EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 9
Year: 2025

PHYTOCHEMICAL INVESTIGATION AND ANTI-ACNE ACTIVITY OF LAGERSTROEMIA SPECIOSA LEAF EXTRACT

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Article Received on 08/07/2025 **Article Revised on 28/07/2025** **Article Accepted on 18/08/2025**

ABSTARCT

Background: Acne vulgaris represents one of the most prevalent dermatological disorders globally, affecting approximately 85% of adolescents and young adults. The increasing resistance to conventional antibiotics and associated adverse effects have necessitated the exploration of natural alternatives with potent anti-acne properties. **Objective:** This comprehensive study investigates the phytochemical composition of Lagerstroemia speciosa (Banaba) leaf extract and evaluates its therapeutic potential against acne through antimicrobial, anti-inflammatory, and antioxidant activity assessments. **Methods:** Fresh leaves of L. speciosa were collected, authenticated, and extracted using ethanol and aqueous solvents. Comprehensive phytochemical screening was conducted using standard qualitative and quantitative methods. Anti-acne activity was evaluated against Propionibacterium acnes using agar well diffusion, minimum inhibitory concentration (MIC), and time-kill assays. Antioxidant potential was assessed through DPPH and ABTS radical scavenging assays. Anti-inflammatory activity was evaluated using carrageenan-induced paw edema model. **Results:** Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, glycosides, terpenoids, and steroids. The ethanolic extract demonstrated significant antimicrobial activity against P. acnes with zone of inhibition ranging from 16±9.2mm to 22±9.1mm at concentrations of 25-100 mg/mL. MIC and MBC values were determined to be 0.325 mg/mL and 0.625 mg/mL, respectively. The extract exhibited potent antioxidant activity with IC50 values of 105-130 µg/mL in DPPH assay and demonstrated notable anti-inflammatory effects with 42% reduction in paw edema. **Conclusion:** L. speciosa leaf extract possesses promising anti-acne properties attributed to its rich phytochemical profile, particularly corosolic acid, ellagic acid, and quercetin. The extract's multifaceted mechanism involving antimicrobial, anti-inflammatory, and antioxidant activities positions it as a viable natural therapeutic alternative for acne management.

KEYWORDS: Lagerstroemia speciosa, anti-acne activity, phytochemical screening, Propionibacterium acnes, corosolic acid, natural therapeutics.

1. INTRODUCTION

1.1 Background

Acne vulgaris, a chronic inflammatory disorder of the pilosebaceous unit, affects millions of individuals worldwide and represents a significant dermatological concern with substantial psychological and social implications (Williams et al., 2012). The pathogenesis of acne involves a complex interplay of factors including increased sebum production, follicular hyperkeratinization, bacterial colonization by Propionibacterium acnes, and inflammatory responses (Zaenglein et al., 2016).

Conventional acne treatments primarily rely on topical and systemic antibiotics, retinoids, and hormonal therapies. However, the emergence of antibiotic-resistant P. acnes strains, coupled with adverse effects associated with long-term antibiotic use, has prompted researchers to explore alternative therapeutic approaches (Dréno et al., 2014; Leyden, 2003). Natural products derived from medicinal plants offer a promising avenue for developing novel anti-acne formulations with reduced side effects and lower propensity for resistance development.

1.2 Lagerstroemia speciosa: Traditional Uses and Therapeutic Potential

Lagerstroemia speciosa (L.) Pers., commonly known as Banaba, Queen's flower, or Pride of India, belongs to the family Lythraceae. This tropical tree species is indigenous to Southeast Asia, including the Philippines, Malaysia, Thailand, and India, where it has been traditionally utilized for various medicinal purposes (Klein et al., 2007). Traditional applications include the

treatment of diabetes, kidney disorders, and inflammatory conditions.

The plant's therapeutic properties are attributed to its diverse phytochemical constituents, including corosolic acid, ellagic acid, gallic acid, quercetin, and various triterpenes. Corosolic acid, in particular, has gained significant attention for its antidiabetic, anti-inflammatory, and antimicrobial properties (Miura et al., 2001; Stohs et al., 2012). Recent investigations have also highlighted the potential of L. speciosa extracts in dermatological applications, particularly for inflammatory skin conditions (Priya et al., 2008).

1.3 Rationale and Objectives

Despite the traditional use of L. speciosa in skin-related ailments, comprehensive scientific evaluation of its anti-acne properties remains limited. The present study aims to bridge this knowledge gap by conducting a thorough investigation of the phytochemical composition and anti-acne potential of L. speciosa leaf extract.

Primary Objectives

1. Conduct comprehensive qualitative and quantitative phytochemical screening of L. speciosa leaf extract
2. Evaluate antimicrobial activity against P. acnes and other acne-associated microorganisms
3. Assess antioxidant potential through multiple in vitro assays
4. Investigate anti-inflammatory properties using appropriate experimental models
5. Identify and quantify major bioactive compounds responsible for therapeutic effects
6. Elucidate potential mechanisms of action for anti-acne activity

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh leaves of Lagerstroemia speciosa were collected during the flowering season (October-November) from authenticated plants growing in the botanical garden of the research institution. The plant material was identified and authenticated by a qualified botanist, and voucher specimens (LS-2023-001) were deposited in the institutional herbarium for future reference.

2.2 Preparation of Plant Extract

2.2.1 Processing of Plant Material Collected leaves were thoroughly washed with distilled water to remove dirt and debris, followed by shade drying at room temperature (25±2°C) for 7-10 days until completely dried. The dried leaves were ground to a fine powder using a mechanical grinder and passed through a 40-mesh sieve to obtain uniform particle size.

2.2.2 Extraction Procedure Two extraction methods were employed to prepare different extracts:

Ethanolic Extract (LSE): 500g of powdered leaf material was subjected to maceration with 2.5L of 70% ethanol for 72 hours at room temperature with occasional stirring. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure.

Aqueous Extract (LSA): 300g of powdered material was extracted with distilled water using the decoction method. The mixture was boiled for 30 minutes, cooled, filtered, and the aqueous filtrate was lyophilized to obtain the dry extract.

The percentage yield of extracts was calculated, and the dried extracts were stored in airtight containers at 4°C until further use.

2.3 Phytochemical Screening

2.3.1 Qualitative Phytochemical Analysis Standard qualitative tests were performed to identify major classes of phytochemicals:

- **Alkaloids:** Mayer's reagent, Wagner's reagent, and Dragendorff's reagent tests
- **Flavonoids:** Alkaline reagent test, Shinoda test, and Lead acetate test
- **Tannins:** Ferric chloride test and Lead acetate test
- **Saponins:** Froth test and Foam test
- **Phenolic compounds:** Ferric chloride test and Folin-Ciocalteu test
- **Glycosides:** Molisch's test and Legal's test
- **Terpenoids:** Salkowski test and Liebermann-Burchard test
- **Steroids:** Liebermann-Burchard test and Salkowski test
- **Carbohydrates:** Benedict's test and Fehling's test

2.3.2 Quantitative Phytochemical Analysis

Total Phenolic Content (TPC): Determined using the Folin-Ciocalteu method with gallic acid as standard (Folin & Ciocalteu, 1927). Results were expressed as mg gallic acid equivalents (GAE) per gram of extract.

Total Flavonoid Content (TFC): Estimated using aluminum chloride colorimetric method with quercetin as standard. Results were expressed as mg quercetin equivalents (QE) per gram of extract.

Total Tannin Content: Measured using tannic acid as standard and expressed as mg tannic acid equivalents (TAE) per gram of extract (Trease & Evans, 2002).

2.4 Antioxidant Activity Assessment

2.4.1 DPPH Radical Scavenging Assay The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Various concentrations of extracts (10-500 µg/mL) were prepared, and the IC50 values were calculated.

2.4.2 ABTS Radical Scavenging Assay 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assay was performed to further evaluate antioxidant potential.

2.4.3 Metal Chelating Activity The ability of extracts to chelate ferrous ions was assessed using the ferrozine method.

2.5 Antimicrobial Activity Evaluation

2.5.1 Test Microorganisms The following microorganisms were used for antimicrobial testing:

- Propionibacterium acnes (ATCC 6919) - Primary target organism
- Staphylococcus aureus (ATCC 25923)
- Staphylococcus epidermidis (ATCC 12228)
- Escherichia coli (ATCC 25922) - Control organism

2.5.2 Agar Well Diffusion Method Antimicrobial activity was assessed using the agar well diffusion method. Mueller-Hinton agar plates were inoculated with standardized bacterial suspensions (1×10^8 CFU/mL). Wells of 6mm diameter were cut, and 100µL of extract solutions at various concentrations (25, 50, 100 mg/mL) were added. Clindamycin (100 µg/mL) served as positive control, while DMSO was used as negative control.

2.5.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Broth microdilution method was employed to determine MIC values using serial two-fold dilutions of extracts (0.0625-32 mg/mL). MBC was determined by subculturing from wells showing no visible growth.

2.5.4 Time-Kill Kinetics The bactericidal kinetics of the most active extract was evaluated against P. acnes at MIC and 2×MIC concentrations over 24 hours.

2.6 Anti-inflammatory Activity Assessment

2.6.1 Carrageenan-Induced Paw Edema Model Male Wistar rats (180-220g) were used for anti-inflammatory studies following institutional ethical guidelines. Paw edema was induced by subplantar injection of 0.1mL of 1% carrageenan solution. Extract treatments (250 and 500 mg/kg) were administered orally 1 hour before carrageenan injection. Diclofenac sodium (10 mg/kg) served as reference standard.

2.6.2 In Vitro Anti-inflammatory Assessment Protein denaturation inhibition assay and membrane stabilization assay were performed to evaluate anti-inflammatory potential in vitro.

2.7 High-Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was conducted to identify and quantify major bioactive compounds in the most active extract. A C18 reverse-phase column was used with gradient elution system comprising water and acetonitrile with 0.1% formic acid.

2.8 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. P-values <0.05 were considered statistically significant.

3. RESULTS

3.1 Extract Yield

The percentage yield of extracts was calculated based on dry weight of plant material:

- Ethanolic extract (LSE): 12.4% w/w
- Aqueous extract (LSA): 8.7% w/w

3.2 Phytochemical Screening Results

3.2.1 Qualitative Phytochemical Analysis

Comprehensive phytochemical screening revealed the presence of diverse secondary metabolites in both extracts, with ethanolic extract showing higher abundance of most compounds.

Table 1: Qualitative Phytochemical Screening of L. speciosa Leaf Extracts.

Secondary Metabolite	Ethanolic Extract (LSE)	Aqueous Extract (LSA)
Alkaloids	+++	++
Flavonoids	+++	+++
Tannins	+++	++
Saponins	++	++
Phenolic compounds	+++	+++
Glycosides	++	+++
Terpenoids	+++	+
Steroids	++	+
Carbohydrates	+	++

Legend:** +++ = **Abundant,** ++ = **Moderate,** + = **Trace amount

3.2.2 Quantitative Phytochemical Analysis

Table 2: Quantitative Phytochemical Content of L. speciosa Leaf Extracts.

Parameter	Ethanolic Extract (LSE)	Aqueous Extract (LSA)
Total Phenolic Content (mg GAE/g)	127.4 ± 3.2	89.6 ± 2.8
Total Flavonoid Content (mg QE/g)	68.9 ± 2.1	45.2 ± 1.9
Total Tannin Content (mg TAE/g)	94.7 ± 2.7	62.3 ± 2.4

3.3 Antioxidant Activity Results

3.3.1 DPPH Radical Scavenging Activity

Both extracts demonstrated concentration-dependent radical scavenging activity. The ethanolic extract showed superior antioxidant potential compared to the aqueous extract.

Table 3: Antioxidant Activity of L. speciosa Leaf Extracts.

Extract	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	Metal Chelating IC50 (µg/mL)
LSE	112.6 ± 4.3	98.4 ± 3.7	156.8 ± 5.2
LSA	164.2 ± 6.1	142.7 ± 4.9	198.4 ± 6.8
Ascorbic acid*	22.4 ± 1.2	19.7 ± 0.9	-
EDTA*	-	-	45.2 ± 2.1

*Standard compounds for comparison

3.4 Antimicrobial Activity Results

3.4.1 Agar Well Diffusion Assay

The antimicrobial screening revealed significant activity against all tested microorganisms, with P. acnes showing highest susceptibility to both extracts.

Table 4: Antimicrobial Activity of L. speciosa Leaf Extracts (Zone of Inhibition in mm).

Microorganism	Extract	25 mg/mL	50 mg/mL	100 mg/mL	Clindamycin (100 µg/mL)
P. acnes	LSE	16.4 ± 1.2	19.7 ± 1.5	23.8 ± 1.8	28.4 ± 2.1
	LSA	11.2 ± 0.9	14.6 ± 1.1	17.9 ± 1.4	-
S. aureus	LSE	14.6 ± 1.0	17.2 ± 1.3	20.5 ± 1.6	24.7 ± 1.9
	LSA	9.8 ± 0.7	12.4 ± 0.9	15.1 ± 1.2	-
S. epidermidis	LSE	13.2 ± 0.8	16.1 ± 1.2	19.4 ± 1.5	23.2 ± 1.7
	LSA	8.7 ± 0.6	11.5 ± 0.8	14.2 ± 1.0	-

3.4.2 MIC and MBC Determination

Table 5: MIC and MBC Values of L. speciosa Leaf Extracts.

Microorganism	Extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC Ratio
P. acnes	LSE	0.312	0.625	2.0
	LSA	0.625	1.25	2.0
S. aureus	LSE	0.625	1.25	2.0
	LSA	1.25	2.5	2.0
S. epidermidis	LSE	0.625	1.25	2.0
	LSA	1.25	2.5	2.0

3.4.3 Time-Kill Kinetics

The time-kill study against P. acnes revealed rapid bactericidal activity of LSE, with >3 log reduction in bacterial count within 6 hours at 2×MIC concentration.

3.5 Anti-inflammatory Activity Results

3.5.1 Carrageenan-Induced Paw Edema

Table 6: Anti-inflammatory Activity of L. speciosa Leaf Extract.

Treatment	Dose	% Inhibition of Edema
Control	-	-
LSE	250 mg/kg	28.4 ± 3.2
LSE	500 mg/kg	41.7 ± 4.1
Diclofenac	10 mg/kg	52.3 ± 3.8

3.5.2 In Vitro Anti-inflammatory Assays

LSE demonstrated significant protein denaturation inhibition (IC50: 187.4 ± 8.2 µg/mL) and membrane stabilization activity (IC50: 164.7 ± 6.9 µg/mL).

3.6 HPLC Analysis Results

HPLC analysis of LSE identified and quantified several bioactive compounds:

Table 7: Major Bioactive Compounds in L. speciosa Ethanolic Extract.

Compound	Retention Time (min)	Content (mg/g extract)
Gallic acid	8.4	12.7 ± 0.8
Corosolic acid	24.6	18.9 ± 1.2
Ellagic acid	19.2	8.4 ± 0.6
Quercetin	26.8	6.2 ± 0.4
Kaempferol	28.1	4.1 ± 0.3

4. DISCUSSION

4.1 Phytochemical Profile and Therapeutic Significance

The comprehensive phytochemical investigation of L. speciosa leaf extracts revealed a rich diversity of bioactive compounds, which aligns with previous reports on this species (Saumya & Basha, 2011; Unno et al., 1997). The abundant presence of phenolic compounds, flavonoids, and tannins in the ethanolic extract correlates with its superior biological activities compared to the aqueous extract. These findings support the traditional use of alcohol-based preparations in folk medicine.

The quantitative analysis demonstrated that LSE contains significantly higher concentrations of total phenolics (127.4 mg GAE/g) and flavonoids (68.9 mg QE/g) compared to previous studies (Perera, 2021; Tanquilut et al., 2009), possibly due to variations in extraction methodology, geographical origin, and seasonal collection. The phenolic content observed is comparable to other well-studied medicinal plants with established antioxidant properties.

4.2 Antioxidant Activity and Skin Health Implications

The potent antioxidant activity exhibited by L. speciosa extracts, particularly LSE (IC50: 112.6 µg/mL in DPPH assay), is attributed to the high phenolic and flavonoid content (Unno et al., 1997). This antioxidant capacity is crucial for acne management as oxidative stress plays a significant role in acne pathogenesis (Del Rosso, 2013). Reactive oxygen species (ROS) generated during inflammatory processes can damage sebocytes and promote comedogenesis.

The metal chelating activity observed (IC50: 156.8 µg/mL) is particularly relevant as transition metals like iron can catalyze lipid peroxidation in sebum, contributing to acne severity. The ability of L. speciosa extracts to scavenge free radicals and chelate metal ions suggests their potential in preventing oxidative damage associated with acne inflammation (Yamaguchi et al., 2006).

4.3 Antimicrobial Activity Against Acne-Associated Pathogens

The antimicrobial evaluation revealed promising activity against P. acnes, the primary pathogenic bacterium implicated in acne vulgaris. The zone of inhibition produced by LSE at 100 mg/mL (23.8 ± 1.8 mm) demonstrated substantial activity, though slightly lower than clindamycin (28.4 ± 2.1 mm). However, the MIC value of 0.312 mg/mL against P. acnes indicates potent antimicrobial efficacy.

The bactericidal nature of the extract (MBC/MIC ratio = 2.0) suggests that L. speciosa can effectively kill bacteria rather than merely inhibiting their growth, which is advantageous for acne treatment. The time-kill kinetics demonstrated rapid bacterial elimination, indicating potential for quick therapeutic response.

The activity against S. aureus and S. epidermidis is also clinically relevant, as these organisms are frequently associated with secondary bacterial infections in acne lesions. The broad-spectrum antimicrobial activity suggests that L. speciosa extracts could address multiple pathogenic factors simultaneously.

4.4 Mechanism of Antimicrobial Action

The antimicrobial activity of L. speciosa extracts can be attributed to multiple bioactive compounds working synergistically. Corosolic acid, identified as a major constituent (18.9 mg/g), has been previously reported to possess antimicrobial properties through membrane disruption and interference with bacterial metabolic processes (Hayashi et al., 2006).

Tannins present in the extract can bind to bacterial proteins and enzymes, disrupting cellular functions (Liu et al., 2000). Flavonoids like quercetin and kaempferol can intercalate with bacterial DNA and inhibit essential enzymes. The combination of these mechanisms likely contributes to the overall antimicrobial efficacy observed.

4.5 Anti-inflammatory Properties and Acne Management

The significant anti-inflammatory activity demonstrated by LSE (41.7% inhibition at 500 mg/kg) is crucial for acne management, as inflammation is a key component of acne pathogenesis (Suzuki et al., 1999). The ability to reduce carrageenan-induced paw edema suggests potential for reducing inflammatory lesions in acne.

The in vitro anti-inflammatory assays further support this activity, with protein denaturation inhibition being particularly relevant as protein denaturation is associated with inflammatory responses in acne (Agrawal et al., 2010). The membrane stabilization activity suggests protection against inflammatory mediator release from immune cells.

4.6 Clinical Implications and Therapeutic Potential

The multifaceted activity profile of L. speciosa extracts - encompassing antimicrobial, anti-inflammatory, and antioxidant properties - positions this natural product as a promising candidate for comprehensive acne management. Unlike conventional single-target therapies, L. speciosa extracts can potentially address multiple pathogenic factors simultaneously.

The presence of corosolic acid, a well-characterized compound with established safety profile, adds credibility to the therapeutic potential. The concentration of corosolic acid found (18.9 mg/g) is within the range reported for biological activity in other studies.

4.7 Comparative Analysis with Existing Treatments

When compared to conventional acne treatments, L. speciosa extracts offer several advantages:

1. **Reduced resistance potential:** The multi-compound nature makes resistance development less likely compared to single-component synthetic antibiotics.
2. **Lower side effects:** Natural extracts generally exhibit fewer adverse effects compared to synthetic drugs like isotretinoin or long-term antibiotic use.
3. **Complementary mechanisms:** The antioxidant and anti-inflammatory activities provide additional benefits beyond antimicrobial action.
4. **Cost-effectiveness:** Plant-based treatments are generally more economical and accessible.

4.8 Formulation Considerations

For practical application, L. speciosa extracts could be incorporated into various topical formulations such as gels, creams, or lotions. The ethanolic extract showed superior activity and could be formulated with appropriate excipients to ensure stability and bioavailability.

Standardization based on corosolic acid content would ensure consistent therapeutic efficacy across different batches. The extract could also be combined with other complementary natural ingredients to enhance overall efficacy.

4.9 Safety Considerations

While L. speciosa has a long history of traditional use, comprehensive safety studies are necessary for therapeutic applications. The plant is generally considered safe based on traditional use patterns, but formal toxicological studies would strengthen the safety profile for modern therapeutic applications.

4.10 Limitations and Future Research Directions

The current study provides valuable insights into the anti-acne potential of L. speciosa extracts, but several limitations should be acknowledged:

1. **In vitro nature:** Most activities were evaluated in vitro; clinical studies are needed to confirm efficacy in human subjects.
2. **Mechanistic understanding:** Detailed molecular mechanisms of action require further investigation.
3. **Standardization:** Development of standardized extraction methods and quality control parameters is necessary.
4. **Formulation optimization:** Research into optimal delivery systems and formulation strategies is needed.

Future research should focus on:

- Clinical trials to evaluate safety and efficacy in human subjects
- Mechanistic studies using molecular techniques
- Development of standardized formulations
- Combination studies with other natural compounds
- Long-term safety assessment

5. CONCLUSION

This comprehensive investigation demonstrates that Lagerstroemia speciosa leaf extract possesses significant anti-acne potential through multiple mechanisms of action. The ethanolic extract showed superior activity compared to the aqueous extract, attributed to higher concentrations of bioactive compounds including corosolic acid, ellagic acid, and various flavonoids.

Key findings include:

1. **Rich phytochemical profile:** The extract contains diverse bioactive compounds including phenolics, flavonoids, tannins, and triterpenes, with corosolic acid being a major constituent.
2. **Potent antimicrobial activity:** Significant activity against P. acnes (MIC: 0.312 mg/mL) and other acne-associated bacteria, demonstrating bactericidal effects.
3. **Strong antioxidant capacity:** Effective radical scavenging and metal chelating activities that can protect against oxidative damage in acne.
4. **Anti-inflammatory properties:** Substantial reduction in inflammatory responses, crucial for managing acne-associated inflammation.
5. **Multi-target approach:** The extract addresses multiple pathogenic factors in acne simultaneously, potentially offering superior therapeutic outcomes compared to single-target treatments.

The results support the traditional use of L. speciosa in skin disorders and provide scientific validation for its development as a natural anti-acne therapeutic. The combination of antimicrobial, anti-inflammatory, and antioxidant activities, along with the presence of well-characterized bioactive compounds, makes L. speciosa extract a promising candidate for modern acne management strategies.

However, clinical studies are essential to translate these promising in vitro findings into practical therapeutic applications. The development of standardized formulations and comprehensive safety evaluation will be crucial steps toward bringing this natural remedy to clinical practice.

This research contributes to the growing body of evidence supporting plant-based approaches to acne management and highlights the potential of L. speciosa as a valuable addition to the arsenal of natural anti-acne therapeutics.

ACKNOWLEDGMENTS

The authors acknowledge the support of the institutional research facilities and express gratitude to the botanical department for plant authentication services. Special thanks to the laboratory staff for their technical assistance throughout the study.

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