

To Study the Anti-Oxidant, Anti-Bacterial and Anti-Microbial Activity of *Sapindus mukorossi* Leaf Extract

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Abstract: *Sapindus mukorossi*, commonly known as soapnut or reetha, is a medicinal plant that has been traditionally used for centuries in India, China, and Southeast Asia. Most scientific studies on this plant have focused on its fruits, while the medicinal potential of its leaves has received comparatively little attention. In recent years, increasing interest in plant-based therapies has encouraged further investigation of *S. mukorossi* leaves due to their unique composition of bioactive compounds. This review summarizes traditional knowledge, botanical information, and recent experimental studies to evaluate the medicinal importance of *S. mukorossi* leaf extracts. Phytochemical studies have consistently identified the presence of flavonoids, saponins, phenolic compounds, and terpenoids, with compounds such as rutin and nicotiflorin reported in high concentrations. These bioactive constituents are associated with various biological activities, including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic effects. Common extraction methods, particularly methanolic Soxhlet extraction, have been shown to effectively isolate these compounds for pharmacological evaluation. Toxicological studies suggest that the saponins present in the leaves are safe for both topical and oral use within recommended limits. Overall, the available evidence indicates that *S. mukorossi* leaves represent a valuable yet underexplored natural resource with potential applications in medicinal, cosmetic, and nutraceutical fields.

Keywords: *Sapindus mukorossi*, Phytochemistry, Saponins, Leaf Extract, Antimicrobial Activity, Antioxidant Activity, Sapindaceae Family.

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I. INTRODUCTION

The use of medicinal plants for the prevention and treatment of disease is deeply rooted in human history. Long before the development of modern pharmaceuticals, plant-based remedies formed the foundation of healthcare systems across cultures. Over time, the systematic study of medicinal plants and their therapeutic properties has expanded scientific understanding and supported the integration of traditional knowledge into contemporary pharmaceutical and medical practices.

Sapindus mukorossi is a deciduous tree native to the tropical and subtropical regions of Asia. It has been widely used in traditional medical systems, particularly Ayurveda and traditional Chinese medicine, for the management of various health conditions. Despite its long-standing use, scientific investigations have primarily concentrated on selected parts of the plant, leaving other components relatively less explored.



Fig 1 *Sapindus mukorossi*

Taxonomically, *Sapindus mukorossi* Gaertn. belongs to the family Sapindaceae. The plant is commonly recognized for its natural cleansing properties, which are attributed to its high saponin content. These properties have led to its

widespread use by local communities as a natural detergent. The plant is known by several common names, including soapnut, soapberry, washnut, reetha, aritha, dodan, and doadni, reflecting its broad geographical distribution and traditional relevance.

In traditional medicine, *S. mukorossi* has been employed for a range of therapeutic purposes. Various parts of the plant have been used as expectorants, emetics, and contraceptives, as well as in the treatment of conditions such as excessive salivation, epilepsy, chlorosis, and migraines. In Ayurvedic practice, soapnut is a common ingredient in herbal shampoos and skin-cleansing formulations and is traditionally used for managing dermatological disorders such as eczema and psoriasis, as well as for improving skin appearance by reducing freckles.

The long history of traditional use, combined with increasing scientific interest in plant-derived bioactive compounds, highlights the importance of further research on *Sapindus mukorossi*. A better understanding of its phytochemical composition and pharmacological properties may support its potential applications in modern medicine, cosmetics, and related industries.

II. OBJECTIVES

The main objective of this review is to critically evaluate the phytochemical composition and pharmacological potential of *Sapindus mukorossi* leaves, with particular emphasis on their therapeutic relevance.

➤ *The Specific Objectives of the Study are as Follows:*

- To examine the phytochemical constituents present in *Sapindus mukorossi* leaves.
- To review the reported antimicrobial and antifungal activities of leaf extracts against pathogenic microorganisms.
- To assess the anti-inflammatory and antioxidant properties associated with *S. mukorossi* leaf extracts.
- To explore the therapeutic potential of major bioactive metabolites, particularly rutin and nicotiflorin.
- To summarize the traditional, historical, and ethnobotanical uses of *Sapindus mukorossi*.
- To review and compare methods used for the extraction of bioactive compounds from the leaves.
- To evaluate the possible medicinal applications of *S. mukorossi* leaves based on available pharmacological evidence.
- To identify research gaps and suggest future directions for scientific investigation.

This review aims to enhance understanding of *Sapindus mukorossi*, a plant known for its saponin-rich composition and diverse biological activities, including anti-inflammatory, antimicrobial, hepatoprotective, and anxiolytic effects.

III. LITERATURE REVIEW

➤ *Historical and Ethnobotanical Uses*

Sapindus mukorossi is a historically significant tree species that has been associated with human use for several centuries, particularly in India and China. References to soapnut appear in ancient historical and cultural records, indicating its importance in early civilizations. According to traditional accounts cited in historical literature, soapnut trees were present in India as early as the sixth century BC. Cultural texts such as the *Historical Dictionary of Ancient India* document the presence and utilization of soapnuts during ancient periods. Additionally, traditional narratives mention that Matsyendra Nath, the founder of Hatha Yoga, is believed to have attained enlightenment beneath a soapnut tree during the ninth or tenth century, highlighting the plant's cultural and spiritual relevance.

Historical observations on the use of soapnuts, soap-making practices, and the washerman community in India between 300 BC and 1900 AD further suggest that *S. mukorossi* was widely used even before documented records. These historical accounts underline the long-standing association between the plant and traditional hygiene, medicine, and daily life.

In northeastern India, particularly in parts of Meghalaya, *Sapindus mukorossi* grows naturally and is deeply integrated into local traditions. Among the Khasi and Jaintia communities, the plant is locally known as "Sohpairah" and "Sohpinrah," respectively. According to residents of Sahsniang and surrounding villages, the tree has been present in the region for more than 150 years. The fruits have been traditionally used as a natural soap for washing clothes and hair for several generations.

Ethnomedicinal practices across different regions report diverse therapeutic uses of *S. mukorossi*. The fruits have been traditionally employed in the treatment of snake bites, scorpion stings, and dandruff. They are also used as expectorants, demulcents, emetics, anthelmintics, and purgatives, and have been applied in conditions such as epilepsy and cholera. In the Indian state of Assam, a paste prepared from the fruit is traditionally used as a febrifuge. Seeds of the plant have been utilized for dental care, including the management of dental caries, and are also used to bleach cardamom seeds to enhance the color and flavor of spices.

Beyond medicinal uses, soapnuts have played an important role in traditional industries. They have been widely used as a natural detergent for washing clothes and are preferred by Indian jewelers for restoring shine to tarnished ornaments. In Kashmir, soapnuts are traditionally considered ideal for washing woolen shawls due to their mild cleansing action and fabric-preserving properties. These ethnobotanical uses collectively demonstrate the multifunctional value of *S. mukorossi* in traditional societies.

➤ *Description and Morphology*

Sapindus mukorossi is a medium to large-sized deciduous tree that typically grows to a height of

approximately 15–20 meters. The trunk is erect and covered with grayish-brown bark, which becomes rough and deeply furrowed as the tree matures. The tree develops a broad,

spreading crown with numerous branches, giving it a distinctive and robust appearance within its natural habitat.



Fig 2 *Sapindus mukorossi* Gaertn Showing Leaves and Immature Fruits

The leaves are compound and alternately arranged along the branches. They are paripinnate and measure approximately 30–50 cm in length. Each leaf consists of 5–10 pairs of leaflets that may be opposite or alternate in arrangement. The leaflets are lanceolate, acuminate, and have entire margins, with a smooth and glabrous surface. Slight curvature or obliqueness is often observed. Seasonal leaf fall occurs during December and January, with the foliage turning yellow before shedding. The tree remains leafless until new growth emerges during March or April.



Fig 4 Flower

Flowering typically occurs in late spring to early summer. The plant produces panicles of small, aromatic, greenish-white to slightly purplish bisexual flowers during May and June. These flowers later develop into drupaceous fruits, which mature between October and November. The fruits remain attached to the tree until January or later, making them highly visible when the tree is leafless.

The mature fruits are round, yellow to orange-brown in color, and measure approximately 2–2.5 cm in diameter. They possess a tough outer pericarp and a fleshy pulp that becomes firm upon drying. Each fruit contains a hard seed embedded in a sticky, viscous matrix rich in saponins. These saponins are responsible for the characteristic foaming and cleansing properties of the soapnut and represent the primary bioactive constituents of pharmacological and commercial interest.



Fig 3 Fruits



Fig 5 Dried fruits of *Sapindus mukorossi* Gaertn. illustrating the pericarp and separated seeds.

Sapindus mukorossi (soapberry) is widely studied due to the presence of triterpenoid saponins in its fruit pericarp. The species produces small drupaceous fruits with a leathery

outer layer that changes in colour from yellow to darker shades during ripening, typically enclosing one to three seeds. The bark shows noticeable variation in colour, ranging from pale yellow to darker tones, and is generally smooth with visible vertical lenticels and irregular fissuring.

➤ *Phytochemistry*

Plants belonging to the family *Sapindaceae* are known to contain a diverse range of secondary metabolites, including saponins, cyanogenic glycosides, and cyclitols. Among these, *Sapindus mukorossi* has been extensively studied due to the widespread presence of saponins across nearly all parts of the plant, with reported concentrations ranging from approximately 10% to 11.5%. These compounds are considered to be the primary contributors to the various pharmacological activities associated with the species.

Phytochemical investigations have revealed a complex chemical profile in *S. mukorossi*, with more than one hundred compounds identified from its fruits, seeds, and leaves. These include flavonoids, triterpenoids, carbohydrates, fatty acids, phenolic compounds, fixed oils, and saponins. The seeds are particularly rich in oil content, accounting for nearly one-quarter of their composition, with triglycerides forming the major fraction. In addition, the fruits have been reported to contain sesquiterpenoid glycosides and several fatty esters derived from tetracyclic triterpenoids.

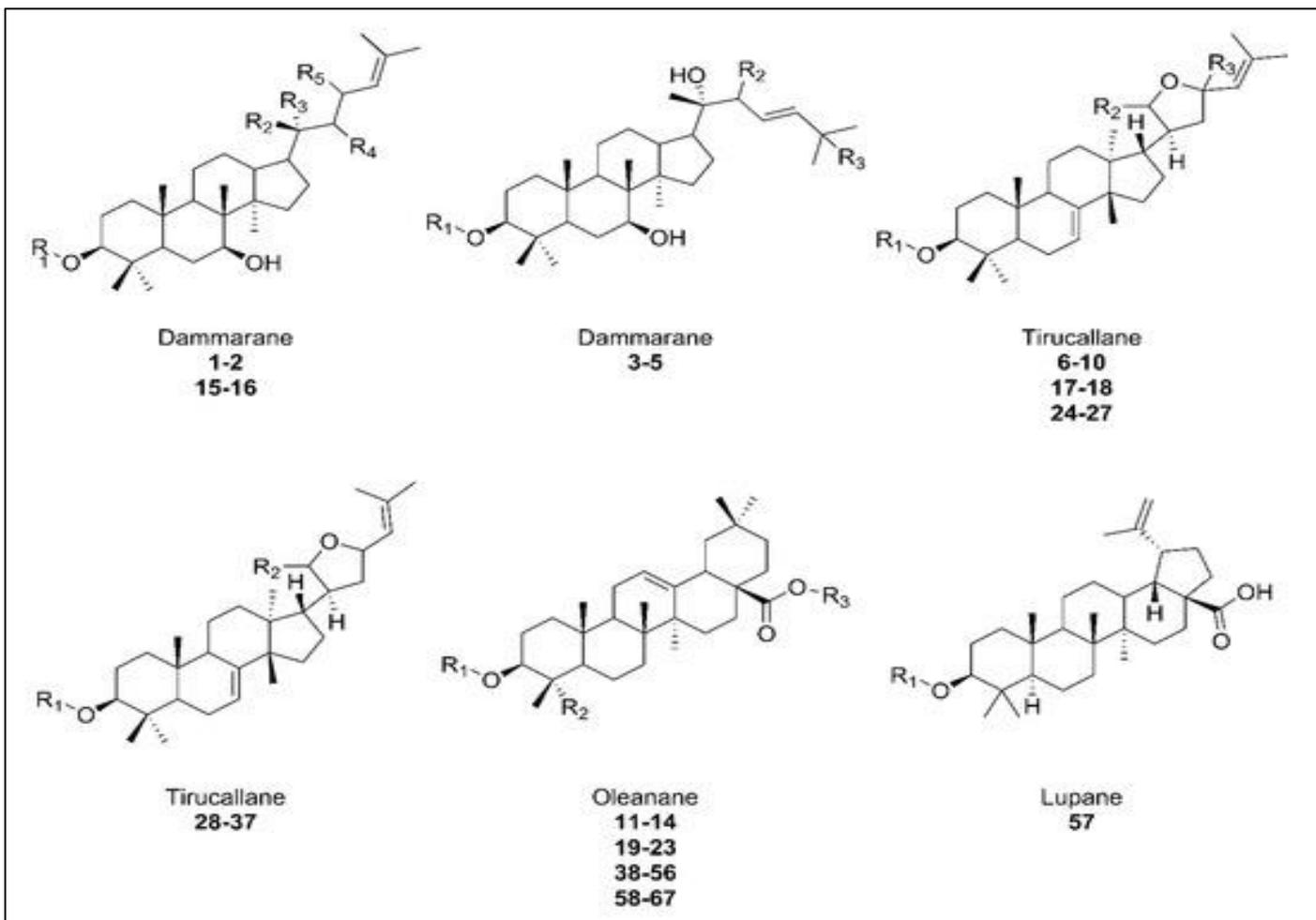


Fig 6 Overview of Saponin Structures Present in *S. mukorossi*.

Various triterpene saponins belonging to oleanane, dammarane, and tirucallane structural types have been isolated from different plant parts such as galls, fruits, and roots, highlighting the chemical diversity of this species. Quantitative analyses further indicate that the distribution of phytochemicals varies significantly among plant organs.

Total saponin content is consistently higher than total flavonoid content across all examined tissues, with the pericarp showing the highest saponin concentration, followed by flowers and leaves. In contrast, flavonoid levels are most prominent in leaves, with comparatively lower concentrations observed in reproductive and woody parts.

Table 1 Major Phytochemical Constituents of *Sapindus mukorossi* and Their Biological Activities

Compound Type	Major Examples	Noted Activities
Saponins	Oleanane, dammarane	Cleansing, antimicrobial
Flavonoids	Rutin, nicotiflorin	Antioxidant, anti-inflammatory
Fatty acids, Phenols	Triglycerides, others	Nutritional, wound healing

➤ Flavonoid Content and Quantification in *Sapindus mukorossi* Leaf Extracts

Although research on *Sapindus mukorossi* has traditionally focused on its fruits due to their high saponin content, increasing attention has recently been directed toward the phytochemical potential of its leaves. Leaf extracts have been investigated for their flavonoid composition as well as for associated antioxidant, antimicrobial, antitumor, and safety-related properties. Phytochemical studies indicate that the leaves contain several biologically significant flavonoids, including quercetin, apigenin, kaempferol, and rutin, which have been isolated using chromatographic techniques such as column chromatography with polyamide sorbents.

Flavonoids constitute an important group of plant secondary metabolites characterized by polyphenolic structures and are widely distributed in plant-derived foods and medicinal materials. Their relevance in nutraceutical, pharmaceutical, and cosmetic applications has been attributed to a broad range of biological activities, including antioxidant, anti-inflammatory, antimutagenic, and anticancer effects, as well as their ability to influence key cellular enzyme systems.

Quantitative analysis of *S. mukorossi* leaf extracts has demonstrated notable variation in flavonoid content depending on sample origin. Among the evaluated extracts, leaf samples collected from Suwon and Daegu exhibited the highest total phenolic content, expressed as tannic acid equivalents. Total flavonoid content, determined using an aluminum chloride colorimetric assay, followed a similar trend, with these samples showing comparatively elevated values when expressed as quercetin equivalents. Advanced analytical techniques, including liquid chromatography–tandem mass spectrometry, enabled the detection of multiple flavonoid compounds, while high-performance liquid chromatography was employed for the quantification of major constituents.

Rutin, nicotiflorin, and related flavonol glycosides were identified as prominent compounds in the leaf extracts, with rutin being the most abundant. The contribution of rutin to the overall flavonoid profile was particularly significant in the Suwon and Daegu samples, accounting for a substantial proportion of the total quantified flavonoids. This predominance suggests that rutin plays a major role in the biological activities attributed to *S. mukorossi* leaf extracts.

Nicotiflorin, a glycosylated derivative of kaempferol, has also attracted attention due to its reported pharmacological relevance. Experimental studies have suggested its inhibitory activity against α -glucosidase, indicating potential antidiabetic effects, while computational approaches have proposed possible interactions with viral enzymes such as SARS-CoV-2 proteases. Rutin, another key flavonoid identified in the leaves, is widely recognized for its anti-inflammatory properties and its ability to modulate oxidative stress and endothelial function, further supporting the therapeutic potential of *S. mukorossi* leaf-derived flavonoids.

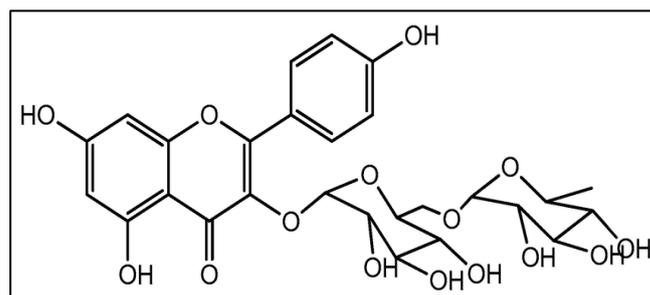


Fig 7 Nicotiflorin

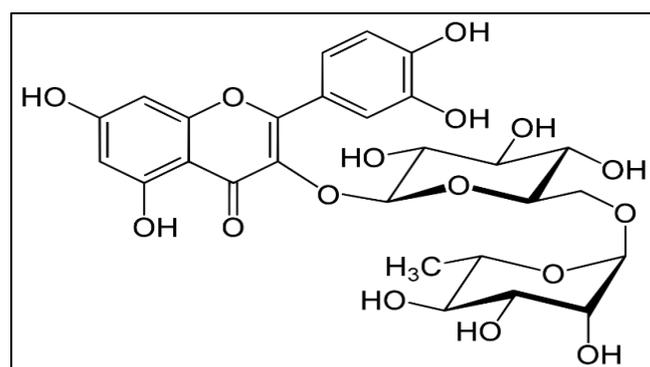


Fig 8 Rutin

➤ Phytochemical Screening and Spectroscopic Analysis of *Sapindus mukorossi* Leaf Extract

Sapindus mukorossi is a medicinally important plant known for producing a wide range of health-promoting secondary metabolites. In order to characterize the chemical constituents of the leaves, preliminary phytochemical screening was conducted, followed by spectroscopic and chromatographic analyses of the methanolic leaf extract.

These approaches were employed to obtain an overall profile of the bioactive compounds present in the extract.

Preliminary phytochemical tests indicated the presence of several major classes of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, saponins, terpenoids, and carbohydrates. To further explore the chemical composition, gas chromatography–mass spectrometry (GC–MS) analysis was carried out on the methanolic extract, enabling the identification of bioactive compounds based on their separation patterns and mass spectral characteristics.

Spectroscopic techniques were also applied to support the phytochemical findings. Ultraviolet–visible (UV–Vis) spectral analysis revealed characteristic absorption peaks, suggesting the presence of various secondary metabolites in the leaf extract. Functional group analysis was performed using Fourier-transform infrared (FTIR) spectroscopy, which provided additional information regarding the chemical nature of the detected compounds. In addition, thin-layer chromatography was used for comparative metabolite profiling, employing a chloroform–methanol solvent system (90:10, v/v) to achieve effective separation of the constituents.

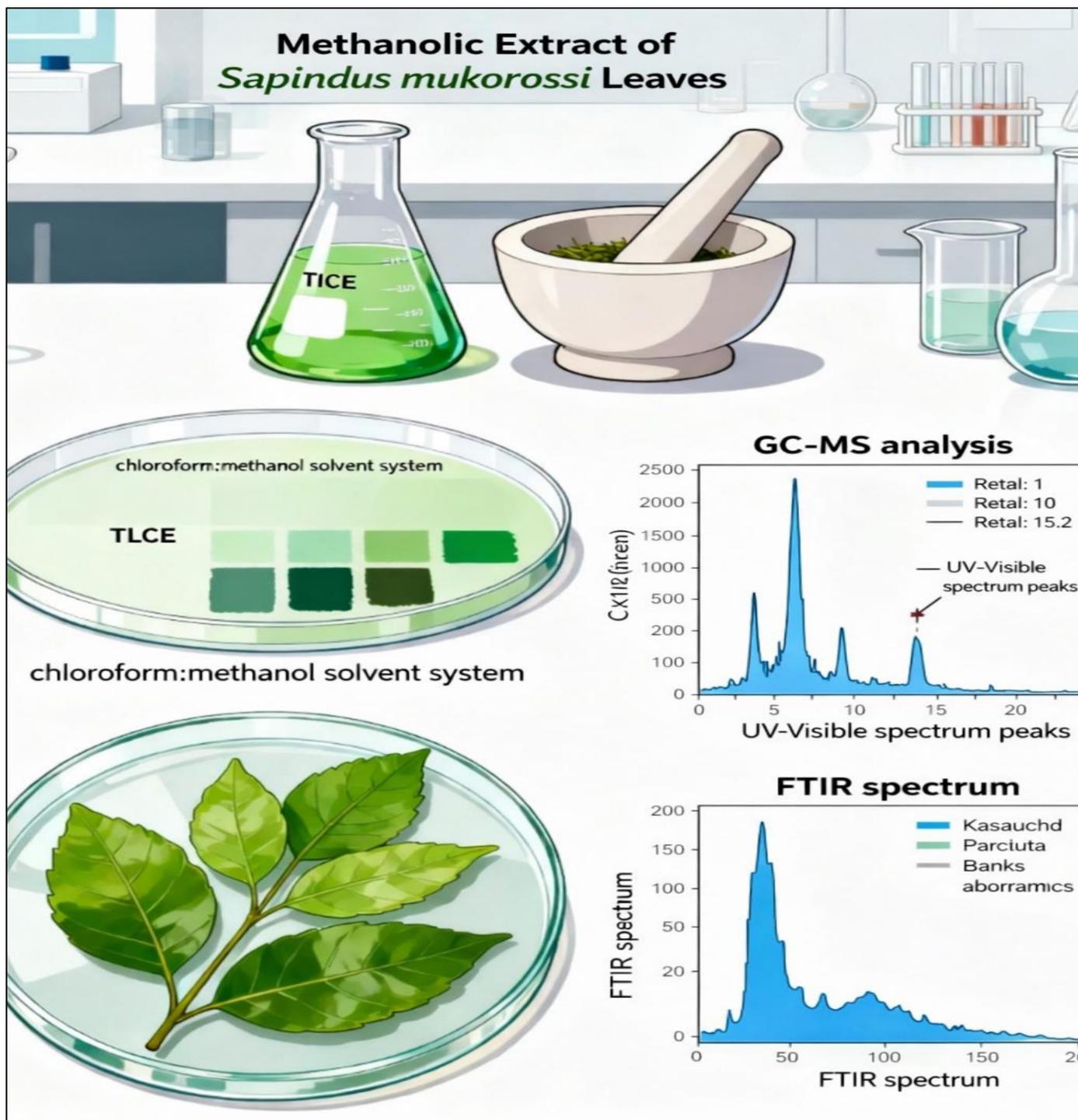


Fig 9 Methanolic Extract of *Sapindus mukorossi* Leaves

➤ *Pharmacological Action:*

Sapindus mukorossi demonstrates a wide spectrum of pharmacological activities, largely attributed to its rich content of triterpenoid saponins, flavonoids, and other bioactive compounds distributed across the leaves, fruits, seeds, and pericarp. Antibacterial effects have been reported for both leaf and pericarp extracts, including inhibition of *Helicobacter pylori* in vitro and in vivo. The pericarp saponins have also been evaluated for spermicidal and contraceptive applications, inducing structural damage to human sperm and being incorporated into topical formulations.

Anti-inflammatory properties are observed in extracts from the whole plant, where saponins reduce carrageenan-induced edema and suppress proinflammatory cytokine production. Anticancer activity has been reported from gall-derived saponins, which promote apoptosis and exhibit cytotoxic effects against various tumor cell lines.

Hepatoprotective effects are noted for pericarp extracts, particularly against chemically induced liver damage, while hydroalcoholic extracts have demonstrated antidiabetic activity through the reduction of blood glucose and lipid levels in experimental models. Stem bark extracts have shown antipyretic and analgesic effects, lowering fever and alleviating pain in animal studies.

Additional pharmacological actions include wound healing, anxiolytic effects, and molluscicidal or insecticidal activity. Seed oil has been shown to accelerate wound closure in rats, and extracts from fruit and seeds display anxiolytic activity in animal models. Moreover, fruit extracts exert toxic and repellent effects against agricultural pests, highlighting their potential as natural biocontrol agents.

Overall, these studies collectively demonstrate the therapeutic versatility of *S. mukorossi*, supporting its use in both traditional and modern medicinal applications.

Table 2 Reported Pharmacological Activities of *Sapindus mukorossi*

Pharmacological Action	Active Part/Extract	Key Activity
Antibacterial	Leaf, pericarp	Inhibits <i>Helicobacter pylori</i> in vitro and in vivo
Spermicidal/contraceptive	Pericarp saponin	Causes structural damage to human sperm, used in creams
Anti-inflammatory	Whole plant, saponins	Reduces carrageenan-induced edema, inhibits cytokines
Anticancer	Gall saponins	Induces apoptosis, cytotoxic against tumor cell lines
Hepatoprotective	Pericarp extract	Protects against carbon tetrachloride-induced damage
Antidiabetic	Hydroalcoholic extract	Lowers blood sugar and lipid levels in diabetic mice
Antipyretic/Analgesic	Stem bark extract	Lowers fever and relieves pain in animal models
Wound healing	Seed oil	Accelerates wound closure in rats
Anxiolytic	Fruit & seed extract	Shows significant activity in animal models
Molluscicidal/Insecticidal	Fruit extract	Toxic and repellent to agricultural pests

➤ *Pharmacological Action (leave extract)*

• *Antioxidant Activity:*

The methanolic extract of the fruit exhibited the highest total phenolic content, measured at 469.00 ± 0.57 mg/g gallic acid equivalent, while the methanolic leaf extract showed the maximum total flavonoid content of 540.11 ± 0.89 mg/g rutin equivalent. Both leaf and fruit aqueous extracts demonstrated significant reducing potential. A positive correlation was observed between the antioxidant activity and the levels of polyphenolic compounds, including total phenolics and flavonoids. Additionally, a strong correlation between antioxidant activity and reducing potential suggests that reducing agents in the extracts are major contributors to the observed antioxidant properties. These findings indicate that extracts from this plant may have potential applications in both pharmaceutical and nutraceutical industries.

• *Antibacterial Activity:*

The antibacterial potential of various *S. mukorossi* Gaertn. extracts was assessed against *Cutibacterium acnes*. Extracts were evaluated based on saponin yield and minimum inhibitory concentration (MIC). Among them, the optimized ethyl acetate extract derived from water extract with isoamyl alcohol (SWFEAI) exhibited the highest saponin content ($7.83 \pm 0.26\%$) and demonstrated the strongest antibacterial activity (MIC = 0.125 mg/mL). Transmission electron

microscopy revealed that SWFEAI caused disruption of the bacterial cell membrane and wall, leading to cell lysis and death. Analysis identified 20 compounds in SWFEAI, including oleanane-type triterpenoid saponins with molecular weights of 734, 750, 882, 924, and 966, which are likely responsible for the antibacterial effects. These results suggest that SWFEAI could serve as a natural anti-acne agent.

• *Antimicrobial Activity:*

Phytomedicines and their derivatives are gaining global attention as alternative therapies. In vitro studies of *S. mukorossi* extracts have demonstrated antimicrobial activity. Although some extracts showed moderate effects, their activity could be enhanced by increasing the concentration or further purifying the extract. The use of plant-derived extracts in a holistic approach offers a promising, cost-effective strategy for treating various ailments and could expand the scope of modern medical therapies.

➤ *Extraction of Leaf Extract*

A *Sapindus mukorossi* leaf extract can be prepared using solvent extraction, most effectively with methanol. The following standardized steps are derived from published phytochemical extraction protocols and research reports;

• *Materials*

- ✓ Fresh or shade-dried *Sapindus mukorossi* leaves
- ✓ Methanol (analytical grade) or distilled water (for aqueous extract)
- ✓ Soxhlet extractor or reflux setup

- ✓ Rotary evaporator or water bath
- ✓ Whatman filter paper (No. 1 or 5)
- ✓ Mortar and pestle or grinder



Fig 10 Extraction of Leaf Extract

Leaves were collected from the Banaras Hindu University campus, Varanasi, in April. Callus and in vitro leaves (IVL) were prepared following Singh et al. [16]. The callus was shade-dried at room temperature for 4–5 days, followed by drying at 40–45°C for 2 hours, and then ground into a coarse powder using a mechanical grinder. Five grams of callus powder were extracted with 150 mL of solvent for

10 hours using a Soxhlet apparatus. Solvents used included ethanol, methanol, and double-distilled water. Extracts were concentrated at 40°C using a rotary evaporator and stored at -20°C. Test samples were prepared in different concentrations in their respective extraction solvents. For in vitro leaves, 200 mg of leaf material was ground in a mortar with solvent to obtain a final extract volume of approximately 20 mL.

• *Procedure*

✓ *Collection and Drying*

Collect mature *S. mukorossi* leaves, wash them with distilled water to remove impurities, and dry them in shade for 7–10 days until crisp.

✓ *Powder Preparation*

Grind the dried leaves into a coarse powder using a clean grinder and sieve through mesh (40–60 µm) to ensure uniformity.

✓ *Extraction*

▪ *Methanolic Extraction (Preferred):*

Weigh 50 g of powdered leaves and place in a Soxhlet apparatus. Add 250–300 mL of methanol and extract at 40–50°C for 5–6 hours or until the solvent in the siphon tube becomes colorless.

▪ *Aqueous Extraction (Alternative):*

Mix equal weight of leaf powder and distilled water at a ratio of 1:3 (w/v). Stir and incubate overnight at 4°C, then filter and freeze-dry to concentrate the extract.

✓ *Filtration and Concentration*

Filter the extract using Whatman filter paper to remove debris. Concentrate the filtrate under reduced pressure using a rotary evaporator at 40–50°C until a thick residue remains.

✓ *Drying and Storage*

Dry the concentrated extract in a desiccator or by freeze-drying. Store in an airtight amber container at 4°C for further use in phytochemical or biological assays.

- Methanol is an effective solvent for extracting flavonoids, saponins, tannins, and terpenoids present in *S. mukorossi* leaves.
- Aqueous extracts are preferable for bioassay or cytotoxicity studies due to their non-toxic nature.

This method yields a standardized crude *Sapindus mukorossi* leaf extract suitable for analysis or formulation research applications.

✓ *Related*

- Optimal solvent and ratio for *Sapindus mukorossi* leaf extraction
- Best temperature and time for hot-water extraction from leaves
- How to purify and concentrate *Sapindus* saponins from extract
- Safe decolorization methods for *Sapindus* leaf extracts
- How to test saponin content and antimicrobial activity of extract

➤ *S. mukorossi Saponin Extract: Safety for Cosmetic Use*

Based on the toxicity classification outlined in the *Hygienic Standard for Cosmetics* (2002), the saponin extract from *S. mukorossi* is categorized as “practically nontoxic”

and “non-dermal irritant.” These findings indicate that the saponin extract is safe for use in cosmetic formulations, supporting its potential as a natural ingredient in personal care products.

IV. RESULTS

Sapindus mukorossi leaf extracts demonstrated notable bioactive properties, including antioxidant, antibacterial, and antimicrobial activities, alongside a favorable safety profile. Methanolic extracts were particularly rich in flavonoids and saponins, while aqueous extracts contained high levels of phenolic compounds. Correlations between polyphenolic content, reducing potential, and antioxidant activity were observed, confirming the functional role of these compounds. The antibacterial effects, especially against *Cutibacterium acnes*, were linked to oleanane-type triterpenoid saponins. Collectively, these findings underscore the leaves of *S. mukorossi* as a promising source of bioactive compounds for both medicinal and commercial applications.

V. CONCLUSION

The leaves of *Sapindus mukorossi* constitute a valuable and underexplored resource for the development of bioactive formulations. Their rich phytochemical composition and diverse pharmacological activities highlight their potential for use in natural product-based applications, particularly within the cosmetic and pharmaceutical industries. Future research focusing on standardization of extracts, optimization of formulations, and clinical validation will be essential to enable their effective and safe commercialization.

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