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Investigation of Phytochemicals and Pharmacognostic Features of *Feronia Limonia* Fruit and Its In Vivo Effects on Oxidative Stress

Research Article

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Abstract

Feronia limonia, commonly known as wood apple, is a medicinal plant widely used in traditional medicine for its therapeutic properties. This study investigates the phytochemical constituents, pharmacognostic characteristics, and in vivo antioxidant potential of *F. limonia* fruit to provide a scientific foundation for its medicinal applications. Phytochemical screening revealed the presence of key bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. Pharmacognostic and physicochemical analyses were performed to establish quality control and standardization parameters for the fruit. In addition, the methanolic extract was evaluated for its antioxidant activity using a carbon tetrachloride (CCl_4)-induced oxidative stress model in Wistar rats. The extract significantly reduced malondialdehyde (MDA) levels and restored antioxidant enzyme activities (SOD, CAT, GSH) in a dose-dependent manner, supported by histopathological findings of hepatoprotection. These results validate the traditional use of *F. limonia* and highlight its potential as a natural source of antioxidant agents for pharmaceutical and nutraceutical development.

Keywords: *Feronia Limonia*, Phytochemical Constituents, Pharmacognostic Properties, Medicinal Plant, Antioxidant activity, Herbal Medicine.

Introduction

Medicinal plants have been a cornerstone of traditional healing systems for centuries, providing essential bioactive compounds for drug development and therapeutic applications. *Feronia limonia* (commonly known as wood apple or elephant apple) is a lesser-explored medicinal fruit belonging to the Rutaceae family, widely distributed in South and Southeast Asia. (1)

The fruit has been traditionally used for its diverse pharmacological properties, including antimicrobial, antioxidant, hepatoprotective, and antidiabetic activities. Despite its widespread ethnomedicinal use, comprehensive scientific investigations on the phytochemical and pharmacognostic properties of *Feronia limonia* remain limited. Phytochemical analysis is essential to identify bioactive constituents such as flavonoids, alkaloids, tannins, and phenolic compounds, which contribute to the fruit's medicinal potential. Meanwhile, pharmacognostic studies provide critical information on the macroscopic, microscopic, and physicochemical characteristics of plant materials, ensuring their authenticity, purity, and quality in herbal medicine formulations. (2)

This study aims to explore the phytochemical constituents and pharmacognostic properties of *Feronia limonia* fruit, providing a scientific basis for its medicinal value. By analysing its bioactive compounds and standardising its pharmacognostic profile, this research seeks to contribute to the validation and potential pharmaceutical application of *Feronia limonia* in modern medicine. (3,4)

Plant Profile

Kingdom	- Plantae
Division	- Magnoliophyta
Class	- Magnoliopsida
Order	-Sapindales
Family	- Rutaceae
Subfamily	- Aurantioideae
Genus	- *Feronia*
Species	- *F. limonia* C.

Materials and Methods

Collection of Plant Material

The plant material was procured and authenticated from Shree Shail Herbs PVT. LTD. Nagpur.

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Figure 1. *Feronia limona* fruit

Organoleptic evaluation (5)

The methanolic and petroleum ether extracts of *Feronia limona* fruit were evaluated for their organoleptic properties, including colour, and weight.

Physicochemical Evaluation (5)

Determination of Extractive Value

A coarse powder of dried plant material was placed in a Soxhlet thimble in the appropriate amount. The plant material was then extracted with 100-250 ml of two different solvents at 60^{0}c °C, in order of polarity, i.e., Methanol and Petroleum ether. Extraction was carried out until 50 cycles were completed for the solvents. The percentage practical yield of each extract was calculated.

Figure 2: Soxhlet Extraction Method

Determination of Total Ash Content

The powdered material (2 g) was accurately weighed and placed in a crucible. The material was spread evenly in a layer and ignited to a constant weight by gradually increasing the heat to 500-600°C until it turned white, indicating the absence of carbon. The remaining ash was allowed to cool in a desiccator. The content of total ash (in mg/g) of air-dried material was calculated as follows:

% Total ash = weight ash/ weight of sample × 100

Determination of Acid-Insoluble Ash

HCl (2 N; 25 ml) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water, and the rinsed contents were added to the crucible. The acid-insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid-insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a desiccator and weighed. The content of the acid-insoluble ash (in mg/g) of air-dried material was calculated as follows:

% Acid-insoluble ash = weight ash/ weight of sample ×100

Determination of Water-Soluble Ash

Water (25 ml) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and added to the crucible. The water-insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water-insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The water-soluble ash content was calculated using the following equation

% Water soluble ash = total ash content-water insoluble residue in total/ ash weight of sample ×100

Foreign Matter Analysis

Foreign matter presence may be due to faulty collection of crude drugs or deliberate mixing. It was separated from the drug so that the results obtained are important parts of the morphology of a particular drug.

Determination of pH

The pH of the 1 % solution of extract was determined by making an appropriate concentration of powdered drug in an aqueous solution, filtering, and checking the pH of the filtrate. A digital pH meter was utilised to ascertain the pH of the mixtures.

Preliminary Phytochemical Screening of *Feronia limonia* Fruit Extracts

Qualitative chemical tests were performed for extracts of plants - Dragondroff's test, Mayer's test, Molish test, Fehling's test, Benedict test, Bortanger test, Saponin Foam test, Sulphuric acid test, etcThe extracts were shown to contain active phytochemical elements such as alkaloids, carbohydrates, glycosides, tannins, and saponins.

Quantitative Estimation of Total Phenolic Content

The Folin – Ciocalteu colorimetric colorimetry method was used to determine the total phenolic content, based on the procedure of Azlim Almey (2010), using Gallic acid as a standard phenolic compound. (7,9,10)

Reagents: Folin Ciocalteu reagent: Dilute the Folin Ciocalteu reagent with an equal volume of distilled

water; 20% sodium carbonate: 20 g sodium carbonate in water; Gallic acid.

Procedure

- Prepare a calibration curve of standard Gallic acid (10-100 µg/ml in water).
- Prepare 1 mg/mL of extract solutions.
- Mix 1 ml of each sample with 0.25 ml of Folin-Ciocalteu's reagent and 1.25 ml of 20% sodium carbonate solution.
- Allow the mixture to react for 40 minutes. At room temperature.
- After the reaction period, the contents are mixed, and the blue colour is at 725 nm in comparison with standards. Calculate the number of total phenols from the calibration curve as a Gallic acid equivalent by the following formula:

$$T = \frac{C.V}{M}$$

Where T = total content of phenolic compounds, milligram per gram plant extract, C the concentration of gallic acid established from the calibration curve, milligram per milliliter, V the volume of extract, milliliter, and M the gram weight of plant extract

Quantitative Estimation of Total Flavonoid Content

The Aluminium Chloride colorimetry method was used to determine the total flavonoid content, based on the procedure of Azlim Almey (2010), using Quercetin as a standard flavonoid compound.

Reagents: Quercetin, ethanol, Aluminium chloride, potassium acetate.

Procedure

- Prepare the calibration curve of standard Quercetin (10- 100 µg/ml in methanol).
- Mix 0.5 ml standard solution with 1.5 ml of 95% ethanol, 0.1 ml of 10% aqueous Aluminium chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water.
- Incubate for 30 min at room temperature. Measure the absorbance of the reaction mixture at 415nm with a UV spectrophotometer.
- To prepare a blank solution, substitute 10% Aluminium chloride with an equal amount of distilled water.
- Similarly, treat 0.5 ml of plant extract samples with Aluminium chloride for the determination of flavonoid content from the calibration curve.

In vitro antioxidant activity of *Feronia limonia*

In the present study, the methanolic extract was tested for free radical scavenging activity at various concentrations using different in vitro methods.

Hydrogen Peroxide Scavenging Assay

Principle: It uses a colour reagent that contains xylenol orange dye in an acidic solution with sorbitol and ammonium iron sulfate that reacts to produce a purple colour in proportion to the concentration of H_2O_2 in the sample being tested.

Reagents

- **Phosphate Buffer Saline (pH 7.4):** Prepare 800 ml of distilled water in a suitable container. Add 20.214 g of Sodium Phosphate Dibasic Heptahydrate and 3.394 g of Sodium Phosphate Monobasic Monohydrate to the solution. Adjust the solution to the final desired pH using HCl or NaOH. Add distilled water until the volume is 1 L.
- **H_2O_2 solution:** Procedure: In this test, varying concentrations of the test substance (50 to 800 µg/ml) were assayed. Test solution: H_2O_2 solution (40 mmol/l in phosphate buffer): phosphate buffer (pH 7.4) at 1:0.6:3.4 ml was added to the test tube. The absorbance of the reacting solution versus blank, including the extract solution plus phosphate buffer (1:4, ml), was checked spectrophotometrically at 230nm. The control consisted of phosphate buffer: H_2O_2 solution (3.4:0.6, ml). The equation was used for % H_2O_2 inhibition.

In vivo antioxidant activity of *Feronia limonia*

The in vivo antioxidant study was designed based on the methodology outlined by Jain et al. (2018), with minor modifications. Wistar rats weighing between 150 and 200 grams were randomly assigned to one of four groups (n = 6 per group): Control, Positive Control (Ascorbic acid at 100 mg/kg), *F. limonia* extract low dose (200 mg/kg), and *F. limonia* extract high dose (400 mg/kg). Oxidative stress was induced by administering carbon tetrachloride (CCl_4) intraperitoneally at a dose of 0.5 mL/kg, diluted in olive oil (1:1 v/v), twice a week for two weeks. Treatment with either the extract or ascorbic acid was given orally, once daily, for a total of 14 days. On day 15, blood and liver samples were collected to evaluate antioxidant markers, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), following standard biochemical protocols.

Experimental Design and Treatment Groups

- Group I: Control (vehicle only)
- Group I: CCl_4 + Positive control (Ascorbic acid, 100 mg/kg/day, orally)
- Group III: CCl_4 + *F. limonia* extract (Low dose: 200 mg/kg/day, orally)
- Group IV: CCl_4 + *F. limonia* extract (High dose: 400 mg/kg/day, orally)

Results

Organoleptic evaluation

Organoleptic evaluation for both the methanolic and petroleum ether extracts was done.

Physicochemical characteristics

The plant extracts were subjected to various evaluation parameters.

Phytochemical screening

The phytochemical screening of the extracts with different chemical tests was performed, and the results are as follows:

Table 1: Organoleptic properties of plant extracts

Sr. No	Solvent used	Colour of the extract	Weight of the extract	The weight of the crude plant taken	Extractive value
1	**Petroleum ether**	Dark green	0.6 gm	50 gm	1.19 % w/ w
2	**Methanol**	Dark brown	4.0375 gm	50 gm	8.05 % w/ w

Table 2: Physicochemical characteristics

Physicochemical Character	*Feronia limonia* crude drug
Total ash (%w/w)	9.47
Acid-insoluble ash (%w/w)	1.24
Water insoluble ash (%w/w)	8.23
Foreign organic matter (%w/w)	1.63
Loss on drying ash (%w/w)	16.28
pH	5.92

Table 3: Phytochemical screening of Methanol and Petroleum ether extract

Sr. No.	Chemical test	Methanol extract	Petroleum ether extract
1	**Alkaloid**		
	Dragondroff's test	+	+
	Mayers test	+	+
2	**Carbohydrate**		
	Molish test	+	+
	Fehlings test	+	+
	Benedict test	+	-
3	**Glycosides**		
	Bortanger test	+	-
4	**Saponin Foam test**	+	+
5	**Tannin**	+	+
6	**Phenolic**	+	+
7	**Flavonoid**	+	-
8	**Saponin**	+	-
9	**Mucilage**	-	-
10	**Lipids/Fats**	-	+

Quantitative estimation of Total Phenolic Content

A linear calibration curve of gallic acid with an R_2 value of 0.9765 was obtained. Figure 3 shows the mean TPC of the plant extract measured using the GAE equation of Y = 0.0018x +0.0061 (R^2 = 0.9765), whereby Y = absorbance at 765nm and X = concentration of total phenolic compounds in mg per ml of the extract. The Methanol extract showed the GAE of (11.23 ±0.13 mg/g), and the petroleum ether extract showed the GAE of (3.74 ±0.13 mg/g).

Quantitative estimation of Total flavonoid content

A linear calibration curve of Quercetine with an R_2 value of 0.9628 was obtained. Figure 4 shows the mean TFC of the plant extract measured using the TFC equation of y = 0.0009x +0.002 (R^2 = 0.9628), whereby Y = absorbance at 415nm and X = concentration of total flavonoid in mg per ml of the extract. The Methanol extract showed the flavonoid content of (4.86 ±0.13 mg/g), and the petroleum ether extract showed the flavonoid content (4.064 ±0.13 mg/g).

Figure 3: Calibration Curve of Gallic Acid

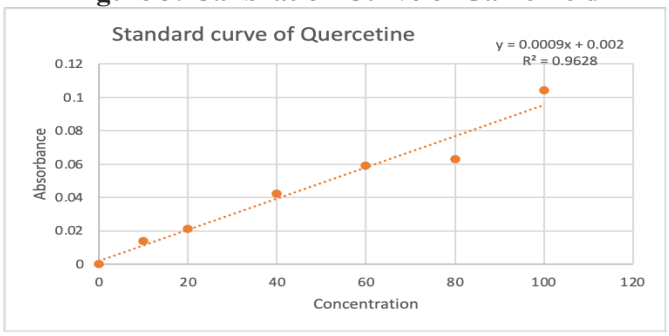
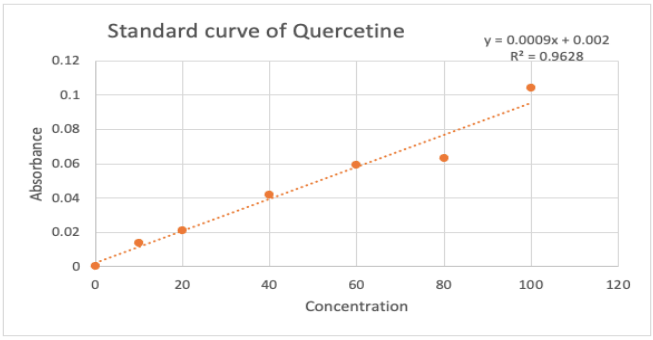


Figure 4: Calibration curve of Quercetine as standard



In vitro antioxidant activity of *Feronia limonia*

Hydrogen Peroxide Scavenging Assay

In the present study, methanolic extract of *Feronia limonia* in various concentrations was tested for its free radical scavenging activity in different in vitro methods. It was concluded that free radicals were scavenged by the test extract.

Table 4: Antioxidant Activity (Hydrogen Peroxide) of Ascorbic Acid

Sr. No.	Concentration (µg)	Absorbance	% Inhibition	IC 50 Value
1	0	0	0	444.20 µg/ ml
2	50	0.589	6.50794	
3	100	0.486	22.8571	
4	200	0.399	36.6667	
5	400	0.356	43.4921	
6	600	0.198	68.5714	
7	800	0.128	79.6825	

Figure 5: Graph of Hydrogen peroxide radical scavenging assay of Ascorbic acid

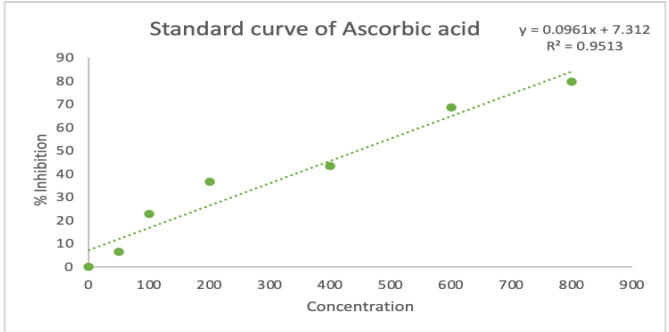


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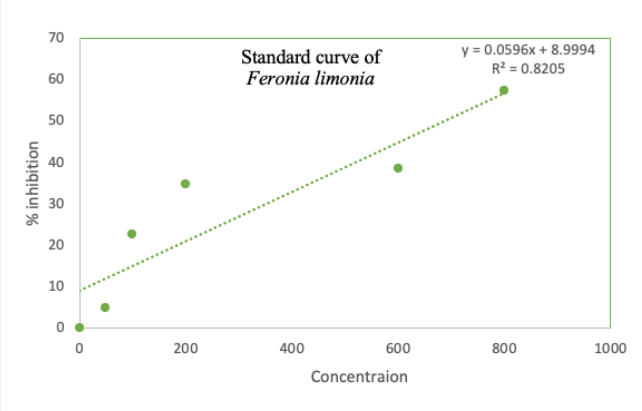
Table 5: Antioxidant Activity (Hydrogen Peroxide) of *Feronia limonia*

Sr. no.	Concen-tration (µg)	Control	Sample	% Inhibition	IC 50
1	0	0	0	0	687.93
2	50	0.63	0.599	4.92063	
3	100	0.63	0.487	22.6984	
4	200	0.63	0.411	34.7619	
5	400	0.63	0.387	38.5714	
6	600	0.63	0.269	57.3016	
7	800	0.63	0.183	70.9524	

Figure 6: Graph of Hydrogen peroxide radical scavenging assay of *Feronia limonia*



In Vivo Antioxidant Study

Effect of *F. limonia* Extract on Lipid Peroxidation (MDA Levels)

The MDA levels were significantly elevated in the CCl_4 group compared to the control group ($p < 0.001$), indicating increased lipid peroxidation. Treatment with *F. limonia* extract at both 200 mg/kg and 400 mg/kg significantly reduced MDA levels in a dose-dependent manner ($p < 0.01$ and $p < 0.001$, respectively), comparable to the standard ascorbic acid group.

Table 6: Effect of *F. limonia* Extract on Lipid Peroxidation (MDA Levels)

Sr. No.	Group	MDA (nmol/mg protein)
1	Control	2.3 ± 0.15
2	CCl_4	6.8 ± 0.24 (↑↑↑ vs. control)
3	CCl_4 + Ascorbic Acid	3.0 ± 0.18 (↓↓ vs. CCl_4)
4	*F. limonia* (200 mg/kg)	4.1 ± 0.21 (↓)
5	*F. limonia (400 mg/kg)*	3.2 ± 0.17 (↓↓)

Antioxidant Enzyme Activities

Superoxide Dismutase (SOD) Activity

CCl_4 significantly reduced SOD activity compared to the control group ($p < 0.001$). Both doses of *F. limonia* significantly increased SOD activity, with the higher dose showing results nearly equivalent to the standard.

Catalase (CAT) Activity

CAT activity was markedly decreased in the CCl_4 group. Treatment with *F. limonia* extract restored CAT levels in a dose-dependent manner ($p < 0.05$ and $p < 0.01$).

Reduced Glutathione (GSH) Levels

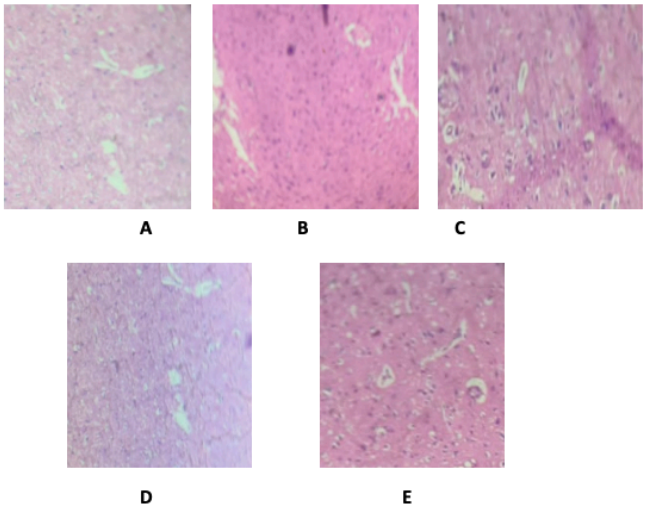
GSH levels were significantly depleted in the CCl_4 group. Both *F. limonia* doses significantly replenished GSH, with the high dose showing the strongest effect ($p < 0.001$).

Table 7: Antioxidant Enzyme Activities

Sr. No.	Group	Group SOD (U/mg protein)	CAT (U/ mg protein)	GSH (U/mg protein)
1	Control	8.2 ± 0.34	62.3 ± 2.4	48.2 ± 1.7
2	CCl_4	3.9 ± 0.29 (↓↓↓)	3.4 ± 0.93 (↓↓↓)	4.5 ± 1.73 (↓↓↓)
3	CCl_4 + Ascorbic Acid	6.32 ± 0.78 (↑↑↑)	7.2 ± 0.88 (↑↑↑)	7.8 ± 0.23 (↑↑↑)
4	*F. limonia* (200 mg/kg)	5.63 ± 0.30 (↑)	5.86 ± 0.36 (↑)	6.01 ± 0.48 (↑)
5	*F. limonia* (400 mg/kg)	7.36 ± 1.26 (↑↑)	7.65 ± 0.67 (↑↑)	6.96 ± 1.83 (↑↑)

Note: Values are presented as mean ± SEM (n = 6). Significance vs. CCl_4 group: $p < 0.05$ (↑/↓), $p < 0.01$ (↑↑/↓↓), $p < 0.001$ (↑↑↑/↓↓↓).CCl_4 group: Hepatocytes showed ballooning degeneration, centrilobular necrosis, and dense inflammatory infiltration.

Figure 7: Representative H&E micrographs from liver tissues collected from rats under a 100X microscope



A- control, B- CCL_4, C- CCL_4 +Ascorbic acid, D- F. *limonia* (200 mg/kg), E- *F. limonia* (400 mg/kg)

A: Control group showing normal hepatic architecture with intact hepatocytes and central veins

B: CCl_4-treated group exhibiting severe hepatocellular necrosis, ballooning degeneration, and inflammatory cell infiltration.

C: CCL_4 +Ascorbic acid treated group exhibiting severe hepatocellular necrosis, ballooning degeneration, and inflammatory cell infiltration.

D: The treatment with *F. limonia* at a dosage of 200 mg/kg demonstrated moderate improvement, resulting in reduced necrosis and inflammation.
E: The treatment with F. limonia at a dosage of 400 mg/kg showed near-normal liver architecture, with only minimal histopathological changes observed.

Statistical Analysis

All data are expressed as mean ± SEM (n = 6). One-way ANOVA followed by Tukey's post hoc test was used for group comparisons. Differences were considered statistically significant at $p < 0.05$. Statistical software used: [GraphPad Prism v9].

Discussion

The phytochemical constituents and pharmacognostic properties of *Feronia limonia* fruit were evaluated using various standard parameters. The powdered fruit was extracted using methanol and petroleum ether. Phytochemical screening revealed the presence of several bioactive compounds, including flavonoids, alkaloids, tannins, saponins, and phenolic compounds. These phytochemicals are well-documented for their antioxidant and hepatoprotective properties. The quantitative estimation of total phenolic content in the methanolic extract yielded a value of 4.064 ± 0.13 mg/g, suggesting that phenolic compounds significantly contribute to the extract's biological activity. The methanolic extract demonstrated promising antioxidant potential in vitro, as assessed by the hydrogen peroxide scavenging assay. This observed activity can be attributed to the presence of polyphenolic constituents, particularly flavonoids and tannins, which are known to neutralise free radicals by donating electrons and inhibiting lipid peroxidation. Building on these in vitro findings, an in vivo study was conducted using a carbon tetrachloride (CCl_4)-induced oxidative stress model in Wistar rats to further evaluate the extract's antioxidant potential under physiological conditions. Administration of carbon tetrachloride resulted in a significant increase in malondialdehyde (MDA) levels and a concurrent decrease in endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), indicating oxidative liver damage. Treatment with *F. limonia* extract significantly reduced these biochemical alterations in a dose-dependent manner. The high-dose group (400 mg/kg) exhibited a protective effect comparable to that of the standard antioxidant, ascorbic acid. Histopathological examination further supported the biochemical data, showing substantial preservation of liver architecture in treated groups compared to the CCl_4 group, which displayed significant necrosis and inflammation. These in vivo findings corroborate earlier reports, including those by Jain et al. (2018), who observed similar hepatoprotective and antioxidant effects of *F. limonia* stem bark extract in a comparable model. The presence of flavonoids and phenolic compounds, identified in the phytochemical analysis, likely underlies the antioxidant mechanism through free radical scavenging, inhibition of lipid peroxidation, and enhancement of endogenous defence enzymes. Overall, the combination of in vitro and in vivo data strongly supports the antioxidant efficacy of *F. limonia* methanolic extract. These results provide a scientific basis for the traditional use of the plant in managing oxidative stress-related disorders and highlight its potential as a natural therapeutic agent.

Conclusion

The study of *Feronia limonia* fruit reveals its potential medicinal applications due to various bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which exhibit antioxidant, antimicrobial, and anti-inflammatory properties. Quantitative analysis showed significant phenolic content, aiding in quality control and standardisation for herbal formulations. An in vivo study indicated that the methanolic extract reduces carbon tetrachloride-induced oxidative stress in Wistar rats, significantly decreasing lipid peroxidation and restoring antioxidant enzymes, especially at a dose of 400 mg/kg. These findings suggest that *Feronia limonia* fruit may serve as a valuable natural resource for pharmaceutical applications. Further research, including chronic toxicity assessments and clinical trials, is necessary to confirm its efficacy and safety.

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