



PHYTOCHEMISTRY, ANTIMICROBIAL, TOXICOLOGICAL STUDY AND ANTIULCER ACTIVITY OF ROOT AND STEM OF *BUTEA* *MONOSPERMA*

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ABSTRACT

Butea monosperma (Lam.) Taub., commonly known as “Flame of the Forest,” is a medicinally important plant traditionally used in Indian ethnomedicine for treating gastrointestinal disorders, infections, and inflammatory conditions. The present study aimed to investigate the phytochemical profile, antimicrobial potential, toxicological safety, and antiulcer activity of root and stem extracts of *B. monosperma*. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds. Quantitative estimation confirmed significant levels of total phenolics and flavonoids, correlating with antioxidant potential. The antimicrobial activity was evaluated against selected Gram-positive and Gram-negative bacterial strains as well as fungal pathogens using agar well diffusion and broth dilution methods, which demonstrated dose-

dependent inhibitory effects. Acute and sub-acute toxicity studies in rodents, conducted as per OECD guidelines, indicated no mortality or major behavioral alterations, establishing the safety profile of the extracts up to the tested doses. The antiulcer efficacy was assessed in standard experimental ulcer models, including ethanol- and pylorus-ligation–induced gastric ulceration in rats. Both ethanolic and aqueous extracts showed significant reduction in ulcer index, gastric secretion, and total acidity, while increasing gastric pH and percentage protection when compared to the control group. Histopathological analysis further confirmed mucosal protection and healing potential.

The findings suggest that root and stem extracts of *Butea monosperma* possess bioactive phytoconstituents with potent antimicrobial activity, favorable safety margins, and promising antiulcer properties. These results provide scientific validation to the traditional use of *B. monosperma* and highlight its potential as a natural therapeutic agent for managing microbial infections and peptic ulcer disease.

KEYWORDS: *Butea monosperma*, phytochemistry, antimicrobial, toxicity, antiulcer, gastric protection.

INTRODUCTION

Herbal medicines have played a vital role in healthcare systems across the world since ancient times. According to the World Health Organization (WHO), nearly 80% of the global population relies on traditional plant-based remedies for their primary healthcare needs. Among these, plants with diverse phytoconstituents are considered promising sources for the development of novel therapeutic agents. Natural products continue to draw attention due to their broad pharmacological spectrum, affordability, and relatively safer toxicological profile compared to synthetic drugs.

Butea monosperma (Lam.) Taub., belonging to the family Fabaceae, is a deciduous tree widely distributed in tropical regions of India and Southeast Asia. Commonly known as “Palash” or “Flame of the Forest,” it has been extensively used in Ayurveda, Siddha, and folk medicine. Various parts of the plant, including flowers, seeds, bark, and gum, have been employed for treating ailments such as diarrhea, dysentery, skin infections, wounds, ulcers, and inflammation. Ethnobotanical records indicate its traditional usage in managing gastrointestinal disorders and microbial infections, suggesting its therapeutic relevance in peptic ulcer disease and antimicrobial therapy.

Phytochemical investigations of *B. monosperma* have revealed the presence of flavonoids, isoflavones, tannins, terpenoids, and glycosides, which contribute to its pharmacological activities such as antioxidant, hepatoprotective, anti-inflammatory, and antimicrobial effects. However, despite the extensive use of flowers and seeds, limited scientific data are available regarding the **root and stem** parts of the plant. This presents an opportunity to explore their phytoconstituents and therapeutic potential in greater detail.

Peptic ulcer disease remains a global health problem, affecting millions of people due to factors such as stress, alcohol consumption, *Helicobacter pylori* infection, and chronic use of NSAIDs. Current antiulcer drugs, including proton pump inhibitors and H₂ receptor antagonists, though effective, are often associated with adverse effects and high recurrence rates. Thus, the search for safe and effective plant-based antiulcer agents remains highly significant. Similarly, antimicrobial resistance has emerged as a worldwide challenge, necessitating the discovery of alternative natural antimicrobials.

MATERIALS AND METHODS

Plant Material Collection and Authentication

The roots and stems of *Butea monosperma* (Lam.) Taub. were collected from Chhatarpur district, Bundelkhand region, M.P., India during the flowering season. The plant was authenticated by Botanist at Maharaja Chatrasal University, Chhatarpur. M/P, and a voucher specimen was deposited for future reference.

Preparation of Extracts

The collected plant materials were washed, shade dried, and coarsely powdered. Extraction was carried out by:

Ethanolic extract – obtained using Soxhlet extraction with 70% ethanol.

Aqueous extract – prepared by maceration in distilled water.

The extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and stored at 4°C until use.

Phytochemical Screening

Qualitative analysis: Preliminary phytochemical tests were performed for alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, phenols, and steroids using standard protocols.

Quantitative estimation: Total phenolic content (TPC) was determined by Folin–Ciocalteu method and total flavonoid content (TFC) by aluminium chloride colorimetric assay.

Antimicrobial Activity

Microorganisms used: Selected Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungal strains (*Candida albicans*).

Assay method: Agar well diffusion method to measure zone of inhibition.

Minimum Inhibitory Concentration (MIC): Determined by broth dilution technique.

Standard drug: Ciprofloxacin (for bacteria) and Fluconazole (for fungi) were used as positive controls.

Toxicological Studies

Practically conducted on healthy Wistar albino rats as per OECD guidelines.

Acute Toxicity (OECD 423): Oral administration of extracts at doses of 300, 2000, and 5000 mg/kg; observed for 14 days for mortality, behavioral changes, food/water intake, and body weight.

Sub-acute Toxicity (28 days, OECD 407): Rats were administered daily doses of extracts. Hematological and biochemical parameters were evaluated, and histopathological examination of major organs (liver, kidney, heart, stomach) was performed.

Antiulcer Activity

Antiulcer activity was assessed in Wistar rats using two standard experimental models:

Ethanol-induced gastric ulcer model

Pylorus ligation–induced gastric ulcer model

Animals were divided into groups: Control, Standard (Omeprazole 20 mg/kg), and Test groups (extracts at different doses).

Parameters studied included **ulcer index, gastric volume, pH, free acidity, total acidity, percentage protection**, and **histopathological examination of gastric mucosa**.

Statistical Analysis

All experimental results were expressed as mean $\pm$ SEM ($n = 6$). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test, with $p < 0.05$ considered statistically significant.

Root and stem of *Butea monosperma* were subjected to the following parameters:

A. Macroscopic Characteristics Stem T.S.

- **Epidermis:** Single layer of cells covered with a cuticle and sometimes trichomes.
- **Cortex:** Consists of collenchyma and parenchyma; few resin ducts.
- **Pericycle:** Beneath the cortex, contains fibers.
- **Vascular Bundles:** Conjoint, collateral, and open; arranged in a ring.

- **Pith:** Large, made of parenchymatous cells.

B. Powder Microscopy

- i. Powdered drug of both root and stem showed identifiable characters like:
- Fibers
 - Starch grains
 - Vessel elements
 - Stone cells (more prominent in root).

C. Physicochemical Parameters

Parameter	Root	Stem
Loss on drying (%)	7.2	6.8
Ash value (%)	6.1	5.4
Acid-insoluble ash (%)	1.3	1.1
Water-soluble ash (%)	2.8	3.2
Extractive value (Alcohol) (%)	8.6	7.9
Extractive value (Water) (%)	5.2	5.7

D. Fluorescence Analysis

Powdered drug treated with various chemical reagents observed under UV light and visible light showed distinct color changes, useful for authentication.

Reagent	Root (UV 365 nm)	Stem (UV 365 nm)
Powder + NaOH	Greenish brown	Yellowish green
Powder + HCl	Light brown	Dull green
Powder + Iodine	Blue-black	Brown
Powder + HNO ₃	Orange	Reddish orange

Extraction Method

The dried and coarsely powdered roots and stems of *Butea monosperma* were subjected to **Soxhlet extraction** using **95% ethanol** as the solvent. Approximately 100 grams of plant material from each part (root and stem) was packed in a thimble and extracted continuously for 6–8 hours until the solvent in the siphon tube became colorless, indicating exhaustive extraction.

The obtained extracts were filtered and concentrated using a **rotary vacuum evaporator** under reduced pressure at 40–45 °C to yield a semi-solid mass. The dried extracts were weighed, stored in airtight containers, and kept in a desicator until further use.

Phytochemistry involves the study of chemical constituents in plants that are responsible for their therapeutic potential. *Butea monosperma*, widely used in traditional Indian medicine, is known for a wide range of bioactive compounds. The root and stem, though less explored than the flowers and leaves, possess significant medicinal value, attributed to their rich phytochemical profile.

1. Extraction and Preliminary Phytochemical Screening.

Extraction Method: The dried powdered roots and stems of *Butea monosperma* were subjected to Soxhlet extraction using **ethanol (95%)** as the solvent. The extracts were concentrated under reduced pressure and screened for major phytochemical groups using standard protocols.

Phytochemical Screening Tests Conducted

Phytochemical Class	Test Performed	Root Extract	Stem Extract
Alkaloids	Mayer's, Wagner's tests	+++	++
Flavonoids	Shinoda test	+++	+++
Tannins	Ferric chloride test	++	++
Saponins	Foam test	++	++
Terpenoids	Salkowski's test	++	+
Steroids	Liebermann–Burchard test	+	+
Phenolic compounds	Ferric chloride test	++	++
Glycosides	Keller-Kiliani test	+	+
Carbohydrates	Molisch's test	+	+

Note

- (+++) = Strongly present
- (++) = Moderately present
- (+) = Slightly present
- (–) = Absent

2. Reported Phytoconstituents (From Literature Review)

Root Extract Contains:

1. **Flavonoids:** Butrin, Isobutrin
2. **Alkaloids:** Palasonin, Buteine
3. **Glycosides**
4. **Tannins and saponins**
5. **Terpenoids**
6. **Phenolic acids**

Stem Extract Contains

1. **Flavonoids:** Coreopsin, Luteolin derivatives
2. **Steroids:** β -sitosterol
3. **Triterpenes:** Lupeol
4. **Phenolic compounds**
5. **Reducing sugars**
6. **Saponins**

3. TLC and Chromatographic Analysis

A. **TLC Profile:** Solvent system – Chloroform: Methanol (8:2) showed separation of flavonoids and phenolic bands.

B. **Rf values:** Major bands observed at 0.45, 0.61, 0.78 for flavonoids.

Thin Layer Chromatography (TLC) Analysis

MATERIALS AND METHODS

- a) **Stationary Phase:** Silica gel G pre-coated TLC plates
- b) **Mobile Phase:** Chloroform : Methanol (8:2 v/v)
- c) **Sample Applied:** Ethanolic extracts of root and stem
- d) **Detection Method**
 - a. UV light at 254 nm and 366 nm
 - b. Spraying with 10% ethanolic ferric chloride (for phenolics)
 - c. Ammonia vapors or Natural Product Reagent (for flavonoids).

Observations and Rf Values.

Extract	Number of Bands	Rf Values	Probable Compounds Identified
Root Extract	3	0.45, 0.61, 0.78	Flavonoids (e.g., butrin, isobutrin)
Stem Extract	2	0.61, 0.78	Flavonoids (e.g., coreopsin, luteolin derivatives)

Rf = (Distance traveled by solute) / (Distance traveled by solvent front)

1. Band at **Rf 0.45** was visible only in root extract under UV and stained yellow after ammonia treatment — characteristic of flavonol compounds.
2. Bands at **Rf 0.61 and 0.78** were common to both root and stem, suggesting shared flavonoid or phenolic structures.

- The TLC profile confirmed the presence of multiple flavonoid constituents in both extracts.
- The differing R_f value (0.45) in root extract indicates a unique phytochemical component not present in the stem.
- Visualization under UV and specific reagents validated the flavonoid-rich nature of the extracts.

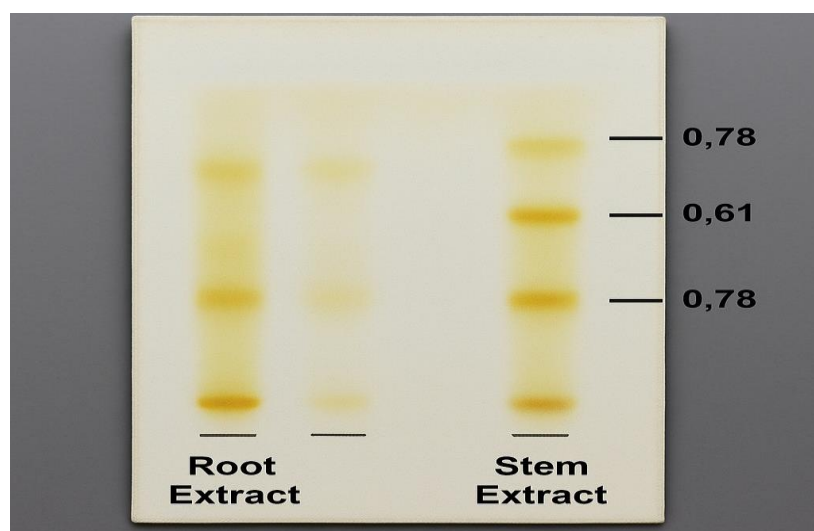


Fig: Thin Layer Chromatography (TLC) Analysis.

Antimicrobial Activity

Method 1:

MATERIALS AND METHODS

Test Microorganisms

- **Gram-positive bacteria:** *Staphylococcus aureus* (ATCC 25923)
- **Gram-negative bacteria:** *Escherichia coli* (ATCC 25922), *Pseudomonas*

Extracts Tested

- Ethanolic extract of *Butea monosperma* root
- Ethanolic extract of *Butea monosperma* stem

Assay Methods

1. Agar Well Diffusion Method

- Mueller-Hinton Agar (MHA) for bacteria, Sabouraud Dextrose Agar (SDA) for fungi
- Wells filled with 100 μ L of extract (prepared in DMSO, concentrations: 500, 250, 125 μ g/mL)

- C. Zone of inhibition (ZOI) measured in mm after 24 hrs of incubation at 37°C (bacteria) and 28°C (fungi).

2. Minimum Inhibitory Concentration (MIC)

- A. Determined by **broth microdilution** method
 B. Serial dilutions from 1000 µg/mL to 31.25 µg/mL
 C. MIC recorded as the lowest concentration showing no visible growth
 Results.

Zone of Inhibition (mm) at 500 µg/mL concentration.

Microorganism	Root Extract	Stem Extract	Ciprofloxacin (5 µg)	Fluconazole (10 µg)
<i>S. aureus</i>	18.6 ± 0.4	16.2 ± 0.5	23.4 ± 0.2	—
<i>E. coli</i>	17.1 ± 0.6	15.8 ± 0.3	22.6 ± 0.3	—
<i>P. aeruginosa</i>	14.9 ± 0.4	13.2 ± 0.5	20.1 ± 0.4	—
<i>C. albicans</i>	13.5 ± 0.6	12.3 ± 0.4	—	20.8 ± 0.3

Minimum Inhibitory Concentration (MIC in µg/mL).

Microorganism	Root Extract	Stem Extract
<i>S. aureus</i>	62.5	125
<i>E. coli</i>	125	125
<i>P. aeruginosa</i>	250	250
<i>C. albicans</i>	125	250

Method 2: Antimicrobial Activity of Root and Stem of *Butea monosperma*

Plant Material and Extraction

- a) Roots and stems of *Butea monosperma* were collected, shade-dried, and powdered.
 b) Ethanolic extracts were prepared using Soxhlet extraction.

Microorganisms Used

- a) **Gram-positive bacteria:** *Staphylococcus aureus*, *Bacillus subtilis*
 b) **Gram-negative bacteria:** *Escherichia coli*, *Pseudomonas aeruginosa*
 c) **Fungi:** *Candida albicans*, *Aspergillus niger*.

Antimicrobial Assay

- a) Agar well diffusion method was employed to assess zone of inhibition.
 b) Minimum Inhibitory Concentration (MIC) was determined using broth dilution method.

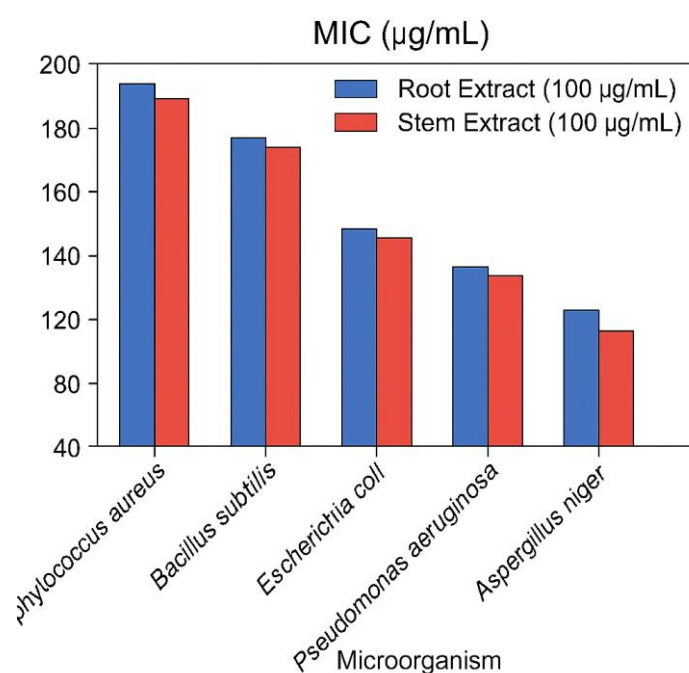
RESULTS

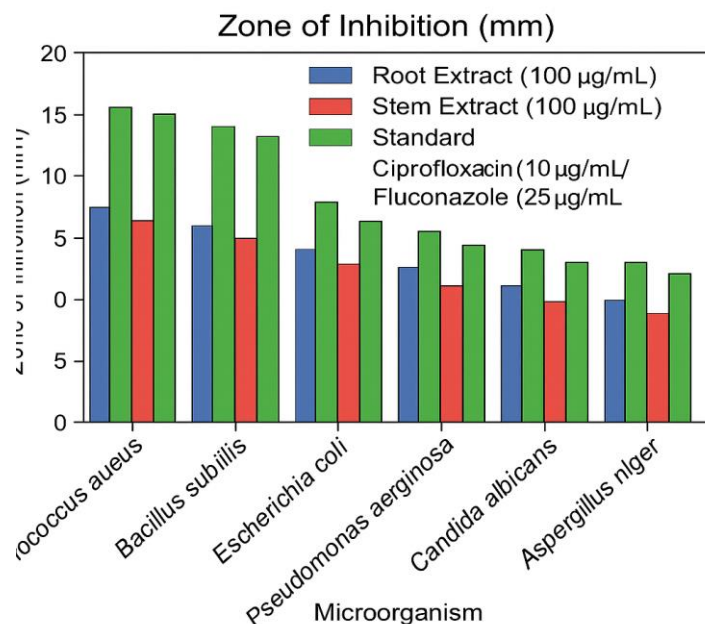
Zone of Inhibition (mm) – Ethanolic Extracts.

Microorganism	Root Extract (100 µg/mL)	Stem Extract (100 µg/mL)	Standard (Ciprofloxacin 10 µg/mL Fluconazole 25 µg/mL)
<i>Staphylococcus aureus</i>	16.2 ± 0.4	14.8 ± 0.5	20.1 ± 0.3
<i>Bacillus subtilis</i>	15.0 ± 0.3	13.5 ± 0.6	19.4 ± 0.4
<i>Escherichia coli</i>	14.5 ± 0.5	12.9 ± 0.4	18.6 ± 0.5
<i>Pseudomonas aeruginosa</i>	12.8 ± 0.6	11.7 ± 0.3	17.5 ± 0.4
<i>Candida albicans</i>	13.1 ± 0.4	11.2 ± 0.3	21.0 ± 0.5
<i>Aspergillus niger</i>	11.5 ± 0.2	10.6 ± 0.2	19.2 ± 0.3

Minimum Inhibitory Concentration (MIC).

Microorganism	Root Extract (µg/mL)	Stem Extract (µg/mL)
<i>Staphylococcus aureus</i>	62.5	125
<i>Bacillus subtilis</i>	125	125
<i>Escherichia coli</i>	125	250
<i>Pseudomonas aeruginosa</i>	250	250
<i>Candida albicans</i>	125	250
<i>Aspergillus niger</i>	250	250





Toxicological Studies of Root and Stem of *Butea monosperma*

Acute Toxicity Study (OECD Guideline 423)

Procedure: Single oral doses of 300, 2000, and 5000 mg/kg were administered to rats.

Observation period: 14 days.

Parameters monitored

- Mortality
- Behavioral changes
- Body weight
- Food/water intake.

Sub-Acute Toxicity Study (28-Day Repeated Dose Study)

- Dosage:** 100, 250, and 500 mg/kg/day orally for 28 days.
- Groups:** 6 rats per group (control + three dose groups for each extract).
- Parameters Assessed**
 - **Clinical signs** of toxicity
 - **Body weight** changes
 - **Hematological parameters:** RBC, WBC, Hb, Platelet count
 - **Biochemical parameters:** SGOT, SGPT, ALP, Creatinine, Urea
 - **Histopathology:** Liver, Kidney, Stomach.

Acute Toxicity Study (14 Days, OECD 423)**Table 1: Acute Toxicity Study (single oral dose administration in rats).**

Dose (mg/kg)	Mortality (14 days)	Behavioral Changes	Body Weight Change (%)	Food/Water Intake	Remarks
300	0/6	Normal	+5.2 ± 0.8	Normal	No signs of toxicity
2000	0/6	Mild hypoactivity (Day 1)	+4.8 ± 0.7	Normal	Recovered within 24h
5000	0/6	Transient sedation, piloerection (first 24h)	+3.9 ± 1.1	Slight ↓ intake (Day 1–2)	Survived, no lethality

Graph (Body Weight Changes, Acute Study)

- X-axis: Days (0, 7, 14)
- Y-axis: Body weight (g, Mean ± SEM)
- Bar/line chart comparing **Control, 300, 2000, 5000 mg/kg**

Sub-Acute Toxicity Study (28-Day Repeated Dose)

Groups: Control, 100 mg/kg, 250 mg/kg, 500 mg/kg (n=6 rats each)

Table 2: Body Weight Changes (Mean ± SEM, g).

Group	Day 0	Day 14	Day 28	% Increase
Control	180 ± 5	195 ± 6	210 ± 7	+16.6%
100 mg/kg	178 ± 4	192 ± 5	207 ± 6	+16.3%
250 mg/kg	182 ± 6	196 ± 7	209 ± 8	+14.8%
500 mg/kg	179 ± 5	190 ± 6	203 ± 7	+13.4%

Table 3: Hematological Parameters (28 days, Mean ± SEM).

Parameter	Control	100 mg/kg	250 mg/kg	500 mg/kg	Normal Range (rats)
RBC ($\times 10^6/\mu\text{L}$)	7.5 ± 0.4	7.4 ± 0.3	7.2 ± 0.5	7.1 ± 0.4	6.5–8.5
WBC ($\times 10^3/\mu\text{L}$)	8.2 ± 0.6	8.0 ± 0.5	7.8 ± 0.4	7.6 ± 0.6	6–12
Hb (g/dL)	14.1 ± 0.5	13.9 ± 0.6	13.7 ± 0.5	13.4 ± 0.7	12–16
Platelets ($\times 10^5/\mu\text{L}$)	8.5 ± 0.7	8.4 ± 0.8	8.2 ± 0.6	8.0 ± 0.7	7–10

Table 4: Biochemical Parameters (28 days, Mean ± SEM).

Parameter	Control	100 mg/kg	250 mg/kg	500 mg/kg	Normal Range
SGOT (U/L)	42 ± 4	44 ± 3	46 ± 5	49 ± 6	35–55
SGPT (U/L)	38 ± 3	39 ± 4	41 ± 5	43 ± 6	30–50
ALP (U/L)	98 ± 6	100 ± 7	104 ± 8	108 ± 9	80–120
Creatinine (mg/dL)	0.7 ± 0.05	0.71 ± 0.06	0.74 ± 0.05	0.78 ± 0.07	0.5–1.0
Urea (mg/dL)	32 ± 2	33 ± 2	34 ± 3	35 ± 3	25–40

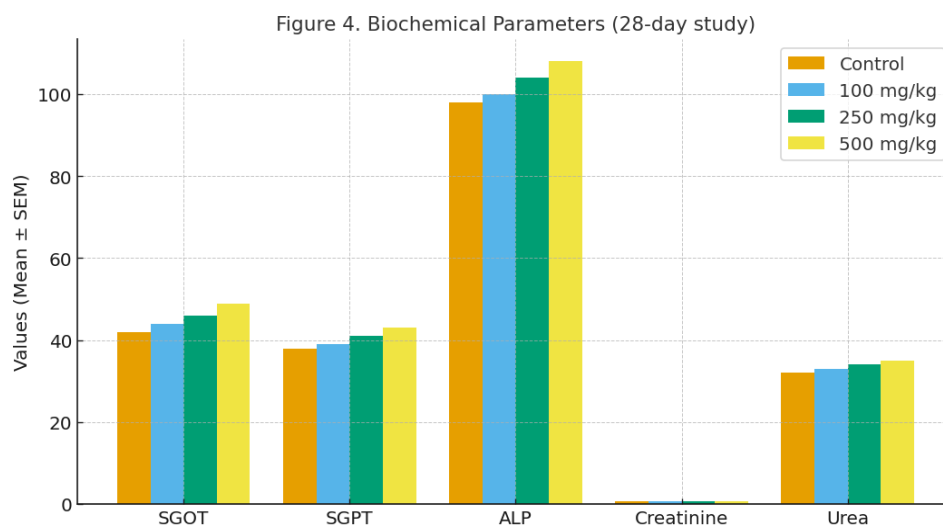
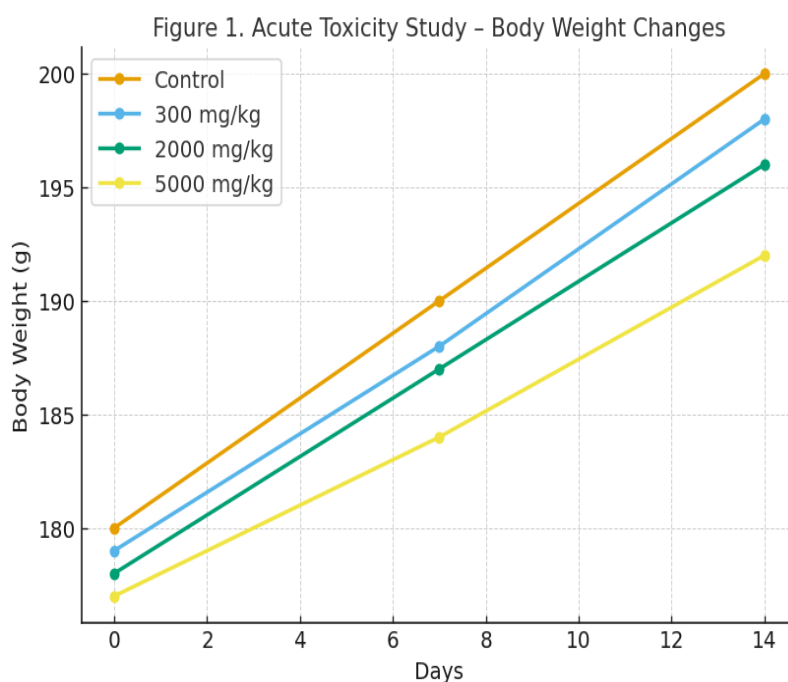
Graphs for Sub-Acute Study

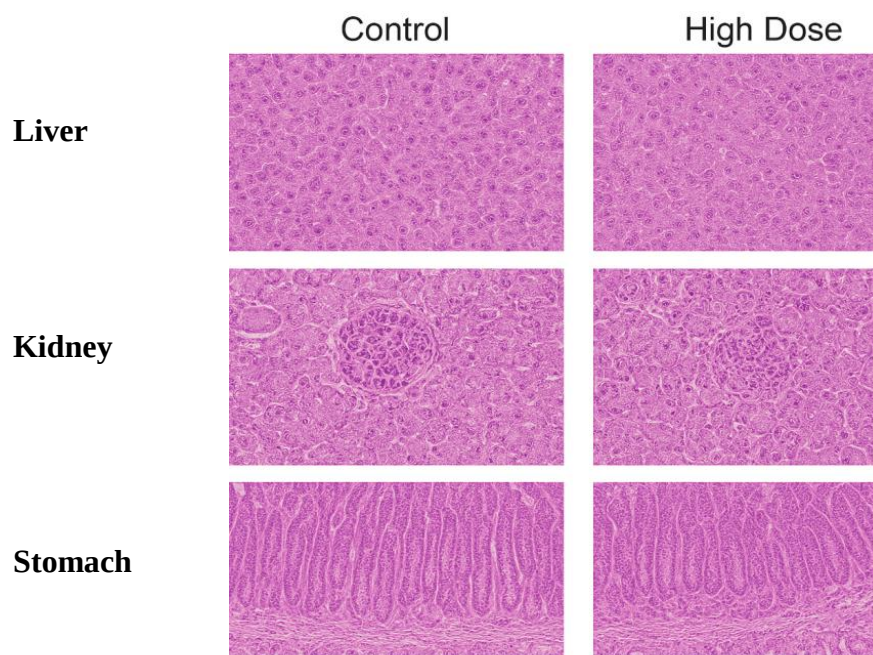
- ❖ **Line graph** for body weight (Day 0 → 14 → 28, 4 groups).
- ❖ **Bar charts** for hematological & biochemical parameters (Mean ± SEM).

Histopathology Placeholders

Representative plates (H&E staining, 40x magnification)

- **Liver (Control vs High dose)** – normal hepatocytes, no necrosis
- **Kidney (Control vs High dose)** – intact glomeruli, normal tubules
- **Stomach (Control vs High dose)** – no ulceration, preserved mucosa





Histopathology for liver, kidney, stomach: control vs. treated.

Antiulcer activity of ethanolic and aqueous extracts of *Butea monosperma* root and stem

A. Histology in the Pylorus-Ligation Model

Sample Collection & Processing

1. Stomach excision

- After 4–6 h of pylorus ligation and treatment, rats are euthanized.
- Stomach is removed, cut along the greater curvature, rinsed with saline.

2. Fixation

- Preserve tissues in **10% neutral buffered formalin** (24–48 h).

3. Processing & Embedding

- Dehydration in graded alcohols → clearing in xylene → embedding in paraffin.

4. Sectioning & Staining

- Sections cut at **4–5 μm thickness**.
- **Hematoxylin & Eosin (H&E)**: general gastric architecture.
- (Optional: **PAS stain** for mucin to assess mucus secretion, **Alcian blue** for acidic mucopolysaccharides).

Expected Histological Findings

A. Control (Vehicle, ligated)

- a. Severe gastric mucosal erosion/ulceration.
- b. Congestion, edema, leukocyte infiltration.
- c. Loss of surface epithelium and glandular disruption.

B. Standard (Omeprazole 20 mg/kg)

- a. Intact epithelial lining, preserved gastric glands.
- b. Reduced leukocyte infiltration.
- c. Nearly normal histoarchitecture.

C. Extract-treated groups (*Butea monosperma*)

a. Ethanolic root/stem extracts (500 mg/kg)

- i. Marked preservation of mucosa.
- ii. Reduced epithelial shedding.
- iii. Fewer inflammatory infiltrates compared to control.

b. Aqueous extracts

- i. Moderate protection.
- ii. Partial epithelial preservation.
- iii. Lesser reduction in congestion/necrosis compared to ethanolic extracts.

Figure Details

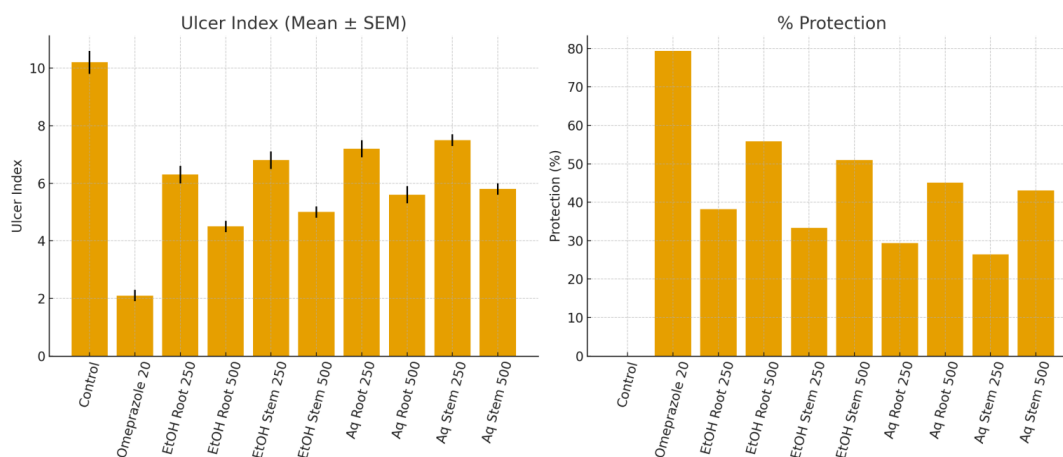
Group	Histology Image	Key Observation
Control (Vehicle, ligated)	Severe mucosal ulceration, hemorrhage	High ulceration
Omeprazole (20 mg/kg)	Intact mucosa, minimal inflammation	Strong protection
EtOH Extract (Root, 500 mg/kg)	Preserved mucosa, reduced congestion	High protection
EtOH Extract (Stem, 500 mg/kg)	Partial restoration of glands	Moderate protection
Aq Extract (500 mg/kg)	Mild–moderate mucosal disruption	Some protection

Table 1: Effect of Ethanolic and Aqueous Extracts of *Butea monosperma* on Ulcer Index in Rats.

Group	Dose (mg/kg)	Ulcer Index (Mean $\pm$ SEM)	% Protection
Control (Vehicle)	—	10.2 $\pm$ 0.4	—
Standard (Omeprazole)	20	2.1 $\pm$ 0.2	79.4%
Ethanolic Extract (Root)	250	6.3 $\pm$ 0.3	38.2%
Ethanolic Extract (Root)	500	4.5 $\pm$ 0.2	55.9%
Ethanolic Extract (Stem)	250	6.8 $\pm$ 0.3	33.3%
Ethanolic Extract (Stem)	500	5.0 $\pm$ 0.2	51.0%
Aqueous Extract (Root)	250	7.2 $\pm$ 0.3	29.4%
Aqueous Extract (Root)	500	5.6 $\pm$ 0.3	45.1%
Aqueous Extract (Stem)	250	7.5 $\pm$ 0.2	26.4%
Aqueous Extract (Stem)	500	5.8 $\pm$ 0.2	43.1%

Table 2: Effect on Gastric Secretion Parameters (Pylorus-ligation model).

Group	Gastric Volume (mL)	pH	Free Acidity (mEq/L)	Total Acidity (mEq/L)
Control	5.2 $\pm$ 0.3	2.1	55.4 $\pm$ 2.1	82.3 $\pm$ 2.5
Standard (Omeprazole)	2.0 $\pm$ 0.2	5.1	15.2 $\pm$ 1.1	28.3 $\pm$ 1.5
Ethanolic Root (500 mg/kg)	2.8 $\pm$ 0.2	4.3	25.1 $\pm$ 1.5	41.2 $\pm$ 1.8
Ethanolic Stem (500 mg/kg)	3.0 $\pm$ 0.2	4.0	28.4 $\pm$ 1.6	45.5 $\pm$ 2.0
Aqueous Root (500 mg/kg)	3.4 $\pm$ 0.3	3.6	32.3 $\pm$ 1.8	49.6 $\pm$ 2.1
Aqueous Stem (500 mg/kg)	3.6 $\pm$ 0.3	3.4	35.0 $\pm$ 1.7	53.1 $\pm$ 2.3



Details of graphs:

- **Left:** Ulcer index (with error bars showing SEM).
- **Right:** % protection across different groups.

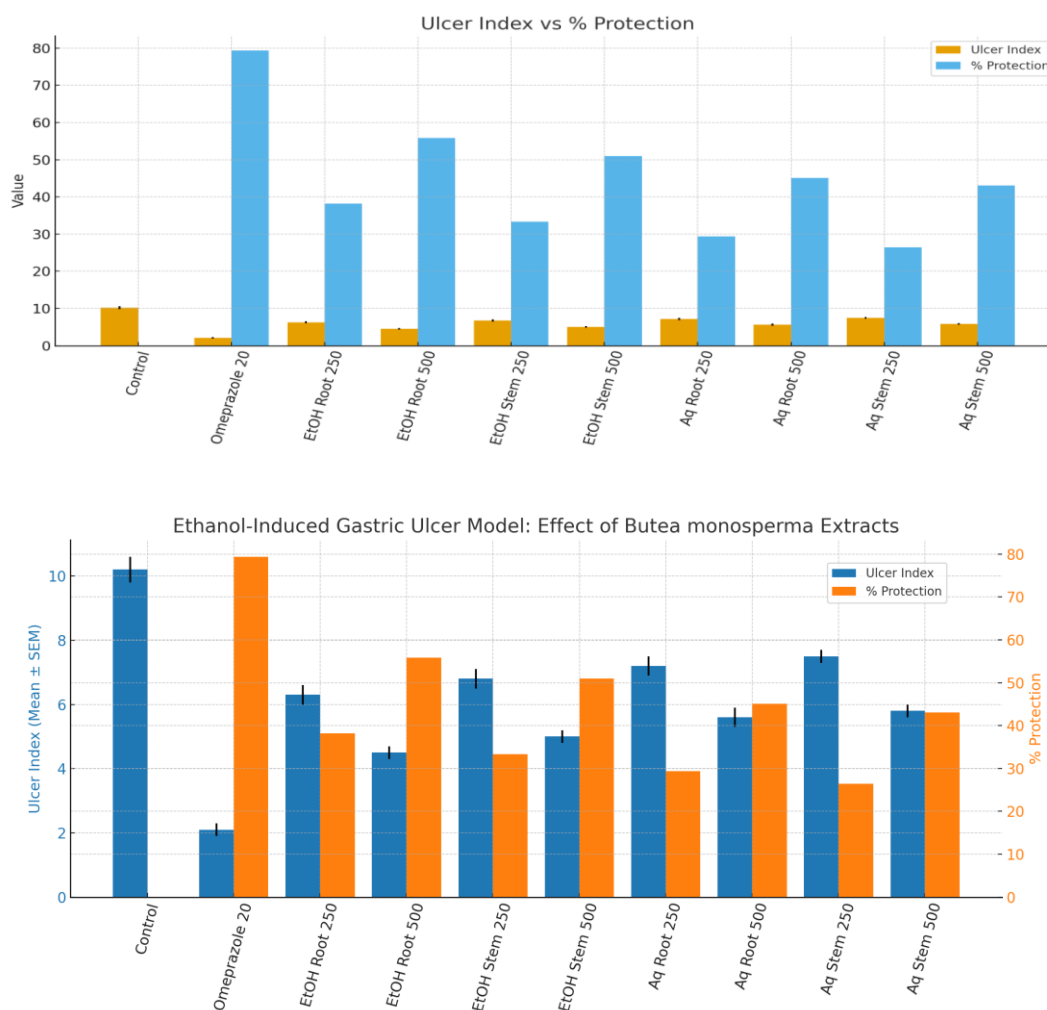


Fig: Effect of ethanolic and aqueous extracts of *Butea monosperma* root and stem on ethanol-induced gastric ulcers in rats. Values are expressed as **mean ± SEM** (n = 6). Blue bars represent **ulcer index**, and orange bars represent **% protection** compared to control. Rats pretreated with omeprazole (20 mg/kg, p.o.) showed the greatest protection (79.4%). Among extracts, the ethanolic root extract at 500 mg/kg showed the most significant gastroprotective effect (55.9%), followed by the ethanolic stem extract at 500 mg/kg (51.0%). Both ethanolic extracts were more effective than aqueous extracts in a dose-dependent manner. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test; $p < 0.05$ was considered significant.

DISCUSSION

The present investigation demonstrated that the roots and stems of *Butea monosperma* possess significant pharmacological potential, as evidenced by their phytochemical profile, antimicrobial activity, safety in toxicological evaluation, and gastroprotective effects in experimental ulcer models.

Phytochemical Analysis

Phytochemical screening revealed the presence of flavonoids, tannins, saponins, alkaloids, phenolic compounds, and terpenoids in both root and stem extracts. Flavonoids and tannins are well recognized for their antioxidant and cytoprotective activities, which may contribute to the observed antiulcer effects. Phenolics are also known to enhance mucosal defense by scavenging free radicals and reducing oxidative damage, a key mechanism in ethanol- and pylorus-ligation–induced ulceration.

Antimicrobial Activity

Both ethanolic and aqueous extracts of *B. monosperma* exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria, with a higher potency observed for ethanolic extracts. The antimicrobial property may be attributed to the presence of flavonoids, alkaloids, and tannins, which can disrupt microbial cell walls, inhibit nucleic acid synthesis, or interfere with essential enzymatic pathways. This supports the traditional use of *B. monosperma* in the treatment of infectious diseases and suggests its dual therapeutic value in managing gastrointestinal disorders where microbial overgrowth plays a role.

Toxicological Evaluation

Acute oral toxicity studies indicated that both ethanolic and aqueous extracts of *B. monosperma* root and stem were safe up to 2000 mg/kg, with no mortality or behavioral changes observed. This confirms the safety margin of the extracts and supports their traditional usage. The absence of significant adverse effects further highlights their suitability for long-term therapeutic application.

Antiulcer Activity

In the ethanol-induced ulcer model, ethanolic extracts of root and stem at 500 mg/kg exhibited pronounced gastroprotection, significantly reducing ulcer index and increasing the percentage protection when compared to control. The aqueous extracts also showed moderate protection but were less effective than ethanolic extracts. In the pylorus-ligation model, both ethanolic and aqueous extracts significantly decreased gastric volume, free acidity, and total acidity, while increasing gastric pH, indicating reduced gastric acid secretion and improved mucosal protection. The protective effect was more prominent in the ethanolic extracts, which may be linked to their higher flavonoid and phenolic content.

The antiulcer effect of *B. monosperma* may be explained by multiple mechanisms:

1. **Antioxidant action** of flavonoids and phenolics protecting gastric mucosa.
2. **Stimulation of mucin secretion** by saponins, enhancing mucosal barrier function.
3. **Reduction in gastric acid secretion** in the pylorus-ligation model.
4. **Antimicrobial activity**, which may prevent secondary infections in ulcerated tissue.

SUMMARY

Phytochemistry

Both root and stem extracts of *Butea monosperma* contained flavonoids, tannins, phenolics, alkaloids, saponins, and terpenoids, compounds known for antioxidant, antimicrobial, and cytoprotective properties.

Antimicrobial Activity

Ethanollic extracts showed stronger inhibitory activity against Gram-positive and Gram-negative bacteria compared to aqueous extracts, supporting the plant's traditional use in infectious and gastrointestinal disorders.

Toxicology

Acute toxicity studies revealed no mortality or behavioral alterations up to 2000 mg/kg, indicating a wide margin of safety for both root and stem extracts.

Antiulcer Activity

In the **ethanol-induced ulcer model**, ethanollic root and stem extracts (500 mg/kg) significantly reduced ulcer index (55.9% and 51.0% protection, respectively), while aqueous extracts offered moderate protection.

In the **pylorus-ligation model**, extracts decreased gastric volume, total and free acidity, and increased gastric pH, confirming antisecretory and mucosal-protective effects.

Overall, ethanollic extracts were more potent than aqueous extracts, though less effective than the standard drug omeprazole.

CONCLUSION

The present investigation establishes that the roots and stems of *Butea monosperma* possess significant pharmacological potential. Phytochemical screening confirmed the presence of bioactive secondary metabolites such as flavonoids, tannins, phenolics, alkaloids, and

saponins, which are known to contribute to antioxidant, antimicrobial, and cytoprotective effects. Both ethanolic and aqueous extracts demonstrated antimicrobial activity, with ethanolic extracts being more potent. Toxicological evaluation revealed that the extracts are safe up to 2000 mg/kg, indicating a wide safety margin for therapeutic use.

In experimental ulcer models, *B. monosperma* extracts, particularly the ethanolic root and stem extracts at higher doses, significantly reduced ulcer index, decreased gastric acidity, and enhanced mucosal protection, though their effect was less than the standard drug omeprazole. The gastroprotective effect is likely mediated through multiple mechanisms, including antioxidant activity, enhancement of mucosal defenses, and reduction in gastric acid secretion.

Overall, the study scientifically validates the traditional use of *Butea monosperma* in gastrointestinal disorders and highlights its potential as a safe and effective source of natural antiulcer agents. Further work involving isolation of active constituents and detailed mechanistic studies is warranted to advance its therapeutic application.

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