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Tridax procumbens L. Extracts: Ethnomedicinal Wound Healing Investigation

Research Article

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Abstract

This research aims to assess the wound healing activity and antimicrobial potential of various extracts (Methanol, Ethanol, n-Hexane, Chloroform, and Aqueous) derived from *Tridax procumbens* L. a plant traditionally used in wound treatment by tribal communities. The study includes evaluating the antimicrobial activity of the extracts using the agar well diffusion method and preparing plant extract-infused gauze and ointment. Different solvents were used to extract the plant compounds using the Soxhlet apparatus. Antimicrobial activity of the extracts was tested against microorganisms isolated from infected wound samples, cultivated on various media (Nutrient agar, Cetrimide agar, Blood agar and MacConkey agar. Minimum inhibitory concentration was determined using the agar well diffusion method, identifying the antimicrobial efficacy of the plant extracts. The ethanolic extract of *Tridax procumbens* L. exhibited the highest antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, among other test organisms (*Streptococcus, Pseudomonas, Proteus* sp.,) isolated from the infected wound samples. Phytochemical tests were conducted to identify bioactive compounds responsible for wound healing, which were subsequently isolated using column chromatography and purified via thin layer chromatography. FTIR analysis was performed to determine the functional groups present and HPLC was conducted for further characterization. The presence of flavonoids, tannins and terpenoids in the plant extracts was identified as the active ingredients responsible for wound healing. Furthermore, a gauze infused with plant extracts was prepared to facilitate wound healing and an ointment was formulated using the ethanolic extract of *Tridax procumbens* L. and an ointment base.

Keywords: *Tridax procumbens* L., Wound healing, Antimicrobial activity, Plant extracts, Phytochemical analysis, Bioactive compounds, Ointment.

Introduction

Throughout history, plants have played a significant role in traditional medicine for the treatment of various ailments. Their therapeutic potential is attributed to the diverse array of bioactive compounds they contain (6). *Tridax procumbens* L. are Coat Button (8), It is a medicinal plant that has been recognized for its pharmacological properties, including antioxidant, antiviral, anti-diabetic, and wound healing activities (2). These properties have sparked scientific interest in exploring the potential of *Tridax procumbens* L. as a natural remedy for wound care. Wound healing is a complex biological process involving multiple stages and interactions between different cellular and molecular components (1). In recent years, there has been a growing focus on discovering novel wound healing agents from natural sources, including medicinal plants. *Tridax procumbens* L. holds promise in this regard, as it has been traditionally used for treating wounds and infections. In this study, we aimed to investigate the wound healing potential of *Tridax procumbens* L. and identify the bioactive compounds responsible for its therapeutic effects. Our objective was to assess the antimicrobial activity of *Tridax procumbens* L. against common wound pathogens and explore its potential application in the development of wound healing formulations. Phytochemical tests to identify the presence of various bioactive compounds in *Tridax procumbens* L. extracts (7,10). Furthermore, we evaluated the antimicrobial activity of the plant extract against *Staphylococcus aureus* and *Escherichia coli*, two major pathogens associated with wound infections. Additionally, we prepared a bandage and ointment using the plant extract and assessed their efficacy in inhibiting microbial growth and promoting wound healing. The findings of this study have the potential to contribute to the development of natural wound healing therapies, offering a safer and more sustainable alternative to conventional treatments. Understanding the bioactive components and their mechanisms of action in *Tridax*

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procumbens L. can provide valuable insights into its therapeutic potential and support its integration into clinical practice. Overall, this research aims to explore the wound healing properties of *Tridax procumbens* L. identify its active compounds, and investigate their antimicrobial activity. The knowledge gained from this study may pave the way for further research, ultimately leading to the development of novel wound healing interventions derived from natural sources like *Tridax procumbens* L.

Materials and Methods

Collection of Plant Material

Fresh parts of *Tridax procumbens* L. were collected in January 2023 from Hindusthan College of Arts & Science in Coimbatore, Tamil Nadu, India. The plant material was thoroughly washed with distilled water and shade-dried for a week.

Extraction of Plant Material

The dried leaves were ground into a coarse powder. Fifty grams of the powder were packed in Whatman filter paper and inserted into a Soxhlet extractor. The extraction was performed using solvents such as aqueous, ethanol, methanol, chloroform, and n-hexane. The extraction process was carried out in the Soxhlet extractor for 24 hours at a temperature ranging from 40-70°C, depending on the solvent used. Additionally, another extraction method was employed using a shaker. Approximately 15 grams of powder were extracted with solvents such as ethanol, methanol, chloroform, hexane and water. The plant material-solvent mixture was centrifuged at 2000 rpm for 15 minutes. After centrifugation, the extract was filtered using Whatman filter paper and the obtained plant extract was collected and stored for further studies.

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, the samples were inoculated onto different culture media, including 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. A loopful of microbial colonies was taken from the culture plate and smeared on a clean sterile glass slide. 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 *Tridax procumbens* L. 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. 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.

Phytochemical Analysis of Plant Extract

The extracts obtained from *Tridax procumbens* L. using the Soxhlet extraction method were subjected to qualitative evaluation for the presence of chemical constituents. Standard procedures were followed for phytochemical tests, including screening for tannins, carbohydrates, proteins, saponins, amino acids, steroids and flavonoids (4).

Column Chromatography

Column chromatography, a preparative technique used to purify compounds based on their polarity and hydrophobicity was employed. 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 analyzed using thin-layer chromatography to detect the separated compounds.

Thin Layer Chromatography

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

FTIR (Fourier Transform Infrared)

The dried powder of the methanol extract of the plant material 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 Tridax procumbens L. The conditions, including the column type, mobile phase composition, gradient program and detection wavelength were specified. The obtained peak values were recorded.

Preparation of Bandage

A gauze infused with the methanolic extract of *Tridax procumbens* L. 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 *Tridax procumbens* L. 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 *Tridax* extract facilitated the wound healing process.

Results and Discussion

The collected plant material from *Tridax procumbens* L. was carefully dried and finely crushed into a powder. This powder was then subjected to extraction using different solvents in a Soxhlet apparatus. The solvents used included methanol, ethanol, n-hexane, chloroform and water. The extraction process aimed to obtain the bioactive compounds present in the plant material. The extracts obtained were collected into conical flasks and stored for further analysis. To isolate the pathogens, present in the wound a simple swab sample was taken from the infected wound site. The swab sample was then inoculated onto various culture media, including blood agar, McConkey agar, nutrient agar, and cetrimide agar. These media support the growth of different types of microorganisms. The inoculated plates were incubated at the appropriate temperature for 24 hours. After incubation, the plates were examined for the presence of visible colonies. Microscopic examination of the colonies on different media was performed to determine their characteristics.

Figure 1: Growth of microbial colonies on different media

a: Cetrimide **b: Nutrient agar** **c: Mac conkey** **d: Blood agar**

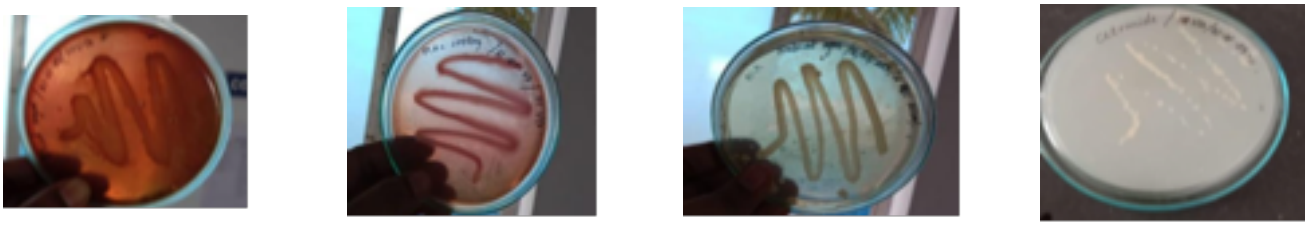


Figure 2: Shows the microscopic examination results of colonies grown on

a: Cetrimide **b: Nutrient agar** **c: Blood Agar** **D: Mac Conkey agar after Grams staining**

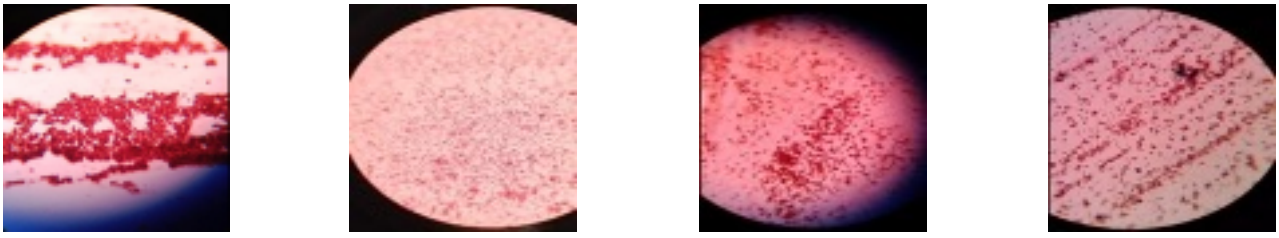


Figure 3 : Shows positive result for (a) Coagulase and (b) Catalase tests

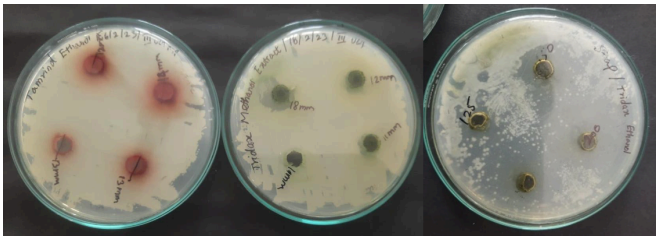
In this study, the microscopic examination revealed the presence of purple-colored, cocci-shaped organisms. This observation indicated the prevalence of *Staphylococcus aureus* a common wound pathogen. To confirm the identity of the pathogen, biochemical tests such as catalase and coagulase tests were conducted. The positive results obtained from these tests further confirmed the presence of *Staphylococcus aureus* in the wound sample. The antimicrobial activity of the extracts obtained from *Tridax procumbens* L. was evaluated using the agar well diffusion method. In this method, wells were made in agar plates and the extracts were

added to the wells. The plates were then incubated to allow the diffusion of the extract into the agar. After incubation, the plates were examined for the formation of zones of inhibition around the wells, indicating the antimicrobial activity of the extract reported by (3). The results of the antimicrobial activity test showed that the methanolic extract of *Tridax procumbens* L. exhibited effective zones of inhibition against both *Staphylococcus aureus* and *Escherichia coli*. The zones of inhibition indicated that the extract had the ability to inhibit the growth of these bacterial pathogens (11). Conversely, the extracts did not show inhibitory activity against *Proteus, Pseudomonas, Streptococcus* and *Klebsiella*. These findings highlight the selective antimicrobial activity of the *Tridax procumbens* L. extract.

Table 1 : Antimicrobial activity of different extracts of *Tridax procumbens* L. by agar well diffusion method (inhibitory zones formation)

Organisms/solvent	Methanol (5mg/ml)	Ethanol (10mg/ml)	n-hexane (25mg/ml)	Chloroform (25mg/ml)	Aqueous 25mg/ml)
Staphylococcus aureus	+	+	+	+	+
Escherichia coli	+	+	+	+	+
Proteus	-	-	-	-	-
Pseudomonas	-	-	-	-	-
Streptococcus	-	-	-	-	-
Klebsiella	-	-	-	-	-

Figure 4: Shows inhibitory zone towards *Staphylococci* and *E coli* by methanolic extract of *Tridax procumbens* L.



Phytochemical analysis was conducted to identify the presence of various bioactive constituents in the methanolic extract of *Tridax procumbens* L. The analysis revealed the presence of several important phytochemicals, including carbohydrates, flavonoids, alkaloids, proteins, tannins, triterpenoids and phenol. These compounds are known for their potential therapeutic properties and contribute to the overall bioactivity of the plant extract.

Table 2: Shows the presence and absence of phytochemical constituents in methanolic extract of *Tridax procumbens* L.

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

Column chromatography is a technique used to separate mixtures into individual components based on their affinity for the stationary phase. Through this process, different fractions were obtained and each containing specific compounds.

Thin layer chromatography (TLC) was then employed to assess the purity of the separated compounds. TLC involves the separation of the compounds based on their differential migration on a thin layer of adsorbent material. The Rf (retention factor) values obtained from TLC analysis indicated the relative mobility of the compounds. The Rf value of 0.54 suggested that the solvent used in the mobile phase had a moderate affinity for the alkaloids present in the purified compound from column chromatography.

Fourier-transform infrared (FTIR) analysis carried out in Karunya university, Coimbatore and performed to identify the functional groups present in the purified compound obtained from column chromatography. The FTIR spectrum obtained exhibited specific peak values at 1055.06 cm-1, 1382.96 cm-1, 2924.09 cm-1, and 3452.58 cm-1. These peaks corresponded to the presence of alcohols, alkanes, nitro compounds and alkyl & aryl compounds, respectively. The identification of these chemical bonds indicated the presence of flavonoids, tannins, saponins and terpenoids in the purified compound.

Figure 5: Shows the purification process by column chromatography & TLC

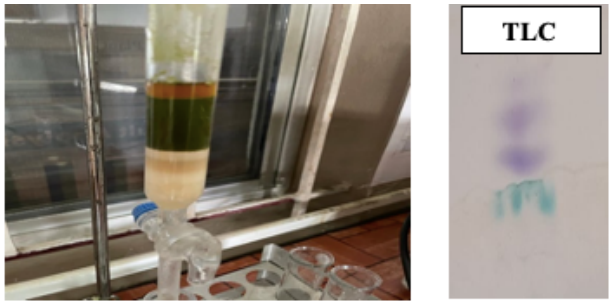
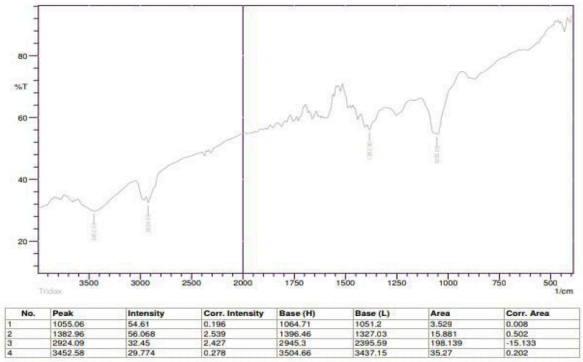
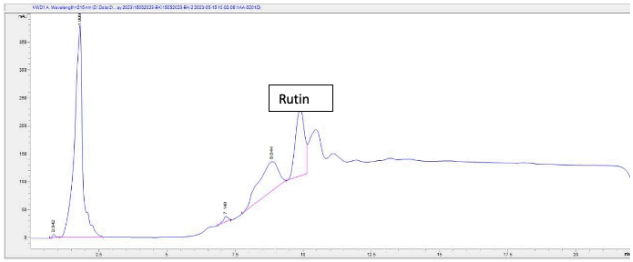


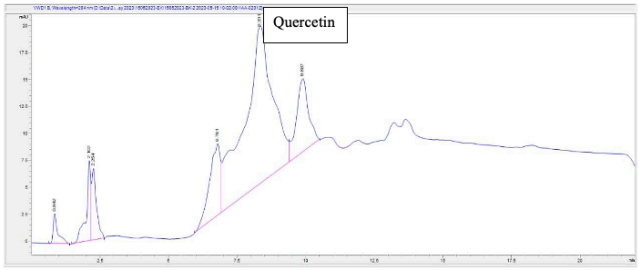
Figure 6: Shows the result of FTIR analysis for the *Tridax procumbens L.* plant



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1055.06	54.61	0.196	1064.71	1051.2	3.529	0.008
2	1382.96	56.068	2.539	1396.46	1327.03	15.881	0.502
3	2924.09	32.45	2.427	2945.3	2395.59	198.139	-15.133
4	3452.58	29.774	0.278	3504.66	3437.15	35.27	0.302

Figure 7: Shows the peak values obtained by the HPLC of *Tridax procumbens L.* extract



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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=215 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.842 BB    0.1175   47.49510    6.19019   0.3464
   2   1.809 BV R  0.2688 7855.29248  380.15356  57.2982
   3   7.148 BB    0.1874  135.30974   10.15851   0.9870
   4   8.844 BB    0.7330 2789.45508   52.91952  20.3469
   5   9.880 BV    0.3637 2881.94873  121.09378  21.0215

Totals :                  1.37095e4   570.51557
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High-performance liquid chromatography (HPLC) done at Karunya university, Coimbatore and this was conducted to evaluate the purity of the compounds present in the extracts. HPLC is a powerful analytical technique used to separate, identify and quantify individual components in a mixture. The HPLC analysis of the *Tridax procumbens L.* extract revealed the presence of specific compounds, such as rutin and quercetin. These compounds are known for their potential wound healing properties.

To assess the practical application of *Tridax procumbens* L. in wound healing, two formulations were prepared: a bandage and an ointment. The bandage was prepared using the infusion of the methanolic extract of *Tridax procumbens* L.

Figure 8: shows gauze infused with methanolic extract of *Tridax procumbens* L. and antimicrobial activity

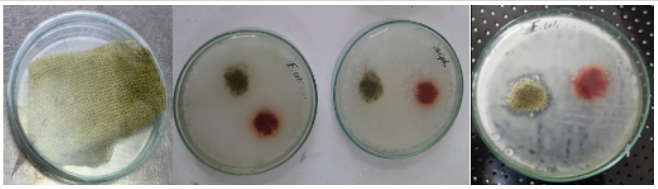
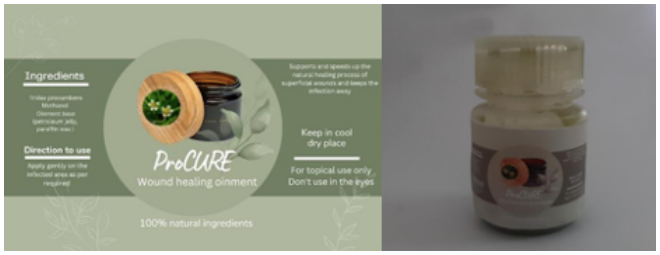


Figure 9: shows the ointment prepared using the methanolic extract of *Tridax procumbens* L. Procure



This ointment was specifically formulated to facilitate wound healing by creating a moist environment, promoting tissue regeneration and preventing microbial growth. The incorporation of the plant extract into the ointment base aimed to harness the potential wound healing properties of *Tridax procumbens* L.

Conclusion

From ancient times, plants have been utilized for treating various diseases. *Tridax procumbens* L. is known for its pharmacological properties, including antioxidant, antiviral, anti-diabetic, and wound healing activities (9). This study aimed to identify the compounds responsible for its wound healing properties through phytochemical tests. The antimicrobial activity of *Tridax procumbens* L. was evaluated using different solvents against common wound pathogens. The results showed significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, emphasizing its potential for wound infection treatment. The wound healing potential of *Tridax procumbens* L. is attributed to bioactive compounds, such as flavonoids, tannins, saponins and terpenoids, which were identified through phytochemical analysis same

reported in his review (5) These compounds have well-known therapeutic effects. Furthermore, practical applications were explored by developing a bandage and ointment using the plant extract. The bandage exhibited antimicrobial activity, while the ointment created a favourable wound healing environment by maintaining moisture and protecting against pathogens. In conclusion, *Tridax procumbens* L. offers potential therapeutic benefits due to its diverse pharmacological properties, particularly wound healing activity. The presence of bioactive compounds, including flavonoids, tannins, saponins and terpenoids, contribute to its wound healing potential. The plant extract's antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* further supports its relevance in wound care. The development of a bandage and ointment utilizing *Tridax procumbens* L. provides practical applications for harnessing its healing properties. These findings support the traditional use of the plant and warrant further research to explore its efficacy, safety, and optimal dosage for wound healing treatments. *Tridax procumbens* L. holds promise as a natural alternative in wound care (4) and may contribute to the development of novel therapeutic interventions.

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