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Identification of bioactive compounds in *Tamarindus indica* L.: extraction, characterization and implications for wound healing applications

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Abstract

This study thoroughly investigates the wound healing potential of various extracts derived from *Tamarindus indica* bark, commonly known as the tamarind tree, by assessing their antimicrobial efficacy using the agar well diffusion method. Recognized for its wound healing attributes, tamarind bark extract proves effective when applied topically to cuts, abscesses and wounds due to its antimicrobial properties. The antimicrobial effectiveness of the extracts, obtained using diverse solvents including ethanol, methanol, hexane, chloroform and aqueous solutions, was systematically evaluated against microorganisms isolated from infected wound samples. Extraction of the bark extract was meticulously performed using the Soxhlet method. Through rigorous employment of the agar well diffusion method, minimum inhibitory concentrations were determined, revealing the antimicrobial activity across various plant extracts. Furthermore, comprehensive phytochemical analyses confirmed the presence of vital bioactive compounds in the extracts, notably emphasizing the richness of alkaloids, steroids, quinone and phenol, attributing these constituents to the robust wound healing properties observed. Separation and identification of these compounds were facilitated through column chromatography, thin layer chromatography and FTIR, HPLC analyses. Particularly, the ethanolic extract displayed substantial antimicrobial activity against a range of test organisms, including *Staphylococcus aureus* and *Escherichia coli*, isolated from infected wound samples. This research underscores the prominence of flavonoids, alkaloids, quinone and phenol in *Tamarindus indica* bark extracts for wound healing, accentuating their potential to significantly enhance wound care and management.

Key words : *Tamarindus indica* L., Phytochemical analysis, Bioactive compounds, Antimicrobial activity, Wound healing

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Tamarindus indica L., commonly referred to as the tamarind tree, holds a significant position within the family Leguminosae (Fabaceae). This venerable fruit tree species is renowned for its diverse applications in traditional medicine, nutrition, industry, and economics. Every part of the *T. indica* plant—the roots, body, fruit, bark, and leaves—boasts rich nutritional content and finds extensive usage in both traditional remedies[6] and contemporary industries. The tamarind fruit, known for its distinct balance of acidity and sweetness, undergoes flavor and composition variations throughout its growing season, adding to its allure and versatility. A comprehensive analysis by the World Health Organization (WHO) underscores the nutritional prowess of tamarind fruit, highlighting it as a superior source of essential amino acids, with the exception of tryptophan (82%). The seeds of T. indica share similar nutritional attributes, making them a pivotal protein source, especially in regions grappling with protein malnutrition. Beyond nutritional benefits, tamarind seeds and fruit are being explored for their potential pharmacological properties. In traditional medicine, T. indica has played a vital role in addressing a spectrum of health concerns, including wound healing, abdominal pain, diarrhoea, dysentery, parasitic infections, fever, malaria and respiratory problems[5]. Its rich medicinal legacy is further underscored by its potent anti-inflammatory action, marking Tamarindus indica as a highly commercialized medicinal plant[4]. This research endeavors to comprehensively investigate the diverse attributes of *Tamarindus indica*, shedding light on its nutritional content, medicinal potential, and economic relevance. By delving into these aspects, this study seeks to offer valuable insights into maximizing the utility of this botanical treasure for the betterment of human health and well-being.

Collection of plant material :

Fresh parts of *Tamarindus indica* bark were meticulously collected from Kanyakumari, Tamil Nadu, India. The collected materials underwent thorough washing with distilled water and were left to dry in the shade for a duration of two weeks.

Extraction of Tamarindus indica bark :

The dried *Tamarindus indica* bark was ground into a coarse powder. Subsequently, 50 g of the powdered bark was utilized for the extraction process using different solvents such as Ethanol, Methanol, Chloroform, Hexane and Aqueous. The extraction was carried out employing two distinct methods: Soxhlet apparatus for 24 hours with varying temperatures (40-70°C) based on the solvents, and a shaker for 24 hours. The solvents were then centrifuged at 2000rpm for 15 minutes and filtered with the aid of filter paper.

Phytochemical analysis of plant extract :

The extracts obtained from *Tamarindus indica* using the Soxhlet extraction method were subjected to qualitative evaluation for the presence of various chemical constituents. Standard procedures were followed for phytochemical tests, encompassing screening for tannins, carbohydrates, proteins, saponins, amino acids, steroids and flavonoids.

Separation and analysis techniques :
Column Chromatography :

Column chromatography, a preparative technique employed to purify compounds based on their polarity and hydrophobicity, was utilized. The plant extract-silica gel slurry was loaded onto a column, and different solvent fractions (hexane, chloroform and methanol) were collected and concentrated. The fractions were subsequently analysed using thin-layer chromatography to detect the separated compounds.

Thin Layer Chromatography (TLC):

Thin-layer chromatography (TLC) was performed utilizing a thin, uniform layer of silica gel coated onto a glass plate. The silica gel served as the stationary phase and appropriate liquid solvents were used as the mobile phase to develop the TLC plate. The Rf value for each compound was calculated using the relevant formula.

FTIR (Fourier Transform Infrared) :

The dried powder of the methanol extract of *Tamarindus indica* was used for FTIR analysis. The range of wavenumbers examined and specific peaks or functional groups of interest were recorded.

HPLC (High-Performance Liquid Chromatography) :

HPLC was performed on the methanolic extract of *Tamarindus indica*. The conditions, including the column type, mobile phase composition, gradient program, and detection wavelength, were specified. The obtained peak values were recorded.

Collection of wound sample :

Wound samples were collected from infected wounds of patients at Kovai Medical College and Hospitals in Coimbatore. Sterile cotton swabs were used to collect the infected wound samples, which were then transferred to clean sterile glass tubes.

Isolation and identification of wound pathogen :

To identify the organisms, present in the wound samples, were inoculated onto different culture media, including MRSA, cetrimide, blood agar, nutrient agar and MacConkey agar. The inoculated media were incubated at 37°C for 24 hours. After incubation, the growth of colonies was observed on different media and subjected to Gram staining and microscopic examination.

Morphological & biochemical characterization:

Microbial colonies were subjected to Gram staining and observed under a microscope. Gram staining was performed on the slide, and the morphology of the bacteria was identified by microscopic examination. For gram-positive organisms, biochemical tests such as catalase and coagulase were carried out.

Antimicrobial activity :

The antimicrobial activity of *Tamarindus indica* was evaluated against both gram-positive and gram-negative test microorganisms, including *Staphylococcus aureus*, *Proteus* sp., *Escherichia coli*, *Streptococcus* sp., *Pseudomonas* sp., and *Klebsiella* sp., commonly found in infected wounds[3]. The agar well diffusion method was used to assess antimicrobial activity. The organic solvent extracts

of the plant, at concentrations ranging from 1 mg/ml to 50 mg/ml were added to wells bored on previously inoculated Muller-Hinton agar plates. The plates were incubated at 37°C for 24 hours and the zones of inhibition (in mm) were measured.

Preparation of bandage :

A gauze infused with the methanolic extract of *Tamarindus indica* was prepared. The bioactive components in the plant extract facilitated wound healing by exerting anti-inflammatory effects on the skin and promoting tissue regeneration. The gauze was soaked in the methanolic extract until fully saturated and then dried in a hot air oven at 60°C for approximately 30 minutes, ensuring sterility.

Antimicrobial activity of bandage :

The antimicrobial activity of the plant extract-infused gauze was evaluated. Muller-Hinton agar plates were prepared and maintained at 4°C. Different test organisms, including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* and *Klebsiella* were subjected to antimicrobial screening using the plant extract-infused gauze. The formation of effective zones of inhibition was observed.

Preparation of ointment :

An ointment for wound healing was prepared using the methanolic extract of *Tamarindus indica*. The preferred ointment base (*e.g.*, paraffin wax, beeswax, petroleum jelly) was mixed with the plant extract. The bioactive components, such as flavonoids and tannins present in the *Tamarindus indica* extract, facilitated the wound healing process.

Extraction of Tamarindus indica bark :

Fresh parts of *Tamarindus indica* bark were collected from Kanyakumari, Tamil Nadu, India, and underwent a rigorous process of washing with distilled water, followed by air-drying in the shade for two weeks (Figure 2). The dried bark was then ground into coarse powder, a critical step before extraction. Utilizing different solvents (Ethanol, Methanol, Chloroform, Hexane, and Aqueous), two extraction methods were employed: the traditional Soxhlet extraction and a shaker-based extraction process. The Soxhlet extraction involved using 50 g of the powdered bark, while the shaker method utilized about 15g of powder for each solvent. The extraction duration for Soxhlet was 24 hours, with varying temperatures (40-70°C) depending on the solvent used. The shaker method involved a 24-hour cycle, after which the solvent was centrifuged and filtered using filter paper[7].

Phytochemical studies :

The phytochemical composition of the crude product obtained from *Tamarindus indica* bark, utilizing the Soxhlet extraction technique, was qualitatively evaluated for the presence of various phytochemicals[1]. The phytochemical screening indicated the presence of alkaloids, tannins, saponins, glycosides, flavonoids, anthraquinones, reducing sugars, terpenoids and phenols (Figure 1). These phytochemical constituents are known for their potential therapeutic properties, including wound healing (ref). To further characterize the phytoconstituents present in the Tamarindus indica bark extract,

a series of standard phytochemical tests were conducted (Table-1). The results showed a diverse range of phytochemicals present in the bark extract, including alkaloids, flavonoids, proteins, tannins, saponins, and more[1]. Flavonoids, in particular, are recognized for their wound healing properties[8].

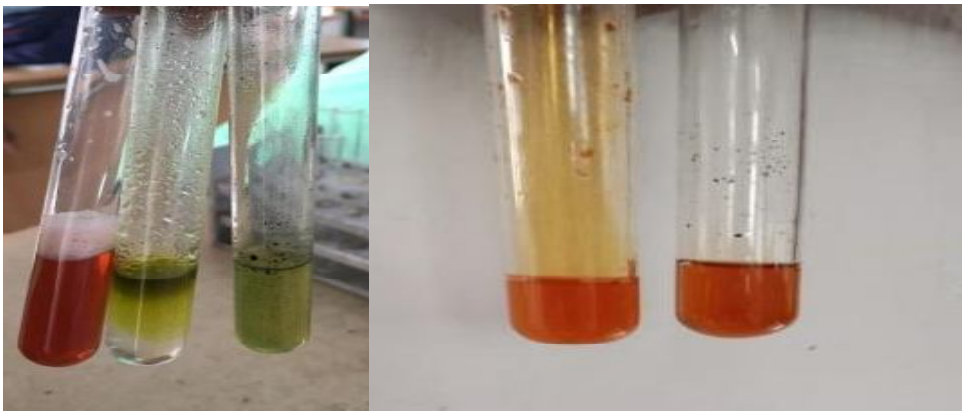


Figure 1: Shows phytochemical tests performed for *Tamarindus indica* bark extract showing positive results (carbohydrate, tannins, proteins & flavonoids

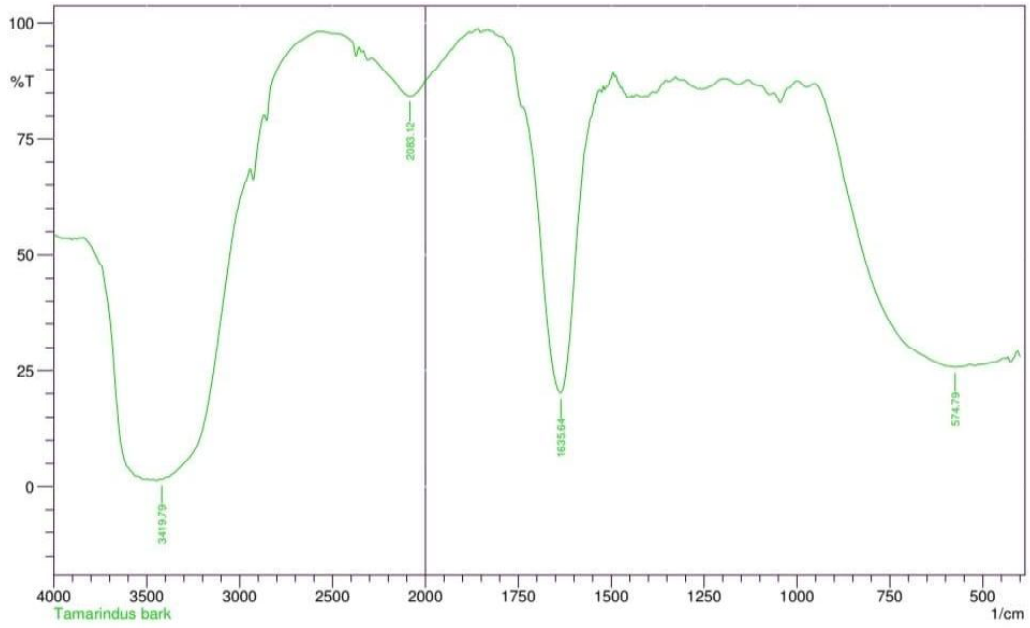
Table-1. shows the result of phytochemical tests

Phytoconstituents	Ethanol	Methanol	Chloroform	n-hexane	Aqueous
Carbohydrate	+	+	+	+	+
Flavonoids	+	+	-	-	-
Alkaloids	+	+	+	+	+
Proteins	+	+	+	+	+
Tannins	-	-	-	-	-
Saponins	+	+	-	+	+
Steroids	+	+	+	+	+
Terpenoids	+	+	-	-	-
Glycoside	+	+	+	-	-
Quinone	+	+	+	+	+
Triterpenoid's	-	-	-	-	-
Phenol	+	+	+	-	+

Characterization of compounds[6]*:*

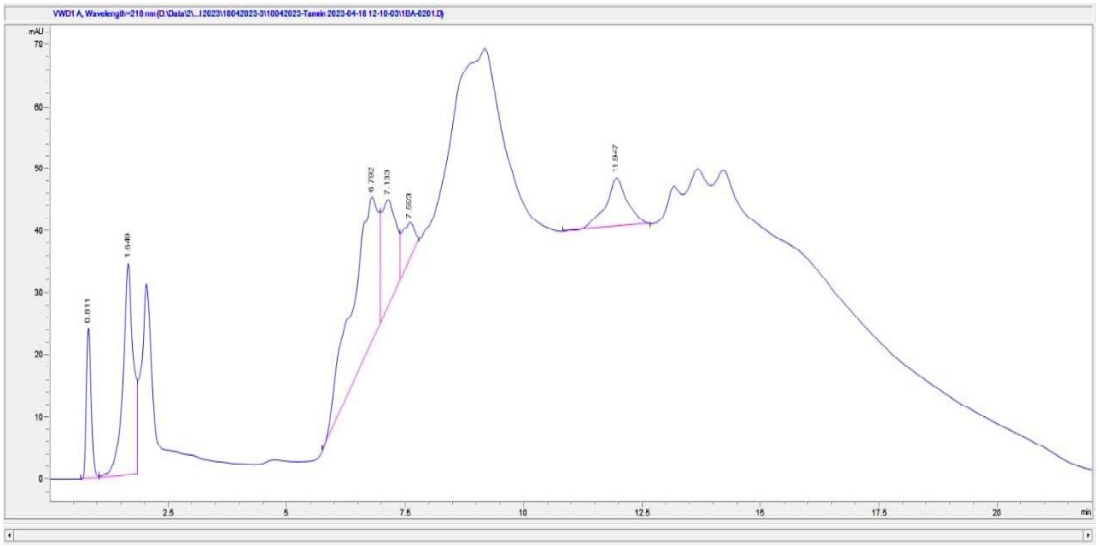
Column chromatography and thin layer chromatography were employed to isolate and analyse the compounds present in the methanolic extract (Figure 2). Additionally, FTIR and HPLC analyses were performed to provide insights into the chemical composition of the extract (Figures 3 and 4). The presence of specific chemical bonds and compounds was confirmed through these techniques, contributing to a comprehensive understanding of the extract's properties and potential therapeutic applications.

Figure 2: Shows the column chromatography performed for the methanolic extract of *Tamarindus indica*



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	574.79	25.741	2.213	952.84	561.29	150.35	23.445
2	1635.64	20.163	63.619	1737.86	1531.48	65.714	49.851
3	2083.12	84.285	0.284	2291.43	2077.33	11.757	0.192
4	3419.79	1.506	1.43	3429.43	2943.37	422.564	-53.327

Figure 3 : Shows the peak value obtained through FTIR analysis



Signal 1: VWD1 A, Wavelength=218 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.811	BV	0.1110	173.21637	24.35765	7.1628
2	1.649	VV	0.2134	531.72925	34.19679	21.9880
3	6.792	BV	0.5187	976.43335	23.50319	40.3773
4	7.133	VV	0.2810	358.86539	17.37174	14.8397
5	7.593	VB	0.2725	119.54546	5.99755	4.9434
6	11.947	BB	0.4515	258.48184	7.86285	10.6887
Totals :				2418.27166	113.28978	

Figure 4 : Shows the peak obtained through HPLC analysis

Antimicrobial activity of extracts of Tamarindus indica:

The antimicrobial potential of the *Tamarindus indica* bark extract was assessed against both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli, Klebsiella, Proteus, Pseudomonas, Streptococcus*) bacteria. The results showcased varying levels of antimicrobial activity across different solvents, with notable efficacy against *Staphylococcus aureus* and *Escherichia coli*[2,3]. This antimicrobial activity makes *Tamarindus indica* bark a promising candidate for wound healing applications.

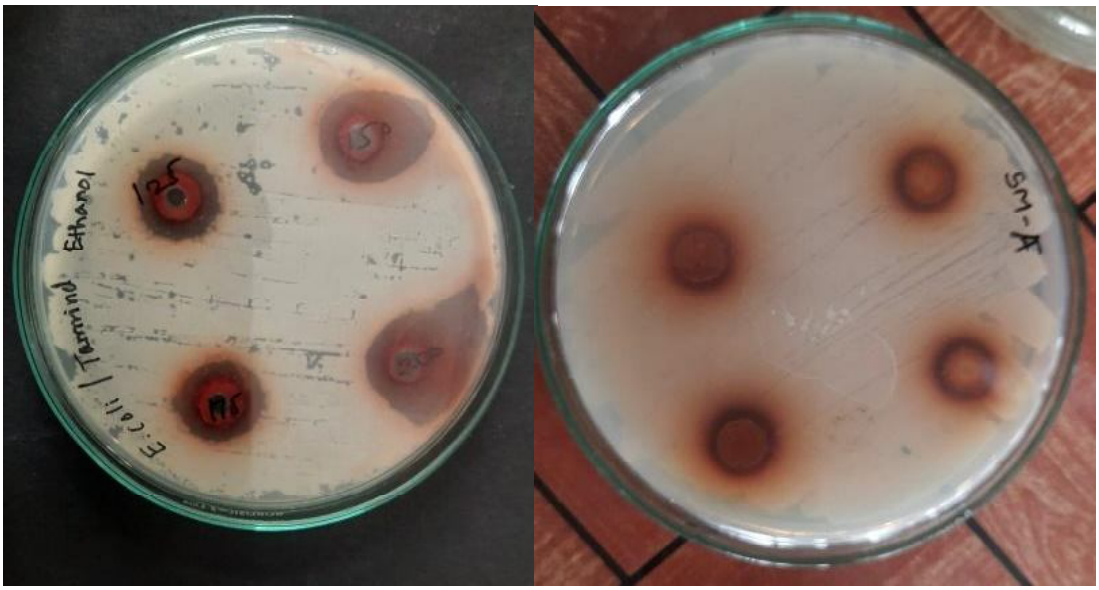


Figure 5: Shows ethanolic extracts of *T. Indicia* (tree bark) shows zone of inhibition towards *Staphylococcus aureus* and *E coli*

Formulation of wound healing products :

Leveraging the bioactive components present in the ethanolic extract of *Tamarindus indica*, a bandage and ointment were prepared for potential wound healing applications[8,9] (Figure 6 & 7). The bandage, soaked in the ethanolic extract, displayed significant antimicrobial activity against *Staphylococcus and Escherichia coli.* This demonstrates the potential of utilizing *Tamarindus indica* bark extract in wound dressings to prevent infections and accelerate wound healing (ref).

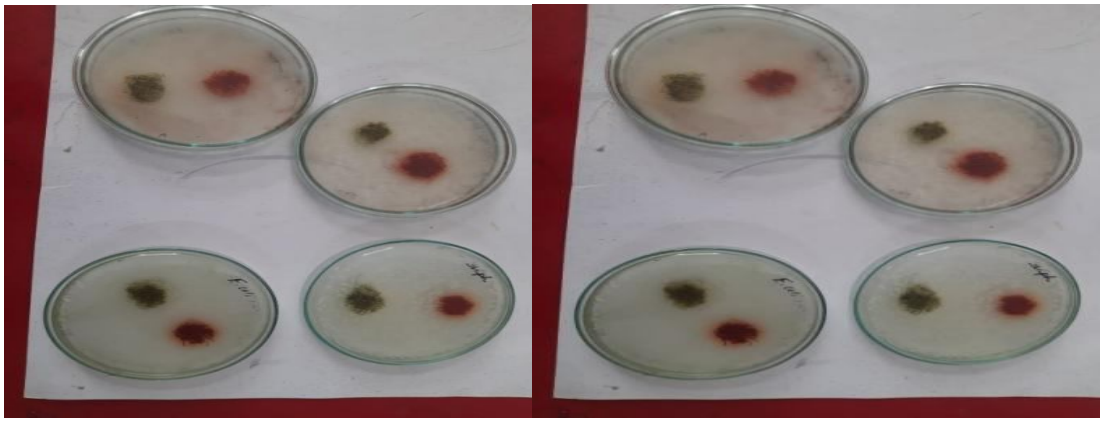


Figure 6: shows the antimicrobial activity of gauze prepared with ethanolic extract of *Tamarindus indica*

Figure 7 : shows the ointment prepared using the ethanolic extract of *Tamarindus indica*

In this study, we explored the therapeutic potential of Tamarindus indica bark extract, a rich source of bioactive compounds. The extraction process revealed a diverse phyto-chemical composition, including alkaloids, flavonoids, and tannins, known for their wound healing properties. Notably, the extract demonstrated significant antimicrobial activity against key bacteria, presenting promising opportunities for combatting infections and supporting wound healing. Isolation and characterization of compounds through various techniques provided valuable insights into the extract's chemical composition, enhancing our understanding of its potential applications. The formulation of a bandage and ointment infused with the ethanolic extract showcased a practical approach to utilizing the extract in wound healing products, exhibiting substantial antimicrobial efficacy. Overall, *Tamarindus indica* bark extract holds significant promise as a natural remedy for wound care, highlighting the need for further research and clinical exploration to fully unlock its therapeutic benefits.

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