



FORMULATION AND EVALUATION OF THE ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF AN ALOE VERA HYDROGEL LOADED WITH LEMON BASIL (*OCIMUM CITRIODORUM*) EXTRACT

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ABSTRACT

Background: *Ocimum citriodorum* (lemon basil) is an aromatic medicinal plant containing phenolic, flavonoid, terpenoid and volatile constituents that may contribute to antioxidant and anti-inflammatory effects. The present study evaluated an *Ocimum citriodorum* leaf extract incorporated into a gel (LBET+GEL) using in vitro antioxidant and anti-inflammatory assays. **Methods:** The supplied LBET+GEL sample was evaluated for appearance, odour, consistency, pH, solubility and physical behaviour following centrifugation. Antioxidant activity was assessed by the DPPH radical-scavenging assay using ascorbic acid as the reference standard. Anti-inflammatory activity was evaluated by the egg-albumin denaturation method using diclofenac sodium as the reference standard. Percentage inhibition and IC₅₀ values were obtained from the reported concentration-response data. The gel was described as green, odourless and viscous, with a reported pH of 8 and stable physical characteristics. In the DPPH assay, mean inhibition values of 70.6%, 84.6% and 83.3% were reported at 5, 10 and 15 mg, respectively, with an IC₅₀ of 7.48 mg, compared with 3.373 mg for ascorbic acid. In the albumin-denaturation assay, mean inhibition values of 62.6%, 56.6% and 48.6% were reported at 5, 10 and 15 mg, respectively, with an IC₅₀ of 10.59 mg, compared with 12.129 mg for diclofenac sodium. LBET+GEL demonstrated appreciable in vitro antioxidant activity and measurable albumin-denaturation inhibitory activity. The findings support further phytochemical, formulation, stability and in vivo investigations to establish the pharmacological relevance and reproducibility of the preparation.

KEYWORDS: *Ocimum citriodorum*; lemon basil; herbal gel; DPPH; antioxidant; albumin denaturation; anti-inflammatory; IC₅₀.

1. INTRODUCTION

Herbal medicines represent an important component of traditional healthcare and continue to attract scientific interest because medicinal plants provide chemically diverse compounds with potential biological activity. Oxidative stress arises when the generation of reactive oxygen species and related reactive species exceeds the capacity of endogenous antioxidant systems. Reactive species can participate in normal cellular signalling at controlled concentrations, whereas excessive levels can

damage lipids, proteins and nucleic acids. Plant-derived phenolics, flavonoids, terpenoids, vitamins and related constituents are therefore frequently investigated as potential antioxidant agents.

Inflammation is a protective biological response to tissue injury, chemical insult or microbial challenge. Although necessary for repair, persistent or excessive inflammatory responses can contribute to tissue damage and chronic disease. Protein denaturation is one

experimental model used for preliminary screening of anti-inflammatory activity, and inhibition of heat-induced albumin denaturation provides an in vitro indication of protein-stabilizing activity.

Ocimum citriodorum, commonly known as lemon basil, belongs to the family Lamiaceae. The leaves contain volatile and non-volatile constituents including citral, linalool, geraniol, limonene, thymol and phenylpropanoid compounds.

Previous studies have reported antioxidant, antimicrobial, phytochemical and essential-oil-related activities for *O. citriodorum*. A 2020 study reported

relevant antioxidant and other bioactivities for lemon-basil extracts, while a separate study of micropropagated *O. citriodorum* reported radical-scavenging activity.

The present work therefore focuses on the in vitro antioxidant and anti-inflammatory properties of LBET+GEL, with emphasis on DPPH radical scavenging and inhibition of albumin denaturation.

Parameter	Details
Botanical name	<i>Ocimum citriodorum</i>
Common name	Lemon basil
Family	Lamiaceae
Plant type	Aromatic medicinal herb
Plant part used	Leaves
Major constituents	Citral, linalool, geraniol, limonene, thymol and phenylpropanoids
Major activities reported	Antioxidant, antimicrobial and potential anti-inflammatory activities
Main use in the present study	Leaf extract incorporated into a herbal gel



2. RATIONALE AND OBJECTIVES

2.1 Rationale

The biological effects of medicinal plants are influenced by plant identity, plant part, cultivation conditions, extraction method, chemical composition and formulation. The literature summarized in the source indicates substantial chemical variability among *Ocimum* species and chemotypes. In *O. citriodorum*, geraniol/neral-rich and methyl-chavicol-rich chemotypes have been described, emphasizing the importance of botanical and chemical standardization.

A gel dosage form can provide a convenient vehicle for botanical extracts and can facilitate topical or localized administration. However, a finished herbal gel should be characterized for basic physical properties and its biological activity should be demonstrated using reproducible analytical assays. The present study addresses these preliminary requirements through physical evaluation, DPPH radical-scavenging testing and albumin-denaturation testing.

2.2 AIM

To investigate the in vitro antioxidant and anti-inflammatory pharmacological properties of *Ocimum citriodorum* leaf extract incorporated into a gel.

2.3 Objectives

- ✚ To collect, identify and botanically authenticate the leaf material of *Ocimum citriodorum*.
- ✚ To evaluate the physical characteristics of the LBET+GEL preparation.
- ✚ To determine the in vitro antioxidant potential using the DPPH radical-scavenging assay.
- ✚ To assess anti-inflammatory potential using the egg-albumin denaturation assay.
- ✚ To compare the activity of LBET+GEL with appropriate reference standards and determine IC₅₀ values.

2.4 Study significance

The study provides preliminary experimental evidence for the biological activity of an *O. citriodorum*-based gel. The results may serve as a basis for subsequent

phytochemical standardization, formulation optimization, stability testing, toxicity assessment and in vivo pharmacological studies.

3. REVIEW OF LITERATURE

3.1 Phytochemical and biological profile of *Ocimum citriodorum*

The literature indicates that *O. citriodorum* has been investigated using hydroethanolic extracts, essential oils,

leaf and flower preparations, seed-derived materials and tissue-culture approaches. Reported areas of investigation include volatile composition, antioxidant activity, antimicrobial activity, essential-oil production, genetic diversity, micropropagation and potential anti-inflammatory activity.

Plant material	Preparation	Principal investigation	Reference in source
Whole plant	Hydroethanolic extract	Phytochemical and bioactive properties	Majdi et al., 2020
Leaves and flowers	Extract	Volatile composition	Al-Kateb & Mottram, 2014
Whole plant	Plant extract	Essential-oil chemistry and bioactivities	Pandey et al., 2014
Leaf explants	In vitro culture	Regeneration/micropropagation	Tripathi et al., 2014
Leaf explants	In vitro culture	Antioxidant activity	Santhi et al., 2020
Leaves	Hydroalcoholic extract	Phytochemical and antifungal potential	Guterres et al., 2022
Stalks	Stalk extract	Potential anti-inflammatory constituents	Kaliyaperumal et al., 2024

Essential-oil composition varies with geographical origin, growth stage, plant part and environmental conditions. Such variation can influence biological activity and highlights the importance of controlling raw-material quality.

3.2 Antioxidant activity of *O. citriodorum*

Santhi and co-workers reported radical-scavenging activity in *O. citriodorum* material, with the source higher activity for mother-plant and in vitro plant extracts than for in vitro callus extract. Majdi et al. reported relevant antioxidant activity in extracts of cinnamon basil and lemon basil, with rosmarinic acid described as a major phenolic compound and linalool as a predominant volatile constituent.

3.3 Anti-inflammatory relevance

Inflammation as a complex biological response involving vascular and cellular changes and identifies nitric oxide as one mediator associated with inflammatory processes. Plant-derived compounds are therefore investigated for their ability to modulate oxidative and inflammatory pathways.

Kaliyaperumal et al. investigated constituents of *O. basilicum citriodorum* and used chromatographic and computational approaches to explore potential anti-inflammatory effects. The source reports identification of multiple bioactive compounds and interactions with a protein target, providing a mechanistic rationale for continued investigation of lemon basil-derived preparations.

3.4 DPPH assay

DPPH is a stable nitrogen-centred radical commonly used for preliminary screening of radical-scavenging activity. Antioxidants donate hydrogen atoms or electrons to reduce DPPH, producing a decrease in the characteristic purple colour. In the present study, a methanolic DPPH solution was used and absorbance was measured at 520 nm.

3.5 Albumin denaturation assay

The albumin-denaturation assay evaluates the capacity of a test sample to protect protein structure under heat-induced denaturing conditions. The present assay used bovine albumin, controlled pH and temperature conditions, and spectrophotometric measurement at 660 nm, with diclofenac sodium as the standard.

4. MATERIALS AND METHODS

4.1 Sample collection

The LBET+GEL sample investigated in the study was obtained from Yazhini Biotech Laboratory, Srivilliputhur, Tamil Nadu, India.

4.2 Preliminary physical evaluation

The gel was examined for colour, odour, consistency, ease of application/spreadability, pH, solubility and behaviour following centrifugation. The preparation was green, odourless and viscous, with a pH of 8, stable physical characteristics, dissolution in the specified solvent and physical separation following centrifugation. The pH of the LBET+GEL preparation was determined during physical evaluation and was recorded as 8. The remaining physical characteristics were assessed under the same experimental conditions.

4.3 DPPH radical-scavenging assay

A DPPH solution at 6×10^{-5} M in methanol was prepared according to the supplied method. Test solutions were evaluated in triplicate. Equal volumes of test solution and DPPH solution were mixed, incubated at room temperature for 30 min and measured spectrophotometrically at 520 nm. Ascorbic acid was used as the positive control and distilled water as the negative control.

The percentage inhibition was calculated after correction for the sample blank using: % inhibition = $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$. IC₅₀ values were determined from concentration-response data using nonlinear regression in GraphPad Prism 9, as reported.

4.4 Egg-albumin denaturation assay

The anti-inflammatory activity was assessed by the egg-albumin/bovine-albumin denaturation method. The supplied procedure describes preparation of the reaction mixture with 5% bovine albumin, adjustment to approximately pH 6.3, incubation at 37 °C for 20 min followed by heating at 57 °C for 30 min, cooling and transfer to a 96-well plate. Absorbance was measured at 660 nm. Diclofenac sodium at 1000 µg/mL was used as the standard and distilled water as the control.

Percentage inhibition of albumin denaturation was calculated as: % inhibition = $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$. The assay is a preliminary in

vitro model and should not be interpreted as direct evidence of clinical anti-inflammatory efficacy.

4.5 Experimental design and data handling

The LBET+GEL concentrations evaluated in the DPPH assay were 5, 10 and 15 mg. Three observations were recorded at each concentration. Mean values and dispersion were calculated from the replicate observations. The same concentration range was used for the albumin-denaturation assay.

Assay	Test concentrations	Replicates	Reference standard	Detection wavelength
DPPH radical scavenging	5, 10, 15 mg	3	Ascorbic acid	520 nm
Albumin denaturation	5, 10, 15 mg	3	Diclofenac sodium	660 nm

4.6 Ethical and experimental considerations

The present work is based on in vitro analytical assays and did not involve human clinical intervention or animal experimentation.

5. RESULTS

5.1 Physical evaluation of LBET+GEL.

Parameter	Reported observation
Colour	Green
Odour	Odourless
Consistency	Viscous, gel-like
pH	8 (reported in abstract)
Solubility	Dissolved in the specified solvent
Centrifugation behaviour	Physical separation observed
Overall physical stability	Reported as stable

The physical evaluation indicated a green, odourless and viscous gel preparation with a pH of 8. The preparation dissolved in the specified solvent and showed physical separation following centrifugation.

inhibition values of 70.6%, 84.6% and 83.3% at 5, 10 and 15 mg, respectively. The corresponding reported IC₅₀ was 7.48 mg. Ascorbic acid showed a reported IC₅₀ of 3.373 mg.

5.2 DPPH radical-scavenging activity

LBET+GEL showed concentration-dependent antioxidant activity across the tested range, with mean

Concentration (mg)	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Mean (%)
5	68	72	72	70.6 ± 2.30
10	86	82	86	84.6 ± 2.30
15	82	82	86	83.3 ± 2.30

5.3 DPPH assay: comparison with standard.

Sample	Concentration (mg)	Mean inhibition (%)	IC ₅₀ (mg)
LBET+GEL	5	71	7.48
LBET+GEL	10	85	7.48
LBET+GEL	15	83	7.48
Ascorbic acid	5	91	3.373
Ascorbic acid	10	87	3.373
Ascorbic acid	15	86	3.373
Ascorbic acid	20	92	3.373
Ascorbic acid	25	84	3.373

Figure 1. DPPH radical-scavenging activity of LBET+GEL at different concentrations.

LBET+GEL showed the highest mean inhibition at 10 mg (84.6%), while the 15 mg concentration produced a slightly lower mean inhibition (83.3%). This pattern indicates a high radical-scavenging response across the tested range without a strictly monotonic concentration-response relationship.

5.4 Anti-inflammatory activity by albumin denaturation

LBET+GEL inhibited albumin denaturation at all tested concentrations. The reported mean inhibition values were

Concentration (mg)	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Mean (%)
5	64	60	64	62.6 ± 2.30
10	53	57	60	56.6 ± 3.51
15	46	50	50	48.6 ± 2.30

Figure 2. In vitro albumin-denaturation inhibitory activity of LBET+GEL.

The greatest albumin-denaturation inhibition was observed at 5 mg, followed by lower mean inhibition at

62.6%, 56.6% and 48.6% at 5, 10 and 15 mg, respectively, with an IC₅₀ of 10.59 mg. Diclofenac sodium showed a mean inhibition of 68.3% at the tested standard concentration and a reported IC₅₀ of 12.129 mg.

10 and 15 mg. The observed pattern is assay-specific and indicates that the response did not increase proportionally with concentration across the tested range.

5.5 Anti-inflammatory activity: comparison with diclofenac sodium.

Sample	Concentration (mg)	Mean inhibition (%)	IC ₅₀ (mg)
LBET+GEL	5	63	10.59
LBET+GEL	10	57	10.59
LBET+GEL	15	49	10.59
Diclofenac sodium	5	91	12.129
Diclofenac sodium	10	93	12.129
Diclofenac sodium	15	85	12.129
Diclofenac sodium	20	89	12.129
Diclofenac sodium	25	88	12.129

Figure 3. Comparison of reported DPPH IC₅₀ values.

Figure 4. Comparison of reported albumin-denaturation IC₅₀ values.

6. DISCUSSION

The present study provides preliminary evidence that LBET+GEL possesses both radical-scavenging and protein-stabilizing activity in vitro. The antioxidant results are consistent with the phytochemical background summarized, where *O. citriodorum* is described as containing several volatile and non-volatile compounds of potential biological interest. Terpenoids such as citral, linalool, geraniol, limonene and thymol, together with phenylpropanoid constituents, have been reported in the literature reviewed.

The DPPH results showed high inhibition at 5–15 mg, with the highest reported mean inhibition at 10 mg (84.6%). The IC₅₀ of 7.48 mg was higher than that of ascorbic acid (3.373 mg), indicating that the test preparation was less potent than the reference antioxidant on a concentration basis. Nevertheless, the observed radical-scavenging activity supports the presence of constituents capable of donating hydrogen atoms or electrons to DPPH.

The activity of *O. citriodorum* may plausibly relate to its phenolic and volatile constituents, but the present data do not establish which compound or combination of compounds is responsible. Chemical characterization by chromatographic methods and correlation of marker-compound concentrations with biological activity would be necessary to establish such relationships.

In the albumin-denaturation assay, LBET+GEL produced 62.6% inhibition at 5 mg, decreasing to 48.6% at 15 mg. The reported IC₅₀ of 10.59 mg was numerically lower than the diclofenac sodium IC₅₀ of 12.129 mg. However, the raw inhibition values of diclofenac sodium were higher than those of LBET+GEL at the listed concentrations. Therefore, the IC₅₀ comparison alone should not be interpreted as evidence that LBET+GEL is more clinically effective than diclofenac sodium.

The physical evaluation indicated a green, odourless and viscous preparation. Such basic characteristics are useful for preliminary quality assessment of a herbal gel.

However, a publication-grade formulation study should additionally report quantitative viscosity, spreadability, homogeneity, microbial quality, drug/extract content uniformity, rheological behaviour, accelerated stability and packaging compatibility.

The abstract reports 51% DPPH inhibition at 5 mg with an IC₅₀ of 10.00 mg and 33% anti-inflammatory inhibition at 5 mg with an IC₅₀ of 3.28 mg, whereas the detailed result tables report 71% DPPH inhibition at 5 mg, an IC₅₀ of 7.48 mg, 63% anti-inflammatory inhibition at 5 mg and an IC₅₀ of 10.59 mg.

The numerical tables and detailed Results section provide replicate-level observations and corresponding mean values for the biological assays. The DPPH and albumin-denaturation findings were therefore interpreted using these experimental observations.

The literature also indicates substantial variation in lemon-basil essential-oil composition according to growth stage and environmental conditions. Al-Kateb and Mottram reported marked changes in citral and linalool across growth stages, while other work has demonstrated differences in essential-oil chemotypes and composition among *Ocimum* taxa. Such variability may contribute to differences between preparations and reinforces the need for botanical authentication and chemical standardization..

6.1 Strengths

- Both antioxidant and anti-inflammatory screening models were employed.
- Reference standards were included for comparative assessment.
- Triplicate observations were reported for the tested concentrations.
- The study connects the biological evaluation with published information on *O. citriodorum*.

6.2 Limitations

- The study did not include quantitative phytochemical marker analysis.
- The in vitro assays do not establish clinical efficacy or safety.
- Mechanistic endpoints such as intracellular reactive oxygen species, protein expression and apoptosis were not evaluated.
- Further formulation, stability and in vivo investigations are required to establish reproducibility and pharmacological relevance.

7. CONCLUSION AND FUTURE PERSPECTIVES

7.1 CONCLUSION

The present in vitro investigation demonstrated that the *Ocimum citriodorum* leaf extract gel (LBET+GEL) possesses appreciable antioxidant activity in the DPPH radical-scavenging assay and measurable anti-inflammatory potential in the albumin-denaturation model. The reported DPPH mean inhibition values were

70.6%, 84.6% and 83.3% at 5, 10 and 15 mg, respectively, with an IC₅₀ of 7.48 mg. The corresponding anti-inflammatory mean inhibition values were 62.6%, 56.6% and 48.6%, with an IC₅₀ of 10.59 mg.

The findings are broadly consistent with the published literature summarized in the source, which documents antioxidant activity and diverse phytochemical constituents in *O. citriodorum*. The preparation therefore merits further investigation as a botanical formulation, particularly through standardized phytochemical characterization and more comprehensive pharmacological evaluation.

7.2 Future perspectives

- Confirm botanical identity using authenticated voucher specimens.
- Quantify total phenolic and flavonoid contents and establish chemical marker profiles.
- Characterize major constituents using HPTLC, HPLC, GC-MS or related validated methods.
- Optimize gel composition and determine quantitative viscosity, spreadability and homogeneity.
- Conduct accelerated and long-term stability studies with microbial quality assessment.
- Expand antioxidant testing using complementary assays such as FRAP and ABTS.
- Investigate cellular and in vivo anti-inflammatory activity after appropriate safety assessment.
- Perform validated statistical analysis using replicate-level data and predefined significance criteria.

Declarations

Ethics approval: Not applicable to the in vitro assays conducted in this study.

Consent for publication: Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest related to this study.

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