







Research paper

# Anti-Inflammatory and Antioxidant Properties of *Pavonia zeylonica* Root Extracts

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ARTICLE INFO	ABSTRACT
<p><b>Keywords</b></p> <p>Qualitative Analysis Quantitative Analysis Antioxidant Activity Anti-inflammatory</p>	<p>Various analysis works are perusing stress-related disorders caused by the buildup of free radicals in the body around the world to explore natural antioxidants derived from plants. The objectives of this work were to assess in vitro antioxidant activity and anti-inflammatory activity and to identify phytochemical elements of <i>Pavonia zeylonica</i> in ethanol, petroleum ether, and aqueous root extracts. We evaluated the antioxidant potential and phytochemical contents of petroleum ether, ethanol, and aqueous root extracts of <i>Pavonia zeylonica</i> using DPPH, ABTS radical cation scavenging activity, ferric reducing antioxidant power assay, superoxide radical scavenging activity, and nitric oxide scavenging activity. The extracts flavonoid, tannin, and phenolic contents were also measured using established phytochemical reaction methods. Alkaloids, flavonoids, steroids, anthraquinones, proteins, phenols, saponins, and carbohydrates were discovered by phytochemical analysis. The total phenolic, flavonoid, and tannin contents of the ethanol root extract were quantified as 69.32, 28.39, and 58.45 mg of rutin and gallic acid equivalent/g of extract, respectively. Anti-inflammatory various concentration extracts of the root evaluated using the method of induced hemolysis and heat-induced hemolysis. The antioxidant activity of various concentrations and extracts of the root were evaluated using five test methods. Ethanol extract of the root responded for good antioxidant activity in all experimental methodologies. The findings of the current study suggested that the ethanol root of <i>Pavonia zeylonica</i> is a potential source of natural antioxidants and inflammation disorders.</p>
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## 1. Introduction

Plants have been traditionally used for healing since before recorded history, and they are an essential component for modern medicine. Many popular medications have been manufactured from plant roots. The worldwide market for herbal products growing quickly and major pharmaceutical companies are currently conducting substantial research on plant components for their potential medicinal benefit.

Phytochemicals are chemical compounds that exist naturally in plants. Phytochemicals may be accessible as dietary supplements, but their potential health advantages come from consuming the entire plant. Several phytochemicals have a wide spectrum

of activities that serve to provide protection against long-term illness. Alkaloids, flavonoids, tannins, saponins, polysaccharides, glycosides, phytosterols, phenols, proteins and amino acids, diterpenes, and other phytochemicals have been shown to have both therapeutic and physiological properties.

Traditionally, oxidative stress is addressed using synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Given the current alternative and complementary tactics, medicinal plants have a better possibility of offering powerful, safer, cheaper, and conveniently accessible treatments for oxidative stress-related diseases. Medical plants contain a variety of secondary metabolites with a wide range of pharmacological

effects. Plant antioxidants have been shown to defend the body against diseases, since their ingestion reduces the risk of cancer, heart disease, hypertension, dementia, and strokes. (Beatrice et al., (2020).

Traditionally, the plants were utilized to treat a range of illness ailments. For thousands of years, Indian System Medicine (ISM) has used herbal medications to treat inflammation. One of many herbal medications used to treat inflammation. Herbal remedies have a wide range of therapeutic applications, including wound healing, infection-related inflammation, skin lesions, diarrhea, scabies, venereal disorders, snake bites, ulcers, and more. It is thought to have unique mechanisms for each species of pathogen. Purified natural chemicals from plants can be used as a template to create new-generation anti-inflammatory medications that are less harmful and more effective.

*Pavonia zeylonica* (L) shrub normally found in waste lands. This species is globally distributed in tropical Africa, southwest Arabia, Pakistan, India, Sri Lanka and Mauritius. Within India, it has been recorded in Rajasthan, Maharashtra, Karnataka and Tamil Nadu in waste land. The plant is used in folk medicine, siddha and Ayurveda to treat inflammation, hemorrhage and dysentery activities have been reported. The Leaves and roots are regarded in Ayurveda as cooling demulcent, carminative, diaphoretic, and diuretic. The aim of the present study was to evaluate in vitro antioxidant and anti-inflammatory activities of *Pavonia zeylonica* (L).

## 2. Materials and Methods

### 2.1 Materials

The plants were selected on the basis of their phytochemical screening, quantitative analysis of primary and secondary metabolites and their medicinal uses reported in the literature. The herbs (*Pavonia zeylonica*) were collected from the area of Thirumalayam Palayam of the Coimbatore city and authenticated by *Pavonia zeylonica* (Ref no: BSI/SRC/5/23/2023-24/Tech- 96) by Dr. M.U. Sharief, Scientist 'F' & Head of Office, from Government of India, Ministry of Environment, Forest & Climatic Change, Botanical Survey of India, Southern regional centre, T.N.A.U Campus, Tamil Nadu, India.

### 2.2 Preparation of the plant extracts

The root was cleaned under running tap water to eliminate surface contaminants, and then air dried in the shade. The powdered root samples were extracted with petroleum ether, ethanol, and water using a Soxhlet equipment. The solvent extracts were concentrated using a rotary vacuum evaporator

before being dried. The resulting extract was used for a variety of analyses.

### 2.3 Preliminary Phytochemical Screening

Petroleum ether, ethanol and aqueous extracts were used for preliminary phytochemical analysis using standard procedures (Junaid R Shaikh et al., 2020). The following qualitative tests for both the metabolites were done as follows:

### 2.4 Quantification Analysis

#### 2.4.1 Total Phenolic Content

The total phenolic content was evaluated following the method described by Siddhuraju and Becker (2003). Fifty microliter triplicates of the root extracts (1 mg/1 mL) were added in test tubes and diluted to a volume of 1 ml with distilled water. Then, 0.5 mL of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added continually to each tube. Soon after vortexing the reaction mixture, the test tubes were placed in the dark for forty minutes. After that, and the absorbance at 725 nm was recorded against the reagent blank. The analysis was performed in triplicate, and the results were expressed in gallic acid equivalents.

#### 2.4.2 Total Tannin content

The tannins were analyzed during treatment with Polyvinyl Polypyrrolidone (PVPP) (Siddhuraju and Manian, 2007). In a 100 × 12 mm Eppendorf tube, weigh 100 mg of PVPP. Add 1 mL of distilled water and 1 mL of sample extracts. The components have been vortexed and stored in the freezer at 4°C for 15 minutes. The sample was then centrifuged at 4000 rpm for 10 min at room temperature, with the supernatant collected. This supernatant includes only simple phenolics other than tannins (which would have precipitated alongside the PVPP). The phenolic content of the supernatant was measured following the method described above and expressed as the total amount of non-tannin phenolics.

Tannins = total phenolics - non-tannin phenolics.

#### 2.4.3 Total Flavonoids content

The flavonoid content was quantified using the method given by Zhishen et al. (1999). A 0.5 mL of various solvent extract of root was combined with 2 mL of distilled water and 0.15 mL of 5% NaNO<sub>2</sub>. After 6 minutes, 0.15 mL of 10% AlCl<sub>3</sub> solution was added and left to stand for 6 minutes before adding 2 mL of 4% NaOH solution to the mixture. Water was immediately added to make the final volume 5 mL, and the mixture was properly mixed before being left to stand for another 15 minutes. The mixture's absorbance was measured at 510 nm and compared

to a water blank. The analysis was conducted in triplicate, and the results were represented as rutin equivalent.

## 2.5 In Vitro Anti-Oxidant Activity

### 2.5.1 DPPH (2,2- diphenyl-1-picrylhydrazyl) Scavenging Activity

The extract's antioxidant activity was evaluated in terms of hydrogen donating or radical scavenging capacity using the stable radical DPPH, as per Blois's method (1958). Sample extracts were obtained in various amounts and reduced to 100  $\mu$ L with methanol. The aliquots of samples and standards (BHA, BHT, & rutin) then vigorously shaken in around 5 mL of a 0.1 mM methanolic DPPH solution. To generate the negative control, 100  $\mu$ L of methanol was mixed with to 5 mL of a 0.1 mM methanol solution DPPH. The tubes were left to stand for 20 minutes at 27 °C. The sample's absorbance was measured at 517 nm against the blank (methanol). The samples' radical scavenging activity was represented as IC50, which is the concentration required to block 50% of the DPPH' concentration.

### 2.5.2 ABTS (2,2- azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) Radical Cation Scavenging Activity

The total antioxidant activity of the samples was measured by the ABTS cation radical decolorization assay according to the method of Re *et al.* (1999). ABTS<sup>•+</sup> was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30 °C to give an absorbance of  $0.700 \pm 0.02$  at 734 nm. The stock solution of the sample extracts was diluted such that after introduction of 10  $\mu$ L aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS solution to 10  $\mu$ L of sample or Trolox (final concentration 0–15  $\mu$ M) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated against the blank (ethanol) at 734 nm and then plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as  $\mu$ M/g sample extracts.

### 2.5.3 Ferric Reducing Antioxidant Power Assay (FRAP)

The antioxidant features for different sample extracts have been evaluated using Pulido *et al.*'s (2000) technique. The FRAP reagent (900  $\mu$ L) was newly produced and incubated at 37°C. It was mixed with 90  $\mu$ L of distilled water and 30  $\mu$ L of test sample or methanol (for reagent blank). The test samples and reagent blank were incubated in a water bath at 37°C for 30 minutes. The ultimate dilution of the test sample in the reaction mixture was 1:34. The FRAP reagent was created through the addition of 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM FeCl<sub>3</sub>. 6H<sub>2</sub>O, and 25 mL of 0.3 M acetate buffer (pH 3.6). At the end of incubation, a spectrophotometer was applied to calculate the absorbance at 593 nm against the reagent blank. The calibration curve was developed with methanolic solutions containing known Fe (II) values ranging from 100 to 2000  $\mu$ M (FeSO<sub>4</sub>.7H<sub>2</sub>O). The parameter equivalent concentration was defined as a volume of antioxidant with a ferric-TPTZ reducing capacity equivalent to 1 mM FeSO<sub>4</sub>.7H<sub>2</sub>O. Equivalent concentration was defined as the concentration of antioxidant that resulted in an absorbance increase in the FRAP the experiment comparable to the theoretical absorbance value of a 1 mM Fe (II) solution.

### 2.5.4 Superoxide Radical Scavenging Activity

The test used the capacity of various extracts to inhibit formazan production by scavenging superoxide radicals formed in the riboflavin-light-NBT system (Beauchamp and Fridovich, 1971). Each 3 mL reaction mixture comprised 50 mM sodium phosphate buffer (pH-7.6), 20  $\mu$ g riboflavin, 12 mM EDTA, 0.1 mg NBT, and 40  $\mu$ L aliquots of sample solution or BHA and BHT (standard). The reaction was started by illumination the mixture with sample extract for 90 seconds. Immediately after illumination, the absorbance at 590 nm was measured with respect with a reagent blank. A negative control was formed by identical tubes having a reaction mixture and was maintained in the dark. The scavenging activity towards superoxide anion output was calculated as:

$$\text{Scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where;

$A_0$  is the absorbance of the control, and

$A_1$  is the absorbance of the sample extract/standard.

### 2.5.5 Nitric oxide Scavenging Activity

The process is based on the method (Sreejayan and Rao, 1997), in which sodium nitroprusside in aqueous solution at physiological pH spontaneously creates nitric oxide, which reacts with oxygen to produce nitrite ions, which can be determined with a Greiss

reagent. Nitric oxide scavengers compete with oxygen, preventing nitrite ion formation. For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline (0.2 M, pH-7.4) was mixed with various quantities of sample solutions of extracts of BHT and rutin (standard) and incubated at room temperature for 150 minutes. The negative control involved the same reaction mixture without the sample. Following the incubation period, 0.5 mL of Griess reagent (1% sulfanilamide, 2% H<sub>3</sub>PO<sub>4</sub>, and 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the newly generated chromophore was measured at 546 nm against a blank (phosphate buffer). The scavenging activity (%) has been determined as follows:

$$\text{Scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where;

A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the extract/standard.

## 2.6 In Vito Anti-Inflammatory Activity

### 2.6.1 Membrane stabilization

#### a) Hypotonic Solution- Induced Hemolysis

Alsever's solution was prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in distilled water, then sterilizing the solution (Shinde et al., 1999). Blood was collected from the retinas of Wistar albino rats. The collected blood was mixed with a comparable volume of sterilized Alsever's solution. The blood was centrifuged at 3000 rpm for 10 minutes, and the packed cells were washed three times with isosaline (0.9%, pH 7.2) before being dissolved in 10% (v/v) isosaline. The reaction mixture (4.5 ml) included 1 mL of phosphate buffer (pH-7.4), 2 mL of hyposaline (0.45%), 1 mL of plant extract (1 mg/mL), and 0.5 mL of RBC (red blood cell) suspension. Diclofenac sodium was selected as the reference prescription drug. A reaction mixture without a plant sample was used as a control, and phosphate buffer served as a blank. The assay mixtures were incubated at 37°C for 30 minutes and centrifuged again. The hemoglobin concentration in the supernatant solution was determined spectrophotometrically at 560 nm. The percentage membrane stabilization activity was obtained using the formula.

$$\text{Percentage of inhibition} = \frac{A_c - A_t}{A_c} \times 100$$

where 'Ac' is absorbance of control and 'At' is absorbance of the test.

#### b) Heat - Induced Hemolysis

The method had been previously described by Shinde et al. (1999) and slightly modified and followed by Henneh et al. (2018). The reaction mixture (2 ml)

consisted of 1.0 ml of 10% HRBC and 1 ml of various solvents and plant extracts (1 mg/ml), which were added to each tube and gently mixed. The positive control contained 1.0 ml of HRBC and different doses of diclofenac sodium (10, 30, and 100 µg/ml). The negative control was 1.0 mL of 10% erythrocyte suspension and 1.0 mL of normal saline alone. The experiment had been carried through in triplicate. The resulting solution was heated at 56°C for 30 minutes, withdrawn to the surrounding temperature and centrifuged at 2500 rpm for 10 minutes. The supernatant was collected, and the absorbance of each solution was measured spectrophotometrically (UVmini 1240, Shimadzu) at 560 nm to determine the degree of hemolysis. The % inhibition of hemolysis was estimated using the formula.

$$\text{Percentage of inhibition} = \frac{A_c - A_t}{A_c} \times 100$$

where 'Ac' is absorbance of control and 'At' is absorbance of the test.

## 3. Results and Discussions

The work presented here deals with preliminary phytochemical study, Anti-inflammatory and Antioxidant estimation of petroleum ether, ethanol and aqueous extract of root plant of *Pavonia zeylonica* L.

### 3.1 Preliminary Phytochemical Analysis

The significance in the above statement, secondary metabolite screening of *Pavonia zeylonica* root was studied and the results revealed that the presence of tested phytochemicals such as alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins, anthraquinone, proteins and phenolics in different extracts of petroleum ether, ethanol and aqueous (Table 1).

Phytochemicals	Root		
	Petroleum Ether	Ethanol	Aqueous
Alkaloids	-	+++	+++
Flavonoids	++	+++	+++
Steroids	++	-	-
Terpenoids	-	+	-
Anthraquinone	-	+++	+++
Anthocyanin	-	-	-
Proteins	-	+++	-
Phenolic	-	+	+++
Carbohydrates	-	+++	+++
Saponins	-	-	++
Lignin	-	-	-
Tannins	+	+	-
Cardiac glycoside	-	-	-
Glycoside's test	-	-	-
Coumarins	-	-	-
Volatile oils	-	-	-



### 3.2 Quantitative Analysis

In this study, we have quantified the presence of total phenolics, total tannins and total flavonoids content in various extracts of root of *P. zeylonica*. Quantification results of the present study indicate that ethanol root extract possess high content of phenolics, tannins and flavonoids.

#### 3.2.1 Determination of total phenolic content

Results showed that total phenolics content (Table: 2) were found to be rich in ethanol sample ( $69.32 \pm 1.45$  mg GAE/g extract). Petroleum ether and aqueous also showed significant amount of phenolic content, however which is lesser than ethanol.

#### 3.2.2 Determination of total tannin content

Estimation of total tannin content in various extracts of *P. zeylonica* is showed in (Table:2). Results showed that total tannins were found to be little bit high tannin content ( $58.45 \pm 1.83$ mg TAE/g extract) in ethanol sample and aqueous shows similar quantity level of tannin when compare to petroleum ether.

#### 3.2.3 Determination of total flavonoid content

The total flavonoid content of *P. zeylonica* (Table: 2) the ethanol root extract was found to contain appreciable level of flavonoid content ( $28.39 \pm 0.38$ mg RE/g extract). The difference between petroleum ether and aqueous samples in total flavonoid content is very less, which is found to be  $4.5 \pm 0.07$  mg RE/g extract in petroleum ether and  $6.75 \pm 0.07$ mg RE/g extract of aqueous.

**Table 2** Quantification analysis of various root extract of *Pavonia zeylonica*

Root Solvent Extracts	Quantitative Analysis		
	Total Phenolics	Total Flavonoids	Total Tannins
Petroleum Ether	$19.74 \pm 0.84$	$4.5 \pm 0.07$	$17.76 \pm 0.92$
Ethanol	$69.32 \pm 1.45$	$28.39 \pm 0.38$	$58.45 \pm 1.83$
Aqueous	$55.6 \pm 0.97$	$6.75 \pm 0.07$	$50.39 \pm 0.84$

### 3.3 In Vitro Antioxidant Activity

In antioxidant investigation, the crude root various solvent extract of the plant and the standard showed the antioxidant activity.

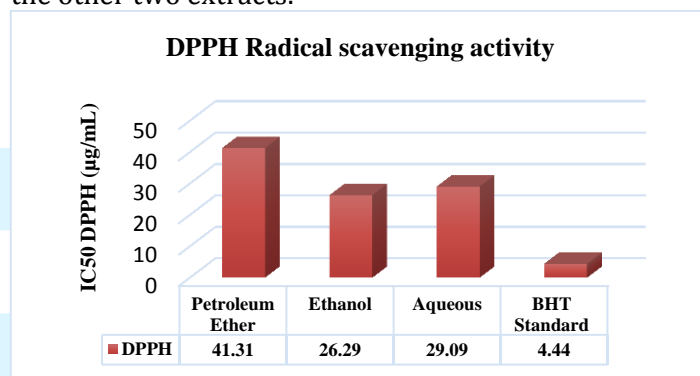
**Table 3** Antioxidant activity in different assay of root extract in *Pavonia zeylonica*

Extracts	DPPH	ABTS	FRAP	Super oxide	Nitric oxide
Petroleum Ether	41.31	$66111.1 \pm 433.68$	$223.7 \pm 0.74$	$20.35 \pm 0.16$	$51.57 \pm 0.47$
Ethanol	26.29	$89166.7 \pm 208.33$	$305.92 \pm 0.74$	$25.14 \pm 0.06$	$16.52 \pm 0.11$
Aqueous	29.09	$72222.2$	$98.02$	$20.96$	$62.25 \pm$

		$\pm 120.28$	$\pm 1.54$	$\pm 0.03$	0.19
BHT	4.44	145347.	506.91	$64.2 \pm$	$82.96 \pm$
Standard		$2 \pm 636.4$	$\pm 3.5$	0.3	0.38

#### 3.3.1 DPPH Radical Scavenging Activity

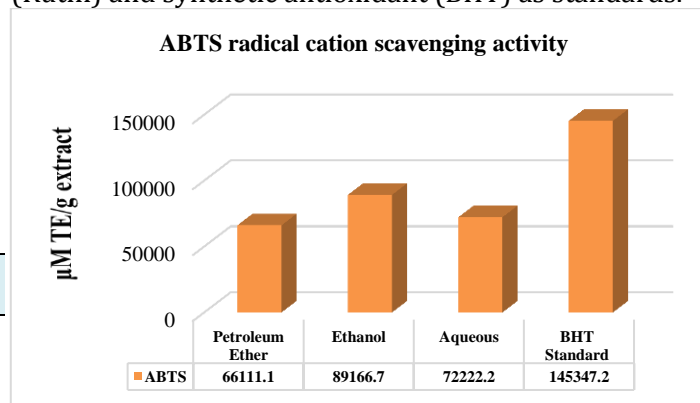
The reactivity of *P. zeylonica* root was analyzed with DPPH (2,2- diphenyl-1-picrylhydrazyl) a stable free radical. As DPPH takes up one electron in the presence of a free radical scavenger, the absorption reduces, and the resulting discoloration is related to the number of electrons gained. (Figure:1) Petroleum ether, ethanolic, and aqueous root extracts of *Pavonia zeylonica* showed IC50 values of  $41.31\mu\text{g/ml}$ ,  $26.29\mu\text{g/ml}$ , and  $29.09\mu\text{g/ml}$ , respectively. The standard concentrations for Rutin and BHT were  $5.56\mu\text{g/ml}$  and  $4.44\mu\text{g/ml}$ , respectively. Thus, three root extracts of *P. zeylonica* show antioxidant activity. However, the ethanolic extract performed better than the other two extracts.



**Fig. 1** In vitro DPPH radical scavenging activity of various root extracts of *P. zeylonica*

#### 3.3.2 ABTS Radical Cation Scavenging Activity

The pre-formed radical cation ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) is generated by oxidation ABTS with potassium persulfate (a blue chromogen) and is reduced in the presence of a hydrogen-donating antioxidant. The (figure:2) showed that the study revealed that *Pavonia Zeylonica* root ethanol extract ( $89166.7 \mu\text{M TE/g extract}$ ) possessed the highest ABTS scavenging activity when compared to other solvents. Natural antioxidant (Rutin) and synthetic antioxidant (BHT) as standards.



**Fig. 2** In vitro ABTS radical cation scavenging activity of various root extracts of *P. zeylonica*

### 3.3.3 Ferric Reducing Antioxidant Power Assay (FRAP)

In this experiment (Fig:3), the transformation of ferric (Fe<sup>3+</sup> -TPTZ) to ferrous (Fe<sup>2+</sup> -TPTZ) iron was studied using the FRAP evaluate to identify the reductive potential of the plant root various extracts. The ethanol extract showed highest ferric reduction antioxidant potential, with a FRAP of 305.92. The FRAP values for both the petroleum ether and aqueous extracts in the lowest ferric reduction antioxidant were 223.7 and 98.02, respectively.

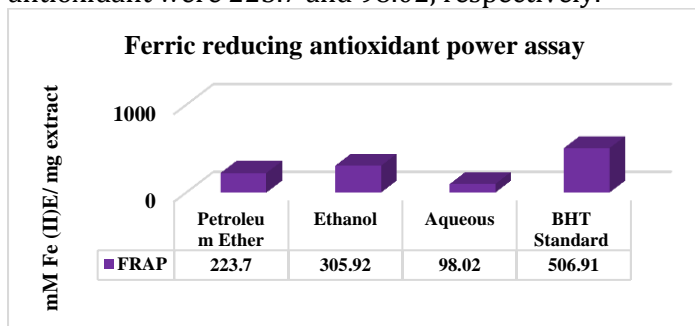


Fig. 3 In vitro Ferric reducing antioxidant power assay of various root extracts of *P. zeylonica*

### 3.3.4 Superoxide Radical Scavenging Activity

Figure 4 shows the superoxide radical scavenging activity of petroleum ether, ethanolic, and aqueous extracts of *Pavonia zeylonica*, as well as ascorbic acid. The values for petroleum ether, ethanolic, and aqueous extracts were 20.35 µg/ml, 25.14 µg/ml, and 20.96 µg/ml, respectively. Ascorbic acid had a value of 55.0 µg/ml. It demonstrated substantial scavenging action for superoxide radicals produced in the riboflavin-light NBT system. The results show that the ethanolic extract has high antioxidant action. When the superoxide radical activity of petroleum ether and aqueous extracts was compared with ascorbic acid, the ethanolic extract was shown to be substantially more active than the petroleum ether and aqueous extract.

### 3.3.5 Nitric oxide Scavenging Activity

The nitric oxide radical scavenging activity of several *Pavonia zeylonica* root extracts was estimated by generating nitric oxide with sodium nitroprusside. Sodium nitroprusside is a key generator of nitric oxide radicals. The extracts scavenge the nitric oxide generated by sodium nitroprusside by blocking chromophore production; thus, absorbance drops as extract concentrations increase. (Fig: 4) The IC<sub>50</sub> values were petroleum ether (51.57 µg/ml), ethanol (16.52 µg/ml), and aqueous (62.25 µg/ml), indicating that aqueous root extract is a more effective NO radical scavenger than its equivalent.

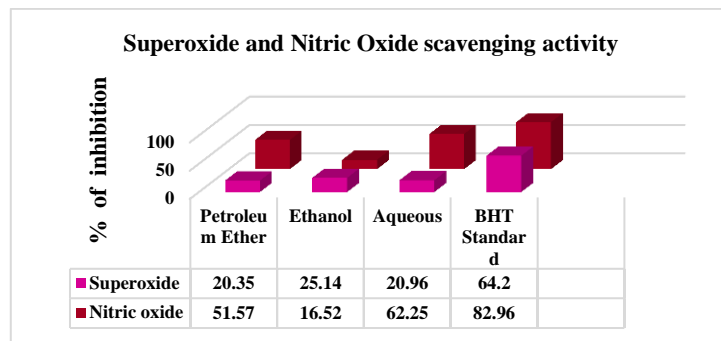


Fig. 4 In vitro Superoxide and Nitric oxide radical scavenging activity of various root extracts of *P. zeylonica*

### 3.4 In Vitro Anti- Inflammatory Activity

#### 3.4.1 Membrane Stabilization

In the present study, the anti-inflammatory activities of root extract of *P. zeylonica* were evaluated using hypotonic solution- induced hemolysis and heat-induced hemolysis.

Table 4 Anti- Inflammatory of Membrane stabilization of *P. zeylonica*

Extracts	Hypotonic solution- Induced hemolysis activity	Heat- Induced Hemolysis activity
Petroleum Ether	32.52	130.22
Ethanol	36.46	49.43
Aqueous	36.81	93.11
Diclofenac Standard	82.41	29.38

#### 1. Hypotonic Solution- Induced Hemolysis

The results (Figure:5) showed that *P. zeylonica* root aqueous extract at a concentration range of 50-250 µg/ml potently protected the erythrocyte membrane against the inflammatory activity of hypotonic solution. The study revealed that *Pavonia zeylonica* root aqueous extract (36.71%) had the highest level of inflammatory activity compared to the other solvent extracts. The lowest concentration was detected in root ethanol extracts (36.56%). Diclofenac (82.41%) was used as the standard. Diclofenac sodium has been shown to show significant protection against the adverse effects of hypotonic solutions.

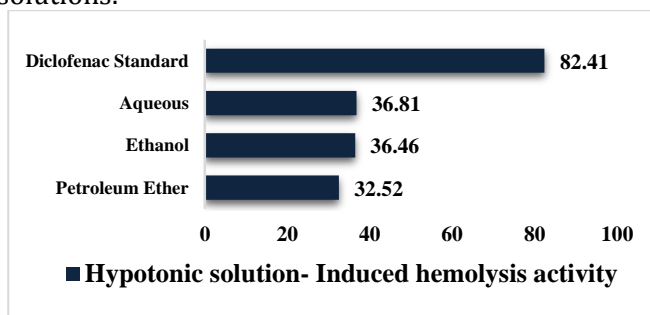
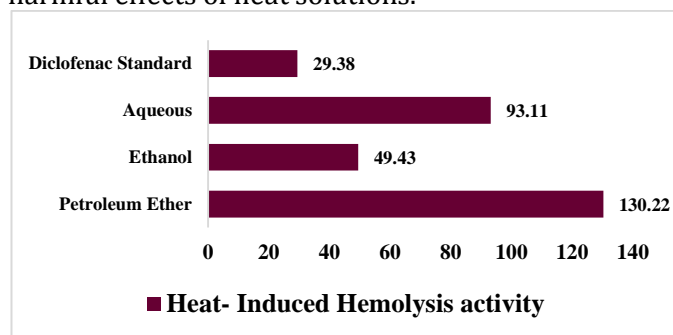


Fig. 5 Membrane stabilization in Hypotonic solution- Induced Hemolysis of different extract of *P. zeylonica*

### 3.4.2 Heat- Induced Hemolysis

The root extract inhibited heat-induced hemolysis in different treatments. The study found that *Pavonia zeylonica* root at concentrations of 50 and 250 µg/ml provided considerable protection (see table:4 & fig: 6). The study found that *Pavonia zeylonica* had the lowest IC50 value and the maximum inflammatory activity. The root ethanol extract (49.43 µg/ml) demonstrated the highest inflammatory activity when compared to other solvent extracts. Ethanol root extract provided significant protection against the harmful effects of heat solutions.



**Fig. 6** Membrane stabilization in Heat-induced Hemolysis of different extract of *P. zeylonica*

## 4. Conclusions

In conclusion, the present study revealed that the in vitro antioxidant and anti-inflammatory activity of the ethanol extract of *Pavonia zeylonica* L. root contains considerable bioactive compounds responsible for significant free radical scavenging activities in the in vitro assays and contributes to potent anti-inflammatory effects in chemically induced. From the observed results, it could be concluded that the ethanol extract of *P. zeylonica* root may be a probable source of natural antioxidants revealed from various in vitro assays. The results of the anti-inflammatory experiments of membrane stabilization methods suggest that *P. zeylonica* root ethanol extract in induced hemolysis and aqueous root extracts in heat-induced hemolysis may be used as an alternative source for the treatment of inflammation disorders.

## Competing Interest Declaration

There are no competing interest

## Author Contribution Declaration

Kalaivani. M and Dr. Vinodhini. S have equally contributed for thematic preparation and editing of the manuscript.

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