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Decoding the cardioprotective mechanisms of *Pterolobium hexapetalum Roth* leaves using an *in-vitro* simulated *in-vivo* model using mammalian liver slice approach

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Abstract
Oxidative stress plays a key role in cardiovascular diseases (CVDs) by producing reactive oxygen species (ROS) that damage cell components. *Pterolobium hexapetalum Roth*, a medicinal plant from traditional Indian systems, has been studied for its potential antioxidant properties. This study aimed to evaluate the antioxidant and cardioprotective effects of *P. hexapetalum* leaf extracts using an *in vitro* model that mimics *in vivo* conditions, with goat liver slices exposed to hydrogen peroxide (H2O2)-induced oxidative stress. Leaves of *P. hexapetalum* were collected, authenticated, and successively extracted with petroleum ether, chloroform, acetone, ethanol, and water. Preliminary phytochemical screening was conducted, followed by *in vitro* antioxidant assays, including enzymatic (superoxide dismutase [SOD], catalase [CAT]) and non-enzymatic (vitamin C) antioxidants in H2O2-stressed goat liver slices. Free radical scavenging was evaluated via the DPPH assay. The ethanolic extract showed the highest yield (12.5%) and contained alkaloids, glycosides, phenolic compounds, and flavonoids. In the liver slice model, ethanolic extract (100 µg/mL) significantly increased SOD (from 2.1±0.2 to 4.5±0.3 units/mg protein), CAT (from 15.2±1.1 to 28.4±1.5 µmoles H2O2/min/mg protein), and vitamin C (from 45.3±2.4 to 78.6±3.1 µg/g) levels compared to H2O2-treated controls ($p < 0.05$). DPPH scavenging showed an IC50 of 883.24 µg/mL, similar to vitamin C (741.20 µg/mL). *P. hexapetalum* ethanolic leaf extract demonstrates strong antioxidant activity, restoring enzymatic and non-enzymatic antioxidants in oxidatively stressed liver tissues, indicating cardioprotective potential. This supports its traditional use and calls for further *in vivo* studies to validate its role in CVD management.

Keywords: *Pterolobium hexapetalum*, antioxidant activity, cardioprotective, liver slices, oxidative stress, DPPH assay

Introduction
The liver, as a central metabolic organ, is crucial for detoxification, nutrient metabolism, and maintaining homeostasis, including glucose and lipid levels [1]. It is highly susceptible to oxidative stress due to its role in first-pass metabolism and exposure to xenobiotics [2]. Oxidative stress, driven by reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, and hydrogen peroxide, contributes to cardiovascular diseases (CVDs) like atherosclerosis, myocardial infarction, and heart failure [3]. Antioxidants neutralise ROS, preventing cellular damage and maintaining redox homeostasis. Natural antioxidants from plants, including vitamins C and E, carotenoids, and polyphenols, offer therapeutic potential with fewer side effects compared to synthetic alternatives [4].
Pterolobium hexapetalum Roth, a shrub from the *Fabaceae* family used in traditional Indian medicine, has been reported for antidiarrheal, antiulcer, and antibacterial activities [5, 6]. However, its antioxidant and cardioprotective effects remain underexplored [7]. This study investigates the phytochemical composition and antioxidant efficacy of *P. hexapetalum* leaf extracts in an *in vitro* simulated *in vivo* model using goat liver slices exposed to H2O2, mimicking oxidative stress in CVDs [8, 9]. The model aligns with ethical guidelines to reduce animal use, as recommended by FRAME.

Materials and Methods

Plant Collection and Authentication

Dried leaves and stems of *P. hexapetalum* were collected from Yercaud Hills, Salem District, Tamil Nadu, India, in August 2020. Dr S. Radha, Research Officer, Central Council for Research in Siddha, Mettur, Tamil Nadu, authenticated the plant.

Extraction Procedure

Powdered leaves (500 g) were extracted successively using a Soxhlet apparatus with petroleum ether (60-80°C), chloroform (61.2°C), acetone (56°C), and ethanol (95% v/v) for 72 hours each. Aqueous extraction was performed by cold maceration with distilled water and chloroform. Extracts were concentrated under reduced pressure using a rotary evaporator and stored in a desiccator [10, 11].

Preliminary Phytochemical Screening

Extracts were screened for alkaloids, carbohydrates, glycosides, saponins, tannins, phenolic compounds, flavonoids, sterols, and fixed oils using standard qualitative tests.

In vitro Antioxidant Studies

Liver Slice Model

Goat liver slices (≈1 mm thick, 250 mg) were incubated in Hanks' Balanced Salt Solution (HBSS) at 37°C with H_2O_2 (2 mL/kg tissue) to induce oxidative stress. Ethanolic extract (100 µg/mL) was added, and tissues were homogenised post-incubation for antioxidant assays [18].

Enzymatic Antioxidants

- **Superoxide Dismutase (SOD):** Measured by inhibition of nitrite formation from hydroxylamine hydrochloride, with absorbance at 543 nm. Activity: units/mg protein (50% inhibition of nitrite formation/min).
- **Catalase (CAT):** Determined by H_2O_2 decomposition, with chromic acetate measured at 590 nm. Activity: µmoles H_2O_2/min/mg protein.

Non-Enzymatic Antioxidant

Vitamin C: Estimated using 2, 4-dinitrophenylhydrazine, with absorbance at 520 nm. Expressed as µg/g sample.

DPPH Radical Scavenging Assay

Extracts (various concentrations) were mixed with 0.2 mM DPPH in methanol. Absorbance was measured at 517 nm after 30 min. Percentage inhibition: $(A_0 - A_1)/A_0 \times 100$. IC_{50} was calculated graphically, with vitamin C as a standard.

Statistical Analysis

Data are mean ± SD (n=3). Differences were analysed using one-way ANOVA followed by Tukey's test ($p < 0.05$ considered significant) [12-17].

Results

Percentage Yield of Extracts

The yields varied with solvent polarity: petroleum ether (4.2%), chloroform (6.8%), acetone (8.5%), ethanol (12.5%), and aqueous (10.2%). Ethanolic extract showed the highest yield, indicating efficient extraction of polar compounds (Table 1).

Table 1: Percentage Yield of *P. hexapetalum* Leaf Extracts.

Solvent	Yield (%)
Petroleum Ether	4.2
Chloroform	6.8
Acetone	8.5
Ethanol	12.5
Aqueous	10.2

Phytochemical Screening

Qualitative analysis revealed diverse constituents (Table 2). Ethanolic extract contained alkaloids, glycosides, phenolic compounds, and flavonoids, suggesting rich antioxidant potential.

Table 2: Phytochemical Constituents in *P. hexapetalum* Extracts.

Constituent	Petroleum Ether	Chloroform	Acetone	Ethanol	Aqueous
Alkaloids	+	-	+	+	-
Carbohydrates	-	+	-	-	-
Glycosides	-	-	-	+	+
Saponins	-	+	+	-	-
Tannins	-	+	+	-	-
Phenolic Compounds	-	-	-	+	-
Flavonoids	-	-	-	+	-
Sterols	-	-	-	-	+
Fixed Oils	+	-	-	-	-

(+ = Present; - = Absent)

Antioxidant Status in Liver Slices: H_2O_2 induction reduced SOD (2.1±0.2 units/mg), CAT (15.2±1.1 µmoles/min/mg), and vitamin C (45.3±2.4 µg/g) compared to controls (SOD: 5.2±0.4; CAT: 32.1±1.8; Vitamin C: 85.4±4.2). Ethanolic extract pretreatment restored levels significantly: SOD (4.5±0.3), CAT (28.4±1.5), Vitamin C (78.6±3.1) ($p < 0.05$) (Table 3, Figure 1).

Table 3: Antioxidant Status in H_2O_2-Induced Goat Liver Slices.

Group	SOD (units/mg protein)	CAT (µmoles H_2O_2/min/mg protein)	Vitamin C (µg/g)
Control	5.2±0.4	32.1±1.8	85.4±4.2
H_2O_2-Induced	2.1±0.2*	15.2±1.1*	45.3±2.4*
Ethanolic Extract + H_2O_2	4.5±0.3**	28.4±1.5**	78.6±3.1**

p< 0.05 vs. Control; ** *p*< 0.05 vs. H_2O_2-Induced (n=3).

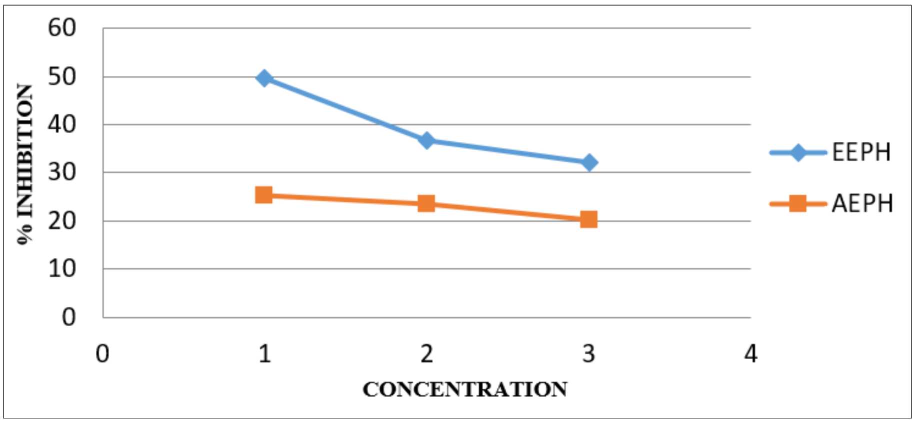
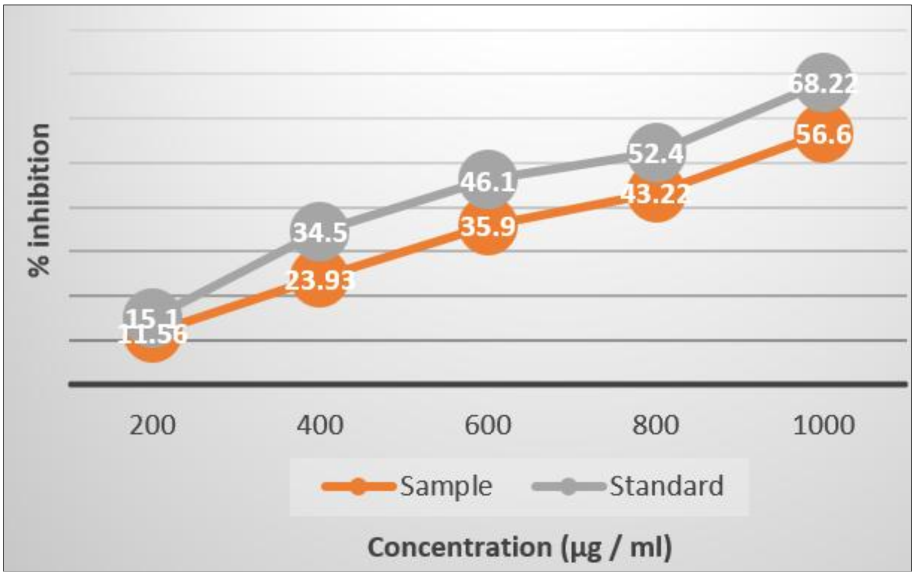


Fig 1: Bar graph showing restoration of SOD, CAT, and Vitamin C levels by ethanolic extract (visualise bars for each group).

DPPH Scavenging Activity: Ethanolic extract showed dose-dependent scavenging: 20% at 200 µg/mL, up to 65% at 1000 µg/mL. IC50: 883.24 µg/mL (vs. vitamin C: 741.20 µg/mL) (Table 4, Figure 2).

Table 4: DPPH Scavenging (%) of Ethanolic Extract.

Concentration (µg/mL)	% Inhibition
200	20.5±1.2
400	35.8±1.5
600	48.2±2.0
800	55.6±2.3
1000	65.1±2.5



IC_{50} value of Sample: 883.24 µg/ml
IC_{50} value of Vitamin C (standard): 741.20µg/ml

Fig 2: Dose-response curve for DPPH inhibition (IC50 marked).

Discussion

The present study demonstrates the potent antioxidant potential of ethanolic extracts from the leaves of *P. hexapetalum*, highlighting its ability to reduce oxidative stress in an ex vivo model using H_2O_2-challenged goat liver slices. Successive extraction with solvents of different polarity revealed that the ethanolic extract not only provided the highest yield (12.5%) but also contained a rich profile of bioactive compounds, including alkaloids, glycosides, phenolic compounds, and flavonoids. This result aligns with the idea that ethanol, as a moderately polar solvent, effectively dissolves a wide range of secondary metabolites from plant materials, often outperforming non-polar (e.g., petroleum ether) or highly polar (e.g., water) solvents in terms of yield and phytochemical diversity. The presence of phenolics and flavonoids in the ethanolic fraction is especially important, as these types of compounds are well-known for their redox-modulating properties, functioning as hydrogen donors, metal chelators, and singlet oxygen quenchers to neutralize reactive oxygen species (ROS).

In the H_2O_2-induced oxidative stress model, the ethanolic extract at 100 µg/mL significantly restored enzymatic antioxidant defences, elevating superoxide dismutase (SOD) activity from 2.1±0.2 to 4.5±0.3 units/mg protein and catalase (CAT) from 15.2±1.1 to 28.4±1.5 µmoles H_2O_2/min/mg protein compared to stressed controls ($p<$ 0.05). These enhancements suggest that the extract may upregulate or stabilise these key enzymes, which play critical roles in the cellular antioxidant cascade: SOD catalyses the dismutation of superoxide radicals to hydrogen peroxide, while CAT decomposes the latter into water and oxygen, thereby preventing lipid peroxidation and cellular damage. The observed restoration could be attributed to the synergistic action of flavonoids and phenolics, which have been shown in similar studies on other medicinal plants to induce gene expression of antioxidant enzymes via pathways such as Nrf2/ARE signalling. Furthermore, the non-enzymatic antioxidant vitamin C levels were markedly increased from 45.3±2.4 to 78.6±3.1 µg/g tissue, indicating that the extract may either directly contribute ascorbate-like compounds or facilitate the regeneration of endogenous vitamin C through electron transfer mechanisms. This dual modulation of enzymatic and non-enzymatic systems underscores the extract's comprehensive protective effect against oxidative insults, which are implicated in various pathological conditions, including hepatic disorders, inflammation, and ageing.

The free radical scavenging capacity of the Ethanolic extract was further corroborated by the DPPH assay, yielding an IC_{50} value of 883.24 µg/mL, which is comparable to that of the standard antioxidant vitamin C (741.20 µg/mL). While the extract's potency is slightly lower than the pure standard, this result is promising for a crude plant extract, as it reflects the collective efficacy of multiple bioactive constituents rather than a single isolated molecule. The DPPH method, which measures the ability to donate hydrogen atoms to stabilise the DPPH radical, provides a reliable indicator of *in vitro* antiradical activity, often correlating with *in vivo* performance. These findings are consistent with reports on other Polygonaceae or related plant families, where ethanolic extracts rich in polyphenols exhibit moderate to strong DPPH inhibition, typically in the range of 500–1000 µg/mL IC_{50}, suggesting that *P. hexapetalum* could serve as a natural source of antioxidants with potential applications in nutraceuticals or pharmacotherapy.

Overall, the results suggest that *P. hexapetalum* leaf extracts are a promising candidate for combating oxidative stress-related ailments, especially those affecting liver function, given the relevance of the ex vivo model to hepatic physiology. However, limitations such as testing only a single concentration (100 µg/mL) and using an animal-derived tissue model warrant further investigation, including dose-response studies, *in vivo* validations in rodent models, and isolation of active compounds for mechanistic understanding. Future research could also examine synergistic formulations with other antioxidants or assess bioavailability to improve translational potential. In conclusion, this study adds to the growing evidence supporting the therapeutic potential of underutilized plants like *P. hexapetalum* in oxidative stress management, paving the way for sustainable herbal interventions.

Conclusion

P. hexapetalum ethanolic leaf extract demonstrates significant antioxidant and cardioprotective potential by enhancing enzymatic (SOD, CAT) and non-enzymatic (vitamin C) defences in oxidatively stressed liver tissues and scavenging DPPH radicals. These findings support its traditional use in managing oxidative stress-related CVDs and highlight its value as a natural therapeutic agent. Future research should explore *in vivo* efficacy, isolation of active compounds, and clinical trials.

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Conflict of Interest

Declared none.

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