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# Bioactive Constituents of Lagerstroemia indica L.: Assessment of Antioxidant and Anti-Inflammatory Potential

Zinah Essam Hameed[1,2], Thukaa Zuhair Abdul-Jalil[2]

[1]Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, Al-Esraa University, Baghdad, Iraq.
[2]Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, Baghdad University, Baghdad, Iraq.

**ABSTRACT: Background**: Lagerstroemia indica L. (Lythraceae) is a medicinal and ornamental plant containing diverse bioactive secondary metabolites. However, information regarding its phytochemical composition and biological activities is limited.

**Objective**: To authenticate Iraqi-grown L. indica, characterize its phytochemical profile and evaluate its antioxidant and anti-inflammatory activities.

**Methods**: We molecularly authenticated Lagerstroemia indica by amplification and sequencing of the chloroplast psbA gene. We sequentially extracted the aerial parts with n-hexane and 85% ethanol, then fractionated the crude ethanolic extract. Phytochemical constituents were chemically characterized. We evaluated the antioxidant activity using DPPH and H2O2 scavenging assays. We assessed the anti-inflammatory activity of the crude ethanolic extract and chloroform fraction in a lipopolysaccharide (LPS)-induced acute kidney injury model by measuring TNF-α and IL-1β levels by ELISA, measuring NF-κB expression by western blot, and examining renal histopathological changes.

**Results**: Preliminary chemical screening revealed a chemically diverse profile comprising flavonoids, flavonoid glycosides, phenolic acids, and other secondary metabolites. The crude ethanolic extract exhibited the highest DPPH and H2O2 scavenging percentages under the experimental conditions. LPS significantly increased TNF-α and IL-1β levels, whereas pre-treatment with either the crude ethanolic extract or the chloroform fraction significantly attenuated these elevations ($p < 0.05$). Histopathological examination further demonstrated improvement in LPS-induced renal alterations following treatment.

**Conclusion**: Iraqi-grown Lagerstroemia indica contains diverse phenolic and flavonoid constituents and exhibits antioxidant and anti-inflammatory activities, supporting further investigation of its bioactive constituents and underlying mechanisms.

## 1. INTRODUCTION

Chronic kidney disease (CKD) is associated with considerable morbidity and mortality, with persistent inflammation and oxidative stress contributing significantly to its onset and progression [1]. Dysregulation of inflammatory and antioxidant signaling pathways contributes to renal injury; activation of nuclear factor kappa B (NF-kB) promotes inflammatory responses, whereas nuclear factor erythroid 2- related factor 2 (Nrf2) regulates cellular antioxidant defenses [2-4]. Despite advances in the management of kidney disease and its complications, effective therapies that directly reverse established renal fibrosis remain limited, emphasizing the need to identify novel therapeutic agents and molecular targets capable of attenuating renal inflammation, oxidative stress, and progressive tissue injury [5, 6].

The therapeutic application of medicinal plants is deeply rooted in traditional knowledge and continues to be widely practiced, particularly in developing countries [7, 8]. Medicinal plants are recognized as valuable sources of structurally diverse secondary metabolites with potential biological and pharmacological activities [9, 10].

Lagerstroemia indica, a member of the family Lythraceae, has been widely utilized in traditional medicine across Asia for the management of various ailments. Different plant parts, including the leaves, bark, flowers, and roots, have been employed for their therapeutic properties. In particular, the plant has traditionally been used as a diuretic and antipyretic to manage fever and urinary disorders [11, 12]. Ethnomedicinally, L. indica has been used for hypertension, gastrointestinal disorders such as diarrhoea, pain, hypercholesterolemia, and diabetes [13].

The medicinal properties of L. indica have been associated with its diverse phytochemical composition. Members of the Lythraceae family are well known to produce a wide range of secondary metabolites, particularly flavonoids, tannins, phenolic compounds, terpenoids, and alkaloids, many of which exhibit important biological activities[14, 15].

Despite the growing interest in the pharmacological properties of this plant, limited information is available regarding the phytochemical constituents and antioxidant activity of Lagerstroemia indica cultivated in Iraq. In addition, solvent fractionation techniques are often used to separate compounds according to their polarity, allowing better identification of the biologically active fraction.

Accordingly, this study aimed to characterize the phytochemical profile of the crude extract and its successive solvent fractions of Iraqi-grown L. indica and to evaluate their antioxidant activity using the DPPH radical scavenging assay and H2O2 scavenging activity assay and to investigate the anti-inflammatory effects of selected preparations in an LPS-induced acute kidney injury model through assessment of pro-inflammatory cytokines TNF-α, IL-1β, and NF-kB.

## 2. MATERIALS AND METHODS

### 2.1. Molecular authentication of Lagerstroemia indica

Genomic DNA was extracted from fresh leaves of Lagerstroemia indica using

a commercial DNA extraction kit according to the manufacturer's instructions. The quality of extracted DNA was evaluated by agarose gel electrophoresis.

Specific primers targeting the chloroplast psbA gene were designed and used for PCR amplification [16, 17].

The primer sequences were as follows:

**Forward primer:** ACCCTGGTGGATCTCTCTCT (605 bp)

**Reverse primer:** CAAGTGATGGAGATACACGATTT (605 bp)

PCR amplification was carried out in a reaction mixture containing genomic DNA, PCR master mix, forward and reverse primers, and nuclease-free water. The amplified PCR products were analyzed by agarose gel electrophoresis and visualized under UV illumination.

The obtained sequences were analysed using the Basic Local Alignment Search Tool (BLAST) available from the National Center for Biotechnology Information (NCBI) database to confirm the identity of the plant material [17].

### 2.2. Extraction and fractionation

The air-dried aerial parts of Lagerstroemia indica were ground into a coarse powder. Approximately 60 gm of the powdered material was exhaustively extracted with n-hexane by repeated maceration using 600 mL portions of solvent. The combined extracts were filtered and concentrated under reduced pressure to obtain the n-hexane fraction (F1).

The remaining plant material was air-dried and extracted with 85% ethanol using a Soxhlet apparatus until exhaustion. The ethanolic extract was filtered and concentrated under reduced pressure to obtain the crude ethanolic extract (C1) [18]. The crude extract was then dispersed in water and successively partitioned with petroleum ether, chloroform, ethyl acetate, and n-butanol to obtain F2-F5, respectively. All fractions were concentrated under reduced pressure using a rotary evaporator [19-21].

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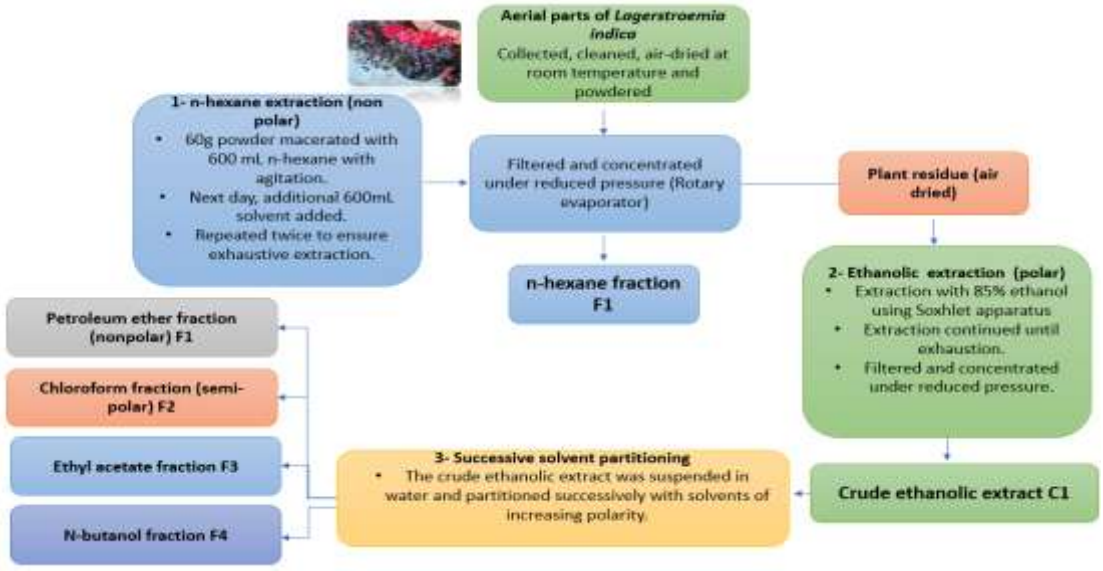



**Figure (1):** Schematic diagram for fractionation of aerial parts of Lagerstroemia indica plant

### 2.3. Preliminary phytochemical examinations of different fractions

Preliminary phytochemical screening was carried out to detect major bioactive secondary metabolites in the powdered plant material and different fractions of Lagerstroemia indica. The investigated phytochemical groups included Alkaloids, saponins, anthraquinones, sterols, terpenoids, tannins, phenolic compounds, and flavonoids. The screening procedures followed standard phytochemical methods [22-24].

#### 2.3.1. Alkaloid test:

Approximately 0.5 to 0.6g of powdered plant and each fraction were mixed with 1% hydrochloric acid, warmed gently, and filtered. About 2 ml of each filtrate was treated separately with Dragendorff's reagent and Mayer's reagent. The formation of an orange precipitate with Dragendorff's reagent or a creamy precipitate with Mayer's reagent indicates the presence of alkaloids.

#### 2.3.2. Saponin test:

About 0.5 g of plant material and each plant fraction were separately dissolved in boiling distilled water, cooled, and shaken vigorously. The formation of a stable persistent froth after standing for approximately to form a froth 30 min indicate the presence of saponins.

#### 2.3.3. Anthraquinones test:

One gram of dried powdered plant material and fraction was boiled with six ml of 1% HCl and then filtered. The filtrate was shaken with five ml benzene, and the benzene layer was separated. A few milliliters of 10% ammonium hydroxide solution were added to benzene extract, the appearance of red, pink, violet coloration in the alkaline layer indicated the presence of anthraquinones.

#### 2.3.4. Sterol test:

A small amount of powdered plant material and each fraction was dissolved in chloroform, and dried anhydrous sodium sulphate. To the chloroform extract, acetic anhydride and concentrated sulfuric acid were added carefully, development of a green color indicated the presence of sterols, while pink to purple coloration suggested the presence of terpenoids or triterpenoids.

#### 2.3.5. Terpenoids test (Salkowski test):

Approximately 5 ml of each fraction was mixed with chloroform, followed by careful addition of concentrated sulfuric acid along the side of test tube. Formation of a reddish-brown coloration at the interface indicating the presence of terpenoids.

**2.3.6. Test for phenolic compounds and flavonoids**

**a. Lead acetate test:**

Five millilitres of each fraction were treated with 1 ml of 10% lead acetate solution; formation of a yellowish-white precipitate indicated the presence of the flavonoids.

**b. Sodium hydroxide test:**

Two millilitres of each fraction were mixed with 1 ml of 2N sodium hydroxide solution; development of a yellow-orange colouration that disappeared upon addition of dilute acid confirmed the presence of flavonoids.

**2.3.7. Tannin test:**

Approximately 0.25g of powdered plant and each fraction were dissolved in distilled water and filtered. A few drops of 1% ferric chloride solution were added to the filtrate. The appearance of green, blue or black colouration indicates the presence of tannins.

**2.4. Evaluation of Lagerstroemia indica antioxidant activity**

**2.4.1. DPPH Radical Scavenging Assay**

The antioxidant activity of the plant extracts and fractions was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following previously reported methods with slight modification [25-27].

A DPPH solution was prepared by dissolving 0.04g of DPPH in 100mL of methanol to obtain a concentration of 400µg/mL

- Preparation of standard and sample solutions

A standard solution of Vitamin C (ascorbic acid) was prepared by dissolving 0.5 g of vitamin C in 100 mL of methanol - distilled water mixture, giving a concentration of 5000 ppm. Serial dilutions were prepared using the dilution equation to obtain concentrations of 30, 60, 120, 250 and 500 ppm.

Similarly, the plant extracts and fractions were prepared in methanol at the same concentrations: 30, 60, 120, 250 and 500 ppm.

- DPPH reaction procedure

An aliquot of the sample and standard solution was mixed with DPPH solution, and the mixture was shaken vigorously. The reaction mixtures were then allowed to stand in the dark at room temperature for 30 minutes.

After incubation, the absorbance of each solution was measured at 517nm using a UV-Visible spectrophotometer[25].

The percentage of DPPH radical scavenging activity was calculated using the following equation:

DPPH scavenging activity (%) = A0-A1/ A0 x 100

Where A0 represents the absorbance of the control (blank) and A1 represents the absorbance of in the presence of sample.

**2.4.2. Hydrogen Peroxide Scavenging Assay**

The hydrogen peroxide ($H_2O_2$) scavenging activity of the plant extract was evaluated according to a previously reported method with slight modifications [28].

A 40 mM Hydrogen peroxide solution in phosphate buffer (pH 7.4) was used. The extract solution (100 µg/ mL) was prepared using distilled water. An aliquot of the extract was then mixed with 0.6 mL of hydrogen peroxide solution.

The reaction mixture was incubated in a room temperature for 10 minutes. Subsequently, the absorbance was measured at 230 nm using UV- Visible spectrophotometer against a blank consisting phosphate buffer without hydrogen peroxide.

The percentage of hydrogen peroxide scavenging activity was calculated using the following equation[29, 30]

% $H_2O_2$ Scavenging = Ac – As / Ac x 100

Where Ac is the absorbance of the control and As represents the absorbance in the presence of the sample.

### 2.5. Nephroprotective effect of Lagerstroemia indica ethanolic extract and chloroform fraction in LPS- acute kidney injury

#### 2.5.1. Experimental design and groups

Male BALB/c albino mice (n = 32) aged 8-10 weeks and weighing 20-30 g were used in this study. Animals were randomly allocated to the experimental groups (n= 6 per group). Male mice within a defined age and weight range were selected minimize biological variability and potential sex-related differences in inflammatory and biochemical responses[3, 31].

The experimental design is illustrated in Figure (2):

1. Control group: received 0.1 mL of normal saline.

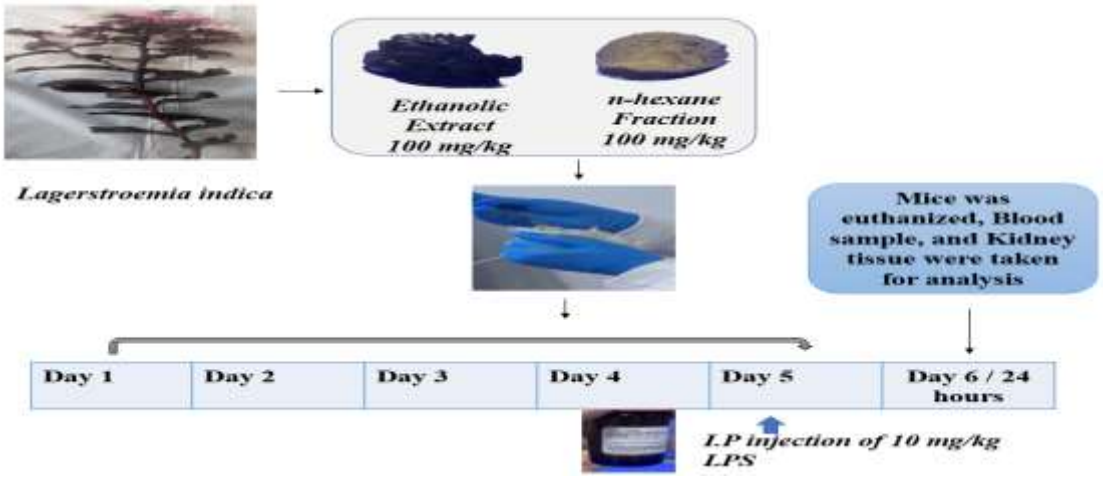
2. LPS group: received a single intraperitoneal (i.p.) injection of LPS at 10 mg/kg [32].

3. Crude ethanolic extract 100 mg group: received crude ethanolic extract orally at 100 mg/kg/day for 5 successive days, followed by a single i.p. injection of LPS (100 mg/kg) on day 5 [33].

4. Chloroform fraction 100 mg group: received chloroform fraction orally at 100 mg/kg/day for 5 successive days, followed by a single i.p. injection of LPS (100 mg/kg) on day 5[32].

LPS at 10 mg/kg (i.p.) was administered 2 h after the final treatment on day 5 and was used to induce acute kidney injury based on previously established experimental protocols [32, 34]. The selected treatment doses were based on previous pharmacological studies and preliminary experimental consideration.

Twenty-four hours after LPS administration, the animals were euthanized under ketamine (80 mg/kg) and xylazine (8 mg/kg) anaesthesia, and biological samples were immediately collected for subsequent analyses.




**Figure(2):** Schematic illustration of experimental design

#### 2.5.2. Protein Quantification by ELISA

Protein levels of TNF- α and IL-1β were quantified using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Briefly, add 50 µL of standards and 40 µL of each sample to the designated wells [35, 36]. Supplement sample wells with 10 µL of the corresponding biotinylated antibodies for TNF-α and IL-1β, then add 50 µL of streptavidin-HRP. The plate was gently mixed, sealed, and incubated at 37°C for 60 minutes. The wells were then washed five times with 300 µL of wash buffer per well. Subsequently, 50 µL each of substrate's A and B were added, and the plate was incubated at 36°C for 10 min [37, 38]. The reaction was terminated by adding stop solution, which changed the colour from blue to yellow. Absorbance was measured at 450 nm using a microplate reader within 10 min, and TNF-α and IL-1β concentrations were determined from corresponding standard curves[39, 40].

#### 2.5.3. Determination of NF-kB expression by western blot analysis

Western blot analysis was performed to evaluate NF-kB protein expression levels in kidney tissues, which were homogenised in ice-cold lysis buffer containing a protease inhibitor cocktail to extract total protein. The homogenates were centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatant containing total protein was collected.

Protein concentration was determined using an appropriate protein assay method according to the manufacturer's instructions. Equal amounts of protein samples were mixed with loading buffer, denatured by heating, and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Subsequently, the separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes.

The membranes were blocked with blocking buffer to minimise nonspecific binding and incubated overnight at 4 °C with primary antibodies against NF-κB; after washing, the membranes were incubated with appropriate horseradish peroxidase (HRP)- conjugated secondary antibodies.

Protein bands were visualised using an enhanced chemiluminescence (ECL) detection system, and band intensities were analysed using image analysis software. We used β-tubulin as an internal loading control to normalise protein expression levels according to the manufacturer's instructions[41, 42].

#### 2.5.4. Histological analysis

Following euthanasia, the left kidney was excised, rinsed with phosphate-buffered saline (PBS, pH 7.4), and fixed in 10% formalin [43, 44]. The tissues were paraffin- embedded, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (A&E). A blind pathologist examined histological sections using light microscopy. Five randomly selected fields per section were evaluated at ×40 magnification. Renal tubular injury, including tubular epithelial damage and loss of the brush border, was assessed using a semi-quantitative scoring system as follows: 0, normal; 1, <25%; 2, 25-50%; 3, 50-75%; and 4, >75 of the area affected [45, 46].

## 3. RESULTS AND DISCUSSION

### 3.1. Molecular authentication of Lagerstroemia indica

PCR amplification of the chloroplast psbA produced a single amplicon of the expected size of approximately 605 bp. The resulting sequence was subjected to BLAST analysis against sequences available in the NCBI database for molecular authentication. Sequence alignment revealed 99% identity with the registered Lagerstroemia indica chloroplast genome sequence (GenBank accession no.NC_030484.1), with an E-value of 0.0. These results supported the molecular authentication of the investigated plant as Lagerstroemia indica.




**Figure (3):** BLAST alignment analysis of the amplified psbA sequence showing 99% similarity with Lagerstroemia indica chloroplast genome sequence (NC_030484.1).

### 3.2. Extraction and fractionation yields

Sequential extraction and solvent partitioning were employed to separate the phytochemical constituents of Lagerstroemia indica according to polarity. Initial extraction with n-hexane was used to recover relatively lipophilic constituents, whereas

subsequent extraction with 85% ethanol provided a border recovery of polar and moderately polar metabolites. The crude ethanolic extract was further fractionated using petroleum ether, chloroform, ethyl acetate, and n-butanol to obtain fractions with distinct polarity [18, 47].

The extraction of 60g of the aerial parts of Lagerstroemia indica yielded 2.39g of the n-hexane extract, corresponding to 3.98% and 9.5g of crude ethanolic extract, representing 15.83% of the starting material (Table (1)).

Successive partitioning of the crude ethanolic extract resulted in different recoveries among the solvent fractions. The n-butanol fraction showed the highest recovery (3.50g, 36.80%), followed by the ethyl acetate (2.98g, 31.36%), chloroform (2.21g, 23.20%) petroleum ether (0.81g, 8.52%) fractions. The greater recovery in the n-butanol and ethyl acetate fractions indicates that a substantial proportion of the ethanol-extractable constituents partitioned into relatively polar and intermediate-polarity solvents.

The differences in fraction yields reflect the differential partitioning of phytochemical constituents according to their phytochemical properties and solvent affinity. Further characterization by UPHPLC-QTOF-MS/MS was therefore performed to determine the phytochemical composition of the individual fractions and to relate their chemical profiles to the biological activities observed in the subsequent assays.

**Table (1):** Extraction and fractionation yields of the aerial parts of Lagerstroemia indica

| Extract/Fraction | Weight (g) | Yield (%) |
|---|---|---|
| n-hexane | 2.39g | 3.9%[a] |
| Ethanol | 9.5g | 15.8%[a] |
| Petroleum ether | 0.81g | 8.52%[b] |
| Chloroform | 2.21g | 23.2%[b] |
| Ethyl acetate | 2.98g | 31.36%[b] |
| n-butanol | 3.50g | 36.8%[b] |

a Yield calculated according to the initial dry plant material (60g).

b Yield calculated according to the crude ethanolic extract (9.5g).

### 3.3. Preliminary phytochemical examination on different fractions

Preliminary phytochemical screening was carried out on the crude ethanolic extract and the solvent fractions of Lagerstroemia indica to determine the distribution of the major classes of secondary metabolites. The results showed qualitative differences in phytochemical composition among the investigated fractions, reflecting the influence of solvent polarity on extracting different constituents.

Phenolic compounds and flavonoids were predominantly detected in the ethyl acetate and n-butanol fractions, whereas terpenoids and phytosterols were mainly concentrated in the petroleum ether fraction. The results of the preliminary phytochemical examination are presented in Table 2.

**Table (2):** Preliminary phytochemical screening of the crude extract and different fractions.

| Phytochemical test | n-hexane fraction | Ethanolic extract | Petroleum ether fraction | Ethyl acetate fraction | Chloroform fraction | n-butanol fraction |
|---|---|---|---|---|---|---|
| **Alkaloid test** | - | + | - | + | - | + |
| **Saponin test** | - | + | - | - | - | + |
| **Anthraquinones test** | - | + | - | + | + | + |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Sterol test** | + | + | + | + | + | - |
| **Terpenoids test (Salkowski test)** | + | + | + | + | + | + |
| **Lead acetate test** | - | + | - | + | + | + |
| **Sodium hydroxide test** | - | + | - | + | + | + |
| **Tannin test** | - | + | - | + | + | + |

(-) = Absence of phytoconstituents, and (+) = Presence of phytoconstituents.

### 3.4. Evaluation of antioxidant activity of Lagerstroemia indica

#### 3.4.1. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the crude ethanolic extract and its petroleum ether, ethyl acetate, chloroform, and n-butanol fractions was evaluated at concentrations ranging from 30 to 500 ppm. All tested samples exhibited a concentration-dependent increase in DPPH radical scavenging activity (Table 3).

At 30 ppm, the crude extract showed the highest scavenging activity (25.80%), followed by the ethyl acetate (22.58%) and n-butanol (22.01%) fractions. The corresponding activity of vitamin C was 12.06%. Scavenging activity increased progressively with concentration, reaching a maximum at 500 ppm.

At 500ppm, the crude ethanolic extract exhibited the highest DPPH radical scavenging activity (86.09%), followed by the ethyl acetate (81.74%), the n-butanol (75.44%), the petroleum ether (72.65%), and the chloroform (70.60%) fractions. Vitamin C showed 68.56% scavenging activity. Thus, under the experimental conditions employed, the crude ethanolic extract consistently produced the highest DPPH scavenging percentage across the tested concentration range[47].

**Table (3)** DPPH radical scavenging activity of crude ethanolic extract and solvent fractions

| Sample | 30 ppm | 60 ppm | 120 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|---|
| Vitamin C | 12.06 | 22.65 | 47.08 | 60.56 | 68.56 |
| Crude extract | 25.80 | 44.98 | 64.89 | 77.80 | 86.09 |
| Petroleum ether | 18.14 | 29.00 | 53.21 | 66.98 | 72.65 |
| Ethyl acetate | 22.58 | 41.47 | 61.56 | 73.41 | 81.74 |
| Chloroform | 16.08 | 26.55 | 50.25 | 64.58 | 70.66 |
| n-butanol | 22.01 | 38.78 | 57.08 | 69.85 | 75.44 |

#### 3.4.2. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of crude ethanolic extract and its solvent fractions is presented in Table 4. All tested samples showed increasing activity with increasing concentration.

The crude ethanolic extract exhibited the highest H2O2 scavenging at all tested concentrations, increasing from 16.66% at 0.5% to 27.89% at 1.5%. Among the solvent fractions, the n-butanol fraction showed the highest activity at 1.5% (25.11%), followed by ethyl acetate (24.26%), petroleum ether (23.00%), and chloroform (22.65%) fractions. Vitamin C showed scavenging activities of 8.89%, 10.55%, and 14.85%at 0.5%, 1.0%, and 1.5%, respectively.

Under the experimental conditions used, the crude ethanolic extract and all investigated solvent fractions showed higher H2O2 scavenging percentages than vitamin C at the corresponding concentrations.

Finally, the crude ethanolic extract showed the highest H2O2 scavenging activity, while the n-butanol and ethyl acetate fractions displayed the highest activities among the fractioned samples.

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**Table (4)** Percentage Hydrogen peroxide H2O2 scavenging activity (%) of the crude extract and its solvent fractions.

| Concentration % | 0.5 % | 1.0 % | 1.5 % |
|---|---|---|---|
| Vitamin C | 8.98 | 10.55 | 14.85 |
| Crude extract | 16.66 | 22.66 | 27.89 |
| Petroleum ether | 12.99 | 14.78 | 23.00 |
| Ethyl acetate | 13.98 | 16.25 | 24.26 |
| Chloroform | 12.49 | 14.25 | 22.65 |
| n-butanol | 14.25 | 17.15 | 25.11 |

### 3.5. Evaluation of the nephroprotective activity of Lagerstroemia indica

#### 3.5.1. The effect of *Lagerstroemia indica* on TNF-α and IL-1β levels

The effects of the crude ethanolic extract and chloroform fraction of Lagerstroemia indica on the pro-inflammatory cytokines TNF-α and IL-1β were evaluated by ELISA.

LPS administration significantly increased TNF-α levels to 420.56 ± 65.05 ng/L compared with the control group (193.76 ± 11.59 ng/L; $p < 0.05$). Pre-treatment with the crude ethanolic extract significantly reduced TNF-α to 184.66 ± 10.43 ng/L, whereas the chloroform fraction reduced it to 269.72 ± 19.87 ng/L compared with the LPS group ($p < 0.05$). Neither treatment group differed significantly from the control group ($p > 0.05$). Although the crude ethanolic extract produced a greater numerical reduction in TNF-α than the chloroform fraction, the difference between the two treatments was not statistically significant ($p > 0.05$).

Similarly, LPS significantly elevated IL-1β levels to 25.27 ± 1.19 ng/L compared with the control group (15.62 ±0.17 ng/L; $p < 0.05$). Pre-treatment with the crude ethanolic extract and chloroform fraction significantly attenuated this increase, resulting in IL-1β levels of 13.55 ± 1.54 and 14.43 ± 2.37 ng/L, respectively ($p < 0.05$ vs LPS). No significant differences were observed among the control, crude ethanolic extract, and the chloroform fraction groups ($p > 0.05$).

Finally, both the crude ethanolic extract and chloroform fraction significantly attenuated the LPS-induced elevation of TNF-α and IL-1β, indicating suppression of the inflammatory response induced by LPS.

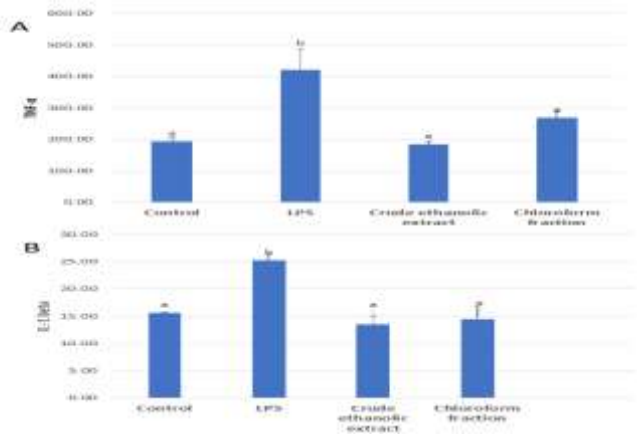



**Figure (4):** Effect of *Lagerstroemia indica* on expressions of inflammation-related parameters (TNF-α and IL-1β) in sepsis-evoked renal inflammation. A. Influence of Lagerstroemia indica on TNF-α. B. Influence of Lagerstroemia indica on IL-1β expression. *Data was expressed as mean ± SEM (n = 8). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple-comparison test. Bars bearing different letters are significantly different at *P < 0.05. Controls (a), LPS (b), Crude (a), chloroform (a).

### 3.5.2. Effect of *Lagerstroemia indica* on renal NF-kB expression.

Western blot analysis showed significant increases in renal NF-kB expression in the LPS model group compared with the normal control group ($p<0.05$). Pre-treatment with either crude ethanolic extract or chloroform fraction significantly reduced the expression of both proteins compared with the LPS group ($p<0.05$). No significant differences were observed between the treated groups and the normal control group. These findings indicate that both crude ethanolic extract and chloroform fraction attenuated LPS-induced inflammatory and apoptotic responses at the protein level (Figure 5).

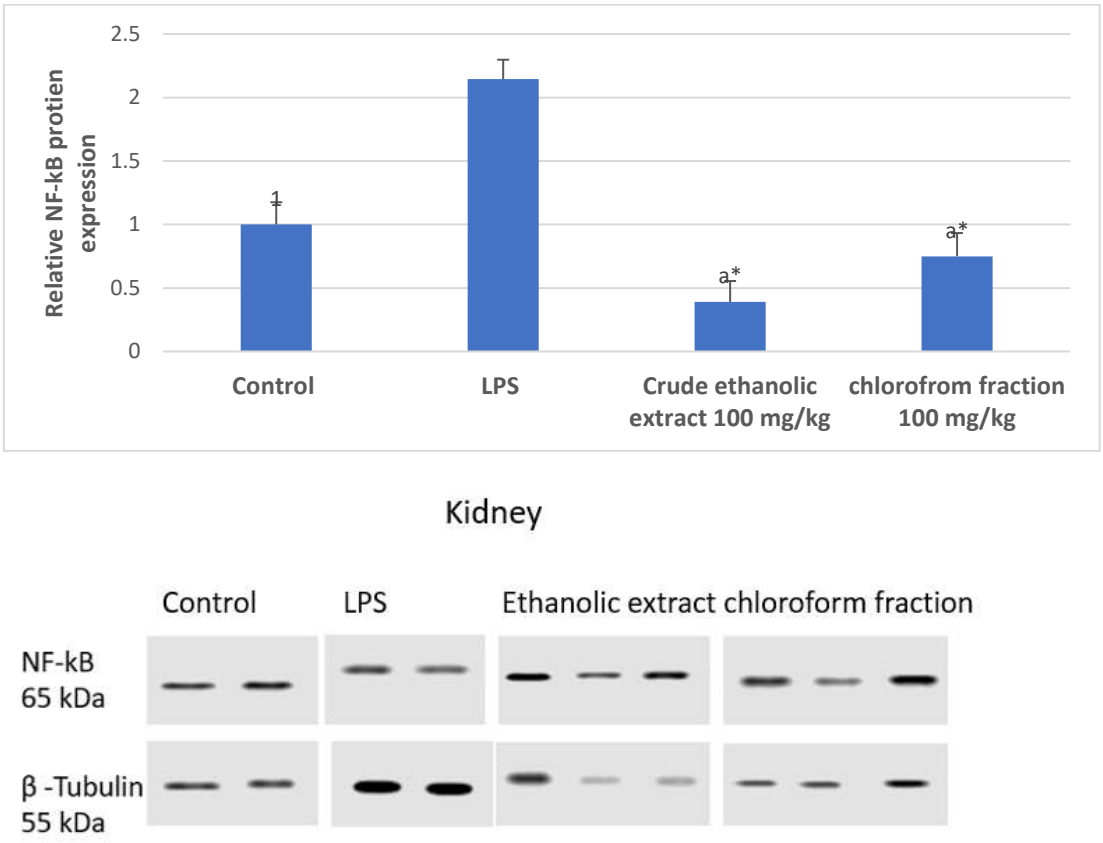



**Figure (5):** Effect of Lagerstroemia indica chloroform fraction on renal NF-kB expression level in renal tissue of LPS-induced AKI *Representative western blot bands and densitometric analysis are showenValues are expressed as relative protein expression mean ± SEM (n=2-3) . * Indicates a significant difference compared with LPS model group ($P<0.05$).

### 3.5.3 Effect of *Lagerstroemia indica* on kidney histology in LPS-induced AKI in mice

Histopathological examination of kidney sections revealed clear differences among the experimental groups (Figure 6). The control group (Figure 6A) showed relatively preserved renal architecture, with intact glomerular and tubular structures and no prominent inflammatory or degenerative changes

In contrast, the LPS group (6B) exhibited pronounced renal histopathological alterations characterized by tubular degeneration and dilatation, epithelial cell swelling, cytoplasmic vacuolation, and marked interstitial inflammatory cell infiltration. These findings were consistent with sever acute tubular injury.

Pre-treatment with the crude ethanolic extract of Lagerstroemia indica (Figure 6C) ameliorated the LPS-induced histopathological alterations, with better preservation of tubular architecture and reduced epithelial degeneration and tubular dilatation than in the LPS group. Nevertheless, mild residual tubular alterations remained evident.

In addition, the chloroform fraction group (Figure 6D) showed improvement in renal morphology compared with the LPS group, characterised by partial preservation of tubular and glomerular architecture and reduced tubular degenerative changes. Residual tubular dilatation and epithelial alterations were still observed.

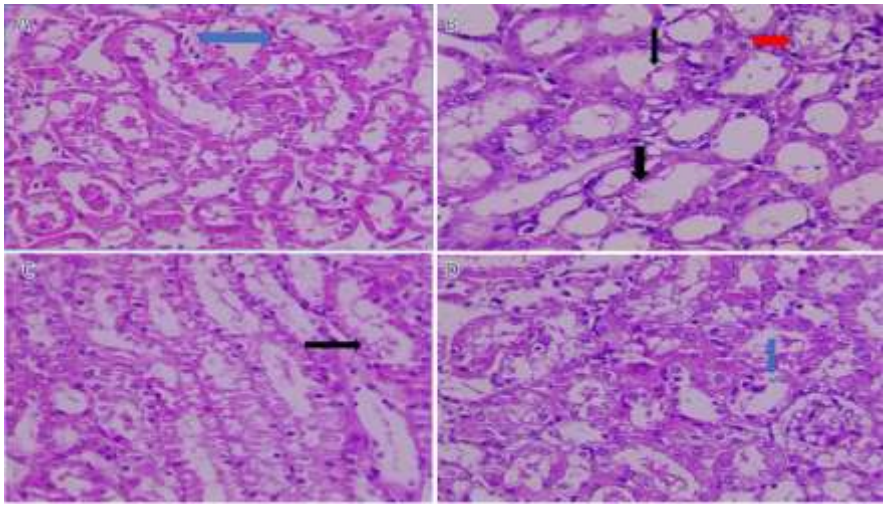


**Figure (6):** Influence of Lagerstroemia indica on renal histology abnormalities. Exemplary photomicrographs of renal slices from every group at a power of 40x. The blue horizontal bars demonstrate undamaged proximal tubules, the black bars sloughed tubular cells, and the red bars brush boundary loss during tubular dilatation. Panel A is the control, panel B is the LPS-evoked renal injury, panel C is the crude ethanolic extract group, panel D is the chloroform fraction group

**3.4 Discussion**

The antioxidant activity observed for *Lagerstroemia indica* may associate with several cases of secondary metabolites detected by preliminary phytochemical screening. The crude ethanolic extract and different solvent fractions showed variations in their phytochemical compositions according to solvent polarity. Phenolic compounds and flavonoids were predominantly detected in the ethyl acetate and n-butanol fractions, whereas terpenoids and phytosterols were mainly associated with the less polar fractions. Such differences in phytochemical distribution may contribute to the variation in biological activity observed among the investigated preparations.

The crude ethanolic extract exhibited the highest DPPH and H2O2 scavenging percentages under the experimental conditions. In the DPPH assay, the crude ethanolic extract showed the highest scavenging activity across the tested concentration range, reaching 86.9% at 500 ppm. Among solvent fractions, the ethyl acetate fraction exhibited the highest DPPH scavenging activity. In the H2O2 scavenging assay, the crude ethanolic extract also demonstrated the highest activity, whereas the n-butanol and ethyl acetate fractions showed the highest activity among the solvent fractions. These findings may reflect the combined contribution of multiple phytochemical classes in the crude ethanolic extract and the preferential distribution of flavonoids and phenolic constituents into the relatively polar fractions. However, because the phytochemical screening was qualitative, we cannot establish the contribution of individual compounds or phytochemical classes to antioxidant activity.

In the in vivo model, LPS administration markedly elevated the pro-inflammatory cytokines TNF-α and IL-1β, indicating a strong inflammatory response in the experimental model. LPS activates innate immune signalling pathways and stimulates the release of pro-inflammatory cytokines, particularly TNF-α and IL-1β [48-50]. Excessive production of these mediators contributes to systemic inflammation and plays an important role in the development and progression of tissue injury [51-53].

Pre-treatment with both the crude ethanolic extract and chloroform fraction of L. indica significantly attenuated the LPS-induced elevations of TNF-α and IL-1β. Moreover, cytokine levels in both treatment groups were not significantly different from those of the control group, suggesting substantial attenuation of the inflammatory response. The crude ethanolic extract produced a greater numerical reduction in TNF-α than the chloroform fraction; however, the difference between the two treatments did not reach statistical significance. Therefore, these findings support anti-inflammatory activity for both preparations but do not establish one treatment's superiority over the other.

The cytokine-level effects were accompanied by changes in renal NF-kB expression. Western blot analysis demonstrates significant increases in both proteins following LPS administration. NF-kB is an important regulator of inflammatory responses. Pre-treatment with either the crude ethanolic extract or chloroform fraction significantly reduced NF-kB expression compared with the LPS group. These findings suggest that the protective effect of the investigated preparations was associated with attenuating LPS-induced inflammatory and apoptotic responses [48, 54]. However, these findings do not establish direct modulation of specific molecular pathways and additional mechanistic studies are required.

The observed anti-inflammatory effects may be related, at least in part, to the classes of secondary metabolites detected by preliminary screening. Flavonoids, phenolic compounds, terpenoids and other plant secondary metabolites have been associated with antioxidant and anti-inflammatory activities. Nevertheless, because we neither isolated nor quantitatively characterised individual constituents in the present study, we cannot attribute the observed biological effects to any specific compound.

The antioxidant activity findings may also be relevant to the observed anti-inflammatory activity because oxidative stress and inflammation are closely interconnected during LPS-induced injury, as increased reactive oxygen species can amplify inflammatory signalling and cytokine production. The demonstrated DPPH and H2O2 scavenging activities of the crude extract and solvent fractions may therefore contribute, at least in part, to their observed anti-inflammatory effects.

Histopathological examination further supported the biochemical and protein marker results. The LPS group showed pronounced renal alterations, including tubular degeneration and dilatation, epithelial cell swelling, cytoplasmic vacuolation, and interstitial inflammatory cell infiltration. Pre-treatment with crude ethanolic extract ameliorated these alterations, preserving tubular architecture and reducing epithelial degeneration and tubular dilatation. Compared with the LPS group, the chloroform fraction improved renal morphology and reduced tubular degenerative changes.

The present study has several limitations. Phytochemical characterization was based primarily on qualitative screening; therefore, it did not identify or quantify the individual constituents responsible for the observed activities. In addition, antioxidant activity was evaluated using in vitro assays, while the in vivo anti-inflammatory assessment was limited to TNF-α and IL-1β levels, NF-kB expression and histopathological changes. Additional biochemical and molecular investigations are required to clarify the mechanisms underlying these activities.

## 4. CONCLUSION

The present study demonstrated that the aerial parts of Lagerstroemia indica cultivated in Iraq contain several classes of secondary metabolites, including phenolic acids, flavonoids, terpenoids and others. The crude ethanolic extract and its solvent fractions exhibited concentration-dependent antioxidant activity in DPPH and H2O2 scavenging assays, with the crude ethanolic extract showing the highest scavenging percentages under the experimental conditions.

In the LPS-induced acute kidney injury model, pre-treatment with the crude ethanolic extract and chloroform fraction significantly attenuated the LPS-induced increases in TNF-α and IL-1β, reduced renal NF-ϰB expression, and improved the associated renal histopathological alterations. These findings indicate that L. indica possesses both antioxidant and anti-inflammatory activities. Further phytochemical characterisation and mechanistic investigations are required to identify the bioactive constituents and clarify the mechanisms underlying these effects.

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## AUTHOR CONTRIBUTIONS

Zinah Essam Hameed: writing original draft preparation, conceptualization, methodological procedures, investigations, data collection, and formal analysis. Thukaa Zuhair Abdul-Jalil: project administration, writing, review and editing, validation, visualization, supervision, and scientific assistance.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ETHICS STATEMENT

The animal component of this study was reviewed and approved by the Research Ethics Committee, College of Pharmacy, University of Baghdad, Iraq (Research Ethics Approval No. REC0224018; approval date: 9 October 2024). The approved protocol covered the research involving experimental animals, and the study was conducted in accordance with the ethical requirements specified by the institutional Research Ethics Committee and applicable animal-welfare principles.

## DATA AVAILABILITY STATEMENT

The corresponding author can provide the data supporting the findings of this study upon reasonable request.

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