studies

When compared to all four extracts, the findings of the cytotoxicity investigation in SH-SY5Y cell lines demonstrated robust anti-AD action in EEPD as compared to memantine as a standard.

## CONCLUSION

ND impose a major global health burden, significantly affecting quality of life. Although several therapeutic agents exist, most provide only symptomatic relief without targeting the underlying pathology. Herbal medicines have long been used for ND management due to their lower side effects, better patient tolerance, and accessibility. As ND is frequently associated with behavioral disturbances, such as depression, anxiety, memory loss, and stress in both adults and children, interest in natural neuroprotective agents continues to increase.

This study evaluated the phytochemical composition and anti-Alzheimer's potential of the 90% EEPD. Preliminary screening confirmed the presence of phytoconstituents, such as flavonoids, tannins, alkaloids, terpenoids, and phenolic compounds in various extracts. *In vitro* antioxidant assays demonstrated that EEPD exhibited the robust free radical scavenging action, with lowest $IC_{50}$ values among all extracts.

Based on these findings, EEPD was selected for detailed analysis. TLC using a 9:1 toluene: ethyl acetate system revealed six major spots, guiding the fractionation through flash chromatography. Thirty-six fractions were collected and consolidated into three major pooled fractions, which LC-MS analysis confirmed to be rich in phenolic compounds.

Evaluation in SH-SY5Y cells showed that EEPD exhibited significant cytoprotective and anti-Alzheimer activity, outperforming other extracts and the standard drug memantine. Overall, EEPD demonstrates strong antioxidant and neuroprotective potential, warranting further molecular and mechanistic studies to identify its active constituents and develop novel therapies from *P. dulce*.

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