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Integrated Pharmacognostic, Phytochemical and HPTLC Analysis of *Litsea chinensis* (Lam.) Pers. (Medalakri) Stem Bark with Evaluation of its Antimicrobial, Antioxidant and Anti-Inflammatory Potential

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Abstract: *Litsea chinensis* (Medalakri), a member of the Lauraceae family, has been traditionally employed to treat inflammatory and infectious disorders. The objective of this investigation was to assess its pharmacognostical, physicochemical, phytochemical, and biological characteristics in order to validate its use and establish standardization parameters. HPTLC was employed to conduct phytochemical profiling and evaluate physicochemical parameters. Concentration-dependent assessments were conducted to evaluate the antimicrobial, antioxidant (DPPH, ABTS), and anti-inflammatory (protein denaturation) activities. Pharmacognostical analysis identified unique macroscopic and microscopic characteristics, such as sclereids, periderm, calcium oxalate crystals, and starch granules, that functioned as diagnostic markers. Physicochemical parameters, including acid-insoluble ash (0.60%), total ash (3.85%), and loss on drying (9.59%), were within acceptable limits, suggesting that the product was of high quality and purity. The presence of a variety of phytoconstituents with multiple bands and peaks was confirmed by HPTLC profiling. The ethanolic extract exhibited antibacterial activity against all tested bacterial strains and antifungal activity against *Candida albicans*, with inhibition zones generally increasing with extract concentration. At 50 mg/mL, the highest antibacterial activity was observed against *Staphylococcus aureus* (23 ± 2.00 mm), followed by *Pseudomonas aeruginosa* (21.67 ± 0.58 mm) and *Escherichia coli* (20.33 ± 0.58 mm). In comparison to ascorbic acid, antioxidant assays (DPPH and ABTS) demonstrated moderate, dose-dependent free radical scavenging activity. Protein denaturation was significantly inhibited in the anti-inflammatory study (57.25% at the greatest concentration). In general, the ethanol extract exhibits the potential to stabilize proteins and mitigate inflammatory responses. The stem bark of *Litsea chinensis* demonstrates promising antimicrobial, antioxidant, and anti-inflammatory properties. These discoveries substantiate its conventional application and underscore its potential as a natural therapeutic agent.

Keywords: *Litsea chinensis*; medalakri; pharmacognosy; HPTLC; antioxidant activity; anti-inflammatory activity; antimicrobial activity; phytochemicals

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1. Introduction

The demand for botanicals and herbal therapeutics is on the rise worldwide, predominantly due to their perceived efficacy and safety. Nevertheless, the increasing demand for herbal products has also resulted in the widespread use of substandard raw materials and adulterants, which may compromise the quality and safety of the product and pose potential health risks [1]. Consequently, it is imperative to guarantee that the plant materials employed in herbal formulations are authenticated and devoid of contaminants. The development of high-quality herbal medicinal products is contingent upon the use of basic materials that are well-characterized and standardized, as well as those that are rich in bioactive phytochemicals [2]. In this process, pharmacognosy is essential, as it utilizes morphological, histological, physicochemical, phytochemical, and molecular methods to authenticate and standardize medicinal plants.

Herbal products exhibit significant potential as adjunct therapies to conventional treatments owing to their relatively low toxicity and cost-effectiveness. They also provide a significant supply of bioactive chemicals that can be transformed into innovative medicinal treatments with improved efficacy [3,4]. Notwithstanding these benefits, ongoing pharmacological study is essential to investigate novel plant species as prospective sources of bioactive chemicals, encompassing those with analgesic, antibacterial, antioxidant, and anti-inflammatory attributes. The genus *Litsea*, part of the Lauraceae family, has garnered considerable scientific attention [5–11]. The Lauraceae family includes around 55 genera and more than 4000 species of aromatic trees and shrubs, primarily found in the tropical and subtropical areas of South America and Southeast Asia [12]. *Litsea* is a genus of approximately 400 species that holds significant economic and ecological importance. These plants are often evergreen or deciduous trees or shrubs distinguished by alternating leaves, umbellate inflorescences, and unique floral morphology. The majority of *Litsea* species are located in tropical and subtropical Asia, with some being found in Australia, the Pacific islands, and certain regions of the Americas [13–15].

Numerous species of *Litsea* have been widely utilized in traditional and indigenous medicine for the treatment of various diseases, including pain, fever, inflammation, gastrointestinal problems, diabetes, asthma, and neurological conditions [16–19]. Over twenty *Litsea* species have been recorded as efficacious analgesics in traditional medicine, and numerous research have corroborated their pharmacological attributes. *Litsea chinensis* (Lam.) Pers., an evergreen plant belonging to the Lauraceae family, is extensively found throughout the tropical and subtropical areas of Asia, encompassing India, Sri Lanka, and Southeast Asia. In traditional medical systems like Unani, it is referred to as Medalakri and has been utilized for the treatment of numerous ailments. Various plant components, especially the bark, leaves, and roots, are extensively employed in traditional medicine for their medicinal attributes [20].

The bark of *L. chinensis* is conventionally utilized as an anti-inflammatory, analgesic, and astringent agent, and is also applied in the management of fever, rheumatism, diarrhoea, dysentery, and fractures. Topical applications of bark paste are frequently employed to manage hemorrhage and facilitate wound healing. Prior research has indicated that extracts of *L. chinensis* demonstrate a diverse array of pharmacological properties, encompassing antibacterial, antioxidant, anti-inflammatory, antipyretic, and anthelmintic actions [21]. Phytochemical analyses have identified that *L. chinensis* comprises an array of bioactive substances, including alkaloids, flavonoids, tannins, terpenoids, and phenolic elements.

Alkaloids like laurotetanine, actinodaphnine, boldine, and norboldine have been detected in the bark and leaves, which are thought to play a role in the documented pharmacological effects [22]. This study seeks to examine the pharmacognostic properties, phytochemical constituents, and biological activities of *L. chinensis*, specifically emphasizing its antibacterial, antioxidant, and anti-inflammatory capabilities. This research may enhance the scientific validation of established applications and facilitate the creation of innovative plant-derived medicinal molecules.

2. Methodology

2.1. Collection and Authentication of Plant Material

The stem bark of *L. chinensis* was obtained from a local market in Chennai, Tamil Nadu, India. Dr. Venkatesan, a botanist at the Regional Research Institute of Unani Medicine (RRIUM), Chennai, verified the authenticity of the plant material. A voucher specimen number DSRU/CHEN/2026/09/P/067 was deposited in the Museum of the Drug Standardisation Research Unit (DSRU), RRIUM, Chennai–600013, for future reference. Equal quantities of the individual market samples were combined and thoroughly mixed to obtain a pooled sample prior to analysis. However, a separate analytical test for the homogeneity of the pooled sample was not performed.

2.2. Pharmacognostical Studies

Standard methods were employed to conduct pharmacognostical evaluations, which included macroscopic, microscopic, and particle analyses [23]. Transverse sections of the stem bark were prepared for microscopic investigations, stained with safranin, and mounted in glycerin. In order to identify lignified tissues and other cellular components, powder microscopy was conducted by treating the pulverized drug with specific reagents, including phloroglucinol–HCl and Jeffrey's reagent [24]. A Nikon Eclipse Ci microscope was employed to acquire microphotographs at varying magnifications.

2.3. Physicochemical Analysis

The coarsely powdered stem bark was subjected to physicochemical analysis to determine parameters such as foreign matter, loss on drying at 105 °C, total ash, acid-insoluble ash at 600 °C, pH, and extractive values (hexane, alcohol, and aqueous) according to standard procedures [25].

2.4. HPTLC Analysis

2.4.1. Sample Preparation

One gram of powdered sample was sonicated with 10 mL of ethanol for 30 min and filtered. The filtrate was transferred into an HPTLC vial for analysis.

2.4.2. Chromatographic Conditions

HPTLC analysis was performed using a CAMAG system (Switzerland). The sample was applied onto pre-coated silica gel 60 F254 TLC aluminum plates (6 × 10 cm) using a CAMAG ATS 4 applicator. The mobile phase consisted of toluene:acetone:formic acid (4.5:4.5:1.0, *v*/*v*/*v*), and the plate was developed in a pre-saturated twin-trough chamber for 30 min [26]. After development, the plate was air-dried and visualized under UV light at 254 nm and 366 nm using a CAMAG UV Visualizer [27]. Densitometric scanning was performed using WINCATS software at a scanning speed of 20 mm/s with a slit dimension of 6 × 0.45 mm. The plate was derivatized using vanillin–sulfuric acid reagent and heated at 105 °C until colored bands appeared. The derivatized plate was photographed under white light and scanned at 520 nm.

2.5. Antimicrobial Activity

2.5.1. Preparation of Extract

Fifty grams of powdered stem bark was extracted by cold maceration using 250 mL of 99.9% ethanol for 24 h at room temperature. The extract was filtered using Whatman filter paper and used for further analysis.

2.5.2. Disc Diffusion Method

Antimicrobial activity was evaluated using the disc diffusion method. Muller–Hinton agar and Sabouraud dextrose agar plates were prepared. Bacterial inoculum was evenly spread using a sterile swab. Sterile discs were impregnated with 50 µL of extract (50 mg/mL) and placed on the agar surface. The plates were incubated at 37 °C for 24 h. The experiments were conducted in triplicate, and the zone of inhibition was measured in millimetres [28].

2.6. Antioxidant Assays

2.6.1. DPPH Radical Scavenging Assay

The DPPH assay was employed to ascertain the antioxidant activity. A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of ethanol. In a 96-well plate, 100 µL of 0.1 mM DPPH solution was mixed with 100 µL of test samples at different concentrations (10, 50, 75, and 100 µg/mL). Ascorbic acid was used as the standard. The mixture was incubated in the dark for 30 min, and absorbance was measured at 517 nm. All experiments were performed in triplicate [29].

$$\% \text{ Inhibition} = [(A_control - A_sample)/A_control] \times 100$$

2.6.2. ABTS Radical Scavenging Assay

The ABTS assay was performed following Arnao et al. (1996). ABTS radical cation (ABTS•$^{+}$) was generated by mixing 7 mM ABTS with 2.4 mM potassium persulfate and incubating in the dark for 14 h. The solution was diluted with ethanol to obtain an absorbance of 0.706 ± 0.01 at 734 nm. Sample solutions (5–100 µL) were mixed with 3.95 mL of ABTS•$^{+}$ solution and incubated for 30 min at room temperature. Absorbance was measured at 734 nm, and ascorbic acid was used as the standard [30].

$$\% \text{ Inhibition} = [(A_control - A_sample)/A_control] \times 100$$

2.7. In Vitro Anti-Inflammatory Assay

The anti-inflammatory activity was evaluated using the protein denaturation method. The reaction mixture contained 1 mL of test sample at different concentrations and 1 mL of 1% bovine serum albumin. The pH was adjusted using phosphate buffer (pH 6.4). The mixture was incubated at 37 °C for 15 min and then heated at 70 °C for 5 min. After cooling, absorbance was measured at 660 nm. Diclofenac sodium was used as the standard drug [31].

$$\% \text{ Inhibition of protein denaturation} = [(A_control - A_sample)/A_control] \times 100$$

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD).

3. Results

3.1. Pharmacognostical Studies

3.1.1. Macroscopic features

Stem bark in pieces 1.5 to 1.6 cm long and 0.1 to 0.5 cm wide; external surface rough, corky, dark brown to reddish brown; internal surface smooth grey to whitish; longitudinally striated and dark brown to black; pink and orange pigments present; abundant lenticels present; fracture short, brittle; odour strong; taste bitter and mucilaginous (Figure 1).



Figure 1. Macroscopic appearance of the Stem bark of *L. chinensis*.

3.1.2. Microscopic Features

T. S. of stem bark shows cork, cortex and phloem rays; the periderm consists of phellem, phellogen and phelloderm; phellem or cork consists of stratified region of cork cells composed of eleven to twelve rows of tangentially elongated tabular cells, followed by an alternative layer of lignified and non-lignified cells of phellogen; phelloderm or secondary cortex consists of six to ten rows of thin walled rectangular parenchyma cells; sclereids in large number of groups present in the secondary cortical region; sclereids very thick cell walled with narrow lumen; Sclereids highly lignified, predominantly present and arranged in irregular either in group or single, some aligned axially, forming long lines or appeared as continuous layer of overlapping plate; rhomboid crystal of calcium oxalate crystals also present in the secondary cortical region; secondary phloem consists of fibres, parenchyma cells, sieve tubes and companion cells; secondary phloem consists of radial rows of parenchyma cells; fibres scattered single or groups of few fibres (2 to 5); uniseriate to multiseriate rays present; phloem rays narrow

in inner side and wider towards the outer side; sclereids present lesser abundantly but size bigger and very thick walled; starch grains present; numerous simple and compound starch present in the parenchyma cells of the secondary cortex and phloem parenchyma cells; presence of abundant mucilage; orange colour pigments present in both the parenchyma cells of the secondary cortex and phloem parenchyma cells were described in Figure 2.

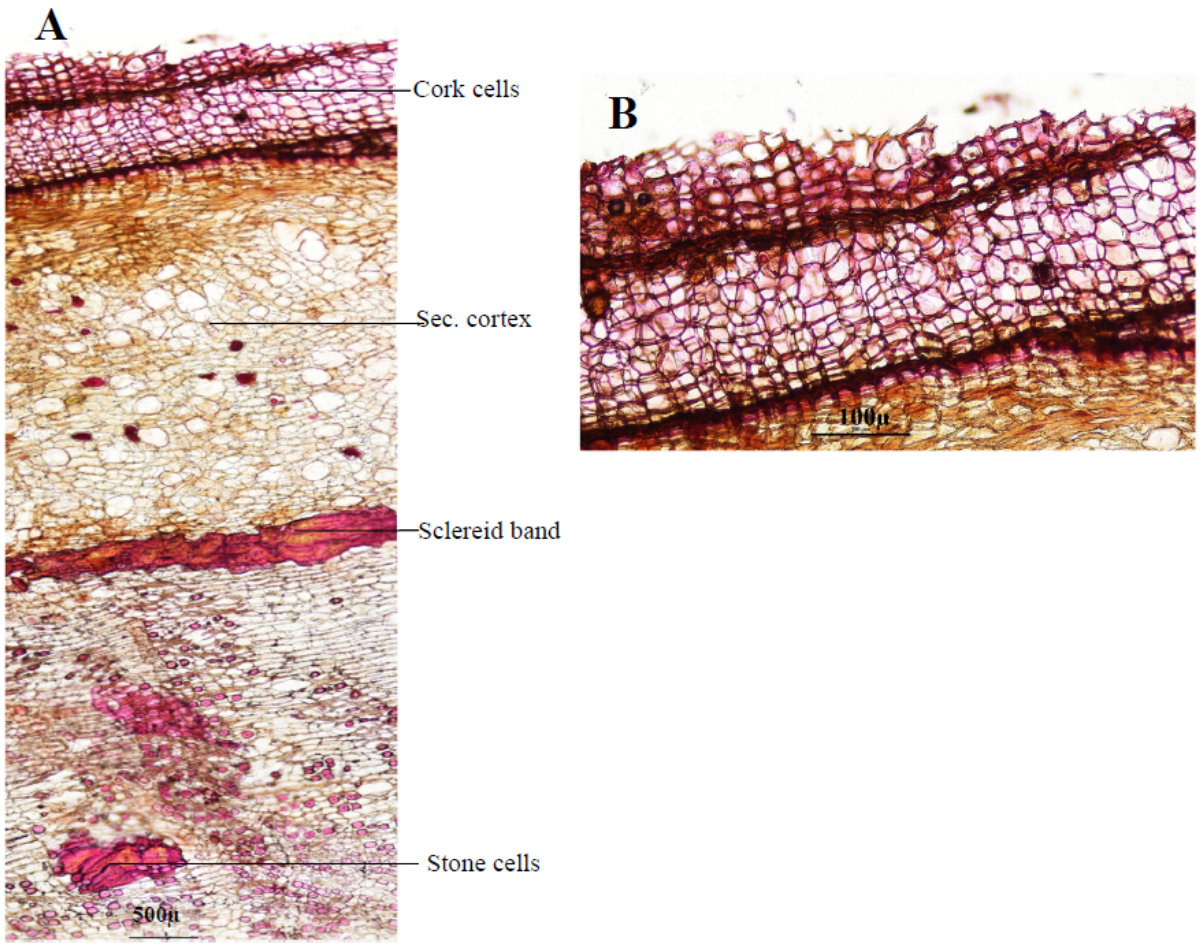


Figure 2. A. Transverse sectional view of *L. chinensis*; B. Cork cells.

3.1.3. Powder Features

Light brown; cork cells in surface view; starch grains numerous, simple and compound, simple starch grains spherical to oval, measuring upto 15 µ; fibres long, thick walled, lignified, very narrow lumen with tapering ends of length upto 750 µ and breadth upto 40 µ; sclereids, lignified, thick walled, round to oblong, laterally compressed with narrow lumen and radiating pit canals of length upto 90 µ and breadth upto 150 µ; parenchyma cells containing tannins and mucilage; rhomboidal crystals upto 30 µ were expressed in Figure 3.

3.2. Physicochemical Parameters

The physicochemical parameters of *Litsea chinensis* stem bark were evaluated according to the guidelines of the World Health Organization (WHO) and Ayurvedic Pharmacopoeia of India (API), and the results are presented in Table 1. No foreign matter was detected, indicating the purity of the raw material. The loss on drying was 9.35 ± 0.03%, indicating the moisture and volatile matter content of the sample. The total ash value was 3.83 ± 0.01%, representing the total inorganic residue, while the acid-insoluble ash value was 0.66 ± 0.01%, indicating the proportion of siliceous matter, such as sand and soil. The extractive values provide an estimate of the constituents soluble in different solvents. The water-soluble extractive value (9.91 ± 0.04%) was considerably higher than the alcohol-soluble (2.23 ± 0.02%) and hexane-soluble extractive values (0.53 ± 0.06%), indicating a greater proportion of water-soluble constituents in the stem bark. The relatively lower alcohol- and hexane-soluble extractive values suggest comparatively lower quantities of constituents soluble in these solvents. The pH of the 5% aqueous solution was 6.77 ± 0.12, indicating a near-neutral reaction. Overall, these physicochemical

parameters provide baseline information for the quality control, standardization, and authentication of *L. chinensis* stem bark and may support further pharmacological investigations.

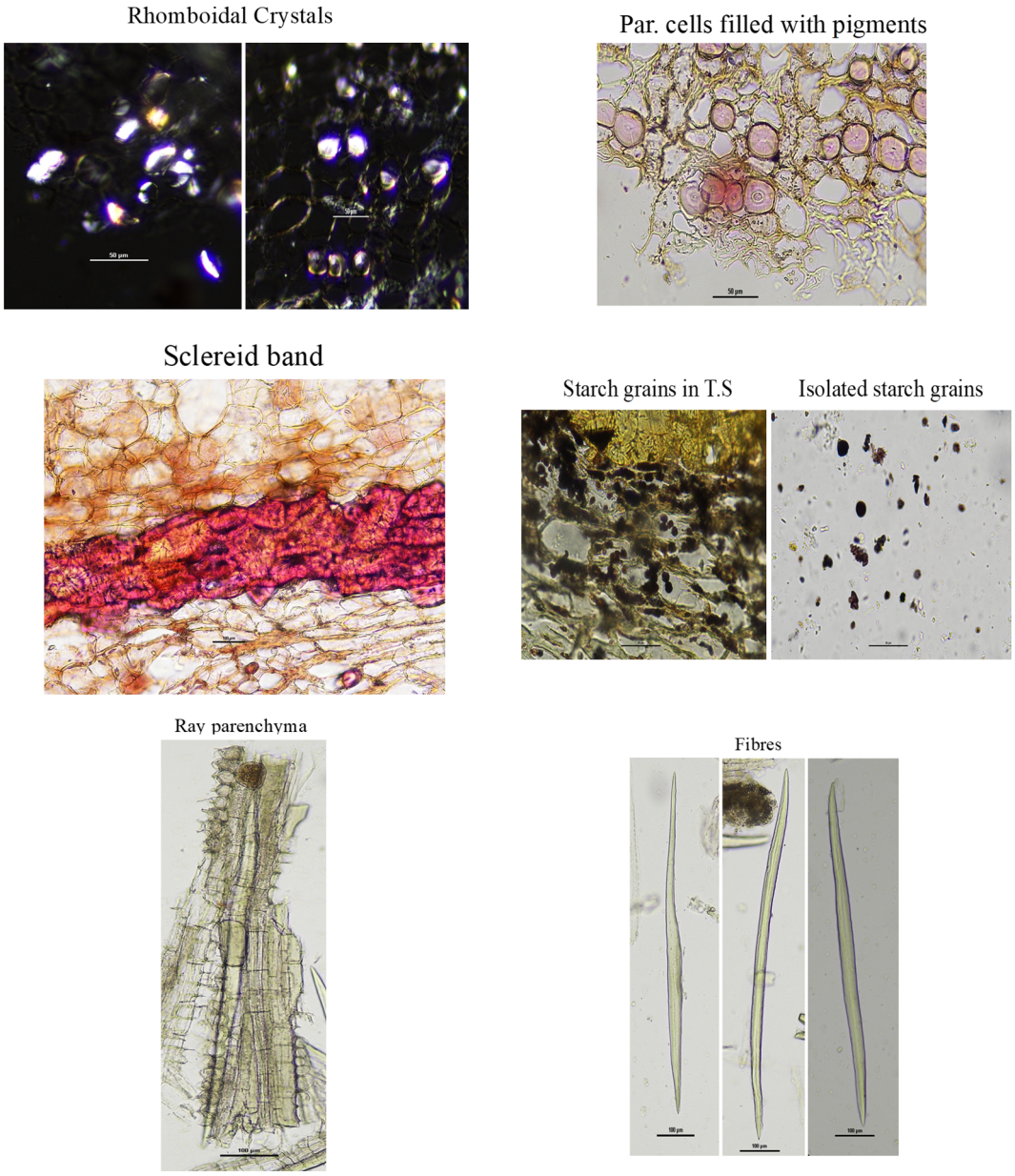


Figure 3. Powder Microscopic features of *L. chinensis* Stem bark.

Table 1. Physicochemical parameters of *L. chinensis* Stem bark.

Parameters	**Results (Mean ± Standard Deviation)**
Foreign Matter	Not detected
Loss on Drying (at 105 °C)	9.35 ± 0.03
Ash (%*w*/*w*)	
Total ash	3.83 ± 0.01
Acid-insoluble ash	0.66 ± 0.01
Extractives (%*w*/*w*)	
(cold percolation method)	2.23 ± 0.02
Alcohol-soluble extractive	
Water-soluble extractive	9.91 ± 0.04
Hexane soluble extractive	0.53 ± 0.06
pH values	
5% Aqueous solution	6.77 ± 0.12

3.3. HPTLC Finger Print Studies of Ethanol Extract

Photo documentation of *L. chinensis* stem bark showed eight well resolved bands at Rf values of 0.01, 0.22, 0.28, 0.33, 0.40, 0.57, 0.66, and 0.85, all appearing green in color under short wave UV light. Under long UV, fifteen bands were observed at Rf values of 0.02 (blue), 0.08 (blue), 0.13 (blue), 0.16 (blue), 0.18 (blue), 0.24 (blue), 0.32 (blue), 0.36 (blue), 0.46 (blue), 0.55 (green), 0.61 (blue), 0.67 (fluorescent blue), 0.81 (fluorescent green), 0.85 (blue) and 0.91 (fluorescent blue). Visualization under white light of the derivatized plate revealed twelve bands at Rf values of 0.01 (grey), 0.08 (green), 0.11 (brown), 0.23 (brown), 0.34 (violet), 0.40 (violet), 0.57 (grey), 0.62 (blue), 0.67 (blue), 0.79 (blue), 0.86 (blue) and 0.93 (blue).

Densitometric scanning at 254 nm exhibited eight peaks corresponding to Rf values and areas of 0.01 (11.07%), 0.22 (4.71%), 0.28 (14.80%), 0.33 (35.07%), 0.40 (9.47%), 0.57 (3.46%), 0.66 (18.66%) and 0.85 (2.79%). The peak at Rf 0.33 became the prominent. Scanning at 366 nm divulged fifteen peaks at Rf values and areas of 0.02 (6.26%), 0.08 (2.59%), 0.13 (6.32%), 0.16 (1.47%), 0.18 (2.91%), 0.24 (7.28%), 0.32 (0.84%), 0.36 (2.71%), 0.46 (9.50%), 0.55 (1.80%), 0.61 (1.75%), 0.67 (22.98%), 0.81 (17.45%), 0.85 (1.34%), and 0.91 (14.81%). The major peaks were observed at 0.67 (22.98%) & 0.81 (17.45%). The fingerprinting at 520 nm of the post derivatized plate contained twelve peaks at Rf values and areas of 0.01 (18.26%), 0.08 (7.62%), 0.11 (4.57%), 0.23 (14.23%), 0.34 (2.18%), 0.40 (1.21%), 0.57 (8.41%), 0.62 (4.99%), 0.67 (16.81%), 0.79 (9.58%), 0.86 (2.19%), and 0.93 (9.94), having 0.01 (18.26%) and 0.67 (16.81%) peaks as major (Figure 4). The existence of numerous distinct peaks at various wavelengths validates the complexity and abundance of phytochemical components in the ethanolic extract. These fingerprint profiles can function as a dependable instrument for the identification, verification, and quality assurance of *L. chinensis*. Furthermore, the significant peaks may indicate key bioactive molecules accountable for the noted pharmacological effects, necessitating additional isolation and characterisation research [32,33].

3.4. Antimicrobial Activity

The antimicrobial efficacy of the ethanolic extract of *Litsea chinensis* stem bark was assessed against specific bacterial strains (Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae) and a fungal strain (Candida albicans) employing the agar disc diffusion technique explained in Table 2.

The extract demonstrated concentration-dependent antimicrobial efficacy, with larger zones of inhibition noted at elevated concentrations (10, 25, and 50 mg/mL). At the maximal dose (50 mg/mL), the extract exhibited the greatest inhibition against *S. aureus* (23 mm), followed by *P. aeruginosa* (21.67 mm) and *E. coli* (20.33 mm). Moderate activity was noted against *K. pneumoniae* (19.33 mm) and *S. pneumoniae* (18 mm), however a comparably lesser inhibition was observed against the fungal strain *C. albicans* (16 mm). The standard medication demonstrated greater zones of inhibition for all examined species, with peak activity against *S. aureus* (27 mm) and *P. aeruginosa* (27 mm), signifying its enhanced antibacterial efficacy. Nevertheless, the plant extract had significant effectiveness, especially against Gram-positive bacteria. The extract exhibited superior antibacterial activity relative to antifungal activity, indicating that bacterial strains are more vulnerable to the phytoconstituents found in *L. chinensis*. The heightened sensitivity of Gram-positive bacteria can be ascribed to their less complex cell wall construction, facilitating the entrance of bioactive substances, whereas Gram-negative bacteria have an outer barrier that restricts compound ingress. The detected antimicrobial activity is attributable to phytochemicals such as flavonoids, alkaloids, tannins, and phenolic compounds, which are recognized for their antimicrobial effects via mechanisms such as cell membrane disruption, enzyme activity inhibition, and nucleic acid synthesis interference. These results corroborate the conventional application of *Litsea chinensis* in addressing microbial illnesses and underscore its potential as a natural source of antibacterial compounds.

Table 2. Evaluation of the antimicrobial activity of *L. chinensis* stem bark extract.

Ethanolic Extract of *L. chinensis* Stem Bark (50 mg/mL)	***S. aureus***	***S. pneumoniae***	***E. coli***	***P. aeruginosa***	***K. pneumoniae***	***C. albicans***
	(Zone of Inhibition mm in dm)					
10	18 ± 1.00	15.33 ± 0.58	15.33 ± 0.58	15 ± 1.0	12.33 ± 0.58	10.67 ± 0.58
25	19.67 ± 0.58	10.67 ± 0.58	17.67 ± 1.15	17.67 ± 1.15	15.33 ± 0.58	12.67 ± 0.58
50	23 ± 2.0	18 ± 1.0	20.33 ± 0.58	21.67 ± 0.58	19.33 ± 0.58	16.00 ± 1.00
Std	27 ± 2.0	21.33 ± 1.53	24 ± 1.0	27.00 ± 1.00	24.00 ± 1.00	19.67 ± 0.58
Standard (Std)	Amp: Ampicillin 10 mcg (Gram positive organisms); Nx: Norfloxacin10 mcg (Gram negative organsisms); Ap: Amphotericin 20 mcg (Fungi)					
Organisms	*SA: Staphylococcus aureus* (ATCC-29213)*; SP: Streptococcus pneumoniae* (MCC-2425)*; PS: Pseudomonas aeruginosa* (ATCC-27853)*; KP: Klebsiella pneumonia* (ATCC-700603); *EC: Escherichia coli* (ATCC 25922) *and CA: Candida albicans* (Clinical Cochin University).					

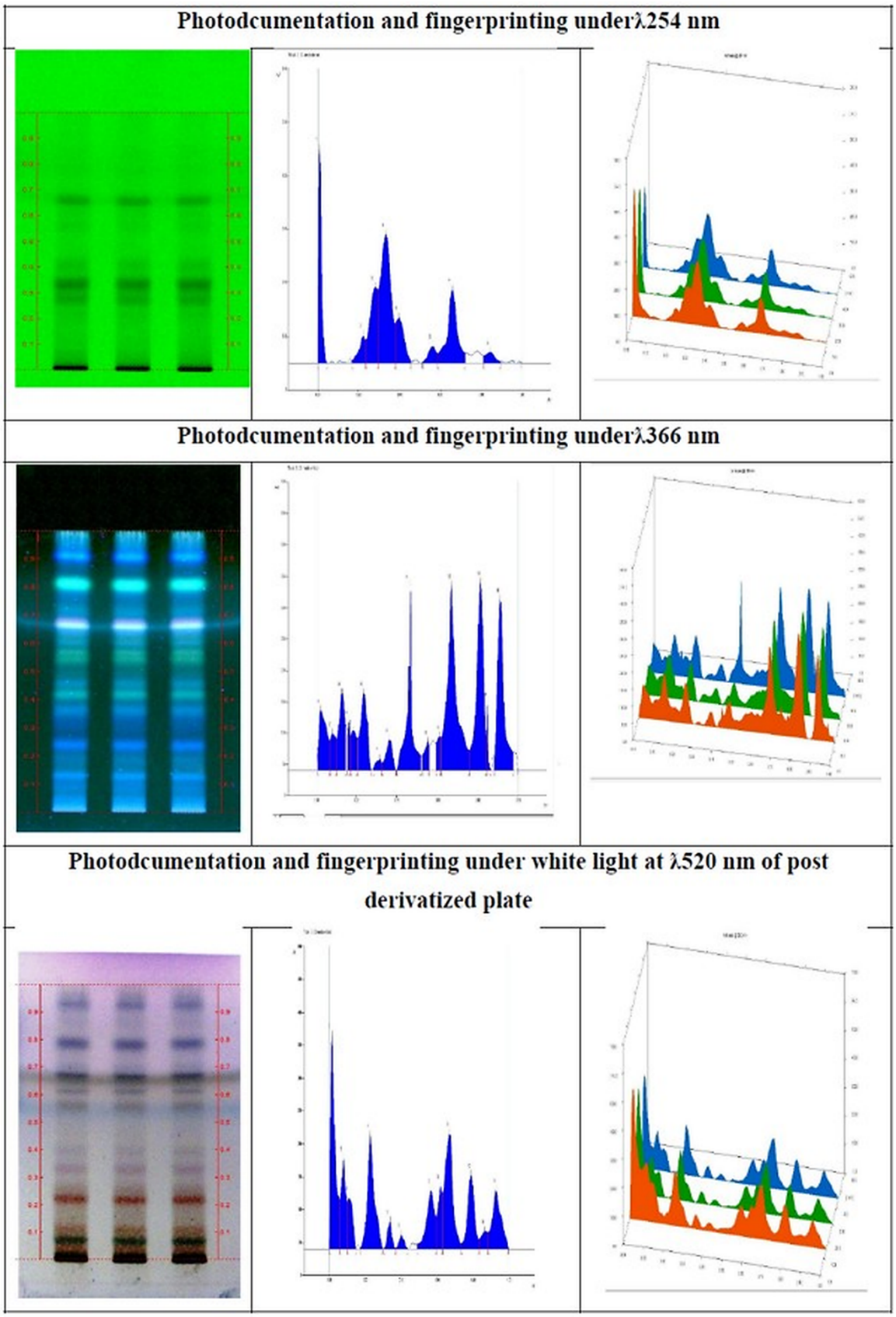


Figure 4. Photo-documentation and HPTLC fingerprinting profile of *L. chinensis* stem extract.

3.5. Antioxidant Activity

The antioxidant capacity of the ethanolic extract of *L. chinensis* stem bark was assessed by DPPH and ABTS radical scavenging assays, with the findings displayed in Tables 3 and 4.

3.5.1. DPPH Radical Scavenging Activity

The extract showed a concentration-dependent increase in DPPH radical scavenging activity and the results were expressed in Table 3. The percentage of inhibition rose from 43.02% at 50 µL to 59.84% at 200 µL with an IC_{50} value of 102.0 ± 0.4 µL. In comparison, the conventional ascorbic acid had much higher activity, ranging from 81.74% to 95.12% across the concentrations tested and an IC_{50} value of 5.5 ± 0.6 µL as determined by non-linear regression analysis. Although the extract's activity was lower than that of the standard, the results show a moderate free radical scavenging capacity, indicating the existence of hydrogen-donating antioxidants such as phenolic and flavonoid compounds.

Table 3. DPPH Radical Scavenging Activity in plant sample and standard Ascorbic in different concentrations.

Concentration	50 µL	100 µL	150 µL	200 µL	IC_{50}
% of inhibition in sample	43.02	49.84	54.03	59.84	102.0 ± 0.4
Standard (Ascorbic acid)	81.74	87.85	90.55	95.12	5.5 ± 0.6

3.5.2. ABTS Radical Scavenging Activity

The ABTS experiment exhibited a dose-dependent antioxidant activity, with the extract displaying inhibition values from 40.01% at 50 µL to 55.84% at 200 µL. The IC_{50} value of the ethanolic extract was 124.9 ± 0.5 µL, as determined by non-linear regression analysis of the concentration–response data, indicating the concentration required to achieve 50% radical scavenging activity. The conventional ascorbic acid demonstrated superior inhibition, varying from 83.25% to 96.15% with an IC_{50} value of 13.63 ± 0.1 µL as determined by non-linear regression analysis. All the results were expressed in the Table 4. The analogous trend noted in both DPPH and ABTS assays validates the extract's consistent antioxidant capacity. The significantly reduced activity in the ABTS assay relative to the DPPH assay may be ascribed to variations in radical types and reaction processes.

The results from both assays clearly indicate that the ethanolic extract of *L. chinensis* possesses significant antioxidant activity in a concentration-dependent manner. The reported activity is due to bioactive phytoconstituents like phenolics, flavonoids, and tannins, which neutralize free radicals by donating electrons or hydrogen atoms. Despite exhibiting lower antioxidant activity than the conventional ascorbic acid, the extract shown significant scavenging capacity, indicating its potential use as a natural antioxidant source.

Table 4. ABTS Radical Scavenging Activity in plant sample and standard Ascorbic acid

Concentration	50 µL	100 µL	150 µL	200 µL	IC_{50}
% of inhibition in sample	40.01	47.49	51.36	55.84	124.9 ± 0.5
Standard (Ascorbic acid)	83.25	87.36	90.54	96.15	13.63 ± 0.1

3.6. Anti-inflammatory Activity

The bovine serum albumin (BSA) protein denaturation assay was employed to assess the anti-inflammatory potential of the ethanolic extract of *Litsea chinensis* stem bark. The results are summarized in Table 5.

At 50 µL, the extract demonstrated a concentration-dependent inhibition of protein denaturation, with a percentage inhibition that increased from 32.14% to 57.25% at 200 µL. In contrast, the standard drug diclofenac sodium exhibited a considerably higher level of inhibition, with a range of 79.21% to 91.06% across the tested concentrations. Nonlinear regression analysis of the concentration–response data yielded an IC_{50} value of 117.30 ± 0.67 µL for the ethanolic extract, whereas diclofenac sodium showed an IC_{50} value of 29.22 ± 0.44 µL. The lower IC_{50} value of diclofenac sodium indicates greater inhibitory activity against BSA protein denaturation under the conditions tested. The concentration-dependent progressive increase in inhibition suggests that the extract has the capacity to stabilize proteins and prevent denaturation, a critical mechanism in inflammation. The extract exhibited moderate and substantial anti-inflammatory potential, despite its lower activity than the standard drug [34]. The presence of bioactive phytoconstituents, including flavonoids, tannins, and phenolic compounds, may contribute to the observed activity, as these compounds have been reported to exhibit anti-inflammatory effects through mechanisms including modulation of inflammatory mediators and stabilization of cellular and lysosomal membranes.

Table 5. Inhibition of Bovine Serum Albumin denaturation assay.

Concentration	50 µL	100 µL	150 µL	200 µL	IC_{50}
% of inhibition in sample	32.14	43.19	52.01	57.25	117.3 ± 0.67
Standard (Diclofenac sodium)	79.21	83.15	87.10	91.06	29.22 ± 0.44

4. Discussion

This study provides a comprehensive evaluation of the pharmacognostical, physicochemical, phytochemical, and biological characteristics of *L. chinensis* (Medalakri) stem bark, thereby validating its traditional medicinal applications and establishing foundational evidence for its standardization. A well-developed periderm, numerous sclereids, calcium oxalate crystals, starch granules, and mucilage-containing cells were identified as unique macroscopic and microscopic characteristics through pharmacognostical characterization. These characteristics serve as critical diagnostic indicators. These morphological characteristics are extensively employed to ensure the authenticity and quality control of herbal pharmaceuticals and are consistent with members of the Lauraceae family. The physicochemical characteristics obtained in this investigation were within acceptable limits, indicating that the raw drug or crude plant material met the highest quality standards. In order to prevent microbiological infection and extend the stability period, it is imperative to maintain a low moisture content. Consequently, low ash measurements indicate that there are minimal inorganic contaminants. The prevalence of polar phytoconstituents, such as phenolics and flavonoids, is suggested by the elevated water-soluble extractive value in comparison to other solvents. These results confirm previous research that *Litsea* species are rich in secondary metabolites, particularly phenolic compounds, which are responsible for a variety of pharmacological effects [35].

HPTLC fingerprinting demonstrated multiple bands at different Rf values, indicating the presence of several chromatographically detectable constituents in the ethanolic extract. The fingerprint profile provides a characteristic chemical pattern that may be useful for quality control and comparison of samples. Previous phytochemical investigations of *Litsea* species have reported the occurrence of diverse classes of secondary metabolites, including phenolic compounds, terpenoids, and alkaloids [36]. However, the present HPTLC analysis primarily provides a fingerprint profile and does not by itself establish the identity or biological activity of individual constituents.

The antioxidant activity of the ethanolic extract, assessed using DPPH and ABTS radical-scavenging assays, increased with increasing concentration under the experimental conditions. The extract showed moderate antioxidant activity compared with the reference standard ascorbic acid. The observed activity is consistent with previous reports describing antioxidant activity in *Litsea* species and ethanolic plant extracts [37]. However, the present assays demonstrate radical-scavenging activity and do not establish the specific compounds or mechanisms responsible for the observed effect.

The ethanolic extract also demonstrated concentration-dependent inhibition of protein denaturation in the in vitro anti-inflammatory assay. This finding indicates anti-inflammatory activity under the experimental conditions used. Similar biological activities have been reported for extracts of *Litsea* species, including effects associated with inflammatory mediators and enzymes [38]. Nevertheless, the present study did not investigate specific molecular pathways or inflammatory mediators; therefore, mechanistic interpretations regarding cyclooxygenase, nitric oxide synthase, or other pathways cannot be conclusively established from the current findings.

Overall, the present study establishes pharmacognostical, physicochemical, HPTLC, antioxidant, and in vitro anti-inflammatory profiles of *L. chinensis* stem bark. The findings provide useful baseline information for authentication, quality assessment, and further investigation of this plant material. Further studies involving isolation and characterization of individual phytoconstituents, mechanistic evaluation, and *in vivo* investigations are warranted to clarify the constituents and mechanisms underlying the observed biological activities.

5. Conclusions

This study evaluated the pharmacognostic, physicochemical, phytochemical, and biological characteristics of Litsea chinensis (Medalakri) stem bark. The macroscopic and microscopic characteristics provided diagnostic features for the identification of the raw drug. The physicochemical parameters established quality-related characteristics of the plant material, while HPTLC fingerprinting demonstrated the presence of detectable phytochemical constituents and provided a reproducible chromatographic profile. The ethanolic extract exhibited concentration-dependent antibacterial activity, with Gram-positive bacteria showing greater sensitivity. It also demonstrated concentration-dependent antioxidant and anti-inflammatory activities under the experimental conditions employed. The findings provide pharmacognostic, physicochemical, phytochemical, and preliminary biological data that can be used for the identification and quality assessment of L. chinensis stem bark. Further studies involving the isolation and characterization of individual constituents, mechanistic investigations, and in vivo evaluation are required to further characterize its biological activities.

Author Contributions

S.A.W.A.: Pharmacognostic Analysis, Collection of resources and data analysis, writing—original draft preparation; M.S.A.: Antimicrobial, Antioxidant and Anti-inflammatory Analysis; A.K.M.: HPTLC Analysis; J.J.C.: Physico-chemical Analysis; M.K.: Review and final editing; A.J.: Supervision, resources, funding acquisition, writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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The data are not publicly available due to ethical, privacy, or confidentiality restrictions. Further access may be considered upon reasonable request, subject to the relevant ethical approvals and institutional requirements.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used QuillBot to assist with paraphrasing and improving the language and readability of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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