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In-vivo cardioprotective activity and *in vitro* antioxidant activity of leaves extract of *Pergularia Deamia*

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Abstract
The present study investigates the *in vivo* cardioprotective and *in vitro* antioxidant activities of the ethanolic extract of *Pergularia daemia* leaves (EEPDL). Cardiac arrhythmia was experimentally induced in *Daphnia magna* using 50 mM lactose to create oxidative stress and calcium imbalance. Treatment with varying concentrations of EEPDL (0.5–2.0 µl/ml) significantly restored normal heart rhythm in a dose-dependent manner, with the highest dose (2 µl/ml) showing a heart rate comparable to metoprolol (25 µg/ml), indicating strong cardioprotective efficacy ($p < 0.001$). The *in vitro* DPPH radical scavenging assay demonstrated potent antioxidant activity with 77.53% inhibition at 100 µg/ml and an IC_{50} of 0.98 µg/ml, suggesting the presence of phenolic and flavonoid compounds responsible for free radical neutralization. These findings highlight that *Pergularia daemia* possesses potent antioxidant and cardioprotective properties, supporting its traditional use in treating oxidative stress–related cardiac disorders.

Keywords: *Pergularia daemia,* cardioprotective activity, antioxidant, DPPH assay, arrhythmia, oxidative stress

Introduction
Cardiovascular diseases (CVDs) are among the foremost causes of mortality and morbidity worldwide, primarily due to myocardial ischemia, arrhythmia, and oxidative stress–related cardiac injury [1]. Arrhythmia refers to an abnormal heart rhythm resulting from altered electrical activity in cardiac tissues, which may lead to impaired cardiac output and, in severe cases, sudden cardiac death [2].
Experimental models using *Daphnia magna* (water flea) have gained popularity in pharmacological and toxicological studies due to their transparent exoskeleton, easily observable cardiac activity, and sensitivity to cardioactive substances [3]. Lactose-induced arrhythmia in *Daphnia magna* serves as a simple, cost-effective, and ethical model for screening potential cardioprotective agents [4].
Pergularia daemia (Family: Asclepiadaceae), commonly known as "Uttaravaruni," is a medicinal plant traditionally used in Ayurveda for treating various ailments such as inflammation, asthma, and cardiac disorders [5]. Phytochemical studies have revealed that the ethanolic extract of *Pergularia daemia* leaves contains flavonoids, alkaloids, saponins, and steroids, which possess significant antioxidant and membrane-stabilizing activities [6, 7]. These phytoconstituents are known to protect cardiac tissues by reducing oxidative stress and improving myocardial function.

Methodology
Plant collection and Authentication
Plants were selected for screening using a focused method based on ethnobotanical information derived from The Natural Medicine Research Center' (NMRC) database of medicinal plants containing the traditional and The medicinal applications of over 500 Israeli plant species were compiled using data-mining techniques and supplemented with information extracted from historical pharmacopeias and medical encyclopedias translated from classical Latin, Hebrew, and Arabic manuscripts.Selected plants were collected from wild sources or harvested from domesticated plants derived and obtained from wild plants. *Pergularia daemia* plant collected from gobichettipalayam, tamil nadu in the month of april 2025. The species for the proposed study was identified & authenticated by Dr. D. Stephen, Professor Department of Botany, American College, Madurai-625002.

Plant Extraction: Simple Maceration Method
The identification and extraction procedures were similar to that earlier reported elsewhere. Leaves of the plant will collect and air-dried separately at room temperature (25 °C) and were pulverise after drying. Of the pulverised sample, 200 g was kept in contact with 500ml Ethanol in a stoppered glass container for 5 days with frequent agitation. The extract was decanting and filter through a cotton plug funnel and Whatman no.1 filter paper. The filtrate from the ethanol extraction was concentrated under vacuum using rotary evaporator [8].

Pharmacological Studies
In- Vitro Antioxidant exertion of EEPDL in DPPH Radical Scavenging Assay [9]
Principle
The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this system is simple and sensitive. This assay is grounded on the proposition that a hydrogen patron is an antioxidant. It measures composites that are radical scavengers. Figure 1, below, shows the medium by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the many stable and commercially available organic nitrogen revolutionaries (1). The antioxidant effect is commensurable to the exposure of DPPH in test samples. Monitoring DPPH with a UV spectrometer has come the most generally used system because of its simplicity and delicacy. DPPH shows a strong

immersion outside at 517 nm(purple). The color turns from grandiloquent to unheroic followed by the conformation of DPPH upon immersion of hydrogen from an antioxidant. This response is stoichiometric with respect to the number of hydrogen tittles absorbed. thus, the antioxidant effect can be fluently estimated by following the drop of UV immersion at 517 nm.

Material needed

mM DPPH result, Ascorbic acid, Methanol

mM DPPH result Dissolve 39 mg of DPPH in 100 ml of methanol and store at-20 °C until demanded. Ascorbic acid (Standard) 1 mg/ ml of Ascorbic acid

Procedure

Compactly, prepare 0.1 mM of DPPH result in methanol and add 100 µl of this result to 300 µl of the result of Sample (TP) at different attention (500, 250, 100, 50, and 10 µg/ ml).

The fusions have to be shaken roundly and allowed to stand at room temperature for 30 twinkles. Also, the absorbance has to be measured at 517 nm using a UV- VIS spectrophotometer. (Ascorbic acid can be used as the reference).

Lower absorbance values of response admixture indicate advanced free revolutionary scavenging exertion. The capability of scavenging the DPPH revolutionary can be calculated by using the following formula.

DPPH scavenging effect (inhibition) = (absorbance of control- absorbance of response admixture)/ absorbance of control) X 100

***In- Vivo* Cardioprotective Activity**

Principle

The principles of determination of cardio defensive agent on D. magna were well enumerated by Bekker, Krijgsman and numerous scientists at the turn of the century. The discovery of cardio defensive medicines made by using D. magna was veritably sensitive, accurate and time bound. Mass webbing of cardio active medicines can be done by using D. magna assay.

Advantage of D. magna [10, 11]

1. Genomic sequence of D. magna shares most with humans.
2. Its myogenic heart, while utmost arthropods hearts are neurogenic.
3. Transparent carapace allows easy observation of heart. So, apply optic styles to fantasize physiological function and to measure several different parameters contemporaneously.
4. Non-invasive system.
5. medicines are directly added to the water in which they swim.
6. In concern of beast weal, no need of ethical concurrence.
7. D. magna has analogous ANS in compared to humans.
8. Economically doable. Easy to culture in lab.
9. lower time consuming
10. More sensitive, accurate results.

Culture of D. magna

attained from the original terrarium in Madurai, Tamil nadu. It was linked and authenticated by DR. A. Vijayaraj, Assistant professor, Department of Marine Biotechnology, star Investigator (DST- SERB), Aquaculture Research Laboratory, Chennai- 603 112. D. magna was dressed by using Elendt- Bias(M4) medium and maintained photoperiod ± 12 hr. spirulina used as a feed in spring water. Aerated for 48 hr to gain O_2 attention not lower than 4mg/ ml. trial was carried out at 20 °C 2 °C and down from the sun [12].

Assesment of Acute Toxin of Eepdl In Using Daphnia magna [13]

Toxicology through ferocious studies has traditionally concentrated on the effect of chemicals on living organisms, which was done by one chemical at a time. similar approach shows the mode of action of numerous chemicals and provides a detailed mechanistic understanding of the molecular targets of toxin for some as the cost of this approach is high. Toxicology studies calculate on the mileage of invertebrate creatures, which is a precious undertaking in both time, and cost with debatable prophetic power in case of safety aspects for mortal.24 hr old Daphnids named for this study. Since babes may be more sensitive than elder one. further over further particularity, simplicity and do n't reproduce.

1. Daphnids in spring water culture transferred to depression (n =20). No food feed throughout the study.
2. guide different attention of test medicine (1, 2, 3, 4, 5, 6 mg/ L of Extract) temperature 20 °C 2 °C maintained.
3. Observe the mortality rate and immobility after 24 hr.
4. LC50was calculated by using probit analysis system.

***In Vivo* Cardioprotective Conditioning of The Eepdl On Daphnia magna [14]**

1. Place the Daphnia in the depression along with drops of water using glass tube with rubber teats.
2. Divided Daphnia into six groups (n =10). Control, lactose convinced, test medicine treated (0.5, 1, 1.5, 2µg/ ml), standard medicine (Metoprolol) treated (20, 25µg/ ml) on lactose convinced heart of D. magna.
3. Heart beat and meter were observed under low power microscope with CCTV and photomicrograph.
4. Results were tabulated.

Results and Discussion

Organoleptic and Morphological Studies of *pergularia daemia* leaves

The fresh Leaves of *pergularia daemia* are subjected to organoleptic and macroscopical studies and result was presented in the table

Table 1: Organoleptic studies of *pergularia daemia* leaves

SI. No	Organoleptic Characters	Observation
1	Colour	Mature leaf - Dark green Tender leaf – Light green
2	Odour	Characteristic odour
3	Taste	Bitter taste

Table 2: Macroscopical studies of *pergularia daemia* leaves

SI. No	Morphological Characters	Observation
1	Shape	broadly ovate
2	Size	Lengh-2-4-inch Width-1/2-3/2 inch
4	Outer surface	glabrous
5	Texture	Smooth

Preparation of Powdered Drug

The preparation of *pergularia daemia* powder begins with shade drying the leaves-a method chosen to gently eliminate moisture while retaining the leaves' natural color, flavor, and nutritional value. Once adequately dried, the leaves are ground into a fine powder using a mixer. However, the grinding process may yield a slightly coarse texture, indicating some variation in particle size. This coarseness can enhance the powder's overall character. After grinding, the course *pergularia daemia* powder is stored in a tightly sealed container to preserve its freshness and quality. These containers are typically airtight to protect the powder from moisture, air, and other contaminants.

Table 3: Organoleptic studies of powdered leaves

S. No	Characters	Observation of leaves
1.	Color	green
2.	Odour	Characteristic odour
3.	Taste	Bitter taste
4.	Nature	Coarse

Discussion

The combined organoleptic and morphological evaluation of *pergularia daemia* leaves confirms the diagnostic features necessary for its proper identification and authentication. Such standardization studies are essential to prevent adulteration and ensure reproducibility in pharmacological research. The green coloration indicates the presence of chlorophyll pigments, which are known for their antioxidant potential. The characteristic odour and taste may be linked to volatile oils and phytochemicals inherent to the species.

Preparation of Ethanolic Extract of *Pergularia Daemia* Leaves

The fresh plants leave of P. daemia were collected. Leaves of the plant were air-dried separately at room temperature (25 °C) and were pulverised after drying. The pulverised sample, 200g was kept in contact with 500 ml ethanol in a stoppered glass container for 5 days with frequent agitation. The extract was decanted and filtered through a cotton plug funnel and Whatman no.1 filter paper. The filtrate from the ethanol extraction was concentrated under vacuum using rotary evaporator.

Table 4: Organoleptic studies of leaf extract of *pergularia daemia*

S. No	Characters	Observation of Leaves
1.	Color	Dark green
2.	Odor	Characteristic odor
3.	Taste	Bitter
4.	Consistency	Sticky
5.	Solubility	Soluble in methanol

Table 5: Maceration Methods of Extraction

S. No	Physicochemical Constants	Results
1.	Acetone extractive	9.8 ± 0.777 %w/w
2.	Chloroform extractive	8.3 ± 0.4 %w/w
3.	Ethanol extractive	25.06 ± 0.427 %w/w
4.	Aqueous extractive	13.405 ± 1.318 %w/w
5.	Benzene extractive	6.756 ± 0.554 %w/w

Pharmacological studies

***In vitro* anti-oxidant activity of *pergularia daemia* by dpph radical scavenging assay**

Ethanolic extract *Pergularia daemia* of was subjected to *in-vitro* antioxidant activity

Table 6: Determination of DPPH radical scavenging assay of ethanolic extract of *Pergularia daemia*

S.No	Tested sample concentration (µg/ml)	OD Value at 517 nm	Percentage of inhibition (%)
1	Control	0.948	0.00
2	20	0.904	4.641
3	40	0.788	16.877
4	60	0.596	37.130
5	80	0.456	51.898
6	100	0.213	77.531
7	Ascorbic acid (50)	0.044	95.29

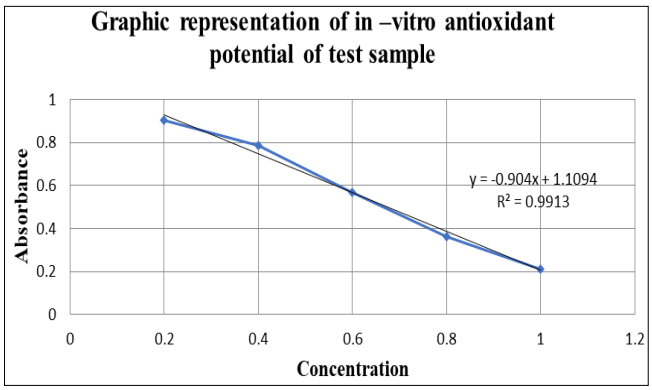

Fig 1: Graphical representation of *in –vitro* antioxidant potential of test sample

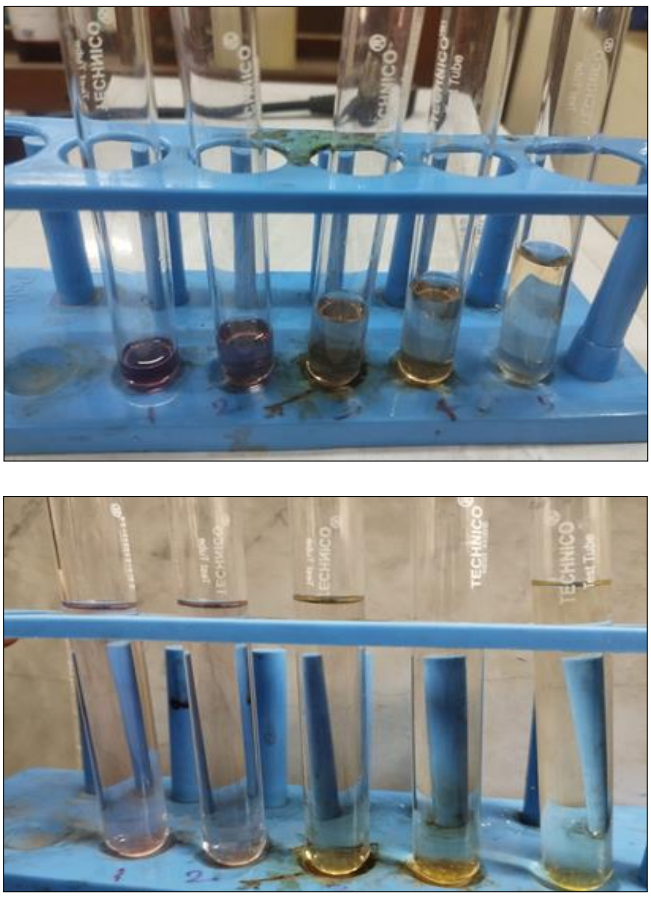

Fig 2: Image representation of assay method

Results

Ic 50 value of EEPDL against DPPH was found to be 0.98 µg/ml and the percentage of inhibition of standard ascorbic acid is 95.29% to that of EEPDL is 77.531 %. The percentage of inhibition occurs in dose-dependent manner.

Discussion

Cardiac arrhythmia is a cardiovascular disorder caused by abnormal heartbeat rhythms due to oxidative stress and myocardial damage. The ethanolic extract of *Pergularia daemia* leaves (EEPDL) showed potent antioxidant activity, with 77.53% DPPH inhibition at 100 µg/ml and an IC_{50} of 0.98 µg/ml, indicating strong free radical scavenging ability. This antioxidant potential helps neutralize ROS, protecting ion channels and maintaining calcium balance in cardiac cells. Bioactive compounds like genkwanin and catechin enhance endogenous antioxidant enzymes such as SOD, CAT, and GPx. These effects collectively prevent oxidative damage and restore electrophysiological stability. Thus, EEPDL exhibits significant cardioprotective potential, supporting its traditional use in managing arrhythmic and oxidative stress–related heart disorders.

Assesment of acute toxicity of *p. Daemia* leaves extract by using *daphnia magna*

We assessed the acute toxicity of p. daemia leaves extract using daphnids. Percentage mortality or immobility of control (spring water).test drug marine leaves extract (1,2,3,4,5,6 µg/ml) were observed. No mortality was observed in control. Gradual increased rate of mortality was observed with increasing concentration of leaves extract. The reading was plotted log concentration in X-axis against probit in Y –axis (Figure). From the graph LC50 value was calculated and found to be 5 µl/ml.

Table 7: Assessment of acute toxicity of EEPDL on *D. magna*

S. No	Concentration(x)	Moratlity (y)
1	1 µl/ml	0
2	2 µl/ml	2
3	3 µl/ml	6
4	4 µl/ml	8
5	5 µl/ml	10
6	6 µl/ml	14

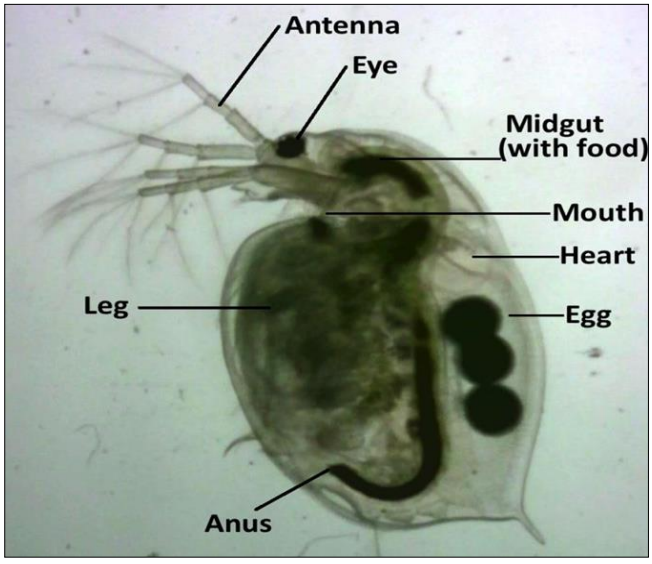

Fig 3: Anatomy of *Daphnia magna*

In vivo cardioprotective activities of the EEPDL on *Daphnia magna*

Cardiac arrhythmia induced by lactose (50mM). After 30 minutes the heartbeat of various concentration of test drug EEPDL (0.5, 1.0, 1.5, 2.0 µl/ml), standard drug metoprolol (20, 25 µg/ml) in triplicate on lactose induced arrhythmic heart of *D. magna* were observed under the microscope. Readings were plotted concentration in X-axis vs heart rate (bpm) in Y-axis (Figure-16). The result was statistically significant ($p<0.001$).

Table 8: Effect of the EEPDL on Lactose induced arrhythmic heart of *D. magna*

S. No	Name of the Substance	Concentration	Heart rate (pbm)
1	Control	-	396.1±0.53
2	Lactose	50mM	243.33±1.24
3	Test drug	0.5 µg/ml	266.7±0.64
		1 µg/ml	349.1±0.7
		1.5 µg/ml	375.3±0.9
		2 µg/ml	393.0±0.7
4	Std drug	20 µg/ml	375.3±0.83
		25 µg/ml	397.3±0.77

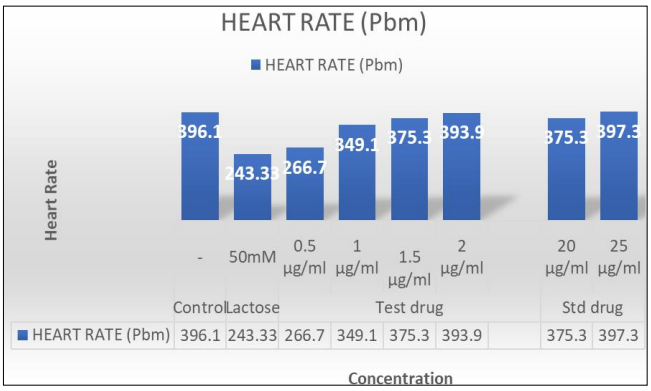

Fig 4: Effect of the EEPDL on Lactose induced arrhythmic heart of *D. magna*

The heartbeat of control, lactose induced and EEPDL treated 0.5, 1, 1.5 and 2 µl/ml and metoprolol 20, 25µg/ml treated were found to be 396.1±0.53, 243.33±1.24, 266.7±0.64, 349.1±0.7, 375.3±0.9 and 393.0±0.7, 375.3±0.83 and 397.3±0.77 bpm respectively. The results were encouraging that it is comparable to that of the standard drug metoprolol. The results were statistically significant ($p<0.001$).

Discussion

The present study evaluated the acute toxicity and cardioprotective potential of the ethanolic extract of *Pergularia daemia* leaves (EEPDL) using *Daphnia magna* as an *in vivo* model. *D. magna* is a well-established bioindicator organism frequently used in ecotoxicological and pharmacological research because of its transparent body, measurable cardiac function, and high sensitivity to pharmacological agents. This simple yet reliable model provides a direct means of assessing cardiac rhythm alterations and the protective efficacy of drug candidates under oxidative or chemical stress conditions.

Acute Toxicity Assessment

The acute toxicity test of EEPDL using *Daphnia magna* revealed that no mortality occurred in the control group, while a gradual, concentration-dependent increase in mortality was observed with increasing doses of the extract (1–6 µl/ml). The LC_{50} value was found to be 5 µl/ml, indicating that EEPDL possesses low toxicity at

pharmacologically relevant concentrations. The extract did not induce abrupt immobility or behavioral toxicity, demonstrating a satisfactory safety margin for biological applications. This finding correlates with the general safety of plant-derived polyphenolic compounds, which often exhibit wide therapeutic windows due to their natural antioxidant composition and low systemic toxicity.

Cardioprotective and Antiarrhythmic Activity

Cardiac arrhythmia was induced in *Daphnia magna* using 50 mM lactose, which disrupts calcium ion balance and causes oxidative stress, leading to irregular heartbeats and reduced cardiac rate. The control group showed a mean heart rate of 396.1 ± 0.53 bpm, while lactose exposure significantly decreased it to 243.33 ± 1.24 bpm. Treatment with ethanolic extract of *Pergularia daemia* leaves (EEPDL) at doses of 0.5–2.0 µl/ml produced a dose-dependent restoration of heart rhythm. The maximum dose (2 µl/ml) restored the heart rate to 393.0 ± 0.7 bpm, comparable to metoprolol (25 µg/ml, 397.3 ± 0.77 bpm). The results were highly significant ($p < 0.001$), confirming strong cardioprotective activity. This effect may be due to the extract's antioxidant polyphenols, such as genkwanin, catechin, and flavone derivatives, which protect against oxidative stress and maintain calcium homeostasis.

The cardioprotective action of EEPDL can be correlated with multiple mechanistic pathways, including antioxidant defense through ROS scavenging and prevention of oxidative damage. It preserves mitochondrial function via BCL2-mediated anti-apoptotic signaling and regulates calcium and ion channel activity to maintain rhythmic contractions. The extract also exhibits anti-inflammatory modulation by inhibiting COX-2 (PTGS2) and suppressing oxidative inflammatory cascades. Additionally, EGFR/ERBB2-mediated survival signaling supports cardiac tissue repair and myocardial recovery. These mechanisms together confirm the strong antiarrhythmic and cardioprotective potential of *Pergularia daemia*, validating its traditional use in heart-related disorders.

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