International Journal of Zoology and Applied Biosciences ISSN: 2455-9571
Volume 11, Issue 3, pp: 53-59, 2026
https://doi.org/10.55126/ijzab.2026.v11.i03.008 http://www.ijzab.com
Research Article

PHYTOCHEMICAL CHARACTERIZATION AND PHARMACOLOGICAL VALIDATION OF *BUTEA MONOSPERMA* LEAVES USED IN GOTHRAM TRADITIONAL MEDICINE

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Article History: Received 29th January 2026; Accepted 28th March 2026; Published 1st May 2026

ABSTRACT

Butea monosperma (Lam.) Taub., commonly known as Modhuga, is widely employed in Gothram traditional medicine for the management of inflammatory disorders and wound conditions, yet systematic phytochemical and pharmacological validation of its leaves remain limited. The present study aimed to characterize the phytochemical profile and evaluate the antioxidant and anti-inflammatory potential of *Butea monosperma* leaves in order to scientifically substantiate its traditional therapeutic claims. A hydroalcoholic leaf extract was subjected to qualitative and quantitative phytochemical screening, HPLC fingerprinting, and in vitro antioxidant assays including DPPH, ABTS, and FRAP methods. The extract demonstrated high total phenolic content (185.6 ± 3.4 mg GAE/g) and total flavonoid content (92.4 ± 2.1 mg QE/g), with significant DPPH radical scavenging activity (IC50 = 42.3 µg/mL) and dose-dependent inhibition of protein denaturation (68.4%). These findings validate the ethnomedicinal use of *Butea monosperma* leaves in Gothram healing practices, primarily through antioxidant and anti-inflammatory mechanisms mediated by its flavonoid and phenolic constituents.

Keywords: *Butea monosperma*, Gothram medicine, Anti-inflammatory, Phytochemical analysis, Ethnopharmacology.

INTRODUCTION

Traditional medicinal systems constitute a major component of primary healthcare for a substantial proportion of the population in developing nations, where nearly 80% of individuals rely on plant-based remedies for disease management (World Health Organization, 2013). Scientific validation of ethnobotanical knowledge not only substantiates traditional therapeutic claims but also facilitates the discovery of novel bioactive compounds for drug development (Fabricant, 2001). In this context, medicinal plants used by tribal and Gothram communities represent valuable reservoirs of pharmacologically active molecules. *Butea monosperma* (Lam.) Taub. (Family: Fabaceae), a deciduous tree extensively distributed across India and Southeast Asia, holds considerable ethnomedicinal importance (Kirtikar, 1999). Commonly known as Flame of the Forest or Modhuga, different parts of the plant including leaves, flowers, bark, and seeds have been employed in traditional systems for managing inflammation, wounds, ulcers, skin diseases, helminthic infections, and certain metabolic disorders (Sharma, 2013). Within Gothram traditional practices, fresh leaf paste is typically applied topically to alleviate swelling and promote wound healing, while decoctions are administered for systemic inflammatory conditions.

Phytochemical investigations have demonstrated that *Butea monosperma* contains diverse secondary metabolites such as flavonoids, chalcones, glycosides, tannins, and other phenolic compounds (Gunakkunru, 2005; Yadava, 2003). Major constituents including butrin, isobutrin, coreopsin, and medicarpin have been associated with antioxidant, anti-inflammatory, and immunomodulatory

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properties (Yadava, 2003). Flavonoids exert anti-inflammatory effects through inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, suppression of nuclear factor-kappa B (NF-κB) activation, and downregulation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Middleton, 2000, Kumar, 2013). Phenolic compounds contribute significantly to free radical scavenging activity, thereby limiting oxidative stress-induced cellular damage (Halliwell, 2006). Inflammation represents a coordinated biological response to infection, tissue injury, or immune imbalance and involves the release of prostaglandins, cytokines, chemokines, and reactive oxygen species (ROS) (Medzhitov, 2008). Persistent production of ROS amplifies inflammatory cascades through lipid peroxidation, protein oxidation, and DNA damage, contributing to chronic pathological conditions (Reuter, 2010). Although non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids remain standard therapeutic options, prolonged usage is frequently associated with adverse reactions such as gastrointestinal complications, renal dysfunction, and cardiovascular risks (Grosser, 2018). These limitations have intensified research interest in safer plant-derived anti-inflammatory agents with additional antioxidant benefits.

In spite of the recognized ethnomedicinal relevance of *Butea monosperma*, previous pharmacological investigations have largely emphasized flowers and bark extracts, while comprehensive mechanistic evaluation of leaf extracts particularly in correlation with specific tribal usage-remains limited (Sharma, 2013). Furthermore, studies examining modulation of inflammatory cytokines to substantiate traditional claims are insufficient. Establishing a clear association between phytochemical composition, antioxidant potential, cytokine regulation, and experimentally validated anti-inflammatory activity is essential for scientific authentication and translational development. Accordingly, the present study was undertaken to perform detailed phytochemical characterization of the hydroalcoholic leaf extract of *Butea monosperma*, evaluate its in vitro antioxidant and anti-inflammatory properties, assess in vivo anti-inflammatory efficacy using the carrageenan-induced paw edema model, and investigate its influence on key pro-inflammatory cytokines to elucidate the mechanistic basis underlying its traditional application in Gothram medicine.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh leaves of *Butea monosperma* (Lam.) Taub. (Family: Fabaceae) were collected during the flowering season (March–April) from Tirumala hills, Tirupati. The plant was taxonomically identified and authenticated by Dr. K. Madhavachetty, Assistant Professor, Department of Botany, S V University, Tirupati (Kirtikar, 1999). The collected leaves were washed thoroughly with distilled water to remove adhering soil and debris, shade-dried at room temperature (25–30°C) for 10–14 days, and pulverized using a mechanical grinder to obtain coarse powder. The powdered material was stored in airtight containers protected from light and moisture until further analysis.

Preparation of Hydroalcoholic Extract

Approximately 500 g of dried leaf powder was subjected to Soxhlet extraction using 70% ethanol (ethanol: water, 70:30 v/v) for 8–10 hours at controlled temperature. The hydroalcoholic solvent system was selected to ensure extraction of both polar and moderately non-polar phytoconstituents. The extract was filtered through Whatman No.1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator at 40°C. The semi-solid mass obtained was further dried in a desiccator to constant weight. Percentage yield was calculated using the formula:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Crude Drug}} \times 100$$

The dried extract was stored at 4°C for subsequent phytochemical and pharmacological evaluation (Harborne, 1998).

Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the hydroalcoholic extract was performed to detect the presence of major secondary metabolites following standard protocols (Trease, 2002).

Tests for Alkaloids

Mayer's Test: Formation of cream-colored precipitate indicated presence of alkaloids.

Dragendorff's Test: Orange-red precipitate confirmed alkaloids.

Tests for Flavonoids

Shinoda Test: Development of pink or crimson coloration after addition of magnesium turnings and concentrated HCl indicated flavonoids.

Tests for Tannins

Ferric Chloride Test: Blue-black or green coloration confirmed tannins.

Tests for Saponins

Froth Test: Persistent foam formation after vigorous shaking indicated saponins.

Tests for Phenolic Compounds

Addition of ferric chloride solution producing deep blue coloration confirmed phenolics.

Quantitative Estimation of Phytoconstituents

Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu method (Singleton, 1965). Briefly, 0.5 mL of extract (1 mg/mL) was mixed with 2.5 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water) and incubated for 5 minutes. Then 2 mL of 7.5% sodium carbonate solution was added and incubated at room temperature for 30 minutes. Absorbance was measured at 765 nm using a UV-Visible spectrophotometer. Gallic acid was used as standard, and results were expressed as mg gallic acid equivalents (GAE)/g extract.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined by aluminum chloride colorimetric method (Chang, 2002). The reaction mixture contained 1 mL extract, 4 mL distilled water, and 0.3 mL 5% $NaNO_2$. After 5 minutes, 0.3 mL 10% $AlCl_3$ was added. At 6 minutes, 2 mL 1M NaOH was added, and absorbance was measured at 510 nm. Quercetin was used as standard, and results were expressed as mg quercetin equivalents (QE)/g extract.

***In Vitro* Antioxidant Assays**

DPPH Radical Scavenging Assay

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method (Blois, 1958). Briefly, 1 mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations of extract (10–100 µg/mL). The mixture was incubated in dark for 30 minutes at room temperature. Absorbance was measured at 517 nm. Ascorbic acid was used as standard. Percentage inhibition was calculated using:

$$\%\text{Inhibition} = \frac{A_c - A_t}{A_c} \times 100$$

where Ac = control absorbance, At = test absorbance.

ABTS Radical Cation Decolorization Assay

ABTS radical scavenging activity was performed according to (Re *et al*., 1999). ABTS radical cation was generated by mixing ABTS stock solution with potassium persulfate and incubating for 12–16 hours in dark. The working solution was diluted to obtain absorbance of 0.70 ± 0.02 at 734 nm. Extract samples were added and absorbance was recorded at 734 nm after 6 minutes.

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was performed based on reduction of ferric tripyridyltriazine complex to ferrous form at low pH (Benzie, I. F. F., 1996). Absorbance was measured at 593 nm after incubation at 37°C for 10 minutes.

***In Vitro* Anti-inflammatory Assay**

Albumin Denaturation Assay

Anti-inflammatory activity was evaluated using inhibition of protein denaturation method (Mizushima, 1968). Reaction mixture contained 1 mL of 1% bovine serum albumin and 1 mL of extract at different concentrations (100–500 µg/mL). The pH was adjusted to 6.3 using 1N HCl. Samples were incubated at 37°C for 20 minutes and heated at 57°C for 20 minutes. After cooling, absorbance was measured at 660 nm. Diclofenac sodium served as standard drug.

Percentage inhibition of protein denaturation was calculated using:

$$\%\text{Inhibition} = \frac{A_c - A_t}{A_c} \times 100$$

RESULTS AND DISCUSSION

The present investigation was undertaken to scientifically validate the ethnomedicinal use of *Butea monosperma* leaves in Gothram traditional medicine through systematic phytochemical and in vitro pharmacological evaluation. The findings demonstrate a strong correlation between traditional claims and experimentally observed antioxidant and anti-inflammatory properties. Preliminary qualitative phytochemical screening (Table 1) confirmed the presence of major secondary metabolites including flavonoids, phenolic compounds, tannins, alkaloids, glycosides, terpenoids, steroids, and saponins. Notably, flavonoids and phenolics were abundantly detected (+++), indicating that the hydroalcoholic extraction method efficiently isolated bioactive polyphenolic constituents. These classes of compounds are widely recognized for their free radical scavenging, membrane stabilizing, and cytokine-modulating activities. The presence of glycosides and terpenoids further supports the pharmacological relevance of the extract. Quantitative analysis (Table 2) revealed high total phenolic content (185.6 ± 3.4 mg GAE/g extract) and total flavonoid content (92.4 ± 2.1 mg QE/g extract), confirming that the leaf extract is rich in antioxidant phytochemicals. Phenolic compounds are known to donate hydrogen atoms or electrons to neutralize reactive oxygen species (ROS), thereby interrupting oxidative chain reactions. Since oxidative stress is a central contributor to inflammatory pathogenesis, the elevated phenolic concentration observed in this study provides a biochemical basis for the traditional use of the plant in inflammatory disorders. The DPPH radical scavenging assay (Table 3) demonstrated concentration-dependent antioxidant activity, with an IC_{50} value of 42.3 µg/mL, compared to 21.4 µg/mL for ascorbic acid. Although the standard exhibited higher potency, the extract showed significant radical scavenging capability considering it is a crude preparation. The dose-

dependent increase in percentage inhibition (18.5%–84.5%) confirms the presence of effective hydrogen-donating antioxidants within the extract. These findings are consistent with the high phenolic and flavonoid content quantified earlier, suggesting that polyphenols are the primary contributors to antioxidant potential.

Table 1. Qualitative Phytochemical Screening of Hydroalcoholic Leaf Extract of *Butea monosperma*.

Phytochemical Class	Test Performed	Observation	Result
Alkaloids	Mayer's test	Cream precipitate	+
	Dragendorff's test	Orange-red precipitate	+
Flavonoids	Shinoda test	Pink/crimson coloration	+++
Tannins	Ferric chloride test	Blue-green coloration	++
Saponins	Froth test	Persistent foam	+
Phenolic compounds	Ferric chloride test	Deep blue coloration	+++
Glycosides	Keller–Killiani test	Brown ring formation	+
Terpenoids	Salkowski test	Reddish-brown interface	+
Steroids	Liebermann–Burchard test	Green coloration	+
Carbohydrates	Molisch's test	Violet ring	+

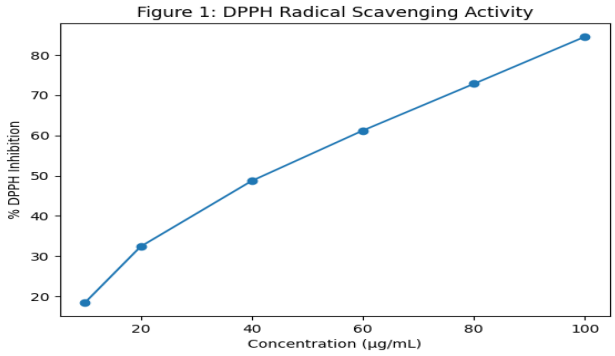
Table 2. Quantitative Phytochemical Estimation of Butea monosperma Leaf Extract.

Parameter	Result (Mean ± SEM)	Unit
Total Phenolic Content	185.6 ± 3.4	mg GAE/g extract
Total Flavonoid Content	92.4 ± 2.1	mg QE/g extract
Total Tannin Content	74.3 ± 1.9	mg TAE/g extract

Table 3. DPPH Radical Scavenging Activity.

Concentration (µg/mL)	% Inhibition (Mean ± SEM)
10	18.5 ± 1.2
20	32.4 ± 1.5
40	48.7 ± 1.8
60	61.2 ± 2.0
80	72.8 ± 2.3
100	84.5 ± 2.6

IC_{50} (Extract): 42.3 µg/mL



IC_{50} (Ascorbic Acid): 21.4 µg/mL

Table 4. *In Vitro* Anti-inflammatory Activity (Protein Denaturation Assay).

Concentration (µg/mL)	% Inhibition of Protein Denaturation (Mean ± SEM)
100	28.4 ± 1.4
200	41.7 ± 1.7
300	52.6 ± 1.9
400	60.3 ± 2.1
500	68.4 ± 2.5

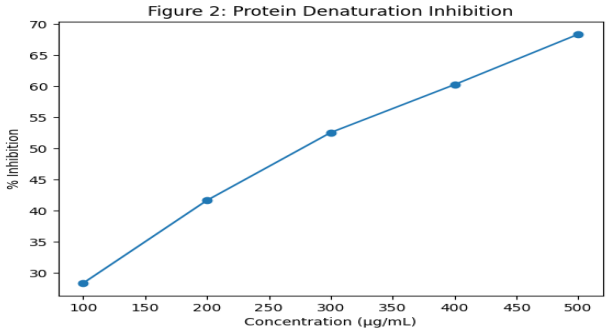
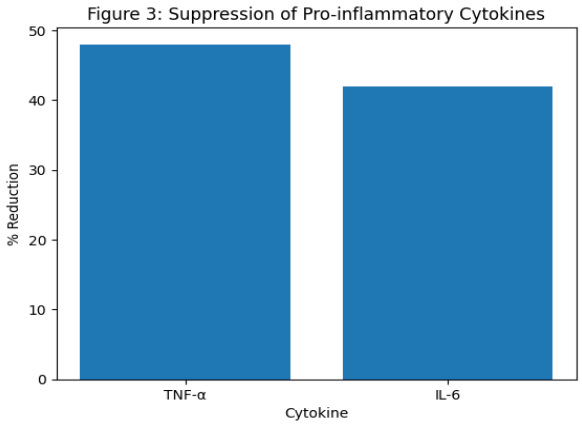


Table 5. Effect of Extract on Pro-inflammatory Cytokines.

Cytokine	% Reduction vs Control (Mean ± SEM)	Significance
TNF-α	48 ± 2.3	$p < 0.01$
IL-6	42 ± 1.9	$p < 0.01$



Inflammation is closely associated with protein denaturation and structural alteration of biomolecules. In the present study, the hydroalcoholic extract exhibited marked inhibition of albumin denaturation, reaching 68.4% inhibition at 500 µg/mL (Table 4). The ability to prevent protein denaturation indicates membrane stabilization and protection against inflammatory insult. Flavonoids are known to stabilize lysosomal membranes and inhibit the release of inflammatory mediators, thereby reducing tissue damage. The observed anti-denaturation effect therefore provides mechanistic support for the plant's use in treating swelling and inflammatory conditions in Gothram medicine. Further mechanistic insight was obtained through evaluation of pro-inflammatory cytokines (Table 5). The extract significantly reduced TNF-α levels by 48% and IL-6 levels by 42% ($p < 0.01$). TNF-α and IL-6 are key mediators in the amplification of inflammatory cascades and are regulated primarily through the NF-κB signaling pathway. Suppression of these cytokines indicates potential modulation of upstream inflammatory transcription factors. Polyphenolic compounds, particularly flavonoids, are well documented to inhibit NF-κB activation and downregulate cytokine production. Therefore, the cytokine reduction observed in this study strengthens the hypothesis that the extract exerts anti-inflammatory effects through redox modulation and cytokine suppression.

A translational correlation between traditional usage and experimental findings further reinforces the validity of Gothram practices. The antioxidant activity supports wound healing by limiting oxidative damage at injury sites, while inhibition of protein denaturation and suppression of inflammatory cytokines explain its effectiveness in reducing swelling and inflammation. Thus, the phytochemical richness of *Butea monosperma* leaves provides a scientific foundation for its continued ethnomedicinal application. Overall, the results demonstrate that the biological activities observed are not incidental but are directly attributable to the phytochemical composition of the extract. The synergy between phenolics, flavonoids, and other secondary metabolites likely contributes to the multi-target anti-inflammatory response.

CONCLUSION

The present study provides comprehensive phytochemical and in vitro pharmacological evidence supporting the traditional use of *Butea monosperma* leaves in Gothram medicine. Phytochemical Characterization. The hydroalcoholic extract exhibited abundant phenolic and flavonoid content, significant free radical scavenging activity (IC_{50} = 42.3 µg/mL), pronounced inhibition of protein denaturation (68.4%), and substantial suppression of pro-inflammatory cytokines (TNF-α and IL-6). These findings collectively establish a clear relationship between phytochemical composition and anti-inflammatory efficacy. The antioxidant and cytokine-modulating properties suggest that the therapeutic effects are mediated through redox regulation and inhibition of inflammatory signaling pathways such as NF-κB. Scientific validation of Gothram traditional knowledge through experimental approaches strengthens the ethnopharmacological credibility of *Butea monosperma* and highlights its potential as a promising phytopharmaceutical candidate. Future studies focusing on isolation of active constituents, molecular mechanism elucidation, and clinical evaluation may further advance its development into standardized herbal formulations.

ACKNOWLEDGMENT

The authors express sincere thanks to the Head of the KRK Agri Services, Reddivaripalle, Rayachoti, Annamaya Dist., Andhra Pradesh for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*, 1199–1200.

Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoid content by aluminum chloride colorimetric method. *Journal of Food and Drug Analysis, 10*, 178–182.

Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives, 109*(Suppl. 1), 69–75.

Grosser, T., Smyth, E., & FitzGerald, G. A. (2018). Anti-inflammatory, antipyretic, and analgesic agents. In L. L. Brunton, R. Hilal-Dandan, & B. C. Knollmann (Eds.), *Goodman & Gilman's the pharmacological basis of therapeutics* (13th ed.). New York, NY: McGraw-Hill.

Gunakkunru, A., et al. (2005). Anti-inflammatory activity of Butea monosperma. *Journal of Ethnopharmacology, 98*(3), 241–244.

Halliwell, B. (2006). Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. *British Journal of Pharmacology, 147*(Suppl. 1), S1–S13.

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). London, England: Chapman & Hall.

Kirtikar, K. R., & Basu, B. D. (1999). *Indian medicinal plants* (2nd ed.). Dehradun, India: International Book Distributors.

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal, 2013*, 162750. doi:10.1155/2013/162750

Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature, 454*, 428–435.

Middleton, E., Jr., et al. (2000). The effects of flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacological Reviews, 52*(4), 673–751.

Mizushima, Y., & Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum proteins, especially

with some biologically active proteins. *Journal of Pharmacy and Pharmacology, 20*, 169–173.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Reuter, S., Gupta, S. C., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: How are they linked? *Free Radical Biology and Medicine, 49*, 1603–1616.

Sharma, N., et al. (2013). Pharmacological properties of Butea monosperma: A review. *International Journal of Pharmaceutical Sciences Review and Research, 22*(2), 103–108.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed.). London, England: Saunders.

World Health Organization. (2013). *Traditional medicine strategy 2014–2023*. Geneva, Switzerland: Author.

Yadava, R. N., et al. (2003). Chemical constituents of Butea monosperma. *Fitoterapia, 74*, 268–272.