

Original Research Article

In vitro pharmacognostic and antioxidant properties of *Lawsonia inermis* aerial parts and extract

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Sent for review: 16 May 2025

Revised accepted: 2 September 2025

Abstract

Purpose: To assess the pharmacognostic and antioxidant activities of the leaf and flower extracts of *Lawsonia inermis* Lythraceae.

Methods: Pharmacognostic analysis involved wet and dry analyses, such as determination of total ash, extractive values, moisture content and phytochemical analysis, while antioxidant activity of plant extracts (100 - 500 µg/mL) was determined through ferric reducing antioxidant power assay. Ascorbic acid in different concentrations (100 - 500 µg/mL) was used as the standard. Apart from fluorescence studies, high-performance thin-layer chromatography (HPTLC) was used to identify novel compounds.

Results: For the pharmacognostic analysis, total ash values of leaf and flowers were 5.57 ± 0.06 and 3.10 ± 0.03 %, respectively. The results indicated that the total ash value was significantly greater in the leaf than flower ($p < 0.05$). Low moisture content of the flower extract implied a lesser tendency of bacterial, fungal, or yeast contamination. The phytochemical evaluation revealed the presence of secondary metabolites in leaf and flower extracts of *L. inermis*. The greater proportion of these metabolites in the leaf extract may be ascribed to significantly higher reducing power potential ($p < 0.05$).

Conclusion: The leaf extract possesses superior antioxidant activity compared to the flower extract of *Lawsonia inermis* and this could be due to the occurrence of a diversity of phytochemicals in the leaves.

Keywords: Antioxidant activity, *Lawsonia inermis* L., Physicochemical analysis, Pharmacognostic study

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Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Lawsonia inermis, a colorant shrub, also known as henna, belongs to the Lythraceae family [1]. It is well known worldwide as a natural dye used to colour hair, beard and decorate hands and feet. For centuries, henna leaves have played a significant role in body art and textile dyeing, commonly used to colour the hands, hair, nails, and fabrics [2]. Essential oil derived from the flowers is valued for its aromatic properties [3]. *Lawsonia inermis* is also considered a

therapeutic plant. Plants have been studied as prospective source of drugs for curing numerous ailments and disorders [4]. According to the Indian Ayurvedic Pharmacopoeia, the leaves of *L. inermis* are recommended for treating pruritus, bleeding disorders, dysuria, and various skin conditions [5]. The leaves are traditionally used for diseases such as scabies, boils, ophthalmia, syphilitic sores, amenorrhea, lumbago, headaches, and hemicrania as they are rich in bioactive compounds, including flavonoids, steroids, xanthenes, polyphenols, coumarins,

triterpenoids, fatty acids, alkaloids, quinones, tannins, catechin, leucocyanidin, epicatechin, and quercetin. Some of these phytochemicals possess pharmacological activities, including antifungal, antitumor, wound healing, antioxidant, anti-inflammatory, analgesic, antiparasitic, hepatoprotective, and hypoglycemic effects [6].

Applied topically, the leaves help treat wounds, ulcers, and prevent skin inflammation. They are further used in managing febrile illnesses, leukoderma, and cephalalgia. The effect of the dye generated from *L. inermis* promotes hair growth [7]. The bark of the plant has been used in traditional medicine for the treatment of skin diseases, urinary calculi, spleen and liver enlargement, and jaundice [3].

Henna also demonstrates a wide range of pharmacological properties. Rahiman *et al* [8] investigated the antibacterial effects of henna both *in vitro* and *in vivo*, finding that its extracts effectively inhibited various pathogenic microorganisms. Elansary *et al* [9] reported significant antioxidant activity in leaf extracts of *L. inermis*, noting a marked reduction in oxidative stress across all tested cancer cell lines compared to controls. Barupal *et al* [10] attributed the antiparasitic effects of henna to the presence of bioactive phytochemicals in its ethanolic extract.

The present study is justified by the need to scientifically validate and compare the pharmacognostic, phytochemical, and antioxidant properties of the leaf and flower extracts of *Lawsonia inermis* L. With the increasing global interest in plant-based therapeutics, it is essential to establish standardized data on the physicochemical parameters, bioactive constituents, and antioxidant potential of the plant. This study not only addresses the gap in current literature but also supports drug discovery and quality control efforts by employing advanced analytical techniques such as High-Performance Thin-Layer Chromatography (HPTLC). The findings are expected to enhance the scientific understanding of *L. inermis* and support its further exploration as a source of natural therapeutic agents.

EXPERIMENTAL

Identification and collection

Fresh leaves and flowers of *Lawsonia inermis* were collected from fields in Coimbatore, India, in January 2023. They were identified by the Botanical Survey of India, Southern Regional

Centre herbarium, located at TNAU campus, Coimbatore, Tamil Nadu. A voucher specimen (no. BSI/SRC/5/23/2025-26/Tech./538) of the studied plant has been deposited and preserved for future reference in the Department of Chemistry, Nehru Institute of Technology, Coimbatore, Tamil Nadu, India. The leaves and flowers from the field were harvested, washed with water and dried in the shade. After drying, leaves and flowers were separately powdered and sieved through a mesh (No. 60). The coarse powder was stored properly and used for pharmacognostic work.

Preparation of ethanol crude extracts

Pulverized leaf (100 g) and flower (100 g) samples were separately soaked in 1 L of absolute ethanol, 1:10 (w/v). The mixtures were placed in a rotary shaker kept at 110 rpm for 48 hours. After extraction, each plant mixture was filtered using a Whatman No. 1 filter. The resulting filtrates were individually concentrated and stored. These concentrated extracts were preserved at -40 °C for further use.

Morphological studies

The morphological study of *L. inermis* leaf includes evaluation of its size, shape, apex, margin, base, surface, colour, taste and odour.

Pharmacognostic analysis

The properties of the plant extracts, including acid-insoluble residue, total residue and water-soluble residue values, were determined following the method described earlier [11], in order to assess the purity of the crude extracts.

Extraction process

Dried plant materials were individually extracted using ethanol, methanol, toluene, water and ethyl acetate. For all the extracts, the volume of solvent was 100 mL. Each extract was strained individually and then evaporated to dryness.

Loss on drying

1 g of each test sample (powdered extract) was accurately weighed into a smooth porcelain dish and dehydrated in an oven at $100 \pm 5^\circ\text{C}$ until a constant weight was attained. The loss of mass was then calculated [12].

Total ash value

The determination of ash content was conducted in accordance with ISO 18122:2022; Technical

Committee ISO/TC 238, 2022 [13]. The empty silica crucible with lid was first weighed and then subjected to ignition in a furnace for an hour. Subsequently, 1 g of each powdered sample was accurately weighed and placed in the crucible. The crucible was then covered with its lid, reweighed, and ignited with the sample at approximately 450 °C for a duration of 3 h. After each one-hour interval during the ignition process, the crucible was removed, allowed to cool to room temperature in a desiccator, and weighed along with the lid and the sample. This procedure was repeated at each interval to monitor mass changes throughout the ignition period, and calculated using Eq 1.

$$\text{Ash content (\%)} = \left\{ \frac{(m_3 - m_1)}{(m_2 - m_1)} \right\} \times 100 \times \left\{ \frac{100}{(100 - M_{ad})} \right\} \dots\dots\dots (1)$$

where m_1 is the mass in g of the empty crucible; m_2 is the mass in g of the crucible and the test sample; m_3 is the mass in g of the crucible and ash; M_{ad} is the percentage moisture content of the test sample as determined.

Water-insoluble residue

The total residue obtained was boiled with 30 mL of distilled water and subsequently strained through residue-free filter paper. The filter paper, containing the remaining insoluble matter, was then placed in a clean, pre-weighed crucible and incinerated at 455 °C until all carbonaceous material was eliminated. The crucible was then allowed to cool to room temperature in a desiccator and then weighed. The water-insoluble residue was determined by the mass difference. Following this, the measurement of water-soluble residue was carried out [14].

Acid-insoluble residue content

The total residue was boiled with 30 mL of 2N hydrochloric acid. The resulting mixture was filtered using ashless strainer paper. The filter paper, containing the acid-insoluble residue, was then transferred to a pre-weighed silica container and ignited in a muffle heater at 455°C until all carbon was removed. After cooling in a desiccator, the container was weighed. The acid-insoluble residue content was calculated as a percentage of the total residue [15].

Extractive value

The dry powdered plant materials (5 g) were subjected to maceration using 100 mL of distilled water as a solvent. For each extraction, 2 grams of the crudely pulverised plant material was weighed and transferred into a dehydrated 250

mL conical flask. Each flask was then filled with 35 mL of the respective solvent (toluene, ethyl acetate, ethanol, or methanol), sealed with a cork and kept aside for 24 hours at room temperature with intermittent shaking. Following maceration, the mixtures were filtered using Whatman No. 1 filter paper into 50 mL graduated cylinders. The filtrates were subsequently transferred to pre-weighed Petri dishes and concentrated to dryness *via* complete solvent evaporation.

Fluorescence analysis

Fluorescence, a type of luminescence, occurs when a molecule absorbs photons and becomes excited to a higher electronic state before returning to the ground state by emitting light. For fluorescence analysis, the method described by Chase and Pratt was used [16].

Phytochemical analysis

Preliminary phytochemical screening of *Lawsonia inermis* leaf and flower extracts was carried out using standard protocols, as described in previous studies [17].

Evaluation of antioxidant activity

Reducing ability assay

The reducing power of leaf and flower extracts was evaluated by the method described earlier with slight modifications [19,20]. Antioxidant compounds reduce ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}), which subsequently reacts with ferric chloride to form ferric-ferrous complex exhibiting maximum absorbance at 700 nm. Different concentrations (100, 200, 300, 400, and 500 µg/mL) of herbal leaf and flower ethanolic extracts were added to phosphate buffer (2.6 mL) and potassium ferricyanide solution (2.6 mL). The resulting mixture was incubated at 55 °C in a water bath for 30 min. After cooling, 2.6 mL of 11 % trichloroacetic acid CCl_3COOH was added and the mixture was centrifuged at 3100 rpm for 10 mins [20]. Subsequently, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of freshly prepared ferric chloride. The absorbance was measured at 700 nm. A standard solution was prepared similarly, excluding the plant extract. Ascorbic acid in different concentrations (100, 200, 300, 400, and 500 µg/mL) was used as the reference standard. An increase in the absorption maximum of the reaction mixture indicated a high reducing ability. The reducing ability was measured by varying both the extract concentration and the incubation time.

High-performance thin-layer chromatography fingerprinting [21]

Sample preparation

Accurately weighed 1 g of the fine powder sample was transferred into a 250 mL flat-bottomed flask. Subsequently, 10 mL of methanol was added and the mixture was refluxed on a water bath maintained at $80 \pm 2^\circ\text{C}$ for 35 min. The resulting extract was filtered through Whatman No. 1 filter paper.

Sample application

A 10 μL volume of the prepared sample solution was applied to a 10-12 mm band on a precoated silica gel thin-layer chromatography (TLC) plate (E-Merck), maintaining a 12-mm distance from the bottom edge of the plate. A pencil mark was drawn at a height of 8.5 cm from the application point to indicate the solvent front.

Preparation of development tank

A CAMAG twin-trough development chamber (10 $\times$ 10 cm) was employed for the chromatographic procedure. One side of the internal compartment was fitted with appropriately sized Whatman No. 1 filter paper. Approximately 20 mL of chloroform: methanol (90:10, v/v) solvent mixture was prepared and carefully introduced into the chamber along the side containing the filter paper to facilitate vapor equilibration.

Tank saturation

The development tank was covered and allowed to equilibrate for 30 minutes to ensure saturation of the atmosphere with solvent vapours. After this period, the lid was carefully opened, and the spotted chromatographic plate was placed on the opposite side of the chamber, away from the solvent reservoir and filter paper. The lid was then gently closed, and the chamber was tilted to allow the solvent to flow into the trough containing the plate until the solvent front reached the pre-marked pencil line. Upon completion, the plate was promptly removed and dried in air.

The TLC plate was visualized under UV light at 254 and 366 nm and later scanned using HPTLC.

Statistical analysis

Statistical analysis was performed on the phytochemical and solvent extraction data obtained from *L. inermis* leaves and flowers to

assess the significance of observed differences. Independent two-sample t-tests were conducted using the mean and standard deviation (SD) values for each parameter ($n = 3$ per group). The analysis covered physicochemical constants such as total ash, water-soluble and acid-insoluble values, as well as solvent extraction yields using toluene, ethyl acetate, ethanol, methanol, and aqueous solvents. Also, statistical analysis was conducted to compare the antioxidant activity, measured by absorbance at 700 nm, of *L. inermis* leaf extract, flower extract, and ascorbic acid across concentrations ranging from 100 to 500 $\mu\text{g/mL}$.

RESULTS

Morphological characteristics

The leaves of *Lawsonia inermis* L. are simple, 2 to 6 cm in length, 1 to 1.5 cm in width (Figure 1). The leaf is lanceolate in shape, with a mucronate apex, entire margin, tapering base, and a short, glabrous petiole (Table 1). The shade of the leaf is greenish-brown to cloudy green. Leaves are slightly astringent in taste and when crushed, they produce an aromatic odour. However, chopped leaf is light bronze green in colour with a different smell and unpleasant taste.

Table 1: Macroscopic characteristics of the leaf *Lawsonia inermis*

Macroscopic characteristic	Leaf
Colour	Green
Surface	Smooth
Venation	Reticulate unicostate
Size (cm)	2.2 - 5.9
Margin	Entire
Shape	Lanceolate
Touch	Smooth

Physicochemical characteristics

The results of the quantitative investigation (total residue, acid unsolvable residue, water insoluble residue, total solvable extractive, and moisture content at 105°C) are tabulated in Table 2. Total residue value defines the authenticity and purity of the test samples. Total ash value for the leaf and flower extracts is 5.57 ± 0.06 and $3.10 \pm 0.03\%$, respectively. Loss on drying was 66.19 and 43.55 % w/w for leaf and flower extract, respectively.

The mean extractive value is higher in leaf samples (25.05 %) compared to flower samples (15.41 %) across all solvents (Table 3).

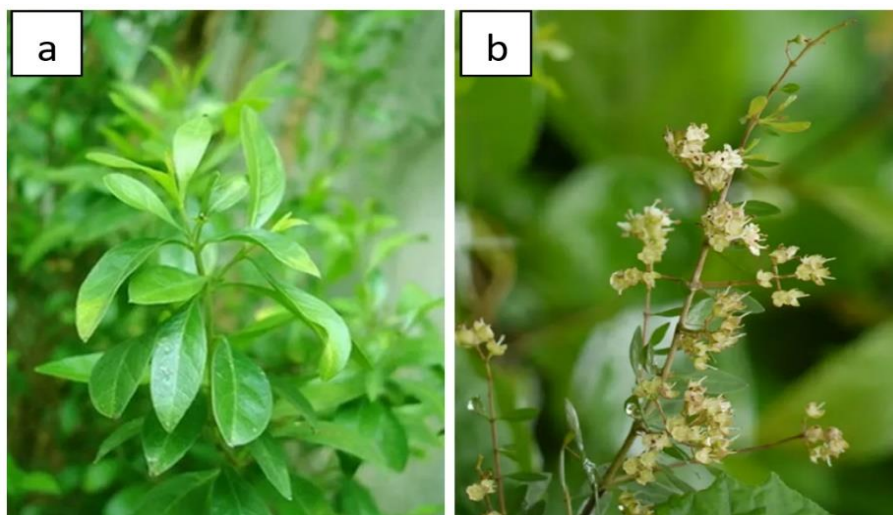


Figure 1: Image shows leaves (a) and flowers (b) of *Lawsonia inermis* L

Table 2: Physicochemical parameters of *Lawsonia inermis*

Parameter	Leaf	Flowers
Loss on drying (%)	66.19±0.15*	43.55±0.11
Total ash value (%)	5.57±0.06*	3.10±0.03
Water soluble value (%)	2.47±0.01*	1.21±0.01
Acid-insoluble value (%)	0.20±0.05*	0.49±0.03

* $p < 0.05$; mean $\pm$ SD; $n = 3$

Table 3: Total extractive value of *Lawsonia inermis*

Solvent	Leaf (%)	Flower (%)
Toluene	7.01±0.01	5.02±0.00
Ethyl acetate	20.40±0.01	18.02±0.03
Ethanol	32.73±0.01	21.01±0.04
Methanol	34.00±0.09	14.01±0.006
Aqueous	33.13±0.05	17.01±0.003

Values are mean $\pm$ SD

Leaf extracts show consistently higher yields in all solvents, indicating that the leaves likely

contain higher quantities of extractable phytochemicals. The low SD values for each measurement indicate high precision.

Phytochemical composition of leaf and flower extracts

The preliminary phytochemical properties of the different extracts of the leaves and flowers of *Lawsonia inermis* is shown in Table 4.

Antioxidant activity

In this study, the reduction of the yellow-colored reaction mixture to varying shades of sea green and cobalt blue reflected the reducing power of the plant extracts. This color change is attributed to the conversion of ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}) in the presence of electron-donating antioxidants. The resulting ferrous complex reacts to form Prussian blue, which is quantified by measuring absorbance at 700 nm.

Table 4: Preliminary phytochemical composition of *Lawsonia inermis* in different solvents

Test parameter	Toluene		Ethanol		Ethyl Acetate		Methanol		Aqueous	
	L	F	L	F	L	F	L	F	L	F
Alkaloids	+	+	+	-	+	-	+	-	+	-
Anthraquinone	-	-	+	-	+	-	+	-	+	+
Amino acids	-	-	-	+	-	-	-	-	-	-
Proteins	-	+	+	-	-	+	+	+	-	-
Carbohydrates	-	+	+	-	-	-	+	-	-	+
Glycosides	+	-	+	+	+	-	+	-	+	-
Phenols	-	+	-	-	-	+	-	-	+	-
Flavonoids	-	+	+	-	-	-	+	-	+	+
Steroids	-	-	+	+	-	-	+	+	+	-
Tannins	-	-	+	-	-	+	+	-	+	-
Saponins	-	-	-	-	-	-	-	-	+	+

L = leaf extract; F = flower extract

A strong absorbance observed at this wavelength indicates a high reducing potential of the sample, confirming its significant antioxidant activity.

Reducing ability of leaf and flower extract

The reducing ability of leaf extract of *L. inermis* is shown in Table 5. The reducing power of *Lawsonia inermis* leaf and flower extracts increased in a concentration-dependent manner, as indicated by rising absorbance values at 700 nm. Across all concentrations (100 – 500 µg/mL), the leaf extract consistently showed higher absorbance than the flower extract, suggesting superior reducing capacity. At 500 µg/mL, the leaf extract reached an absorbance of 0.674 ± 0.003 , compared to 0.437 ± 0.02 for the flower extract. Ascorbic acid, used as a standard, showed the highest reducing power at all concentrations, with an absorbance of 0.971 ± 0.05 at 500 µg/mL. The significant increase ($p < 0.05$) in absorbance with concentration, particularly at 400 and 500 µg/mL,

confirms the strong antioxidant potential of the leaf extract.

Fluorescence characteristics

Since leaves showed better efficiency in all the above studies than flowers, only leaf extract was considered for fluorescence analysis. The powdered leaf was screened for fluorescent characteristics. The observations of fluorescence analysis under normal daylight and UV light are presented in Table 6.

HPTLC Fingerprinting

Compounds in *Lawsonia inermis* plant leaf ethanolic extracts showed two main bands observed at Rf values of 0.82 and 0.85 under UV 254 nm. Figure 2 A shows the HPTLC fingerprint under UV 254 nm and Figure 2 B shows the 3D densitograms of *Lawsonia inermis* leaf extract. Figure 3 A shows the HPTLC fingerprint under UV 366 nm and Figure 3 B shows the 3D densitograms of *Lawsonia inermis* leaf extract.

Table 5: Reducing ability of *Lawsonia inermis* plant extracts (absorbance values at $\lambda = 700$ nm)

Extract concentration (µg/mL)	Leaf extract	Flowers extract	Ascorbic acid
100	0.150 ± 0.01	0.109 ± 0.07	0.201 ± 0.01
200	0.251 ± 0.007	0.182 ± 0.05	0.408 ± 0.00
300	0.387 ± 0.007	0.284 ± 0.09	0.613 ± 0.04
400	$0.560 \pm 0.003^*$	$0.401 \pm 0.06^*$	0.799 ± 0.01
500	$0.674 \pm 0.003^*$	$0.437 \pm 0.02^*$	0.971 ± 0.05

Note: * $P < 0.05$ (n = 3); values are mean $\pm$ SD

Table 6: Fluorescence investigation of *Lawsonia inermis* leaf powder

Fluorescence test	Observations in/under		
	Day light	UV 254nm	UV 366nm
Fine powder	Greenish brown	Green	Black
Nitrocellulose + Fine powder	Greenish brown	Green	Black
1N NaOH in methanol + Fine powder	Dark brown	Black	Black
1N NaOH in methanol + nitrocellulose in amyl acetate + Fine powder	Dark brown	Black	Black
1N HCl + Fine powder	Green Dark	Green Dark	-
1N HCl + Nitrocellulose in amyl acetate + Fine powder	Green Dark	Green	Dark brown
1N NaOH in water + Fine powder	Dark brown	Dark brown	Black
1N NaOH in water + Fine powder dried and mounted in nitrocellulose in amyl acetate	Dark brown	Dark brown	Black
HNO ₃ + Fine powder (1:1)	Yellowish	Brown	Green Black
H ₂ SO ₄ + Fine powder (1:1)	Dark brown	Black	Black
1 % Picric acid + Fine powder	Yellowish	Brown	Greenish brown
Acetic acid + Fine powder	Brown	Black	Black
5 % Iodine + Fine powder	Brown	Dark brown	Black
5 % FeCl ₃ + Fine powder	Dark green	Dark brown	Black
25 % NH ₃ + HNO ₃ + Fine powder	Yellowish brown	Green Dark	brown
Methanol + Fine powder	Dark green	Black	Dark brown
Conc. HNO ₃ + Fine powder	Brown	Green	Black
10 % Potassium dichromate solution + Fine powder	Dark brown	Black	Black
50 % KOH + Fine powder	Brown	Dark green	Dark brown
Conc. H ₂ SO ₄ + Fine powder	Dark brown	Black	Black

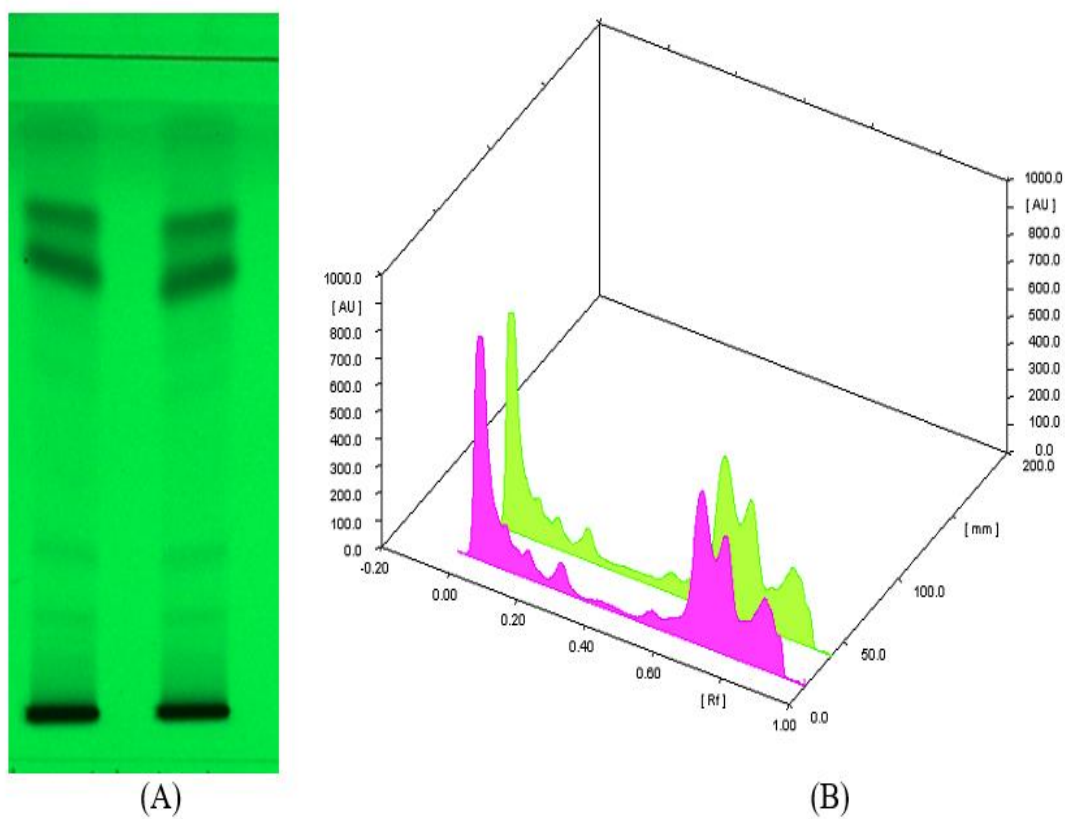


Figure 2: (A) HPTLC fingerprint under UV 254 nm and (B) three-dimensional (3D) densitograms of *Lawsonia inermis* leaf extract.

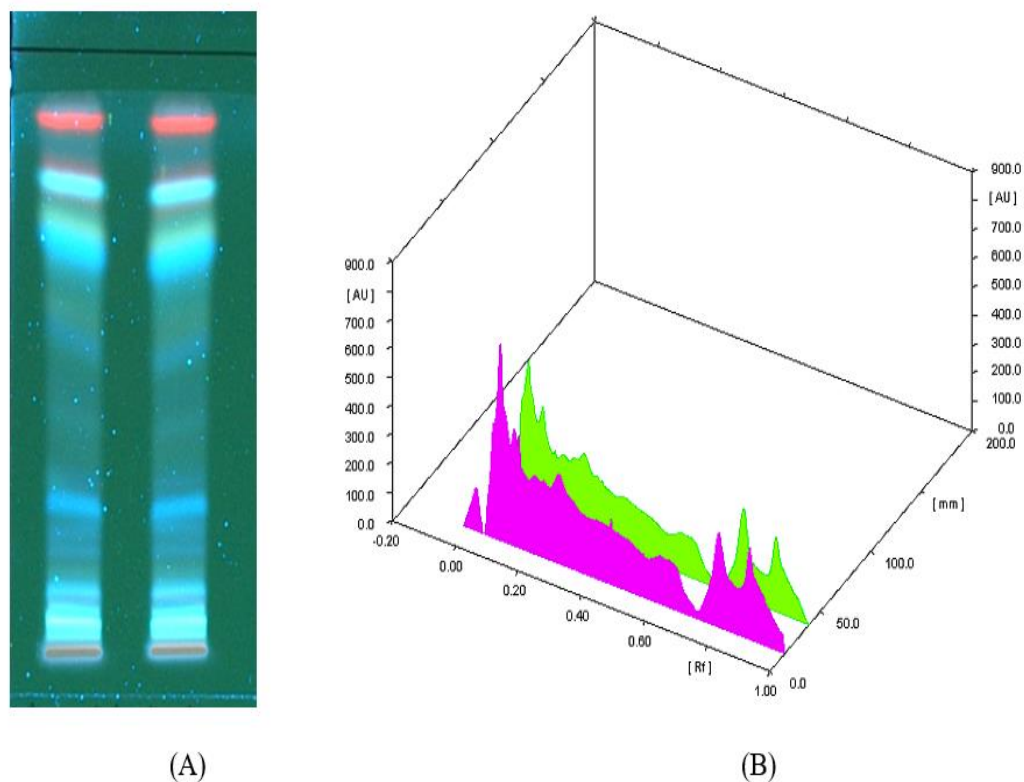


Figure 3: (A) HPTLC fingerprint under UV 366 nm and (B) three dimensional (3D) densitograms of *Lawsonia inermis* leaf samples

DISCUSSION

The findings of this study highlight the superior antioxidant potential of *Lawsonia inermis* leaf extract compared to its flower extract, as demonstrated by the reducing power assay. Absorbance values at 700 nm increased with concentration for both extracts, indicating a dose-dependent antioxidant activity. However, the leaf extract consistently showed significantly higher absorbance ($p < 0.05$), reaching 0.674 ± 0.003 at $500 \mu\text{g/mL}$, while the flower extract exhibited a lower value of 0.437 ± 0.02 . Ascorbic acid, used as the standard antioxidant, displayed the highest activity at all concentrations, validating the reliability of the assay.

The higher reducing capacity of the leaf extract is likely correlated with its greater phytochemical content. Total ash analysis revealed a significantly higher ash content in the leaf extract ($5.57 \pm 0.06 \%$) than in the flower extract ($3.10 \pm 0.03 \%$). Since total ash is a measure of the mineral content and inorganic matter in plant material, these results suggest that the leaves of *L. inermis* are richer in essential bioactive components and basic nutritional elements. This also supports the traditional use of the leaf in various herbal formulations. In the phytochemical investigation of *L. inermis* leaf, Jain et al [22] reported a total ash content of 14.60% , which is about 3 times higher than that found in the current study.

The total extractive data suggest that leaf samples are more suitable for extraction of bioactive compounds compared to flowers, especially when using polar solvents like methanol, aqueous, and ethanol. Methanol and aqueous solvents appear to be the most efficient for extracting compounds from leaves.

The relatively low moisture content observed in the flower extract suggests reduced susceptibility to microbial contamination, which may be relevant for storage and formulation stability. Nevertheless, the leaf extract exhibited higher total extractive values, which reflect the amount of active constituents, especially secondary metabolites, extracted from the plant. These metabolites, such as flavonoids, phenolics, and tannins, are known contributors to antioxidant activity and are presumably present in greater concentration in the leaf extract. Similarly, Jain et al [22] reported a good quantity of carbohydrate, tannins, phenolic compounds, glycosides, gums and mucilage in alcoholic extract and aqueous extracts of henna leaves.

The presence of diverse phytochemicals was further supported by HPTLC, which produced distinct qualitative fingerprints for the leaf and flower extracts. The HPTLC profiling aids in the identification and authentication of plant materials by offering characteristic band patterns with specific solvent systems. The fingerprint patterns observed in this study indicate the presence of novel compounds, particularly in the ethanolic extract of the leaves. This strengthens the potential of *L. inermis* leaf as a source of bioactive compounds suitable for pharmaceutical development. Furthermore, such phytochemical fingerprinting not only supports compound discovery but also ensures consistency in quality and efficacy of herbal preparations.

All tested parameters showed statistically significant differences ($p < 0.05$), with extremely low p-values indicating strong to very strong evidence of higher extractive content and physicochemical differences in leaves compared to flowers. Statistical analysis on the antioxidant activity of *Lawsonia inermis* leaf extract, flower extract, and ascorbic acid across concentrations ranging from 100 to $500 \mu\text{g/mL}$ was performed. Independent t-tests revealed that at lower concentrations ($100 - 300 \mu\text{g/mL}$), the leaf and flower extracts did not differ significantly ($p > 0.05$). But both the extracts showed statistically significant differences when compared to ascorbic acid, particularly from $200 \mu\text{g/mL}$ onwards ($p < 0.01$). At higher concentrations (400 and $500 \mu\text{g/mL}$), the leaf extract showed significantly higher absorbance than the flower extract ($p < 0.05$), and both extracts differed significantly from ascorbic acid ($p < 0.001$). These results indicate that the antioxidant potential of the leaf extract is consistently stronger than that of the flower extract and more closely resembles the activity of ascorbic acid at higher concentrations. At higher concentrations ($\geq 400 \mu\text{g/mL}$), the leaf extract is significantly different from both the flower extract and ascorbic acid. Similarly, Philip et al [23] reported that ethanolic extracts from seeds of *L. inermis* exhibited strong reducing activity, with concentrations ranging from $10 - 500 \mu\text{g}$ demonstrating greater ferric-reducing power than ascorbic acid. This effective reduction is due to the extract's potential efficiency in preventing oxidation.

CONCLUSION

There are significant differences in extractive values and physicochemical parameters between the two plant parts. The higher antioxidant capacity of the leaf extract has been attributed to the richness and diversity of its phytochemicals,

many of which are known to contribute to redox-based protective mechanisms. High-performance thin-layer chromatography profiling further validated the presence of unique compounds in the leaf extract. These findings suggest *L. inermis* leaf extract as a promising natural source of antioxidant compounds and support its use in traditional medicine, pharmaceutical development, and nutraceutical formulations.

DECLARATIONS

Acknowledgement/Funding

None.

Use of Artificial intelligence/Large language models

We declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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