

PHYTOCHEMICAL CHARACTERIZATION AND STANDARDIZATION OF TAMARINDUS INDICA

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ABSTRACT

Tamarindus indica is a commonly used medicinal plant with a rich phytochemical composition and a variety of pharmacological qualities. The goal of the current study is to conduct a thorough inquiry that includes Tamarindus indica authentication, isolation, extraction, separation, phytochemical screening, and standardization. To guarantee botanical identification and purity, the plant sample was authenticated using both macroscopic and microscopic assessment. A wide range of phytoconstituents were extracted using appropriate solvents with increasing polarity. To separate and isolate bioactive components from the crude extracts, chromatographic techniques such thin-layer chromatography (TLC) and column chromatography were used. Major secondary metabolites that are known to contribute to the plant's medicinal potential, including alkaloids, flavonoids, tannins, saponins, glycosides,

and phenolic compounds, were found during preliminary phytochemical screening. Advanced analytical techniques were used to further characterize the isolated chemicals. By analyzing physicochemical parameters such as ash values, extractive values, moisture content, and fluorescence analysis, the plant extract was standardized. Furthermore, chromatographic fingerprinting was created to guarantee the herbal material's quality, repeatability, and consistency. The study supports the use of Tamarindus indica in both traditional and

contemporary medicine by offering a scientific foundation for its identification, quality assurance, and therapeutic application.

KEYWORDS:- Tamarind indica, Extraction, TLC, Antimalarial.

INTRODUCTION

Tamarind, or *Tamarindus indica* L., is a tropical evergreen tree that is a member of the Fabaceae (Leguminosae) family. It is extensively found throughout the world's tropical and subtropical climates, especially in Mexico, Thailand, and India. The plant is thought to have originated in Africa before being brought to the Indian subcontinent, where it has developed into a significant culinary and medicinal herb.^[5]

The big, long-lived *Tamarindus indica* tree yields edible pods with a sour pulp that is used in traditional medicine and as a food ingredient. The fruit pulp, seeds, bark, and leaves are among the various plant parts with important medicinal qualities. The herb has been used to treat microbiological infections, fever, inflammation, constipation, and digestive issues in traditional medical systems like Ayurveda.

The study of crude pharmaceuticals derived from natural sources, including plants, animals, and minerals, with a focus on their macroscopic, microscopic, physicochemical, and phytochemical properties, is known as pharmacognosy. In addition to preventing adulteration and substitution, pharmacognostic evaluation is essential for the identification, authenticity, and quality control of medicinal plants.

As a result, the pharmacognostic study of *Tamarindus indica* is crucial for determining the plant material's phytochemical components, standardization criteria, and diagnostic characteristics. These investigations support its safe and efficient use in herbal medicine and add to the scientific justification of its traditional usage.^[3]

Plant Profile

Synonyms: Imli (Hindi), Tamarind, Indian Date, and *Tamarindus* Biological Source: Tamarind is made from the dried, ripe fruit pulp of *Tamarindus indica*, a member of the Fabaceae (Leguminosae) family.^[1]

Macroscopic Characters

Fruit: Brown, straight or curved pod (legume) Pericarp: Hard, brittle outer shell; length: 5–15 cm Pulp: Sweet-sour, sticky, reddish-brown Three to ten firm, glossy brown seeds are found inside the pulp. Odor: A faint, distinctive Taste: Sweet and acidic.^[8]



Fig. Tamarind Indica Leaves & Fruits.

Tamarindus indica is classified as follows in taxonomy

Kingdom-Plantae Subkingdom-Tracheobionta (Vascular plants)

Division: Magnoliophyta (flowering plants and angiosperms)

Class: Dicotyledons (Magnoliopsida)

Fabales Order

Family: Fabaceae (family of legumes)

Caesalpinioideae subfamily

Tamarindus is the genus.^[11]

Chemical Constituents: Tamarindus indica is classified as follows in taxonomy

5-Hydroxymethyl furfural (HMF): Found to be the primary constituent (about 30.74%) in fruit pulp chloroform extracts that showed the strongest antimalarial activity against Plasmodium falciparum.

The leaves contain triterpenes, such as lupanone and lupeol, which have antibacterial and possibly antiparasitic properties.

Polyphenols and Flavonoids: The fruit and seeds include compounds such catechin, epicatechin, and procyanidin B2 that have anti-inflammatory and antioxidant properties. By stabilizing red blood cell membranes, these compounds may promote the overall antimalarial action.

Tannins: Found in fruit and bark, they are said to have antiparasitic properties by attaching to free proteins or glycoproteins outside of the parasite, killing it.

Volatile Constituents: The fruit pulp's therapeutic qualities are attributed to the presence of carboxylic acids and furan derivatives. The following are important components of tamarind pulp: Acids that are organic Citric acid, malic acid, and tartaric acid (major) Carbohydrates: pectin, fructose, and glucose Other substances Polysaccharides, tannins, flavonoids, and potassium bitartrate Tamarind seed polysaccharide, proteins, and fixed oil are found in seeds.^[13]

Pharmacological Uses

Studies have shown that many components of the plant, especially the leaves and fruit pulp, have strong antimalarial and antiplasmodial properties. *Tamarindus indica*, or tamarind, is utilized as an antimalarial medication in traditional medicine throughout Africa and Asia.

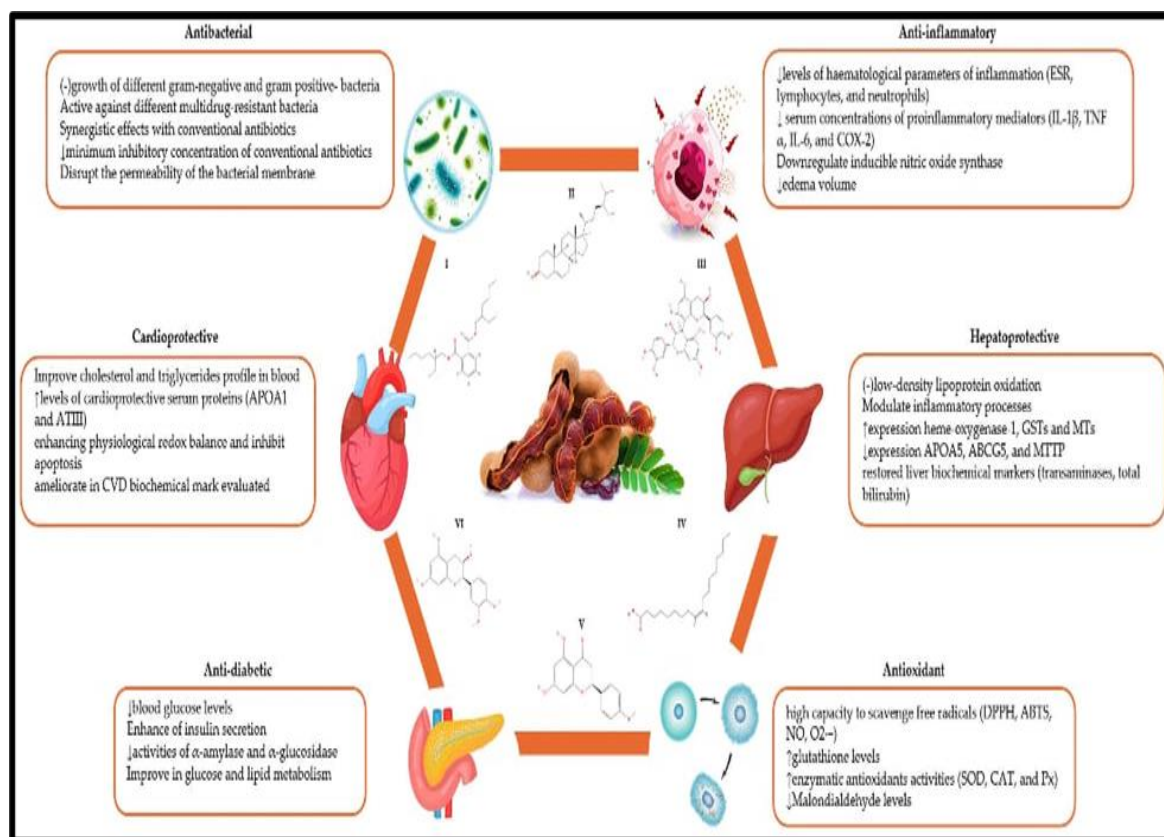


Fig. Therapeutic Uses of Tamarind Indica.

Antimalarial and Antiplasmodial Properties Plasmodium berghei, a parasite used to simulate malaria in mice, can be suppressed by up to 81.09% at a dose of 650 mg/kg, according to research on the aqueous extract of tamarind fruits.

Leaf and Fruit Efficacy: Traditional medicine makes use of both fruit pulp and leaves. When compared to control groups, ethanol extracts of leaves have demonstrated a notable in vivo decrease in parasitaemia (up to 85.71%).

Mechanism of Action: Phytochemicals such as tannins, flavonoids, saponins, and alkaloids are probably responsible for the antimalarial action. These substances function as antiparasitic agents; research indicates that they attach to the parasite's surface glycoproteins or free proteins, killing it.

Febrifuge Properties: In places like Madagascar, Sudan, and the Sahel, tamarind fruit is frequently used as a febrifuge (fever-reducer), which helps control malaria symptoms.

Immune System Support: The plant's high vitamin C and B content helps strengthen the body's defenses against illness.

Tamarind exhibits a number of therapeutic properties: Laxative: slightly relieves constipation; digestive stimulant: enhances appetite and digestion; antipyretic: lowers fever; antioxidant: because of polyphenols and flavonoids; antimicrobial: inhibits certain bacteria and fungus; and hypolipidemic: may help lower cholesterol.^[16]

MATERIAL AND METHODOLOGY

Plant Collection and Authentication

The verification of the plants under research is a crucial component of the technique because there are multiple species from the same genus and they may resemble other plants physically. The proper plant species and plant parts utilized as raw materials for scientific research on therapeutic plants are guaranteed by authentication. The Botanical Survey of India, Pune, identified and verified the plant *T. indicus*, which was obtained from the Department of Pharmacognosy, College of Pharmacy, Chopda (Jalgaon, Maharashtra).^[18]

Extraction of Active Constituents from *T. Indicus* leaves

Powered leaves and fruits weighing about fifty (50) grams each were macerated for twenty-four hours at room temperature in 500 milliliters of distilled water and methanol, respectively. After filtering each preparation through Whatman filter paper, the aqueous filtrate was dried in a water bath at 40°C while the methanol extract was dried in a rotary evaporator at 50°C. The residue was then diluted with 10% Dimethylsulphoxide (DMSO) to create 100 mg/ml of the extract, from which different concentrations of 50, 40, 30, 20, and 10 mg/ml.^[19]

Phytochemical Screening of Crude ethanol and aqueous Extracts of Tamarindus Indica leaf

Test for alkaloids:- 0.5g of each extract was mixed with 5ml of 1% aqueous hydrochloric acid in a steam bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent, and another 1ml portion was treated similarly with Dragendorff's reagent. The presence of alkaloids in the extracts was initially indicated by the observation of turbidity or precipitation using any of these reagents.^[4]

Test for saponins:- a test tube containing around 0.5g of each plant extract was shaken with water. Preliminary evidence for the existence of saponins was recognized as frothing that persists when heated.^[16]

Test for Tannins: Ferric chloride reagent was added to the filtrate after 0.5g of each plant extract was mixed with 1ml of distilled water and filtered. The presence of tannins was indicated by a blue-black, green, or blue-green precipitate.^[8]

Test for anthraquinones:- put about 0.5g of each extract in a dry test tube, add 5ml of chloroform, and shake for five minutes. After filtering the extract, the filtrate was shaken with an equal volume of 100% ammonia solution. The presence of free anthraquinones was indicated by a pink, violet, or red color in the ammoniacal layer (lower layer).^[18]

Test for glycosides:- put 100 grams of each extract in a test tube, add 2.5 milliliters of diluted sulfuric acid, and boil the mixture in a water bath for 15 minutes. After cooling, a 20% potassium hydroxide solution was used to neutralize this. After adding five milliliters of a combination of Fehlings Solutions A and B, the mixture was cooked for three minutes. The hydrolysis of a reducing sugar, a sign of a glycoside, was suggested by a brick-red precipitate.^[12]

Keller Kiliani test for cardiac glycosides: One drop of ferric chloride solution was added to two milliliters of glacial acid and around 0.5 grams of extract diluted to five milliliters in water. One milliliter of pure sulfuric acid was used to underplay this. The presence of a deoxysugar was shown by a brown ring at the contact. characteristic of cardenolides. The brown ring may be accompanied by a violet ring, although in Just above the brown ring, the acetic layer may develop a greenish ring that progressively spreads throughout this layer.^[12]

Lieberman's Test Steroids and Terpenes: One milliliter of acetic anhydride and a small amount of each extract were dissolved in chloroform. was added, followed by two drops of conc. They added sulfuric acid. A shifting shade of pink When upright, a bluish green color indicates the presence of terpenes and steroids.^[14]

Test for Flavonoids: Acetone was used to fully retain two grams of the extract. After evaporation on a water bath, the residue was extracted in warm water. While the mixture was hot, it was filtered. After cooling, the filtrate was utilized in the subsequent experiments.^[1]

Phytochemical Screening of Aqueous and Ethanol Extracts of Tamarindus indica Leaf

Tamarindus indica leaf extracts in both ethanol and water. While ethanol extract displayed alkaloids, flavonoids, saponins, tannins, phenols, glycosides, terpenoids, and steroids, aqueous extract did not. The ethanol extract has more flavonoids than the aqueous extract. is consistent with ethanol's greater polarity, which makes it more effective at extracting flavonoid chemicals. Because of their anti-inflammatory, anti-cancer, and antioxidant qualities, flavonoids are frequently more soluble in organic solvents like ethanol.

This implies that chemicals can be extracted more successfully via ethanol extraction. Significant biological activity may increase the extract's medicinal usefulness. Both the aqueous and ethanol extracts had comparatively significant concentrations of tannins and phenols. This is in line with earlier research showing that phenolic chemicals and tannins are soluble in water and may be effectively extracted by ethanol. Phenols and tannins are widely recognized Tamarindus indica leaf extracts may have medicinal uses due to their antibacterial, anti-inflammatory, and antioxidant qualities.

Another significant class of bioactive substances with anti-inflammatory and hypocholesterolemic properties are saponins, which were found in considerable concentrations in the aqueous extract and low in the ethanol extract (+). Applications needing water-soluble chemicals may benefit from the aqueous extract's apparent higher saponin yield. Steroids are present in the ethanol extract but not in the aqueous extract. suggests that organic solvents are a better way to extract steroids, which is consistent with the lipophilic nature of steroid molecules. Steroids are useful in therapeutic settings because of their well-known anti-inflammatory and antiallergic properties.^[4]

Standardization of Plant Extract

Pure tartaric acid was identified and isolated using the chromatographic method using a solution of crude tartaric acid extract (10 μ l each). Spots were found using a 1% solution of vanillin in methanolic sulfuric acid as chromatogenic reagents, and the separated compounds were identified by seeing them under ultraviolet light at a wavelength of 365 nm. The mobile phase chosen for use was n-Propanol: n Butanol: Ammonia in a ratio of 7:1:2.

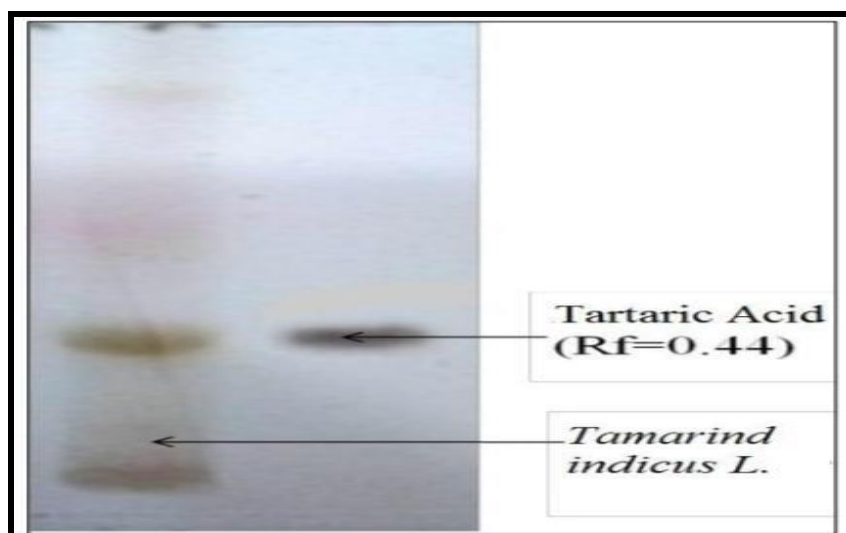


Fig. Thin Layer Chromatography.

To confirm that tartaric acid was present in the extracted sample compared to the standard, the sample's Rf value was calculated.^[2]

Physico-chemical analyses

Powdered dried *T. indica* leaves were used to evaluate a number of factors, such as moisture content, ash values, foaming index, alcohol extractive value, hot water extractive value, cold water extractive value, and swelling index.

Moisture content: A conventional gravimetric method was used to determine the moisture content of *T. indica* leaf powder.^[10] Five grams of precisely weighed powdered material were dried in a hot air oven set at 100 to 105 degrees Celsius until a consistent weight was reached. The results are shown as mean percentage (% w/w) \pm standard error of the mean (SEM), and all measurements were made in triplicate.^[5]

Ash values: Total ash, acid-insoluble ash, and water-soluble ash were among the physicochemical ash characteristics assessed. following the World Health Organization's (WHO) recommendations.^[10] Two grams of powdered leaves were burned at 500–600

degrees Celsius in a silicon crucible that had been previously weighed in order to determine the total ash content. use a muffle furnace until a consistent, carbon-free ash was produced. Boiling the ash allowed for the calculation of acid-insoluble ash. After filtering the mixture for five minutes in 25 milliliters of diluted hydrochloric acid, dry the residue that remained on the filter paper.

After boiling the ash with 25 milliliters of distilled water, the water-soluble ash was filtered. The water-soluble fraction was calculated by deducting the weight of the insoluble materials from the total ash. Based on triplicate analysis, ash levels were given as mean % w/w \pm SEM and expressed as mg/g of air-dried material.^[8]

Values that are extracted:-Alcohol-soluble extractive values, hot water, and cold water were measured in compliance with WHO guidelines. Four grams of leaf powder were refluxed in the hot water extractive test. for one hour using 100 milliliters of pure water. Following cooling, the vol-Ume was readjusted to 100 mL, filtered, and 25 mL of the filtrate were dried on a water bath. To calculate the extractive yield, the residue was weighed. In the cold-wa-Ter procedure, 4 g of sample was macerated with 100 mL of wa-Ter for 24 hours; the first 6 hours were shook occasionally, and the latter 18 hours were left undisturbed. The residue was weighed after the filter (25 mL) was evaporated.^[9]

Alcohol-soluble: The same method was used to determine extractives, substituting water with methanol. All values are displayed as mean % w/w \pm SEM and were acquired in tripli-Cate.^[6]

Swelling index: A 25 mL graduated cylinder was filled with one gram of powdered T. indica leaves. After re-cording the initial volume, 25 milliliters of distilled water were added. The mixture was left undisturbed for three hours at room temperature after being shook every ten minutes for one hour. After noting the final volume, the swelling index was computed by deducting the initial volume from the final volume. The data are shown as mean \pm SEM, and each test was conducted in triplicate.^[6]

Foaming index: In a 500 mL conical flask, 1 g of leaf powder and 100 mL of distilled water were boiled for 30 minutes to calculate the foaming index. The volume was adjusted to 100 mL following chilling and filtration. Serial quantities between 1 and 10 milliliters The decoction was moved into several test tubes with stoppers and diluted with distilled water to a

volume of 10 mL. After a vigorous 15-second shake, each tube was allowed to stand for 15 minutes. The height of the stable foam generated was used to measure the foam height and establish the foaming index.^[7]

CONCLUSION

The extraction, separation, pharmacognostic assessment, and phytochemical screening of *Tamarindus indica* were all effectively completed in this study. Important morphological and microscopic traits that are helpful for the identification and authentication of the plant material were established by pharmacognostic investigations. The various chemical components found in the crude medicine might be isolated thanks to the extraction and separation methods.

Numerous secondary metabolites, including flavonoids, tannins, alkaloids, saponins, and phenolic chemicals, which are recognized for their medicinal qualities, were found during preliminary phytochemical screening. These results confirm *Tamarindus indica*'s therapeutic value, provide its traditional use a scientific foundation, and offer helpful data for additional pharmacological and phytochemical research.

Important details regarding the identification and chemical components of the plant were revealed by the *Tamarindus indica* study, which included extraction, separation, pharmacognostic assessment, and phytochemical screening. Phytochemical screening verified the existence of many bioactive components, including flavonoids, tannins, alkaloids, and saponins, while pharmacognostic analysis assisted in the authenticity of the crude medicine.

These chemicals were shown to be present in the plant extract by the extraction and separation methods. As a result, the study validates *Tamarindus indica*'s therapeutic efficacy and lays the groundwork for future investigations and pharmaceutical uses.

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RESULT

Tamarindus indica's pharmacognostic analysis identified unique macroscopic and microscopic traits that aid in the crude drug's identification and authentication. The macroscopic analysis revealed that the plant material had the distinctive texture, color, and smell of *Tamarindus indica*. Diagnostic characteristics such as epidermal cells, parenchymatous tissues, vascular bundles, fibers, and starch grains were found under a microscope. These characteristics are helpful indicators for accurately identifying and standardizing the plant material.

Using an appropriate solvent extraction technique, the powdered plant material was successfully extracted. The resulting extract had a semisolid consistency, a distinct odor, and a dark brown color. The extract's % yield showed that the chosen solvent was successful in removing *Tamarindus indica*'s phytoconstituents. The plant extract was submitted to additional separation techniques, such as thin-layer chromatography (TLC) or other appropriate procedures, which led to the separation of several components, demonstrating the presence of diverse chemical ingredients in the extract.

Several significant secondary metabolites were found in the extract after preliminary phytochemical screening. The presence of flavonoids, tannins, alkaloids, saponins, glycosides, and phenolic substances was verified by qualitative chemical testing. The traditional medical benefits of *Tamarindus indica* are supported by these phytoconstituents' known biological activities, which include antioxidant, antibacterial, and anti-inflammatory qualities.

Tamarindus indica has a range of bioactive substances, according to the overall findings of the pharmacognostic evaluation, extraction, separation, and phytochemical screening. These results offer valuable information for the plant's identification, quality assurance, and more pharmacological research.

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