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Preparation And Exploration Of *Terminalia Chebula* Based Ointment For Wound Healing

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Abstract: *Terminalia chebula* has been widely recognized for its medicinal properties, including antimicrobial, antioxidant and wound healing activities. This study focuses on the preparation and exploration of herbal ointment incorporating *Terminalia chebula* extract for the potential and dermatological applications. The ointment was prepared using a stable base and evaluated for physiochemical parameters such as spreadability, ph and stability. Additionally, its antimicrobial and wound healing efficacy were assessed through in vivo and invitro studies. Preliminary studies suggests that the formulations exhibit promising therapeutic effects, making it a potential candidate for testing skin related ailments. Further studies on its long-term stability and clinical effectiveness are recommended to establish its commercial viability.

Key Words: *Terminalia chebula , antioxidant , spreadability, phase separation.*

1. INTRODUCTION :

Terminalia chebula is a widely used medicinal plants in Indian traditional medicine, particularly in Ayurveda. It is a medium to large sized tree found across tropical and subtropical regions of Asia, including china and Tibet. In India, it grows in forests across northern states like Uttar Pradesh and Bengal, as well as in Tamil Nādu, Karnataka, and Southern Maharashtra. Commonly known as black myrobalan in English and harad in Hindi, *Terminalia chebula* belongs to the genus which includes around 250 species distributed across tropical regions worldwide. The fruit *T. Chebula* is highly valued in traditional medicine. It is referred to as the “king of medicine” inn Tibetan healing practices and is considered one of the most potent medicinal fruits in Ayurveda and other folk medicinal systems. *Terminalia chebula*, commonly known as ‘Kadukkaai’ in Tamil Nadu, is widely used in traditional medicine by tribal communities to treat various ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, *candidiasis*, urinary tract infections, and wound infections. Studies have shown that extracts from *T. chebula* exhibit antibacterial activity against several bacterial strains. In addition, plant extracts from different parts including roots, flowers, leaves, and seeds are known for their antibacterial properties and have been applied to cotton-based materials for wound care and healthcare applications. The demand for herbal treatments is growing not only in India but also worldwide, leading to increased research on Ayurvedic plant-based medicines [1] .

Wound healing is a complex and well coordinated process involving biochemical and cellular activities that promote tissue growth and regeneration. It relies on a network of blood cells, cytokines, and growth factors working together to restore injured skin or tissue to its normal state. The primary goal of wound care is to facilitate healing as quickly as possible while minimizing pain, discomfort and scarring. For effective recovery, the healing process must take place in an environment that supports tissue repair and generation

2. METHODOLOGY

Collection and extraction of *Terminalia chebula*:

Terminalia chebula sample was collected from the ayurvedic medical shop in Tirupur. The *Terminalia chebula* samples were dried, and ground into fine powder. The extraction was done by a magnetic stirrer in an ethanol solution [2].

Qualitative analysis of phytochemical screening of *Terminalia chebula*

The phytochemical analysis test carried out for the *Terminalia chebula* sample. The colour change was observed for various phytochemical compounds such as tannins, alkaloids, saponins, steroids, terpenoids, glycosides, flavonoids, phenol, quinones, proteins, carbohydrates [3].

Uv – spectroscopy of the *Terminalia chebula* extract

Different coloured pigments vary in the wavelength of light that they absorb. Most pigments are conjugated compounds wth alternating double and single bonds and typically absorb light in the visible region. The conjugated part of the dye molecule can be very short , meaning that there is a low degree.of conjugation and few alternating double and single bonds, or long. Meaning that there is a high degree of conjugation with many alternating double and single bonds . The extracts were examined under the visible UV-visible spectrum. The extracts were scanned in the wavelength ranging from 200-1100 nm using a systronic Spectrophotometer. The blank was kept as distilled water. The sample was added to the cuvette and the readings were taken for the absorbance. The peak value of the UV-visible was recorded [3].

FTIR spectroscopy analysis of the *Terminalia chebula* extract

Fourier- transform infrared spectroscopy (FT/IR-4600typeA). Turn on FTIR, and wait for 3 characteristic beeps from the instrument. Ensure the instrument is calibrated and run a background scan. Clean the sample holder. Apply one drop or a small micro spatula portion (15-20 mg) to fill the dwell 50% full. Make sure not to scratch the crystal with the spatula or any other metal. Apply the press to the sample, screw it up. Select Monitor Sample from the Measure menu and read the spectra, Save the spectra, Power off the equipment after measurement [4].

Formulation of ointment from *Terminalia chebula* extract

The wound healing ointment was prepared by using *Terminalia chebula* extract along with beeswax, potassium sorbate, liquid paraffin, gelatin and glycerol. At first, the beeswax is melted by using double boiling method. Simultaneously, gelatin and glycerol were dissolved in water. Once both phases reached a uniform consistency, they were combined to ensure proper emulsification. Liquid paraffin was then added to enhance the ointment texture and spreadability. Potassium sorbate was incorporated as a preservative to prevent microbial growth. Finally, the *Terminalia chebula* extract was carefully mixed into the formulation and the ointment was allowed to cool, forming a smooth and stable emulsion. The prepared ointment was then stored in a sterile container for further evaluation .

Analysis of protein denaturation activity for the ointment [5]

The in vitro bioassay consisted of assaying the effect of the extract by denaturation of protein and measuring the absorbance. In vitro Anti-inflammatory assay by protein denaturation activity was performed.

Solution preparation (PBS & BSA):

The reagents such as PBS (Phosphate buffer saline) solution and BSA (Bovine serum albumin) standard was prepared by adding 1 gm of phosphate buffer saline in 100 ml of distilled water and 0.1 gm of Bovine serum albumin was added in 10 ml of distilled water.

Antioxidant activity (DPPH assay) of the Ointment

Antioxidant activity was carried by (DPPH) assay using a modified method of brand William's. DPPH (oxidized form) is a stable free radical with purple colour. In the presence of a antioxidant which can donate an electron to DPPH radicals' decays, antioxidants activity was done by the occurrence of polyphenols is responsible for the hydrophilic antioxidant's activity of *Teminalia chebula* for the calculation of radicals scavenging % and IC50 activity by DPPH assay [6] .

The reagents required are,

1. DPPH solution (2,2-diphenyl-1-picrylhydrazyl)

2. Methanol

3. The total free radical scavenging capacity of the *Terminalia chebula* sample was estimated using the stable DPPH radicals, which is absorption maximum at 580 nm. A solution of the radical is prepared by dissolving. The extract of *Terminalia chebula* of 100µl, 200µl, 300µl, 400µl, 500µl) was added to 3 ml of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 minutes in a dark place. The absorbance of the reaction was measured at 560 nm. Blank was also measured. All the determination was performed in triplicate. The capability to scavenge the DPPH radicals was calculated using the following equation. Radicals scavenging activity inhibition % =(ab-as) / (ab×100). Where, ab - absorbance of blank at t = 0 mins, As - absorbance of the antioxidants a t= 30 mins. A calibration curve was plotted with % DPPH scavenged versus concentration of standard antioxidants and IC 50 value are also estimated .

Evaluation of the ointment

The formulated ointment looks elegant, very soft to touch, free from grittiness, irritant, and such facts detected. This ointment has a yellow- coloured appearance with characteristics pleasant- type odour. Physical evaluation of ointments ensures they are aesthetically pleasing, stable, and effective. Proper formulation and stability testing are essential to maintaining the quality and performance of the ointment [7].

3.RESULT

Collection of samples (*Terminalia chebula*):

The *Terminalia chebula* sample was collected from the ayurvedic medical shop in Tirupur.

Ethanolic extraction of *Terminalia chebula*

The *Terminalia chebula* seeds were removed and dried in sunlight to remove moisture content. After that, the crushed seeds are in powder form. The powder sample of seeds was kept in the magnetic stirrer for extraction. The extraction was done by a magnetic stirrer in an ethanol solution. (Diagram 1).

Diagram1: 1 *Terminalia chebula* extraction

Qualitative phytochemical analysis for the *Terminalia chebula* extract

Phytochemical screening of *Terminalia chebula* revealed the presence of tannins, alkaloids, steroids, and proteins. These findings are consistent with those reported by The total phenolic content in the three different extracts was measured using a standard curve based on gallic acid. Results showed that the phenolic content ranged from 867.2 to 1041.8 mg of gallic acid per gram of extract, with the water extract containing the highest concentration of phenolic compounds among the three. Triterpenoid content was evaluated through a colorimetric method using ursolic acid as the standard. The total triterpenoid content ranged from 0.8 to 4.2 mg of ursolic acid per gram of extract. Interestingly, the water extract had the lowest triterpenoid content, while the methanol extract had the highest. This aligns with the fact that triterpenoids are low-polarity compounds, and nine oleanane-type triter penoids have been isolated from the

methanol extract of *T. chebula*. Regarding tannins, the total tannin content varied from 33.9% to 40.3% per milligram of extract, with the water extract again showing the highest content. The water extract of *Terminalia chebula* showed the highest total phenolic and tannin contents compared to the methanol and 95% ethanol extracts. This likely explains why the water extract also had the best extraction yield. (Table 1)

Table: 1 Phytohemical analysis of *Terminalia chebula*

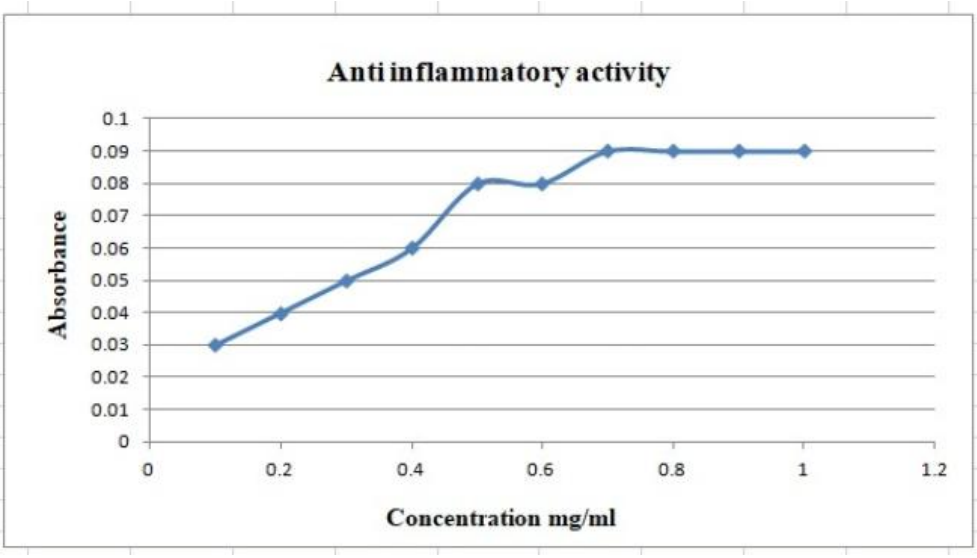
S.NO	TEST	RESULT
1	Tannins	+
2	Alkaloids	+
3	Saponins	+
4	Steroids	-
5	Terpenoids	-
6	Glycoside	-
7	Phenol	-
8	Quinone	-
9	Proteins	+
10	Carbohydrates	-
11	Flavonoids	-
12	Iodine alkaloids	-

(+) indicates positive, (-) indicates negative.

Protein denaturation activity of the formulated ointment

The protein denaturation method was utilized to measure the anti-inflammatory properties of the ointment obtained from *Terminalia chebula.*(Diagram 2)

Diagram 2: Anti-inflammatory activity for formulated ointment

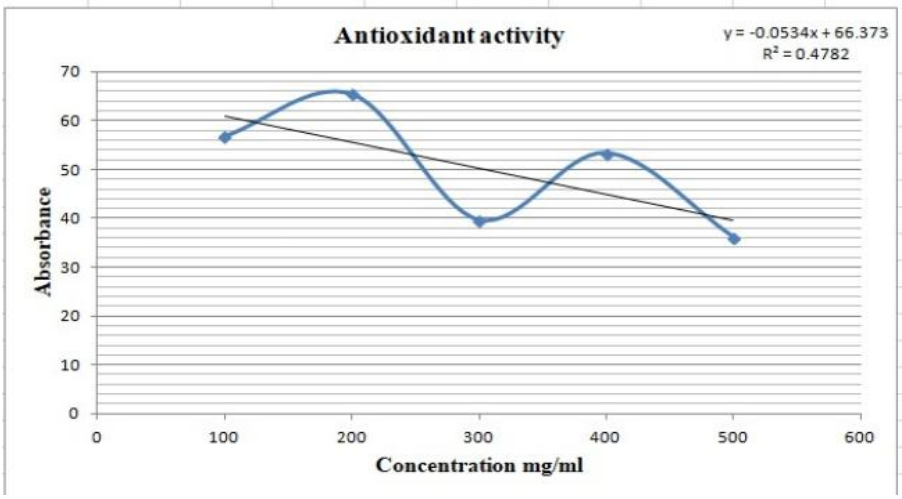


Antioxidant activity of the ointment

The DPPH method assayed the antioxidant activity of *Terminalia chebula* ointment. In this research, six different extracts and four pure compounds from *Terminalia chebula* were tested for their antioxidant activity. All of them showed antioxidant properties, although their potency varied. Recent research has also highlighted several other biological activities of *T. chebula*, including anticancer, antidiabetic, antimutagenic, antibacterial, antifungal, and antiviral effects. Many of these activities may be partially linked to its antioxidant potential. For instance, the antioxidant properties of the methanolic extract of *T. chebula* have been suggested to help alleviate long-term complications in diabetic rats by reducing oxidative stress. Similarly, its antimutagenic and anticancer effects may be associated with its

ability to counteract oxidative damage, as antioxidants are known to interfere with carcinogenesis. In addition, since free radical scavengers have been shown to improve survival rates in influenza-infected mice, the antiviral effects of *T. chebula* might also be related to its antioxidant capacity.(Diagram 3)

Diagram 3: Antioxidant activity for formulated ointment



When the pure compounds were assessed for antioxidant activity, some interesting findings emerged. The results showed that each compound had a somewhat specific mode of action. For example, casuarinin was effective at scavenging free radicals, but it showed only moderate to weak activity in preventing superoxide formation and lipid peroxidation. On the other hand, chebulanin was more effective at inhibiting lipid peroxidation and superoxide generation than in scavenging free radicals. Chebulinic acid's antioxidant effect, however, was mainly due to its strong free radical scavenging activity, rather than its ability to inhibit superoxide formation or lipid peroxidation[8]

UV spectroscopy for the *Terminalia chebula* extract:

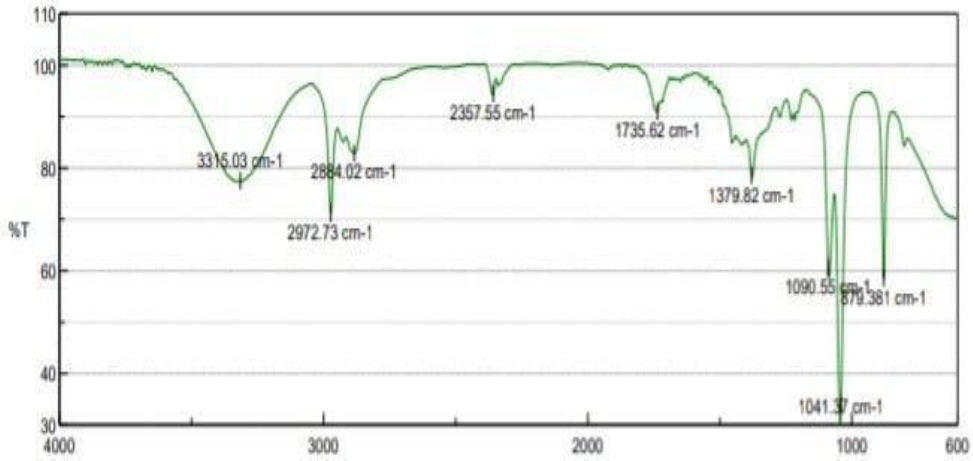
Spectroscopic method has become a powerful tool for secondary metabolite profiling as well as for qualitative and quantitative analysis of the pharmaceutical and biological material. The UV Spectroscopy was characterized for the *Terminalia chebula* extract. The peaks were observed in the wavelength range of 600-700 nm. The peak of absorbance for *Terminalia chebula* at 0.4126 in wavelength of 664 nm. The UV spectroscopy analysis of *Terminalia chebula* extract revealed a characteristic absorption peak at 664 nm indicating the presence of bioactive compounds such as flavonoids, tannin. Similar peak was observed those reported in , where peak was Observed at 430 nm.

FTIR-spectrum analysis of the sample

The FTIR spectrum was utilized to determine the functional group of the active components by analyzing the peak value in the region of infrared radiation. The results of the FTIR spectrum, along with its peak values and corresponding functional groups, are presented in the *Terminalia chebula* extract was analysed using FTIR to separate the functional groups based on their peak ratios. The results of the analysis confirmed the presence of several functional groups including Alcohol, Halogen, Ethers, Alkanes, Alkenes, Nitrile and Carboxylic acid compounds will be present. These functional groups were identified by their corresponding peaks at 879.381cm^{-1}, 1041.37cm^{-1}, 1090.55cm^{-1}, 1379.82cm^{-1}, 1735.62cm^{-1}, 2357.55cm, 2884.02cm, 2972.73cm, 3315.03cm respectively. Peak 1 of the FTIR spectrum of standard *Terminalia chebula* from graph 5 represented the characteristics of C-Cl stretching vibration on 879.381 cm, the similar bonds were observed in the sample also. So, the similar Peak absorption revealed the presence of standard as well as sample. In Peak 2, the absorption bond was observed in a wide range from 1041.37- 1045.28 corresponding to C-O stretching. The peak 3 revealed the presence of C-O Stretching (Ether group), the similar peak was Observed in the sample also. The Peak 4 revealed the presence of 32 C-H Stretching (Alkane group), the similar peak was observed in the sample also. Peak 5 revealed the presence of C=C stretching (alkene group), The similar Peak was Observed in the sample also. Peak 6 revealed the presence of C-H stretching (Alkane group), the similar peak was Observed in the sample also. In Peak 7, the absorption bond was observed in a wide range from 3240.41 - 3423.99 cm-1 corresponds to

C-O stretching. Similar peaks were observed from [9]. Maximum peak has similarity between standard and the sample. (Diagram 4)

Diagram 4: FTIR for *Terminalia chebula* extract



Product formulation (ointment)

The wound healing ointment was prepared by using *Terminalia chebula* extract along with beeswax, potassium sorbate, liquid paraffin, gelatin and glycerol. Initially, the beeswax (15gm) is melted using gentle heat to form an oil phase. Simultaneously, gelatin (5gm) and glycerol (6ml) were dissolved in water to create aqueous phase. Once both phases reached a uniform consistency, they were combined with continuous stirring to ensure proper emulsification. Liquid paraffin (8ml) was then added to enhance the ointment texture and spread ability. Potassium sorbate (1gm) was incorporated as a preservative to prevent microbial growth. Finally, the *Terminalia chebula* (12ml) extract was carefully mixed into the formulation and the ointment was allowed to cool, forming a smooth and stable emulsion. The prepared ointment was then stored in a sterile container for further evaluation .

Antimicrobial activity test for formulated ointment:

The antimicrobial activity for formulated ointment shows different concentrations. The antimicrobial activity of the formulated ointment was evaluated using various test cultures, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas*, *Klebsiella*, and *Candida albicans*. Nutrient agar plates were prepared, and the test organisms were evenly swabbed across the surface. Wells were then created on the agar, and the ointment was added in different concentrations (25 µl, 50 µl, 75 µl, and 100 µl). The plates were incubated at 37°C for 24 to 48 hours. After incubation, zones of inhibition were observed and measured. Notably, a clear zone was seen around the *Pseudomonas* colonies, indicating significant antibacterial activity. Among the tested organisms, the ointment demonstrated the highest effectiveness against *Pseudomonas*, showing a large zone of inhibition. These results suggest that *Terminalia chebula* possesses strong antibacterial properties, particularly against wound-infecting organisms [10]. (Table 2)

Table 2: Antimicrobial activity for formulated ointment.

S.no	Organisms	25µl	50µl	75µl	100 µl
1	*Pseudomonas*	22mm	25mm	27mm	29mm
2	*E.coli*	20mm	22mm	24mm	26mm
3	*Klebsiella*	22mm	24mm	25mm	27mm

4	*S.aureus*	15mm	16mm	18mm	20mm
5	*C.albicans*	16mm	17mm	19mm	21mm

4. CONCLUSION

The formulated *Terminalia chebula* extract ointment demonstrated promising wound healing potential. With the Ph of 5.23, it remains within the ideal range of skin application, supporting a favourable healing environment. The stability tests, including heating at 50 ^{o}c and centrifugation at 3000rpm for 30 minutes showed no phase separation, indicating good physical stability. These results suggests that the ointment formulation is stable and may be suitable for wound healing applications. Thus, *Terminalia chebula* ointment offers a natural cost effective and effective solution for wound care making it a potential alternative to synthetic drugs in dermatology and traditional in the medicine ointment.

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