

EVALUATION OF ANTIFUNGAL ACTIVITY OF TAMARINDUS INDICA L. SEED EXTRACT AND DEVELOPMENT OF HERBAL OINTMENT

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Abstract:

The emergence of antifungal resistance and adverse effects associated with existing antifungal agents have prompted the search for alternative therapeutic options derived from natural sources. *Tamarindus indica* L., a medicinal plant widely used in traditional medicine, was evaluated for its *in vitro* antifungal activity against selected pathogenic fungal species. The antifungal efficacy of the plant extract was assessed using the agar well diffusion method against *Candida albicans*, *Fusarium oxysporum*, and *Phialophora verrucosa*. The extract was tested at concentrations of 500, 250, 100, and 50 µg/ml on Potato Dextrose Agar medium, while Amphotericin B served as the reference standard. Antifungal activity was quantified by measuring the zones of inhibition following incubation at 28°C for 72 hours. The results demonstrated a concentration-dependent inhibition of fungal growth, with statistically significant activity observed at higher concentrations ($p < 0.05$). The findings of this study substantiate the antifungal potential of *Tamarindus indica* L. And warrant further investigation to identify its bioactive constituents and underlying mechanisms of action.

Keywords: Agar well diffusion method; Seed extract; *Candida albicans*; *Fusarium oxysporum*; *Phialophora verrucosa*.

1. Introduction:

Herbal plants have played an indispensable role in human civilization, serving as sources of nutrition, medicine, and cultural identity. They are widely dispersed across the globe and encompass a diverse category of species used for culinary, aromatic, and therapeutic purposes. Botanically, herbs are defined as plants lacking woody stems, characterized by soft green tissues, and are often employed in fresh or dried form. Unlike spices, which are derived from roots, bark, seeds, or fruits, herbs primarily consist of leafy or flowering parts. Their versatility has ensured their relevance across traditional medicine, modern pharmacology, and nutraceutical industries.

The history of medicinal plants dates back thousands of years. Ancient civilizations such as the Sumerians, Egyptians, and Indians documented herbal remedies, highlighting their profound knowledge of plant-based therapies. The Ebers Papyrus (1550 BCE) listed over 850 plant remedies, while Indian texts like the Atharvaveda and Charaka Samhita emphasized the holistic use of herbs in maintaining health. These records underscore the continuity of herbal medicine from antiquity to modern times ^[1].

Traditional systems of medicine including Ayurveda, Siddha, and Unani continue to hold immense importance in South Asia. Ayurveda emphasizes the balance of

Doshas (Vata, Pitta, Kapha) and Mahabhutas (five elements), while Siddha and Unani rely on humoral and elemental theories. These systems integrate herbal interventions, dietary practices, and lifestyle modifications, offering preventive and therapeutic strategies. Their resurgence in contemporary research highlights the potential for integration with modern medicine, particularly in addressing chronic diseases and lifestyle disorders [2].

Herbal drugs remain a cornerstone of pharmaceutical innovation. Approximately 25-30% of modern medicines are derived from plants, reflecting their enduring relevance. Bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids exhibit antioxidant, antimicrobial, and anti-inflammatory properties. Advances in extraction and formulation technologies have enabled herbal medicines to complement conventional therapies, particularly in managing diabetes, cardiovascular diseases, and liver disorders. However, challenges such as standardization, dosage optimization, and clinical validation persist, necessitating rigorous scientific evaluation.

Among medicinal plants, *Tamarindus indica* L. (tamarind) occupies a unique position. Indigenous to tropical Africa but extensively cultivated in India and South Asia, tamarind has been integrated into traditional medicine, cuisine, and trade for centuries. Its vernacular names Imli (Hindi), Puli (Malayalam), Chinch (Marathi), and Chinta (Telugu) reflect its widespread cultural assimilation. Archaeological records from Egypt (400 BCE) and references in ancient Indian texts suggest its long-standing medicinal and nutritional significance [3].

Botanically classified under the Fabaceae family, tamarind is a large evergreen tree with pinnate leaves and indehiscent pods containing sticky pulp and hard seeds. Morphological variations in fruit size, pulp weight, and seed characteristics have been documented across genotypes, reflecting its adaptability and genetic diversity. Nutritionally, tamarind seeds are rich in protein (13.6%), dietary fibre (21.6%), and essential minerals such as calcium, phosphorus, magnesium, and potassium. These attributes make tamarind a valuable dietary component in resource-limited settings [4].



Fig. No 1.1: Tamarind

Phytochemical investigations reveal that tamarind seeds contain flavonoids (quercetin, luteolin), procyanidins, catechins, phenolic acids, terpenoids, tannins, and saponins. These compounds contribute to its pharmacological activities, including antioxidant, hepatoprotective, antimicrobial, and hypoglycemic effects. The seed coat, abundant in catechins and polyphenols, has demonstrated antibacterial and anti-melanogenic properties, making it relevant for pharmaceutical and cosmetic applications.

Of particular interest is the antifungal potential of tamarind. Fungal infections, Thus, tamarind represents a vital link between traditional knowledge and modern pharmacological innovation. Its nutritional richness, diverse phytochemical profile, and antifungal properties highlight its potential as a nutraceutical and therapeutic agent. Continued research into its bioactive constituents, mechanisms of action, and clinical applications may pave the way for developing standardized herbal formulations that address pressing health challenges, including antimicrobial resistance.

2. Materials and methods

2.1 Collection Authentication of The Plant Specimen

The plant specimen was collected from the village in Salem district, Tamil Nadu, and the seed material was authenticated at Botanical Survey of India, Coimbatore.

2.2 The Plant Materials

Mature seeds of *Tamarindus indica* L. Are typically obtained from healthy tamarind pods collected from a village. The seeds are cleaned to remove pulp and impurities. They are then dried under suitable controlled conditions until they reach a

ranging from superficial dermatophytosis to systemic candidiasis and aspergillosis, have increased due to widespread antibiotic use and immunosuppression. Resistance to conventional antifungal agents such as azoles and echinocandins underscores the urgent need for novel, safe, and cost-effective alternatives. Extracts of tamarind seeds, bark, and leaves have shown activity against *Candida albicans*, *Aspergillus niger*, and *Trichophyton mentagrophytes*. The mechanism is attributed to phenolic compounds and flavonoids that disrupt fungal cell wall integrity and inhibit spore germination [5].

stable moisture level. Once dry, the seeds are grind into a fine powder using appropriate equipment. This powder serves as the raw material for extract preparation.

2.3 The Extract

2.3.1 Extraction

The process of separating secondary metabolites or bioactive compounds (such as alkaloids, flavonoids, essential oils, terpenoids, and phenolics) is called extraction of medicinal herbs [6]. By using suitable solvents and adhering to a standard extraction technique [7].

2.3.2 Pre -Extraction Plant Sample Preparation

In medicinal plant research, the first step is to prepare plant samples to protect its bioactive compounds. Dried parts like husk or fibres can be used, and the quality of phytochemicals depends on pre-treatments such as drying and grinding [8].

The difference in extraction method will affect the amount of extract and the composition of secondary metabolites depending on:

- Type of extraction
- Extraction time

- Temperature
- Weight product
- Liquid concentration
- Polarity

2.4 Maceration

The extraction process involves placing coarsely powdered drug material (such as leaves, stem bark, or root bark) into a container, then adding menstruum until the

material is fully submerged. The container is sealed and left undisturbed for at least three or seven days [9]. The mixture is occasionally stirred, and if stored in a bottle, it should be shaken periodically to ensure complete extraction. Once extraction is complete, the liquid is separated through decantation or filtration. The micelle is then retrieved from the menstruum by evaporation, typically using an oven or a water bath.



Fig No 2.1 Maceration process of hydroalcoholic, aqueous, ethanolic extracts.

2.4.1 Procedure

Aqueous maceration:

Weigh 100 g of tamarind seed powder and transfer it into a 1 L round-bottom flask. Add 600 ml of distilled water and 3 ml of chloroform to prevent microbial growth. The mixture was soaked for 7 days with occasional stirring.

Ethanolic maceration:

Weigh 100 g of tamarind seed powder and place it in a 1 L round-bottom flask. Add 600 ml of ethanol and soak the material for 7 days with occasional shaking.

Hydro-alcoholic maceration:

Weigh 100 g of tamarind seed powder and keep it in a 1 L round-bottom flask. Add

600 ml of hydro-alcoholic solvent (300 ml distilled water + 300 ml ethanol). The mixture was soaked for 7 days with intermittent stirring.

Mechanism Process:

Maceration helps to soften and break the plant cell wall, allowing the release of soluble Phytochemicals into the solvent. After 7 days, the mixture was filtered to Obtain the extract. The choice of solvent determines the type of Phytoconstituents extracted. Maceration was selected because it is simple, Economical, suitable for bulk extraction, and causes less degradation of Phytochemicals compared to methods such as Soxhlet extraction, decoction, or Reflux.

Table No. 2.1: Extractive Values

S. No	Solvent
1	Water (5.90%)
2	Hydroalcoholic (8.70%)
3	Ethyl acetate (3.60%)
4	Chloroform (2.40%)

3. Pharmacological Studies

3.1 Antifungal Activity Study

Fungal infections, collectively known as mycoses, constitute an important category of infectious diseases caused by a broad range of pathogenic and opportunistic fungi. These organisms are eukaryotic, non-photosynthetic microorganisms characterized by rigid cell walls primarily composed of chitin and exhibit various morphological forms, including yeasts, Molds, and dimorphic fungi. Fungal infections may originate from environmental exposure or develop from the body's normal microbial flora when the host's immune system is compromised [10].

Based on the site of infection and mode of transmission, fungal diseases are clinically categorized as superficial, cutaneous, subcutaneous, systemic (endemic), or opportunistic mycoses [11]. Superficial and cutaneous mycoses typically affect the skin, hair, and nails and are generally mild, whereas systemic and opportunistic infections can spread to internal organs, resulting in serious and potentially fatal conditions. Opportunistic fungal infections such as candidiasis, aspergillosis, and cryptococcosis are increasingly observed among immunocompromised patients [12].

The worldwide incidence of fungal infections has increased significantly due to factors including the rising prevalence of HIV/AIDS, extensive use of immunosuppressive drugs, organ transplantation, cancer chemotherapy, prolonged administration of broad spectrum antibiotics, and the expanding elderly population. However, despite their growing prevalence, fungal infections often remain underdiagnosed and inadequately treated owing to nonspecific clinical symptoms and limited access to advanced diagnostic facilities, particularly in resource-limited settings [13].

The diagnosis of fungal infections is based on clinical assessment in combination with laboratory investigations such as direct microscopic examination, culture techniques, histopathological analysis, serological tests, and molecular diagnostic methods [14]. Although significant progress has been made in antifungal therapy, several challenges persist, including drug-related toxicity, increasing antifungal resistance, limited availability of effective agents, and high treatment costs. Consequently, timely diagnosis and appropriate therapeutic intervention are crucial for reducing the morbidity and mortality associated with fungal infections [15].

Natural products have historically played a vital role in drug discovery and development. Numerous plant-based substances, including tea tree oil, clove oil, turmeric, neem, and tamarind, have been reported to exhibit noteworthy antifungal properties and hold promise for therapeutic applications [16].

3.1.1 Antifungal Activity of *Tamarindus indica* Linn. Seed Extract Against Selected Fungi

3.1.1.1 Test Organisms

- *Candida albicans*
- *Fusarium oxysporum*
- *Phialophora verrucosa*

3.1.1.2 Preparation of Potato Dextrose Agar (PDA) Medium

Potato dextrose agar medium was prepared by dissolving 20 g of potato infusion, 2 g of dextrose, and 1.5 g of agar in 100 mL of

Principle

The disc diffusion method is based on the diffusion of an antifungal agent from an impregnated disc into the agar medium seeded with test organisms. The antifungal agent inhibits fungal growth around the disc, producing a clear zone of inhibition. The diameter of this zone, measured in millimeters, indicates the antifungal efficacy of the test sample [17].

3.1.1.3.1 Materials Used

Potato dextrose agar medium, Amphotericin B antimycotic solution (standard drug), test samples, sterile paper discs, Petri plates, test tubes, beakers, conical flasks, spirit lamp, and double-distilled water [18].

4. Formulation And Evaluation of Herbal Ointment

4.1 Preparation of Ointment Base

The ointment base was prepared by melting beeswax on a water bath. Shea butter was

distilled water. The medium was sterilized by autoclaving at 121°C under 15 lbs pressure for 15 minutes. After sterilization, the molten medium was mixed thoroughly and poured aseptically into sterile 100 mm Petri plates (25–30 mL per plate) and allowed to solidify [19].

3.1.1.3 Determination of Antifungal Activity by Disc Diffusion Method

3.1.1.3.2 Procedure

Antifungal activity was evaluated using the disc diffusion method. Sterile Petri plates containing approximately 20 mL of PDA medium were prepared and allowed to solidify. The agar surface was uniformly inoculated with freshly prepared fungal suspensions using sterile cotton swabs. Sterile paper discs (6 mm diameter) impregnated with predetermined concentrations of the test samples were aseptically placed on the inoculated agar surface. Amphotericin B served as the positive control, while discs containing only the solvent were used as negative controls. The plates were incubated under appropriate conditions, and antifungal activity was assessed by measuring the diameter of the zones of inhibition. All experiments were conducted in triplicate, and results were expressed as mean \pm standard deviation [20].

added to the molten beeswax and mixed until completely melted. Coconut oil and olive oil were then added with continuous stirring to obtain a smooth, uniform mixture. The base was allowed to cool with gentle stirring to ensure even consistency.

Table No. 4.1: Formulation ointment base

S. No	Name of Ingredients	Quantity to be taken
1	Coconut oil	15g
2	Olive oil	15g
3	Bees wax	5g
4	Shea butter	12g

4.2 Preparation of Herbal Ointment**Table No. 4.2: Formulation Ingredients**

S. No	Ingredient	Quantity(50g)	Role in Formulation
1	Tamarindus indica extract	5 g (10% w/w)	Anti-inflammatory, antioxidant
2	Beeswax	7 g	Stiffening agent, protective barrier
3	Shea butter	8 g	Emollient, skin healing agent
4	Coconut oil	12 g	Emollient, antimicrobial
5	Olive oil	15 g	Moisturizer, anti-inflammatory
6	Neem oil	1 g	Antimicrobial, anti-inflammatory
7	Vitamin E	0.5 g	Antioxidant, skin conditioner

4.2.1 Preparation of Tamarindus indica Extract

- Fresh Tamarindus indica pulp was cleaned to remove seeds and fibres.
- The pulp was shade-dried and coarsely powdered.
- The powdered material was subjected to aqueous extraction by maceration for 24 hours with intermittent stirring.
- The extract was filtered through muslin cloth followed by Whatman No.1 filter paper.
- The filtrate was concentrated on a water bath to obtain a semi-solid extract.

- The extract was stored in an airtight container until further use.

4.2.2 Incorporation of Extract, Additives, and Final Adjustments

The concentrated Tamarindus indica extract (5–10% w/w) was incorporated into the ointment base using geometric dilution. Neem oil (1–2% v/w) and Vitamin E (0.5–1% v/w) were added as an antifungal enhancer and antioxidant, respectively. The formulation was evaluated for smoothness, homogeneity, and pH (5.5–6.5), and consistency adjustments were made as required.

4.2.3 Filling, Packaging, and Storage

The prepared ointment was filled into sterilized aluminium tubes, sealed, labelled, and stored in a cool, dry place away from direct sunlight. Aluminium tubes were selected to prevent oxidation and photodegradation. The shelf life of the formulation was estimated to be 3–6 months under refrigerated conditions.

5. Result And Discussion

5.1 Authentication of The Plant Specimen

The collected plant specimen was authenticated as *Tamarindus indica* L. (Family: FABACEAE) by **Dr. S. S. Hameed, Scientist 'F' & Head of Office**, Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, Tamil Nadu.

Authentication No.: BSI/SRC/5/23/2025-26/Tech/732.

5.2 The Plant Material

Mature seeds of *Tamarindus indica* Linn. were collected from ripe pods obtained from well-grown trees in cultivated areas. The plant material was authenticated by a qualified botanist. The seeds were separated manually from the pulp, thoroughly washed with water to remove adhering impurities, and shade dried at room temperature. The dried seeds were coarsely powdered using a mechanical grinder. The powdered seed material was stored in airtight containers and used for subsequent pharmacognostical, physicochemical, anti-inflammatory, and antioxidant studies.

5.3 Pharmacognostical Studies:

Plant: *Tamarindus Indica* Linn. (Seed)

Family: Fabaceae

5.3.1 Macroscopic parameters

- **Colour:** Reddish-brown to dark brown
- **Odour:** Odourless
- **Taste:** Slightly bitter and astringent
- **Texture:** Hard and glossy
- **Shape:** Irregularly polygonal to ovoid
- **Surface:** Smooth, hard seed coat
- **Size:** Approximately 1–1.5 cm in length
- **Seed nature:** Ex albuminous with hard Testa

5.4 Physicochemical Constants Determination

5.4.1 Extractive values

The dried seed powder of *Tamarindus indica* Linn. was subjected to extraction using solvents of different polarity to determine extractive values. The solvents used include polar (water, ethanol), intermediate polar (ethyl acetate), and non-polar (chloroform). The extractive values reflect the presence of bioactive constituents such as polyphenols, flavonoids, tannins, fatty acids, and carbohydrates, which contribute to the antioxidant and anti-inflammatory activities of the seed. % Soluble Extractive Value (w/w)

Table No 5.1: Percentage soluble extractive values of *Tamarindus indica* Linn. Seeds

S. No	Solvent	Colour	Odour	Consistency
1	Water (5.90%)	Brownish	Characteristic	Sticky
2	Hydroalcoholic (8.70%)	Dark brown	Characteristic	Sticky
3	Ethyl acetate (3.60%)	Light brown	Characteristic	Semi-solid
4	Chloroform (2.40%)	Greenish	Characteristic	Semi-solid

5.4.2 Ash values

The powdered seed material of *Tamarindus indica* Linn. was evaluated for ash values to determine the inorganic content and purity of the crude drug.

Table No 5.2: Ash values of *Tamarindus indica* Linn. seeds

S. No	Types of Ash	Percentage (% w/w)
1	Total ash	3.85%
2	Acid insoluble ash	0.95%

5.4.3 Determination of FOM (Foreign Organic Matter)

The dried seeds of *Tamarindus indica* Linn. were examined for foreign organic matter such as sand particles, seed coat fragments, and other extraneous materials. The foreign organic matter content was found to be 1.20% w/w, indicating acceptable purity of the seed material.

5.4.4 Determination of LOD (Loss on Drying)

The moisture content of *Tamarindus indica* Linn. seed powder was determined by the loss on drying method. The moisture content was found to be 6.50% w/w, which is within acceptable limits and suitable for long-term storage and experimental use.

5.5 Preliminary Phytochemical Analysis of The Extract

Preliminary phytochemical screening of *Tamarindus indica* Linn. seed extract revealed the presence of carbohydrates, proteins, flavonoids, phenols, tannins, saponins, glycosides, fixed oils, and fats, with moderate presence of steroids and trace alkaloids. The presence of polyphenols and flavonoids indicates strong antioxidant potential, while tannins and saponins contribute to anti-inflammatory and protective activities. These findings support the traditional medicinal use of *Tamarindus indica* seeds in herbal formulations.

Table. No: 5.3: Preliminary Phytochemical Analysis of the Extract

S. NO	Phytoconstituents	Hydro-alcoholic Extract
1	Alkaloids	+
2	Carbohydrates	+
3	Tannin	+
4	Flavonoids	+
5	Protein	-
6	Phenols	+

+(Positive) = Presence of constituents: -(Negative) =Absence of constituents

5.6 Pharmacological Studies

5.6.1 Overview of Antifungal Evaluation

The antifungal potential of the test sample TI was assessed using the agar well diffusion method against three pathogenic fungal strains: *Candida albicans*, *Fusarium oxysporum*, and *Phialophora verrucosa*.

The study aimed to determine the efficacy of TI at varying concentrations (500, 250, 100, and 50 µg/ml) by measuring the zone of inhibition in millimeters. Amphotericin B served as the positive control due to its well-established antifungal activity.

The potato dextrose agar (PDA) medium was used to support fungal growth, and the plates were incubated at 28°C for 72 hours. The zones of inhibition were measured and analyzed using GraphPad Prism 6.0 software. The results are presented in both tabular and graphical formats to illustrate the comparative antifungal activity of TI.

5.6.2 AGAR WELL DIFFUSION METHOD

Principle

The anti-fungal agent present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of

zone of inhibition can be measured in millimeters.

Materials Required

Potato dextrose agar medium, Amphotericin B antimycotic solution, test samples, test tubes, beakers conical flask, spirit lamp, double distilled water and petri-plates.

5.6.2.1 DISC DIFFUSION METHOD

A. Potato Dextrose Agar Medium

The potato dextrose agar medium was prepared by dissolving 20 gm of potato infusion, 2 gm of dextrose and 1.5 gm of agar in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten.

Procedure

Petri plates containing 20ml potato dextrose agar medium was seeded with 72 hr culture of fungal strain (*C. albicans*, *F. oxysporum*, *P. verrucosa*) with different concentration of the samples TI (500, 250, 100 and 50 µg/ml) were added. The plates were then incubated at 28°C for 72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed

around the wells. Amphotericin B was used as a positive control. The values were

calculated using Graph Pad Prism 6.0 software (USA).

Results

Fig 5.2: Effect of samples TI against *C. albicans*.

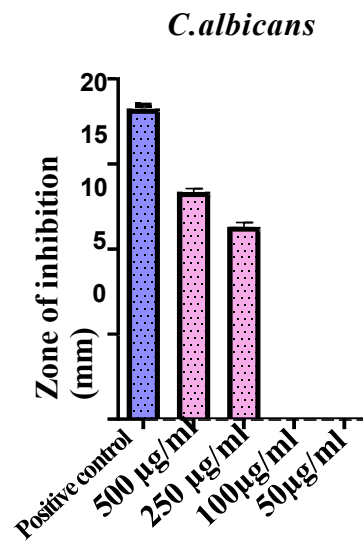
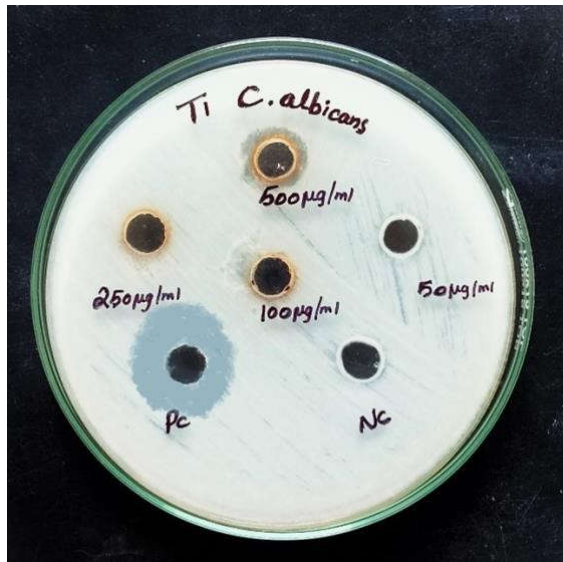


Fig 5.3: Effect of sample TI against *F. oxysporum*.

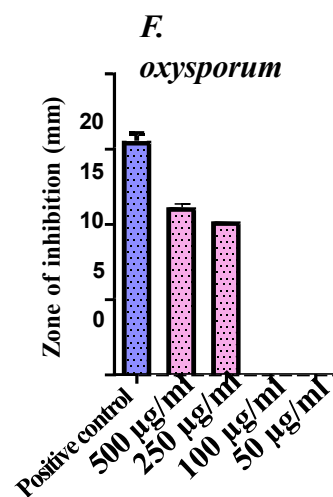
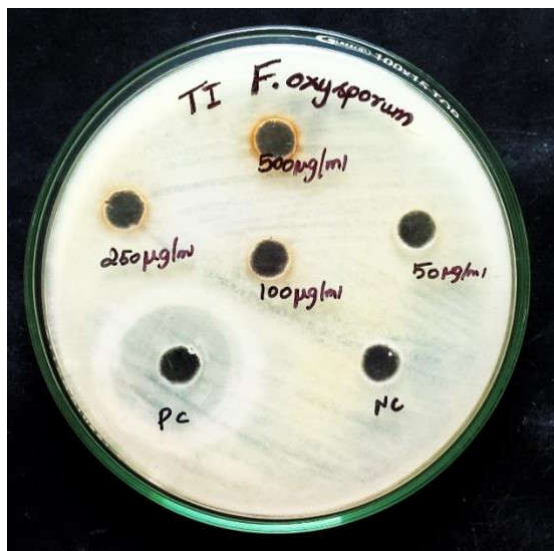
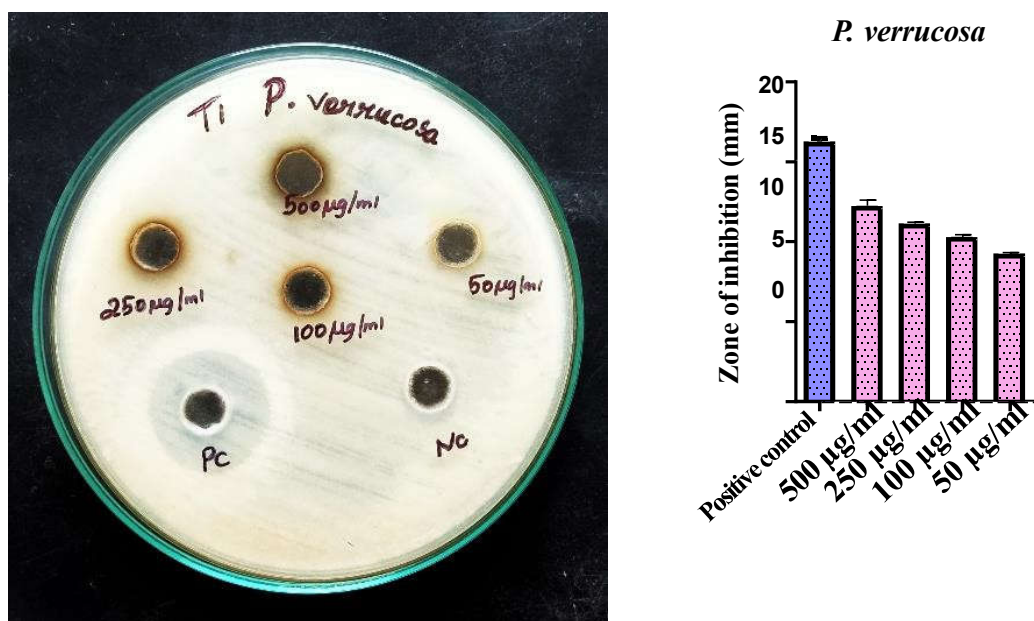


Fig 5.4: Effect of sample TI against *P. verrucosa*.



5.6.3 Tabulated Results and Observations

Table 6.3. Means ± SD of zone of inhibition obtained by sample TI against *C. albicans*, *F. oxysporum*, *P. verrucosa*.

S.N O	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	<i>C. albicans</i>	TI	13.35±0.21	11.3 ± 0.28	0.0±0.0	0.0±0.0	18.25±0.21
2.	<i>F. oxysporum</i>		11.15±0.21	10.2±0.0	0.0±0.0	0.0±0.0	15.55±0.49
3.	<i>P. verrucosa</i>		12.25±0.35	11.15±0.07	10.3±0.14	9.25±0.07	16.25±0.21

SD – Standard Deviation *Significance – p<0.05

Key Observations:

- TI exhibited a dose-dependent antifungal effect, with higher concentrations producing larger zones of inhibition.
- *C. albicans* and *F. oxysporum* showed no inhibition at 100 µg/ml and 50 µg/ml, indicating a higher minimum inhibitory concentration (MIC).
- *P. verrucosa* was the most sensitive strain, showing inhibition even at the lowest concentration tested (50 µg/ml).

5.6.4 Comparative Analysis and Interpretation**5.6.4.1. Efficacy Against *Candida albicans***

- TI demonstrated moderate antifungal activity at 500 µg/ml (13.35 mm) and 250 µg/ml (11.3 mm).
- No inhibition was observed at 100 µg/ml and 50 µg/ml, suggesting that TI is less effective at lower concentrations.
- Compared to Amphotericin B (18.25 mm), TI showed approximately 73% relative efficacy at the highest concentration.

5.6.4.2. Efficacy Against *Fusarium oxysporum*

- TI showed inhibition zones of 11.15 mm and 10.2 mm at 500 and 250 µg/ml, respectively.
- No activity was observed at lower concentrations.
- Amphotericin B showed a 15.55 mm zone, indicating TI's relative efficacy was about 71% at 500 µg/ml.

5.6.4.3. Efficacy Against *Phialophora verrucosa*

- TI exhibited consistent antifungal activity across all concentrations, with inhibition zones ranging from 12.25 mm (500 µg/ml) to 9.25 mm (50 µg/ml).
- This suggests a broader spectrum and stronger potency of TI against *P. verrucosa*.
- Compared to the standard (16.25 mm), TI achieved up to 75% efficacy.

5.6.5. Discussion and Implications

The antifungal screening Of sample TI revealed promising activity, particularly against *P. verrucosa*, where inhibition was observed even at lower concentrations. The results suggest that TI contains bioactive compounds capable of disrupting fungal growth, with a clear concentration-dependent response.

The absence of activity at lower concentrations against *C. albicans* and *F. oxysporum* indicates that the MIC for these strains is higher, possibly due to differences in cell wall composition or resistance mechanisms. The superior response against *P. verrucosa* may be attributed to its higher susceptibility or the specific mode of action of TI.

Implications:

- TI could serve as a potential lead compound for developing antifungal agents, especially for infections caused by *P. verrucosa*.
- Further studies are recommended to isolate the active constituents, determine the MIC and MFC (minimum fungicidal concentration), and evaluate cytotoxicity.
- In vivo studies and formulation development could help assess its therapeutic potential.

5.7 CONCLUSION:

Sample TI exhibits significant antifungal activity, especially at higher concentrations.

Its broad-spectrum efficacy against *P. verrucosa* makes it a promising candidate for further pharmaceutical development.

6. Summary And Conclusion

The present work investigates the antifungal activity and ointment formulation of *Tamarindus indica* Linn. seed extract, situating the study within the broader context of herbal medicine and traditional pharmacology. Since ancient times, herbs have been integral to human health, serving as food, flavouring agents, and therapeutic remedies. Civilizations such as the Sumerians and Egyptians documented herbal practices thousands of years ago, and systems like Ayurveda, Siddha, and Unani in South Asia continue to emphasize holistic healing through plant-based interventions. In modern times, herbal drugs remain highly relevant, with nearly one-third of pharmaceuticals derived directly or indirectly from botanical sources. This continuity between tradition and science underscores the importance of exploring plants like tamarind for their medicinal potential.

Tamarindus indica is a large evergreen tree belonging to the Fabaceae family, native to Africa but widely cultivated in India and South Asia. Its vernacular names—Imli in Hindi, Puli in Malayalam, Chinch in Marathi, Tentuli in Bengali—reflect its deep cultural integration. Morphologically, the tree exhibits pinnate leaves with numerous small leaflets, pods containing sticky pulp, and hard seeds that vary in size and colour. Nutritionally, tamarind seeds are rich in proteins, carbohydrates, dietary fibre, and essential minerals such as calcium, phosphorus, magnesium, and potassium. Medicinally, they contain diverse phytochemicals including flavonoids (quercetin, luteolin), catechins, procyanidins, tannins, terpenoids, and saponins. These compounds are known for antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic, and wound-healing properties.

The study emphasizes the antifungal potential of tamarind seeds. Fungal infections, ranging from superficial dermatophyte infections to systemic candidiasis and aspergillosis, have become increasingly problematic due to widespread antibiotic use and immunosuppression. Rising resistance to conventional antifungal drugs such as azoles and echinocandins highlights the urgent need for safe, cost-effective alternatives. Tamarind seed extracts, particularly those rich in polyphenols and catechins, have demonstrated activity against *Candida albicans*, *Aspergillus niger*, and *Trichophyton* species. The mechanism involves disruption of fungal cell walls and inhibition of spore germination, validating traditional uses and offering scientific promise for modern formulations.

In this research, tamarind seeds were collected from Salem district, authenticated at the Botanical Survey of India, cleaned, dried, powdered, and subjected to extraction. Maceration was chosen as the primary technique due to its simplicity, economy, and ability to preserve phytochemicals. Aqueous, ethanolic, and hydroalcoholic solvents were employed, each influencing the yield and composition of bioactive compounds. Preliminary phytochemical screening confirmed the presence of alkaloids, glycosides, tannins, flavonoids, and phenols, aligning with literature reports. The plan of work included authentication, extraction, phytochemical analysis, pharmacological studies, and formulation of herbal ointments.

Future research should focus on standardizing extraction methods to ensure reproducible yields of bioactive compounds, conducting clinical trials to validate antifungal efficacy in humans, and developing stable ointment formulations with optimized delivery systems. Exploring tamarind seed by-products, such as mucilage and polysaccharides, could further enhance value-added applications in

pharmaceuticals, food, and cosmetics. Integrating ethnobotanical knowledge with modern pharmacological research will not only expand therapeutic options but also promote sustainable use of natural resources.

In summary, *Tamarindus indica* bridges traditional medicine and modern science. Its seeds, rich in phytochemicals, demonstrate significant antifungal activity and can be formulated into effective herbal ointments. This study reinforces the importance of herbal plants in addressing contemporary health challenges and emphasizes the role of tamarind as a natural, multifunctional resource. By validating traditional practices through scientific inquiry, the research contributes to the development of safe, affordable, and sustainable remedies that align with global needs for innovative healthcare solutions.

7. Reference

1. Aly, S.H., et al., 2023. Comparative metabolic study of *Tamarindus indica* L'. various organs based on GC/MS analysis, in silico and in vitro Anti-inflammatory and wound healing activities. *Plants* 12 (1), 87. <https://doi.org/10.3390/plants12010087>.
2. Ankri S, Mirelman D., 1999. Antimicrobial properties of allicin from garlic. *Microbes Infect.* 1999;1(2):125–129.
3. Aneja KR., 2005. Experiments in microbiology, plant pathology and biotechnology. 4th ed. New Delhi: New Age International; 2005.
4. Bhadoriya SS, et al., 2011. *Tamarindus indica*: Extent of explored potential. *Pharmacogn Rev.* 2011;5(9):73-81.
5. Bauer AW, et al., 1966. Antibiotic susceptibility testing by a standardized disc method. *Am J Clin Pathol.* 1966;45(4):493-96.
6. Balouiri M, et al., 2016. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.* 2016;6(2):71–79.
7. Brown GD, et al., 2012. Hidden killers: human fungal infections. *Sci Transl Med.* 2012;4(165):165rv13. Brown, G. D., et al., 2012. Hidden killers: Human fungal infections. *Science Translational Medicine,* 4(165), 165rv13.
8. Burt S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbial.* 2004;94(3):223–253.
9. Biswas K, et al., 2002. Biological activities and medicinal properties of neem (*Azadirachta indica*). *Curr Sci.* 2002;82(11):1336–1345.
10. Bongomin F, et al., 2017. Global and multi-national prevalence of fungal diseases—estimate precision. *J Fungi.* 2017;3(4):57.
11. Cowan MM., 1999. Plant products as antimicrobial agents. *Clin Microbiol Rev.* 1999;12(4):564-82.
12. Cheesbrough M., 2006. District laboratory practice in tropical countries. Part 2. Cambridge: Cambridge University Press; 2006.
13. Cappuccino JG, Sherman N., 2014. *Microbiology: A Laboratory Manual.* 10th ed. Boston: Pearson Education; 2014. p. 77–78.
14. Cock IE., 2008. Antimicrobial activity of *Aloe barbadensis* Miller leaf gel components. *Internet J Microbiol.* 2008;4(2):1–8.
15. Chavan P.P., Gadhe K.S., Sharma Gunjan & Kondamgire R., 2025. “*Tamarindus indica*: Origin, cultivation, and antioxidant potential of a multifunctional medicinal plant.” *Journal of Current Research in Food Science,* 2025;6(1):313–322. DOI.

16. Doughari JH., 2006. Antimicrobial activity of *Tamarindus indica* Linn. *Trop J Pharm Res.* 2006;5(2):597-603.
17. Devi, K. et al., 2023. Antioxidant and antibacterial potential of *Tamarindus indica* seed extract. PubMed ID: 37755709. <https://pubmed.ncbi.nlm.nih.gov/37755709>
18. Fandohan, Belarmain, et al., 2011. “Quantitative Morphological Descriptors Confirm Traditionally Classified Morphotypes of *Tamarindus Indica* L. Fruits.” *GENETIC RESOURCES AND CROP EVOLUTION*, vol. 58, no.2, 2011, pp. 299–309, doi:10.1007/s10722-010-9575-3.
19. Garcia, L., 2011. A comparative study on the antifungal effects of tamarind (*Tamarindus indica*) and garlic (*Allium sativum*) extracts on banana anthracnose. *Journal of Nature Studies.* 10 (2): 96-107.
20. Harborne JB., 1998. *Phytochemical methods.* 3rd ed. London: Chapman and Hall; 1998.