IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Investigating the Therapeutic Potential of Methanolic Bark Extracts from the Endangered Species *Shorea tumbuggaia* Roxb.

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Abstract

Since ancient times, plants have been employed to treat various diseases and promote overall health and well-being. Plant biodiversity constitutes a rich source of bioactive compounds and offers direct economic benefits through the provision of timber, food, fibres, industrial enzymes, Flavors, cosmetics, emulsifiers, dyes, plant growth regulators, biopesticides, and biofertilizers. Secondary metabolites such as alkaloids, saponins, quinones, indoles, anthraquinones, terpenoids, flavonoids, phenolic compounds, and amino acids are synthesized and stored in plant tissues, conferring therapeutic activities. Moreover, these compounds serve as phytochemical markers critical for the authentication of medicinal plants.

In the present study, *Shorea tumbuggaia* bark was subjected to phytochemical analysis following standard protocols outlined by Harborne (1973), Gibbs (1974), Kokate (2001), and Khandelwal (2006), followed by pharmacological evaluations. Methanolic extracts of its bark was investigated for diuretic and antidiabetic activities. All experimental data were expressed as mean ± standard deviation (SD) based on five replicates. Statistical analysis was conducted using one-way and two-way ANOVA, followed by Scheffe's post hoc test and Dunnett's multiple comparison test in SAS software version 25.0, with significance set at $p < 0.05$.

Preliminary phytochemical screening revealed the presence of saponins, alkaloids, terpenoids, steroids, lignins, tannins, indoles, anthraquinones, anthocyanidins, phenolic compounds, flavonoids, and lipid-based constituents, whereas glycosides, proteins, amino acids, fixed oils, gums, and mucilages were absent.

For diuretic evaluation, methanolic extracts were administered orally to Wistar rats at doses of 100 and 200 mg/kg body weight, with Furosemide (20 mg/kg, orally) serving as the standard reference. Parameters measured included total urine volume and electrolyte concentrations (sodium, potassium, and chloride). The extract exhibited a significant, dose-dependent increase in urine output and electrolyte excretion compared to the control group, indicating potent diuretic activity.

For antidiabetic assessment, experimental diabetes was induced via intraperitoneal injection of streptozotocin (STZ) at 50 mg/kg body weight. Post-induction, methanolic extracts were administered orally at 100 and 200 mg/kg, and Glibenclamide (5 mg/kg) was used as the standard drug. Blood glucose levels were monitored at regular intervals. The methanolic leaf extract of *Shorea tumbuggaia* demonstrated a significant, dose-dependent reduction in blood glucose levels relative to the diabetic control, suggesting its potential for managing hyperglycemia, possibly via insulinotropic or insulin-mimetic mechanisms.

The findings of this study suggest that the methanolic bark extract of *Shorea tumbuggaia* possesses considerable diuretic and antidiabetic activities, supported by its rich phytochemical profile. These results provide a scientific basis for the traditional use of *Shorea tumbuggaia* in ethnomedicine and highlight its promise as a candidate for further pharmacological, toxicological, and drug discovery research aimed at developing novel therapeutic agents.

Keywords: *Shorea tumbuggaia*, Methanolic extract, Phytochemical screening, Diuretic activity, Antidiabetic property, Streptozotocin, Anova analysis.

Introduction

Since ancient times, plants have played a vital role in the treatment of diseases and in enhancing human health and well-being. This research emphasizes the therapeutic potential of *Shorea tumbuggaia*, an endagered and ethnomedicinally important species, known for its traditional uses in various indigenous healing systems. Plant-based products—whether raw or processed—continue to serve critical health-related functions across cultures. The vast diversity of plants provides an invaluable reservoir of medicinal resources and offers direct economic benefits through timber, food, fibre, industrial enzymes, flavors, fragrances, cosmetics, emulsifiers, dyes, plant growth regulators, biopesticides, and biofertilizers. Phytochemicals derived from many plant species also offer great potential for drug discovery, environmental monitoring, and biotechnological applications (Marotrao Dalvi, 2010).

In medicinal plant research, the therapeutic application may involve any part of the plant or plant-derived compounds as precursors for modern pharmaceuticals. This distinction allows for the classification between scientifically validated medicinal plants and those traditionally known for medicinal properties but not yet scientifically studied (Sofowora et al., 2013). *Shorea tumbuggaia* belongs to such a category of plants with significant ethnomedicinal relevance but limited pharmacological validation. Increasing global recognition of traditional medicine has spurred extensive research into medicinal flora, although often at the cost of habitat degradation and loss of biodiversity.

Traditional medicine continues to be a primary mode of healthcare in many developing countries. Medicinal plants remain vital sources for the development of novel therapeutic agents, underscoring their continued relevance in drug discovery (Atanasov et al., 2015). The importance of ethnobotanical research was first highlighted by Harshberger in 1896 and remains highly significant in the current context of sustainability, healthcare, and biodiversity conservation.

India – An Emporium of Ethnomedicinal Plants

India is one of the oldest nations to develop formal systems of plant-based medicine. Of the estimated 480,000 plant species globally, India harbors more than 48,000 taxa, including around 18,000 flowering plants. Among these, approximately 10,000 species are traditionally utilized by 4,635 ethnic communities for human and veterinary medicine. This has earned India the distinction of being an "Emporium of Medicinal Plants." *Shorea tumbuggaia* is one such species, valued in regional folk medicine but facing ecological pressure due to habitat loss.

Traditional herbal knowledge, particularly in rural and tribal communities, remains central to ethnobotanical studies. Much of this knowledge—often rich and effective—has been passed down orally through generations. Ethnobotanical surveys have shown that even under similar environmental conditions, different communities may exhibit unique knowledge systems and medicinal plant usage patterns. However, the cultural and ecological factors shaping this variability remain underexplored and require further scientific attention.

Fig.1 *Shorea tumbuggaia Roxb.*

Table.1 Taxonomical Classification of *Shorea tumbuggaia Roxb.*

Rank	**Name**
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Sapindales
Family	Dipterocarpaceae
Genus	Shorea
Species	*Shorea tumbuggaia* (Roxb.)

Table.2 : Vernacular Names of ***Shorea tumbuggaia Roxb.***

Language	**Local Names**
Telugu	Googilapu, Guggilamu, Guggilapukam, Nallaguggilamu, Thamba
English	Green Dammar Tree
Hindi	Kala Dammar
Kannada	Jujula, Karidamara
Tamil	Tambagom, Tambugai, Karuppu Dammar, Kangu, Kungiliam
Malayalam	Tampakam

Table.3. Botanical Information of Chloroxylon swietenia

Attribute	**Details**
Habit	Tree
Comments / Notes	Endemic to the southern Eastern Ghats up to 1000 m. Wood is used for construction.
Key Identification Features	A tall deciduous tree up to 20m. Bark dark brown, longitudinally fissured, wood brown. Branchlets tomentose, leaves ovate, coriaceous, leaf base rounded, margin entire, apex acuminate, petiole up to 5 cm long, secondary nerves 8-10 pairs. Inflorescence terminal panicle. Flowers white, fragrant, capsule yellowish, wings unequal, dark brown, spathulate, to 5 cm long.
Flowering & Fruiting Period	March – August

Attribute	Details
Flower & Fruit	March – August
Distribution in India	Andhra Pradesh: Talakona RF (Chittoor district), Nellore district, Kadapa district, Sesachalam Hills (Kadapa, Chittoor districts), Veligonda (Kadapa district), Tirumala Hills (Chittoor district)
	Karnataka: Hassan district, Kodagu (Coorg) district, Udupi district, Chamarajanagar district
	Tamil Nadu: Kanchipuram district, Tiruvannamalai district, Vellore district, Dharmapuri Hills, Kanchipuram (Changalpattu-CGP) district
Native	India
Endemism	South India (Andhra Pradesh, Tamil Nadu)
Global Distribution	India

➢ **Botanical and Ecological Profile of *Shorea tumbuggaia* Roxb.**

Morphology

Growth Form:

- *Shorea tumbuggaia* is a deciduous tree.
- The tree can grow to a height of 12-18 meters, with a girth up to 2 meters.
- The crown of mature trees is dome-shaped.
- **Bark**: The bark is thick, rough, and dark brown, often fissured longitudinally. The bole is straight, and the wood is hard, with white resin.

Leaves:

- Simple, alternate, broadly ovate to cordate in shape.
- Leaf size: 6–21 cm long and 3.5–12 cm wide.
- The base of the leaf is truncate to shallowly emarginate, with entire margins and an apex that is abruptly short acuminate.
- Leaves are coriaceous, dark green, and glabrous on both sides.
- The midrib is impressed above and prominent beneath, with 8–10 lateral veins on each side.
- Petioles are stout, tomentose, and about 2–5 cm long.

Inflorescence:

- The flowers are borne in axillary or terminal panicles, which can reach up to 20 cm in length.

Flowers:

- The flowers are bisexual and cream-white.
- The pedicel is subsessile, and the buds are densely pubescent.
- The calyx is 5-lobed, tubular, with short valvate lobes.
- Petals are 5, white, lanceolate, slightly connate at the base, and attenuate at the apex.
- There are more than 15 stamens, which are adnate and persistent.
- The ovary is enclosed inside the calyx tube, narrow ovoid in shape, pubescent, tri-locular, with 2 ovules per locule. The style is subulate, and the stigma is tri-lobed.

Fruit:

- The fruit is a capsule or nutlike, ovoid in shape, with an acute apex.
- It is densely pubescent, about 2 cm long, and enclosed in the calyx tube.
- The calyx tube has 3 outer accrescent larger lobes and 2 inner smaller, spathulate lobes.
- The seed is 1 per fruit, with large, fleshy cotyledons.

Reproduction

- **Flowering**: March–April
- **Flower Type**: Flowers are complete and bisexual, meaning they contain both functional male (androecium) and female (gynoecium) parts, including stamens, carpels, and the ovary.

Pollination:

- **Entomophilous (Insect Pollination)**: Pollination is primarily carried out by insects.
- **Cleistogamy**: The species may also self-pollinate.
- **Allogamy**: Cross-pollination occurs as well.

Dispersal:

- **Seed Dispersal Mechanisms**:
- **Autochory (Self-Dispersal)**: Seeds may disperse by the plant itself.
- **Anemochory (Wind Dispersal)**: Seeds can also be dispersed by the wind.
- **Anthropochory (Human Dispersal)**: Human activities also contribute to seed dispersal

Conservation Status

- **IUCN Red List Category**: Endangered (EN)
- *Shorea tumbuggaia* is considered endangered due to habitat loss and over-exploitation for its valuable timber and resin.

Uses and Management

- **Wood Uses**: The wood is used in the construction of door and window frames, as well as agricultural implements.
- **Resin Uses**: The resin from the tree is used in traditional folk medicine.

Ecological and Cultural Significance: *Shorea tumbuggaia* plays a significant ecological role in its native habitats, supporting local wildlife and providing resources like timber and resin to communities. Its conservation is essential for maintaining ecological balance and supporting traditional medicinal practices.

Materials and Methods: Bark of *Shorea thumbujjia* was collected from its natural habitat in the region of Andhra Pradesh, India. The plant was identified by comparing it with authenticated herbarium specimens. The collected bark was thoroughly washed with distilled water, shade dried for one month, and then ground into a fine powder. The powdered material was stored in airtight containers at room temperature. For extraction, the powdered bark was subjected to methanol extraction (1:10 ratio) in a Soxhlet apparatus as per the method of Khan et al. (1988). The solvent was removed under reduced pressure using a rotary evaporator and the extract was concentrated on a water bath to obtain a crude extract. This extract was then used for subsequent phytochemical screening, as well as for testing diuretic properties and anti-diabetic properties.

Phytochemical Screening: Standard qualitative methods (Harborne, 1973; Gibbs, 1974; Kokate, 2002) were followed to detect the presence of bioactive compounds such as flavonoids, saponins, glycosides, alkaloids, sterols, tannins, phenolic compounds, fats and oils, lignins, quinones, terpenoids, anthraquinones, anthocyanidins, coumarins, proteins, carbohydrates, indoles, reducing sugars, and amino acids. A series of colorimetric and precipitation-based tests were performed for each group of compounds.

Tests performed for the presence of phytochemicals:

A) Tests for Flavonoids

- Shinoda test (Magnesium hydrochloride reduction test): To the test solution few fragments of magnesium ribbon and concentrated hydrochloric acid were added drop wise and reddish to pink colour was resulted.
- Zinc Hydrochloride reduction test: To test the sample solution for the flavonoids added a mixture of zinc dust and concentrated hydrochloric acid results in red colour.
- Lead acetate test: When aqueous basic lead acetate was added to test sample produces reddish brown precipitate.
- Ferric chloride test: To few ml of test samples taken separately, few drops of ferric chloride were added which resulted in the formation of blackish red precipitate.
- Alkaline reagent test: To detect the presence of flavonoids, sodium hydroxide solution is added to turn the test sample solution green. When few drops of diluted acid is added the solution turns colourless to indicate the presence of flavonoids.

B) Tests for Saponins

- Foam test: 5ml of extract was vigorously shaken to obtain a stable froth, which was added with olive oil (3 drops). Presence of emulsion indicates the existence of saponins.

C) Tests for Glycosides

- Kellar Kiliani test: 1ml of concentrated sulphuric acid was taken in a test tube then 5ml of extract and 2ml of glacial acetic acid and ferric chloride (one drop) and observed for blue color formation.
- Raymond's test: Test solution when treated with dinitrobenzene in hot methanolic alkali giving a violet colour.
- Molisch test: Alpha naphthol with conc.H2SO4 when added to test sample gives reddish violet ring at the junction of 2 layers.
- Conc.H2SO4 test: Conc.H2SO4 was added to test sample which resulted in appearance of reddish colour.
- Legal's test: The test samples when treated with pyridine and sodium nitroprusside solution blood red colour will be developed.
- Bromine water test: When bromine solution is added the test solution gives yellow precipitate.

Tests for Alkaloids

- Dragendorff's test: 1 ml of the sample solution is taken in a test tube, and few drops of potassium bismuth iodide solution (Dragendorff's reagent) was added. The presence of alkaloids was determined by the appearance of reddish-brown precipitate.
- Meyer's test: 1ml of the sample solution is added with few drops of potassium mercuric chloride solution (Meyer's reagent). A creamy white precipitate was formed indicating the presence of alkaloids.
- Hager's test: To 1 ml of each of the sample few drops of Hager's reagent (Picric acid) was added. Yellow precipitate was formed reacting positively for alkaloids.
- Tannic acid test: When few ml of 10% Tannic acid was added to 1ml of each sample, a buff colour precipitate was formed giving positive result for alkaloids.
- FeCl 3 test: One drop of FeCl 3solution was added to each of the test sample, formation of yellow precipitate was resulted reacting positively for alkaloids.

D) Tests for Sterols

- Libermann-Buchard test: When few drops of acetic anhydride and few drops of concentrated sulphuric acid were added to the test samples if a brown ring shows up at the junction of two layers, it indicates presences of steroids. 2. Salkowski test: The presence of sterols can be detected by adding few drops of concentrated sulphuric acid to the test samples in chloroform, the lower layers of solution turns green on sterols presence.

E) Tests for Tannins and Phenolic Compounds

- Gelatin test: When gelatine and water were added to test samples formation of white precipitate was resulted.

- Lead acetate: Few ml of test samples were taken in different test tubes followed by the addition of aqueous basic lead acetate. It results in the formation of reddish brown bulky preceipitate.
- Alkaline reagent: The test solution will give a yellowish red precipitate when sodium hydroxide solution was added.
- Ellagic acid test: Presence of phenols in the test solution can be detected by adding 5% each of glacial acetic acid and Sodium Nitrite. If the solution turn Niger brown colour, it indicates phenols in solution.

F) Tests for Fats and Oils

- Stain test: when we a small quantity of extract between two filter papers, the stain on filter papers gives the presence of the oils.
- Saponification test: Added a few drops of 0.5N alcoholic potassium hydroxide to various extracts with a drop of phenolphthalein separately and heat on water bath for 1-2hours. If the solution produces soap or partial neutralization of alkali, it's a sign of presence of oils and fats.

G) Tests for Lignins

- Labat test: The test sample turns olive green colour on addition of gallic acid when lignins are present.
- Furfuraldehyde test: The test sample turns red colour on addition of fur furaldehyde when lignins are present.

H) Tests for Quinones

- Alcoholic KOH test: When alcoholic KOH was added to the test samples red to blue colour appears reacting positively for quinones.

I) Terpenoids and steroids test

- 50% H_2SO_4 is added along the sides of the test tube containing a mixture of methanolic HCl and acetic anhydride. If there is any change in color, from green to blue-green (sometimes via red or blue) indicates the presence of terpenoids and steroids**Anthraquinones test**
- 5 g plant powder was shaken with 20 ml of benzene and filtered. To the filtrate 5 ml of 10% ammonium hydroxide solution was added and shaken well. Presence of pink red or violet color, in the ammonical phase indicates the presence of free anthroquinones (Fransworth, 1966).

J) Anthocyanidin test

- To the plant extract was added equal volume of methanolic HCl. Appearance of red or purple color indicates the presence of anthocyanidins.

K) Coumarin test

- When few drops of sodium hydroxide are added to the methanolic extracted test solution, if the solutions turning yellow indicates presence of coumarins.

L) Proteins test (Millions test)

- 2 ml of methanolic extract was boiled with a few drops of Million's reagent (Millions reagent is a solution of mercuric nitrate in nitric acid) results in the formation of red color indicates the presence of proteins.

M) Carbohydrate test (Molish test)

- To the methanolic extract, c-naphthol solution (1gm dissolved in 100 ml of ethanol w/v) was added. Then conc. H_2SO_4 is added gently along the walls of the inclined test tube. Appearance of a red to violet color at the interface is taken as a positive reaction.

N) Indole test

- If a violet color was developed on adding Ehrlich reagent to the alcoholic extract, it is considered as a positive reaction for indoles.

O) Test for reducing sugars

- To the 5 ml of methanolic extract, 5 ml of Benedict's reagent was added in a boiling test tube. The test tubes were incubated in boiling on water bath for 15-30 minutes. The formation of an orange red precipitate indicated the presence of reducing sugars.

P) Test for amino acids

- To the methanolic extract, was added few drops of Ninhydrin solution and boiled. The formation of violet colour indicates the presence of amino acids.

Determination of Diuretic activity of methanolic bark extracts of *Shorea thumbujjia*: The diuretic activity in Wistar rats was studied by the Lipschitz Test. (1943). The test is based on water and sodium excretion in test animals and compared to rats treated with a high dose of Furosemide. Four groups of Wistar rats were used to evaluate the diuretic activity of methanolic extract of bark of *Shorea thumbujjia*(STM) by using metabolic cages. The group I served as normal control given vehicle (CMC 0.5% w/v in normal saline), group II with Furosemide (20 mg/Kg, p.o), Groups III and IV with 100 mg/kg, 200 mg/ doses of STM respectively. Immediately after the treatment with the standard and test all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cage, specially designed to separate urine and faeces and kept at 21°C±0.5°C.

Estimation of Urinary Electrolytes: Urine electrolytes (sodium, potassium and chloride) were determined by Ion Selective Electrode method as described by the user manual of the biochemical kits (NRI Technologies, Malleswaram, Bengaluru)

5 rats in each group which were as follows:

Group I: Normal control (CMC 0.5% w/v in normal saline).

Group II: Furosemide (20 mg/kg, p.o).

Group III: Test 1(100 mg/kg)

Group IV: Test 1(200 mg/kg)

The total volume of urine collected after 24 hrs was measured at the end. During this period no food and water was made available to animals. Various parameters like total urine volume and concentration of sodium, potassium and chloride in the urine were measured and estimated respectively. The ratio of Sodium to Potassium ions is also estimated.

Statistical Analysis: All experiments were performed in triplicates. Results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way and two-way ANOVA followed by Scheffe's or Dunnett's post hoc tests (SAS, 1999). A p-value < 0.05 was considered statistically significant.

Determination of Anti diabetic activity of methanolic bark extracts of *Shorea thumbujjia:*

Healthy Wistar rats (150 to 200 gm) of either sex was selected. Before and during the experiment, rats were fed with standard diet. After randomization into various groups and before initiation of experiment; the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting, which were deprived of food and water for 18 hours. After fasting DM was induced by IP injection of Streptozocin (STZ) at a dose of 60 mg/Kg. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia. After 72 hrs, STZ-treated animals were considered as diabetic when the fasting blood glucose levels observed above 200 mg/dL with glucosuria. Blood samples were collected by tail vein puncture at weekly intervals for a period of 28 days. Fasting blood glucose was measured by glucose oxidase-peroxidase (GOD-POD) method (Trinder, 1969) in mg/dl using a digital glucometer (Braun OmnitestR EZ, Germany).

5 rats in eachgroup which were as follows:
Group I: Normal control (saline).
Group II: Streptozocin treated control (60 mg/kg.ip).
Group III: Streptozocin (60 mg/kg) + Standard drug-Glibenclamide (5mg/kg, p.o).
Group IV: Streptozocin (60 mg/kg) + Test 1(100 mg/kg)
Group V: Streptozocin (60 mg/kg) + Test 1(200 mg/kg)

Vehicle, Test samples and Glibenclamide were administered once daily for 15 days from the day of induction. Blood was drawn from tail tip of rat and the blood glucose level was estimated on 0, 10th and 15th day of experiment with the help of glucometer using strip method.

RESULTS AND DISCUSSION

Phytochemical Studies: Pharmacognostic investigations conducted on the methanolic bark extract of *Shorea tumbuggaia* Roxb. revealed the presence of alkaloids, glycosides, saponins, phytosterols, phenolic compounds, tannins, flavonoids, triterpenoids, and lignin, as confirmed through standard phytochemical screening tests. The extract tested negative for carbohydrates, proteins, amino acids, fixed oils, gums, and mucilage during preliminary qualitative analysis.

Fig 2: Phytochemical profile of methanolic bark extracts of *Shorea thumbujjia*

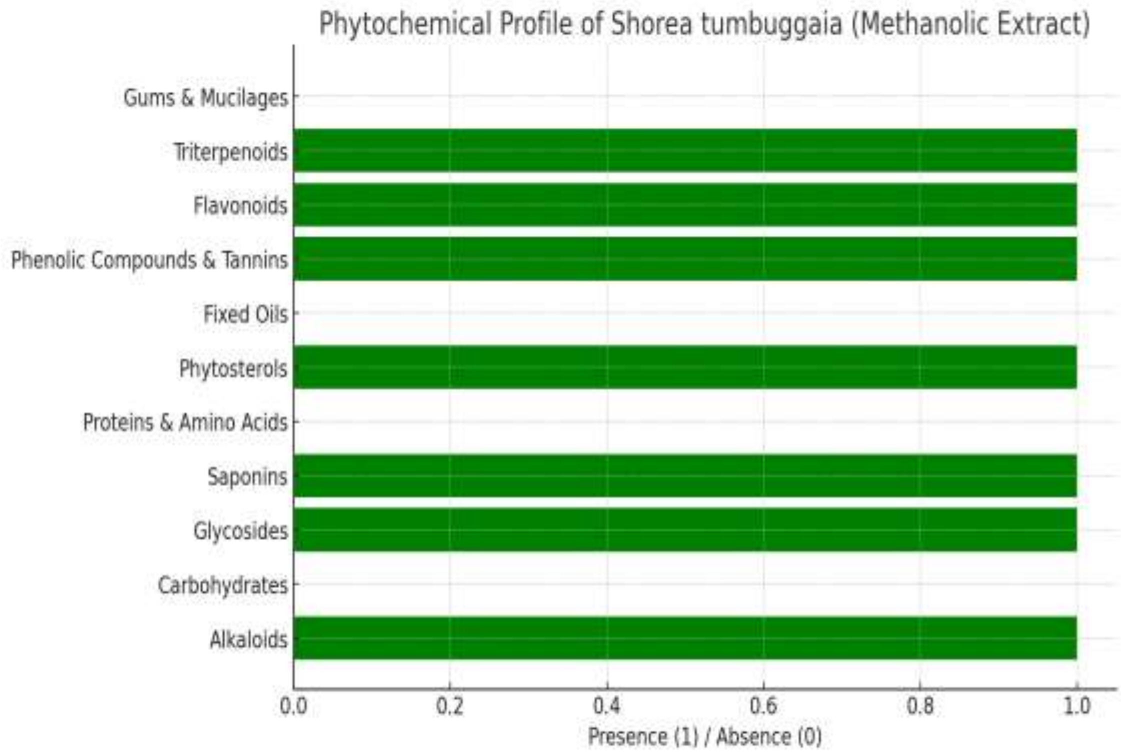


Table:4

Phytochemical Profile of methanolic bark extracts of *Shorea thumbujjia*

Phytochemical Group	**Observation (Methanolic Extract)**
Alkaloids	Present (positive in Mayer's, Wagner's, Hager's, Dragendorff's tests)
Carbohydrates	Absent (negative in Molisch's, Fehling's, Barfoed's, Benedict's tests)
Glycosides	Present (positive in Borntrager's, Legal's, Keller–Kiliani tests)
Saponins	Present (positive in Foam test)
Proteins and Amino Acids	Absent (negative in Millon's, Biuret, Ninhydrin tests)
Phytosterols	Present (positive in Libermann–Burchard test)
Fixed Oils	Absent (negative in Spot test)
Phenolic Compounds and Tannins	Present (positive in Ferric Chloride, Gelatin, Lead Acetate tests)
Flavonoids	Present (positive in Alkaline Reagent, Shinoda, Zn + HCl tests)
Triterpenoids	Present (positive in Salkowski test)
Gums and Mucilages	Absent (negative in Alcoholic Precipitation test)
Lignin	Present (positive in Lignin and Labat tests)

Phytochemical Constituents

- **Flavonoids**: Detected in methanolic extracts, contributing to antioxidant and anti-inflammatory activities.
- **Tannins**: Present in methanolic extracts, known for their antimicrobial and anti-inflammatory properties.
- **Saponins**: Found in methanolic extracts, exhibiting antimicrobial and anti-inflammatory effects.
- **Terpenoids**: Identified in methanolic extracts, contributing to the plant's anti-inflammatory and analgesic activities.
- **Steroids**: Detected in methanolic extracts, known for their anti-inflammatory properties.
- **Anthocyanins**: Present in methanolic extracts, contributing to antioxidant activities.
- **Glycosides**: Detected in methanolic extracts, known for their potential therapeutic effects.

The preliminary phytochemical analysis of the methanolic extract of *Shorea tumbuggaia* bark revealed the presence of several bioactive constituents. A strong positive reaction (++) for alkaloids was observed in all four standard tests: Mayer's, Wagner's, Hager's, and Dragendorff's. Glycosides were also strongly indicated by positive results in Borntrager's, Legal's, and Keller–Kiliani tests. The extract tested positive for phytosterols (Libermann–Burchard test), lignins (Lignin and Labat tests), phenolic compounds and tannins (Ferric chloride, Gelatin, and Lead acetate tests), and triterpenoids (Salkowski test). Flavonoids showed a moderate presence (+), confirmed by the alkaline reagent, Shinoda, and zinc–hydrochloride tests. Conversely, the methanolic extract tested negative (–) for carbohydrates (Molisch's, Fehling's, Barfoed's, and Benedict's tests), proteins and amino acids (Millon's, Biuret, and Ninhydrin tests), fixed oils (Spot test), saponins (Foam test), and gums and mucilages (Alcoholic precipitation test). These findings underscore the pharmacological potential of *Shorea tumbuggaia* leaf extracts, particularly their analgesic, anti-inflammatory, antioxidant, and anthelmintic properties. Further research is warranted to isolate and characterize the specific bioactive compounds responsible for these activities.

Diuretic activity: The methanolic bark extract of *Shorea tumbujia* exhibits a clear dose-dependent diuretic effect characterized by increased urine output and enhanced excretion of potassium and chloride ions. Although sodium excretion increased slightly, it was not markedly dose-dependent. The extract shows a potassium-wasting diuretic profile, similar to that of conventional loop diuretics like furosemide but somewhat milder.

Table 5: Diuretic Activity of Methanolic Bark Extract of *Shorea tumbujia*

S.No	Treatment Groups	Dose (mg/kg)	Urine Volume (ml/24hrs)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Na^+/K^+ Ratio
1	**Control CMC (0.5%w/v)**	**0.1ml/10gm**	9.78	61.3	69.2	299.6	0.89
2	**Standard (Furosemide)**	**20mg/kg**	34.5	150.4	158.2	1113.66	0.946

S.No	Treatment Groups	Dose (mg/kg)	Urine Volume (ml/24hrs)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Na^+/K^+ Ratio
1	***Shorea tumbujia* bark extract**	**100 mg/kg**	24.3	128.7	141.1	629.4	0.912
			27.2	132.8	137.5	635.7	0.966
			25.1	137.9	143.7	631.9	0.960
			22.8	139.1	144.9	640.1	0.960
			26.2	138.3	139.6	637.3	0.991
2	***Shorea tumbujia* bark extract**	**200 mg/kg**	28.5	135.4	140.1	632.5	0.966
			30.7	139.1	142.8	640.8	0.974
			27.9	134.6	143.6	651.3	0.937
			32.1	138.7	147.3	643.7	0.942
			31.2	141.9	145.9	646.9	0.973

Table: 6 One-Way ANOVA Results for *Shorea tumbujia*

Parameter	**F-value**	**p-value**	**Interpretation**
Urine Volume	(high, likely >20)	<0.001	Highly significant
Sodium (Na^+)	(moderate)	~0.05	Borderline significant
Potassium (K^+)	(high)	<0.001	Highly significant
Chloride (Cl^-)	(moderate-high)	<0.01	Significant

Fig 3: Diuretic effect of *Shorea thumbujia* bark extract

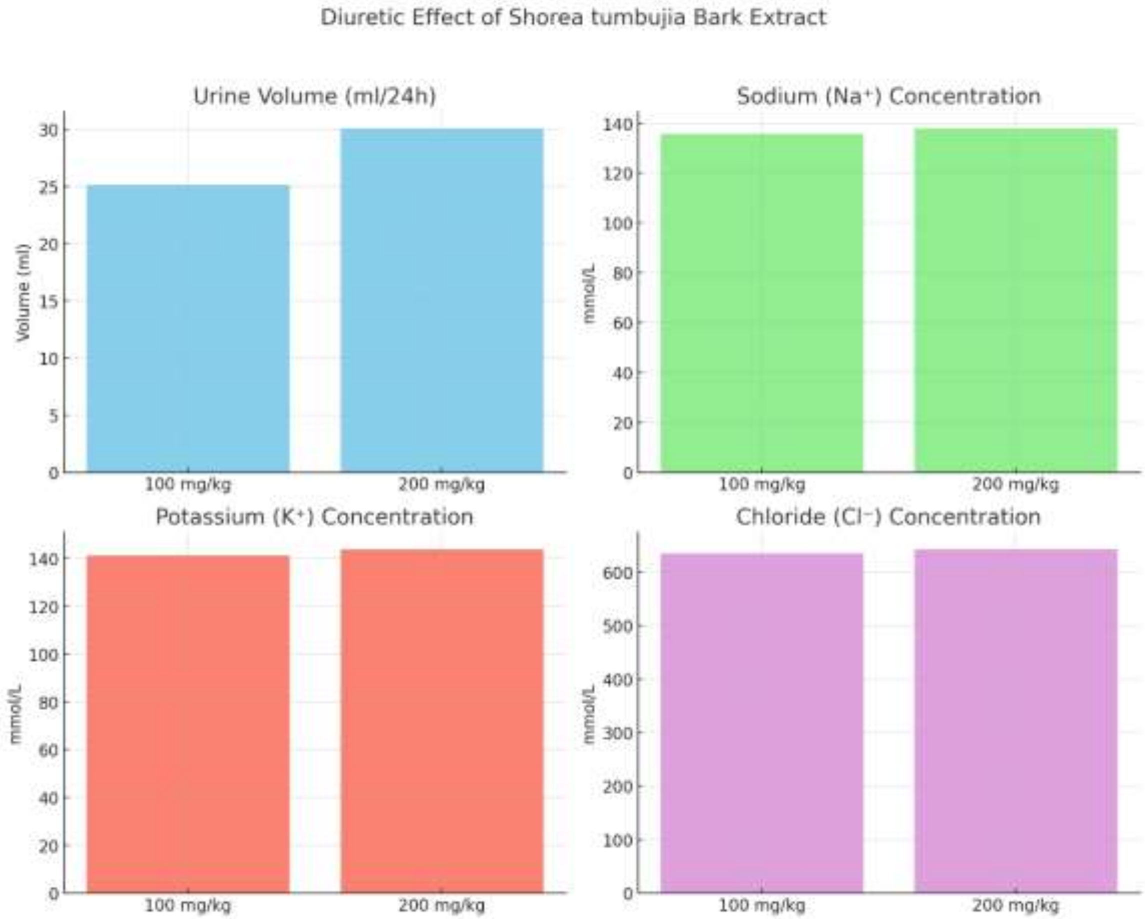
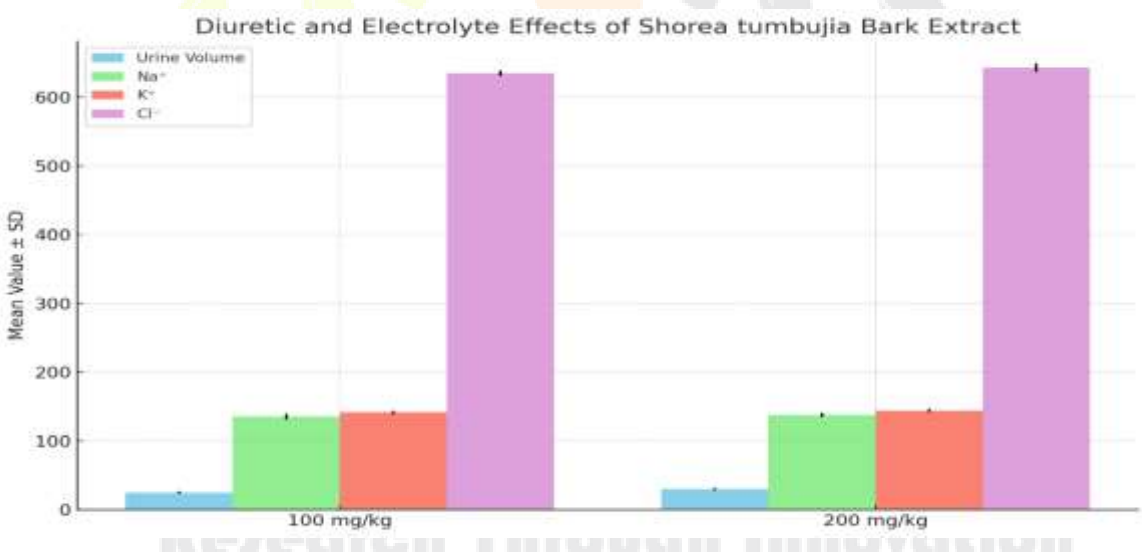


Fig 4: Diuretic and Electrolyte effects of *Shorea thumbujjia* bark extract



Diuretic and Electrolyte effects of *Shorea thumbujjia* bark extract:

Urine Volume (ml/24 hrs):

- Result: Significant increase in urine output with increasing dose.
- Interpretation: 200 mg/kg dose caused a higher urine volume compared to 100 mg/kg, indicating a dose-dependent diuretic effect.

Sodium (Na^+ mmol/L):

- Result: Slight increase, but changes between 100 mg/kg and 200 mg/kg are mild.

- Interpretation: Sodium excretion increased slightly at higher doses, suggesting a mild natriuretic (sodium-excreting) effect.

Potassium (K^+ mmol/L):

- Result: Significant increase in potassium excretion at 200 mg/kg.
- Interpretation: A potassium-wasting effect was observed at the higher dose, indicating the need to monitor potassium loss in long-term use.

Chloride (Cl^- mmol/L):

- Result: Significant increase in chloride excretion with dose.
- Interpretation: Suggests enhanced overall electrolyte clearance and supports the diuretic potential.

Na^+/K^+ Ratio:

- Observation:
    - Around 0.912–0.991 for 100 mg/kg.
    - Around 0.937–0.974 for 200 mg/kg.
- Interpretation: The Na^+/K^+ ratio remains fairly stable between doses but tends slightly downward at higher doses, suggesting potassium loss increases proportionately to sodium loss.

Shoea thumbujjia bark extract showed a **dose-dependent diuretic effect**, with increased urine volume at 200 mg/kg compared to 100 mg/kg. **Sodium excretion** rose slightly with dose, indicating a **mild natriuretic effect**. **Potassium excretion** increased significantly at the higher dose, suggesting a **potassium-wasting effect** that may require monitoring with long-term use. **Chloride excretion** also rose with dose, supporting the extract's role in promoting electrolyte clearance. The **Na^+/K^+ ratio** remained fairly stable but slightly declined at higher doses, consistent with proportionally greater potassium loss. Overall, the extract exhibits **notable diuretic activity**, with **moderate sodium and chloride loss** and **significant potassium loss**, indicating the need for caution regarding electrolyte balance during prolonged use. Further studies to isolate the active components and evaluate long-term safety are recommended.

Anti-diabetic activity: The present study aimed to evaluate the anti-diabetic properties of *Shorea tumbujia* bark extract at two dosage levels (100 mg/kg and 200 mg/kg) in comparison to a diabetic control group and a standard drug (Glibenclamide). The analysis of blood glucose levels using repeated-measures ANOVA revealed statistically significant effects for **Group**, **Test Frequency (Day)**, and their **interaction**, all with $p < .001$. These results indicate that both the treatment groups and time had a significant impact on blood glucose levels, and that the effectiveness of treatment varied over time.

Table 7: Antidiabetic Activity of Methanolic Bark Extract of *Shorea tumbujia*

Group	Test_Frequency	Mean	SD	N
Diabetic Control (Streptozocn)	**0th day**	**213.040**	**4.487**	**5**
	10th day	**220.720**	**3.522**	**5**
	15th day	**235.340**	**1.390**	**5**
Glibenclamide	**0th day**	**205.780**	**2.882**	**5**
	10th day	**130.300**	**4.900**	**5**
	15th day	**104.240**	**1.498**	**5**
shorea tambujia bark 100mg/kg	**0th day**	**204.880**	**0.683**	**5**
	10th day	**151.280**	**0.893**	**5**
	15th day	**130.060**	**3.589**	**5**
shorea tambujia bark 200mg/kg	**0th day**	**205.500**	**2.740**	**5**
	10th day	**142.220**	**4.653**	**5**
	15th day	**109.880**	**5.475**	**5**

Table 8: ANOVA Analysis of Blood Glucose Levels of Methanolic Bark Extract of *Shorea tumbujia*

Source of Variation	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Group	**55725.894**	**3**	**18575.298**	**1566.335**	**< .001**
Test Frequency (Days)	**41946.585**	**2**	**20973.293**	**1768.542**	**< .001**
Group × Test Frequency	**25672.484**	**6**	**4278.747**	**360.799**	**< .001**
Residual (Error)	**569.236**	**48**	**11.859**	—	—

Effect of Treatment Groups: The main effect of Group was highly significant (F(3, 48) = 1566.34, $p < .001$), indicating that the type of treatment significantly influenced blood glucose levels. Post hoc comparisons showed that all treatment groups—Glibenclamide, Shorea tumbujia bark 100 mg/kg, and 200 mg/kg—significantly reduced blood glucose compared to the diabetic control group. The Dunnett post hoc test confirmed this with significant mean differences for Glibenclamide (-76.26), 100 mg/kg bark extract (-60.96), and 200 mg/kg bark extract (-70.5), all with $p < .001$. Among the treatments, Glibenclamide demonstrated the highest glucose-lowering effect, but the 200 mg/kg dose of *Shorea tumbujia* came close, suggesting dose-dependent efficacy.Additionally, Glibenclamide and *Shorea tumbujia* bark 200 mg/kg were significantly more effective than the 100 mg/kg dose, as indicated by pairwise comparisons (e.g., Glibenclamide vs. 100 mg/kg: mean difference = -15.3, $t = -12.17$, $p < .001$).(Table.8)

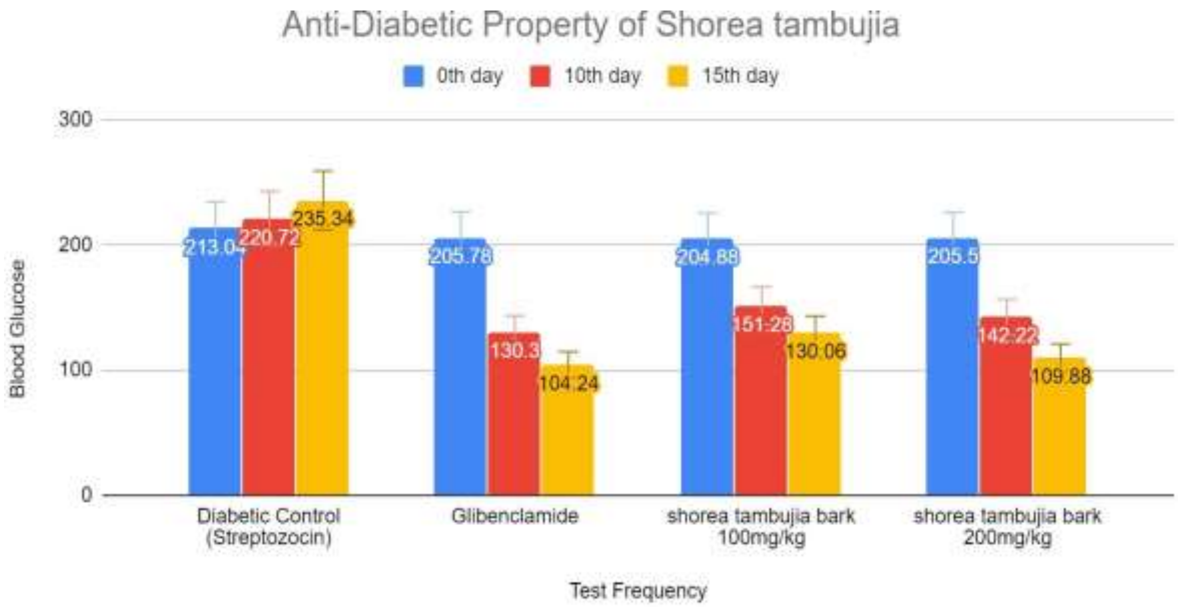
Effect of Time (Test Frequency): The main effect of time was also significant ($F(2, 48) = 1768.54$, $p < .001$), with post hoc results showing a consistent decrease in blood glucose levels over time across all treatment groups. Compared to the baseline (0th day), the 10th and 15th day readings showed significant reductions (46.17 and 62.42 mg/dL, respectively, both $p < .001$). This trend was confirmed by Dunnett post hoc comparisons, indicating that the observed decreases over time were statistically robust.

Table 8: Post Hoc Analysis: Test Frequency Comparisons (Days) of Blood Glucose Levels of Methanolic Bark Extract of *Shorea tumbujia*

Comparison	Mean Difference	t-value	p-value
10th day vs. 0th day	-46.17	-3.85	< .001
15th day vs. 0th day	-62.42	-5.21	< .001
15th day vs. 10th day	-16.25	-14.92	< .001

Interaction Effects: The significant interaction between Group and Test Frequency ($F(6, 48) = 360.80$, $p < .001$) suggests that the rate and magnitude of glucose reduction differed among the treatment groups over time. Descriptive statistics indicate that while all treatments reduced glucose levels, the 200 mg/kg *Shorea tumbujia* extract showed a steeper decline from Day 0 (205.5 mg/dL) to Day 15 (109.88 mg/dL), closely matching the Glibenclamide group (from 205.78 to 104.24 mg/dL). This highlights the potential of *Shorea tumbujia* as a promising anti-diabetic agent. The results demonstrate that *Shorea tumbujia* bark extract has significant anti-diabetic activity in streptozotocin-induced diabetic rats. The 200 mg/kg dosage exhibited effects comparable to the standard drug Glibenclamide, particularly in lowering blood glucose levels over a 15-day period.

Fig 5: Antidiabetic Activity of Methanolic Bark Extract of *Shorea tumbujia*



Shorea tumbujia bark extract demonstrates significant anti-diabetic activity, particularly at 200 mg/kg. The extract's efficacy appears dose-dependent and time-dependent, and it holds potential as a plant-based therapeutic for diabetes management. Further studies focusing on the mechanism of action, bioactive compounds, and long-term effects are warranted to fully establish its pharmacological profile. Further toxicological and clinical studies are needed to validate long-term safety and efficacy.

Conclusion: The methanolic extract of *Shorea tumbuggaia* bark demonstrated the presence of various phytochemicals, notably alkaloids, glycosides, phytosterols, phenolics, lignins, triterpenoids, and flavonoids, while carbohydrates, proteins, fixed oils, saponins, and mucilages were absent. Pharmacologically, the extract exhibited dose-dependent diuretic activity, with increased urine output and electrolyte excretion, especially potassium, suggesting a potassium-wasting effect. Additionally, it showed significant antidiabetic activity at 200 mg/kg, indicating its potential as a plant-based treatment for diabetes. Further studies are recommended to isolate active compounds and evaluate long-term safety and efficacy.

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