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Research

Optimization And Evaluation Of Herbal Extract Loaded Hydrogel

G. Pranitha*[1], Dr. G. Praveen Kumar[1], T. Suresh[2]

[1]Associate Professor, [1]Principal, Professor & HOD; [2]MPharm Final Year Student, Department of Pharmaceutics, Sahasra Institute of Pharmaceutical Sciences, Hanumakonda 506007, Telangana

*Author for Correspondence: Dr. G. Praveen Kumar
Email: prof.dr.gunn@gmail.com

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Abstract

Traditionally considered non-toxic, herbs have been used worldwide to treat a wide range of illnesses by both the general public and practitioners of traditional medicine. Eighty percent of people in developing countries receive their basic medical treatment from traditional medicines, mostly made from plants, according to the World Health Organization.The creation, statistical analysis, evaluation, and comparison of *T. Terrestris* hydrogel and its therapeutic efficacy in wound healing are the main objectives of this research. The phytoconstituents were extracted using a variety of more polar solvents, including ethanol, chloroform, ethyl acetate, and aqueous extracts obtained by cold maceration and continuous hot percolation, respectively. T. Terrestris L. was found to have an absorption maximum (max) of 229 and 226 nm in ethanol and a buffer containing 1% SLS, respectively. The FTIR spectrum of T. Terrestris was obtained, revealing the presence of distinctive peaks. A band at 3379.05 (OH stretching) and 3436.91 (-NH2 stretching) can be seen in the badam gum spectrum. The absorption bands at 2923.88 (-CH2 stretching) and 1118.64 (asymmetric stretching of the C-O-C bridge). The hydrogels were found to have a particle size of 461.3 µm and a PDI of 0.072. Acute toxicity studies showed an LD50 investigation on ethanolic extracts of Tribulus terrestris conducted in mice using the Karbers method. Tribulus terrestris ethanolic extracts had LD50 values of 2660 mg/kg and 2870 mg/kg, respectively. One tenth of the LD50 is used as the ED50, and this is how the ED50 of the extract is determined from the LD50. The effects of L. leaves on newly created wounds were investigated using three different models: excision wound, in vivo, and in vitro. models of dead space wounds and incisions. *T. Terrestris* extracts decreased the area of wounds in different animal groups during a period of 23 days. The several species of L. leaves are listed in Table 6.22. Rats treated with TT-Ethanol had a wound contraction rate ranging from 7.85±3.64 to 46.29±5.68 percent from day 2 to day 12, while rats treated with TT-chloroform had a wound contraction rate ranging from 18.59±3.82 to 80.65±10.59 percent, and rats treated with standard mupirocin cream had a wound contraction rate ranging from 17.53±9.50 to 90.53±10.59 percent. A unique topical gel formulation with promising wound healing activities made of natural components is the study's output.

Keywords: Herbal Extract, Hydrogel, Wound Healing, *T. Terrestris*

INTRODCUTION

Active ingredients found in medicinal plants can aid in the treatment of illness or lessen discomfort. In the majority of developing nations, traditional medicines and medicinal plants are frequently employed as therapeutic agents to maintain good health. Eighty percent of people in developing countries receive their basic medical treatment from traditional medicines, mostly made from plants, according to the World Health Organization.[1,2] The plant's medicinal qualities may be explained by the phytochemicals' anti-oxidant, antibacterial, and antipyretic capabilities. Traditionally considered non-toxic, herbs have been used worldwide to treat a wide range of illnesses by both the general public and practitioners of traditional medicine. The general public and professional groups for traditional medicine have not yet acknowledged the possibility of herb toxicity, even though the literature has repeatedly reported cases of toxicity resulting from the use of herbs.[3]

Ancient secrets have just recently been developed and commercialized in herbal medicine. Many patients who were unhappy with traditional medication or surgery turned to herbal medicine. Since herbal treatments come from natural sources, they are still commonly used today and are seen to be safe.[4,5]

Instead of using the entire plant to extract and synthesize the active ingredients, pharmacologists isolate, extract, and synthesize specific components. In addition to active ingredients, minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavonoids, and other plant-based chemicals are essential for sustaining the medicinal properties of herbs.[6,7] A full plant, with all of its components, requires a considerably larger amount to reach a poisonous level than isolated or synthesized active compounds, which can be dangerous in minute amounts. This is another reason why these ingredients offer an essential natural defense. On the other hand, herbs are powerful remedies.[8] They are not to be taken lightly. From Europe to the Orient, scientists have confirmed the benefits of numerous medicinal herbs. Thanks to modern technologies, science can now identify some of the distinctive characteristics and interactions of plant elements. Thanks to this scientific data, we now understand why certain plants are effective against different conditions.[9,10]

Aim, objective

The creation, statistical analysis, evaluation, and comparison of *T. Terrestris* hydrogel and its therapeutic efficacy in wound healing are the main objectives of this research.

MATERIALS AND METHODS

Chemicals and equipements used in expierments

Tables 1 and 2 below list the chemicals and tools utilized in the formulation and assessment of wound healing gel preparations in experiments.

Table 1: List of Chemicals used

S. No	Name of the Chemicals	Manufacturer
1	*T. Terrestris*	Himalaya drug company, Bangalore, Karnataka.
2	Lecithin	Fisher Scientific, India, Mumbai, INDIA.
3	Polyethylene glycol	Fischer Scientific, Mumbai, INDIA.
4	Ethanol	Sigma-Aldrich Corporation.
5	Sodium benzoate	Thermo Fischer Chemicals, india
6	Dialysis membrane-70	HimediaLab.Pvt.Ltd,Mumbai
7	Cholesterol AR	SD Fine-Chem Ltd, India
8	Potassium dihydrogen phosphate	Merck, India
9	Sodium hydroxide	Merck, India
10	Carbopol® 980NF	Lubrizol Advanced Materials India Private Limited, India
11	Acetonitrile	Thermo Fisher Scientific, India
12	Methanol	Thermo Fisher Scientific, India
13	Chloroform	Thermo Fisher Scientific, India
14	Formic acid	Thermo Fisher Scientific, India

Instruments used

Table 2 : List of Instruments Used

S.No	INSTRUMENTS	MANUFACTURER/ MODEL
1	FTIR Spectrophotometer	Bruker IFS 125HR
2	UV-Visible Spectrophotometer	Elico Version 6.1
3	Hot air oven	Universal Q-5427
4	High precision balance	Wensar AX-200
5	Centrifuge	Remi TROI
6	Magnetic stirrer	Remi 1MLH
7	Brookfield viscometer	LV DVE LDVD
8	Homogenizer	Remi RQT-124A
9	pH meter	CyberScan pH 510 Eutech Instruments
10	Rotavapor	Buchi R-210
11	Probe sonicator	Rivotek™ Ultrasonic sonicator
12	Bath sonicator	Branson CPX 1800 H-E
13	Inverted microscope	Labomed TCM 400
14	Zetasizer	Nano ZS Malvern instruments
15	Micro-Ultracentrifuge	Sorvall MTX 150 Thermo scientific
16	Franz diffusion cell	Franz diffusion cell Perme Gear

Selection of the plant

Nature has consistently given humans access to a wide range of structurally diverse and pharmacologically active chemicals, which have shown to be invaluable in the treatment of life-threatening illnesses or as starting points for the development of new medications.

Global historians have presented evidence that suggests, at least in part, that prehistoric people employed herbs—often in complex ways. They are utilizing the plants to treat a variety of illnesses based on their experience.

T. Terrestris Linn.

The aforementioned plants are widely distributed in the wild and are considered cosmopolitan.

Collection and authentication of plant

In June of 2002, the Tribulus terrestiris plant was harvested from the Namakkal District plains. Botanist Prof. Dr. K. Sigamani, Head of the Department of Botany at Kandasamy Kandar College, Velur, Namakkal District, Tamilnadu, India, identified and verified the collected plants.

STATISTICAL ANALYSIS

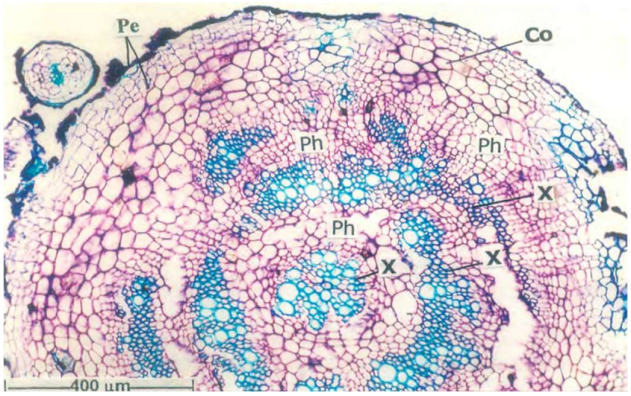
All statistical analyses were carried out using Statistica 13.0, STATSOFT; Statistica, Tulsa, OK, USA. The standard deviation (SD) and mean (M) of the experimental data were reported. To determine whether there was a difference between the groups, a one-way ANOVA was used, followed by Tukey's multiple comparison tests; $p < 0.05$ was deemed significant.

RESULTS & DISCUSSION

Tribulus terrestris Linn.

Leaf

The leaf is slender, with a less noticeable midrib, and undulating surfaces. In sectional view, the abaxial size is slightly protruding into a blunt hump, the adaxial side is inflated, and the midrib is planoconvex. The thickness of the midrib in a vertical plane is 320 lam. On the adaxial side, it has an epidermal layer that resembles the lamina's epidermal cells. The epidermal cells on the abaxial side of the midrib are dilated, round, and have thin walls. Along the adaxial portion of the midrib, the palisade tissue is transcurrent in a horizontal direction. One or two layers of highly dilated parenchyma cells make up the abaxial. A small patch of phloem tissue and a cluster of xylem elements make up the single, top-shaped, collateral vascular bundle. There is a single layer of chloroplast-containing sheath cells in the bundle.



Co=Cortex; Pe=Periderm; Ph=Phloem; X=Xylem

PHYTO CHEMICAL SCEREENING

The phytoconstituents were extracted using a variety of more polar solvents, including ethanol, chloroform, ethyl acetate, and aqueous extracts obtained by cold maceration and continuous hot percolation, respectively. Table 6 displays the color and consistency of different Tribulus terrestris extracts. Table 7 provides the extraction values.

Chemical testing was used to identify the phytoconstituents, and the results indicated that the following extracts included a variety of phytoconstituents.

Table 3. Qualitative chemical tests of various extracts of leaves of Tribulus terrestris.

S. No	Constituents	Tests	$CHC1_3$	Ethyl acetate	Ethanol	Aqueous
1	Alkaloids	Mayer's reagent	-	-	+	-
		Dragondraffs reagent	-	-	+	-
		Hager's reagent	-	-	+	-
		Wagner's reagent	-	-	+	-
2	Sterols	Libermann's sterol test	+	+	+	+
		Libermann's test	+	+	+	+
		Salkowski's test	+	+	+	+
3	Carbohydrate and glycosides	Molish reagent	-	-	+	+
		Fehlings reagent	-	-	+	+
		Barfoed's reagent	-	-	+	+
		Borntrager's reagent	-	-	+	+
		5% KOH	-	-	+	+
4	Fixed oils and fats	Spot test	+	+	-	-
		Saponification	+	+	-	-
5	Phenolic compounds	Extract + FeC13	-	-	-	-
6	Test for Tannins	Gelatin test	-	-	-	-
		FeC13test	-	-	-	-
7	Protein and amino acids	Biuret test	-	-	-	-
		Ninhydrin test	-	-	-	-
		Xanthoprotein test	-	-	-	-
		Millon's reagent	-	-	-	-
8	Triterpenoids and Saponins	Tin + Thionyl chloride	-	-	+	+
		Foam test	+	+	+	+
		Haemolysis test	+	+	+	+
9	Gums and mucilages	Precipitation with 95% alcohol	-	-	-	-
		Molish's test	-	-	-	-
		Ruthenium test	-	-	-	-
10	Flavone and flavonoids	Aqueous NaOH	-	-	+	-
		Con. H2S04	-	-	+	-

Acute toxicity studies

Using a gastric gavage tube, the animals were given several dosages of Tribulus terrestris ethanolic extracts orally. The animals were monitored closely for the first twenty-four hours following the extract administration in case of acute toxicity-related deaths. Tables 10–11 present the findings of an LD50 investigation on ethanolic extracts of Tribulus terrestris conducted in mice using the Karbers method. Tribulus terrestris ethanolic extracts had LD50 values of 2660 mg/kg and 2870 mg/kg, respectively. One tenth of the LD50 is used as the ED50, and this is how the ED50 of the extract is determined from the LD50.

Table 4. Determination of LD50 value of ethanolic extract of Tribulus terrestris by karber's method.

S. No	Groups animal	Dose (mg)	Dose difference (mg) (a)	Mortality	Mean mortality (b)	Product a x b
1	6	500	0	0	0	0
2	6	1000	500	1	0.5	250
3	6	1500	500	2	1.5	750
4	6	2000	500	3	2.5	1250
5	6	2500	500	5	3.5	1750
6	6	3000	500	6	5.5	2750
						6750

LD50 value = 3000 -6750 / 10= 625 mg /kg ED50= 625 mg/kg.

Pharmacological Investigations of Extracts of *T. Terrestris*. L. leaves

Wound Healing Activity of Extracts

Numerous phases, including granulation, collagen maturation, and scar formation, occur concurrently yet independently of one another during the healing process of a wound. As there are now only three in vivo wound research models available, using a single model is insufficient because numerous models provide diverse information. The extract of T. Terrestris has regenerative qualities. The effects of L. leaves on newly created wounds were investigated using three different models: excision wound, in vivo, and in vitro. models of dead space wounds and incisions

Excision wound model

The excision wound model is used to investigate the rate of wound contraction and epithelialization. Contraction and epithelialization are required throughout the healing process since the boundaries of an excised wound are not in contact with each other. Consequently, epithelialization and wound contraction were evaluated using the excision wound model.

Percent wound contraction and period of epithelization

Wound contraction, which starts in the fibroblast stage, causes the wound to shrink during the healing process. Epithelialization is the process by which epithelial cells multiply and move toward the center of a lesion after it has been sustained.

***T. Terrestris*L. leaves Extact**

T. Terrestris extracts decreased the area of wounds in different animal groups during a period of 23 days. The several species of L. leaves are listed in Table 6.22. Rats treated with TT-Ethanol had a wound contraction rate ranging from 7.85±3.64 to 46.29±5.68 percent from day 2 to day 12, while rats treated with TT-chloroform had a wound contraction rate ranging from 18.59±3.82 to 80.65±10.59 percent, and rats treated with standard mupirocin cream had a wound contraction rate ranging from 17.53±9.50 to 90.53±10.59 percent. The mean epithelization time of rats treated with std mupirocin, TT-Ethanol, TT-chloroform, and negative control was 22.33±0.66, 21.33±0.66, 20.66±0.33, 20.49±0.23, 18.51±0.37, 16.28±0.15, and 15.28±0.15 days, respectively. The statistical data analysis showed that the mean epithelization durations and wound contraction of the extract-treated rats were significantly different from those of the negative control groups. Here are several extracts from T. Terrestris. Day 12 showed the fastest rate of wound contraction and the shortest length of epithelization for L. leaves, TT-Ethanol extract, and std mupirocin cream, but they were not comparable.

It's possible that the treated rats' increased fibroblast activity accelerated wound contraction and sped up the formation of epithelial cells. Wound contraction is regulated by specialized myofibroblasts found in granulated tissue. Bacteria and their metabolites inhibit wound contraction and impede healing, which is why they slowed down wound closure in negative control rats [425].

Table 5: Effect of extracts of *T. Terrestris*. L. leaves on rate of wound closure and epithelization period.

Treatment groups	Percentage wound closure (%)						Epithelization period (days)
	Day2	Day4	Day6	Day8	Day10	Day12	
Negative Control	7.85 ± 3.64	16.28± 5.34	24.16± 3.48	30.49± 6.29	35.49± 4.57	46.29± 5.68	20.49 ± 0.23
TT-Ethanol	26.54 ± 9.52	35.6 ± 11.47	54.9 ± 9.63	66.6 ± 12.59	77.34± 13.26	88.68± 11.56	15.28 ± 0.15
TT-Chloroform	18.59 ± 3.82	29.5 ± 9.51	39.9 ± 10.73	50.9 ± 9.85	66.5 ± 12.59	80.65± 10.59	18.51 ± 0.37
TT-Ethyl acetate	11.32± 11.53	19.52 ± 9.68	29.76± 9.74	40.12± 12.86	52.83± 8.53	69.23± 11.64	20.66 ± 0.33
TD-Aqueous	11.62 ± 9.37	20.31 ± 10.92	29.92± 8.58	39.99± 13.92	53.28± 8.27	67.3 ± 10.11	22.33 ± 0.66
Positive Control	17.53± 9.50	45.44 ± 12.46	67.29± 11.22	78.35± 8.59	87.39± 9.86	90.53± 10.59	14.53±2.13

*N=6; Values are represented in Mean ± SEM. ns- not significant, *p≤0.05; **p≤ 0.01, ***p≤ 0.001 versus negative control (one-way ANOVA, followed by Dunnett's test)*

Preformulation Studies

Ultraviolet (UV) spectrum

As the medication is being created, preformulation studies need to provide an analytical method for determining drug concentrations. T. Terrestris L. is an anticoagulant, antifungal, and insect repellant that has been used for wound healing and anticoagulation in India. T. Terrestris is used by Indian traditional healers to treat wounds, boils, and blisters. Because the T. Terrestrisis molecule has a large number of unsaturated bonds, its spectrum falls between 200 and 400 nm. The UV absorption spectra of T. Terrestris L in water and buffers containing 1% sodium lauryl sulphate (SLS) are displayed in Figure 1. T. Terrestris L. was found to have an absorption maximum (max) of 229 and 226 nm in ethanol and a buffer containing 1% SLS, respectively [432].

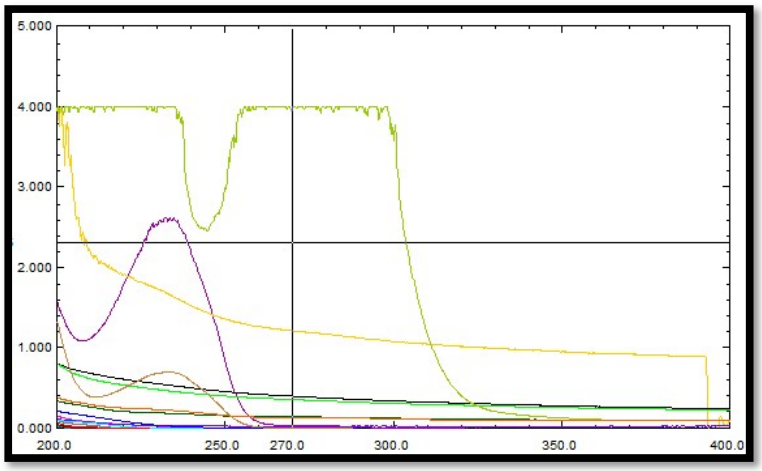


Fig 1: UV scan of *T. Terrestris*. L in different solvents

Before developing a formulation, the drug molecule was initially analyzed to ascertain its physiochemical properties. A new analytical method for formulation, in vitro, and animal research was developed. Throughout the formulation development process, these investigations help identify and address problems. The creation of active pharmaceutical ingredients and their products depends on these studies.

Characterization of *T. Terrestris*. L

Numerous processes were employed to determine the authenticity and purity profile, including:

UV-Visible Spectroscopy

UV spectroscopy is used as a phosphate buffer to determine the peak maxima of a drug at a specific wavelength, which helps determine the purity of a sample. Thus, the drug's peak max (229 nm) in a pH 7.4 buffer solution (1 percent v/v) was calculated, and this scan indicates that the result was consistent with the

extract's reported max (232 nm) in the standard literature. This allows for the verification of the drug's purity (Fig. 2).

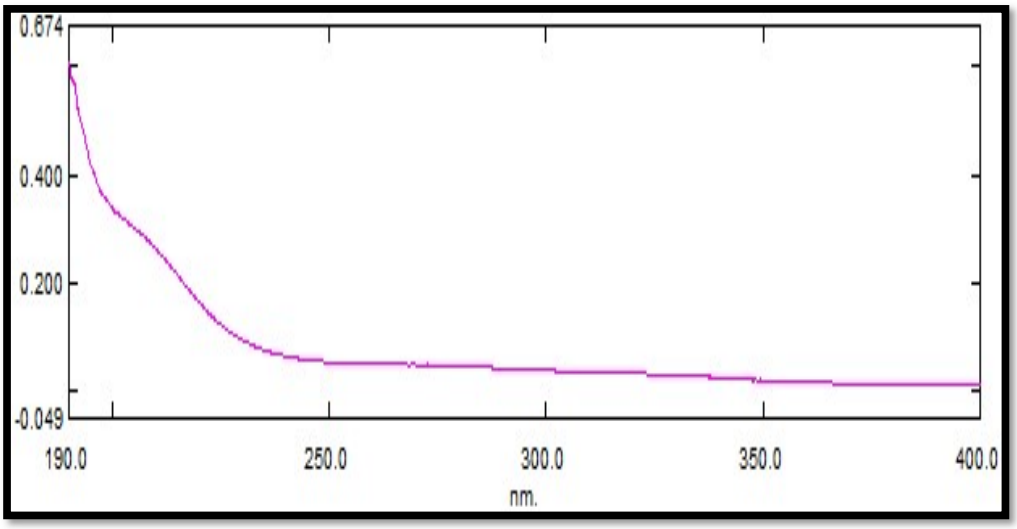


Fig 2: UV-Visible Spectroscopy

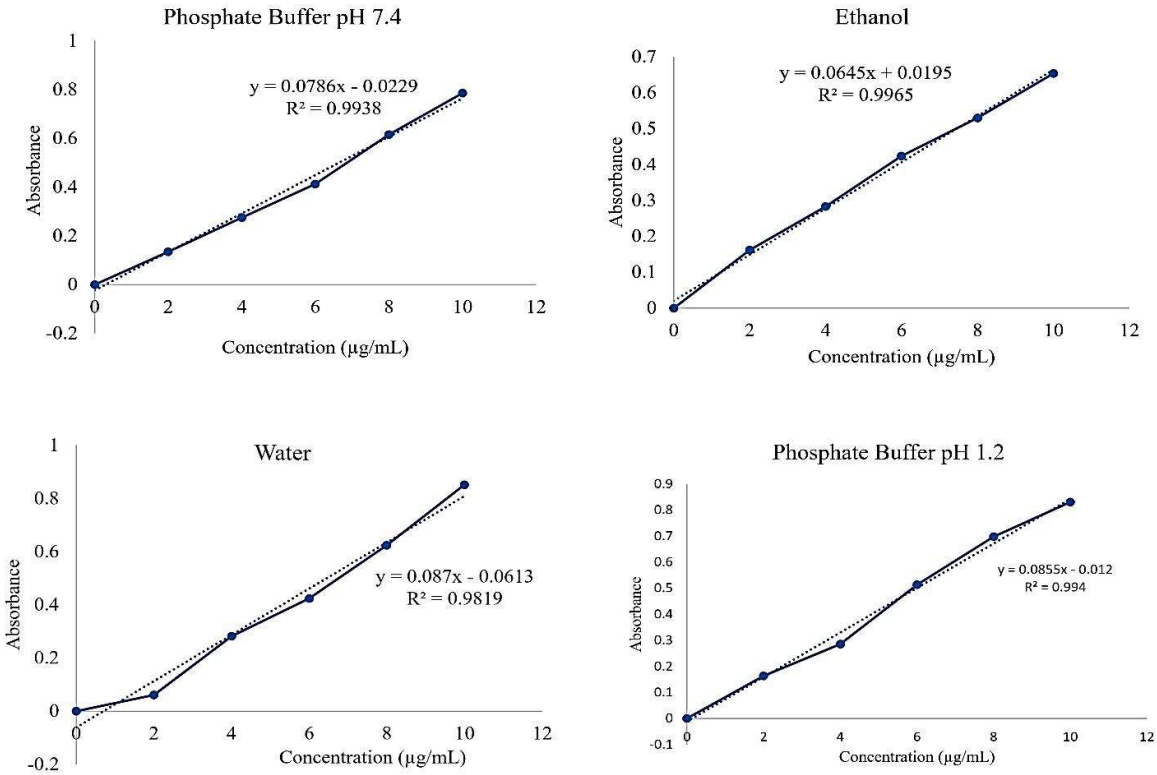


Fig 3: Calibration cure of *T. Terrestris*. L on different mediums

Beer's law was followed throughout the method, and T. Terrestris concentrations between 2 and 10 g/mL may be employed. L aqueous leaf extract from many mixes of samples. With a correlation coefficient of 0.995, it was found that the aqueous extract content and the corresponding absorbance readings were favorably associated. The following equation illustrates the link between concentration and absorbance:

Y= 0.0966x-0.1292
where x is the concentration of T. terrestris and y is the absorbance at 229 nm. concentration of L's water-based extract

Solubility
There are leaf extracts that are freely soluble in DMSO, water, and buffer solutions with pH values of 1.2, 6.8, and 7.4. The leaf extract was discovered to be insoluble in ether, pet, n-butanol, methanol, chloroform, and ethanol or diethyl ether. Water insoluble, but somewhat soluble in ether.

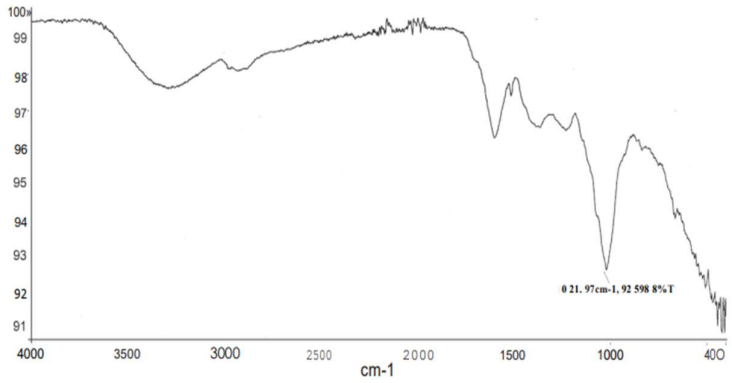


Fig 4: Solubility determination of leaf extract with different solvents

Compatibility study of FTIR

The FTIR spectrum of T. Terrestris was obtained, revealing the presence of distinctive peaks. Table No. 7 displayed the descriptions of the observed peak.

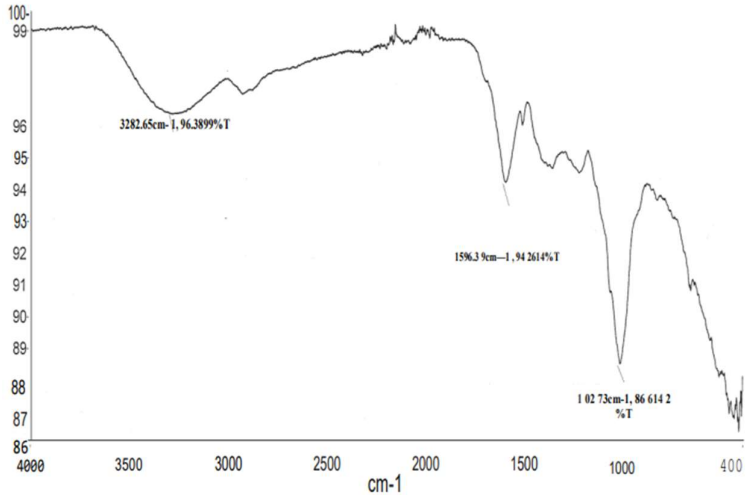


Fig 5: FTIR structure of *T. Terrestris*

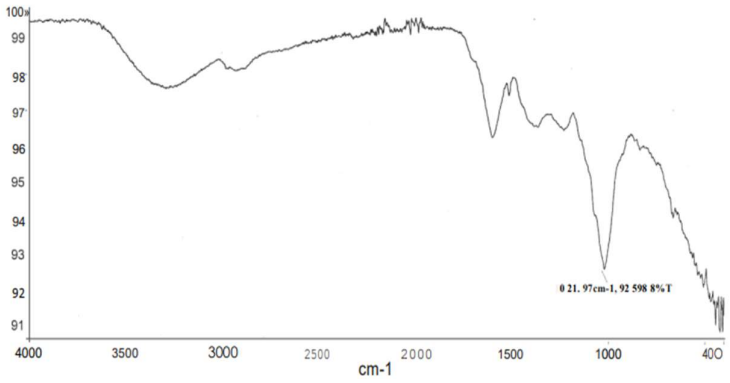


Fig 6: FTIR structure of Badam gum

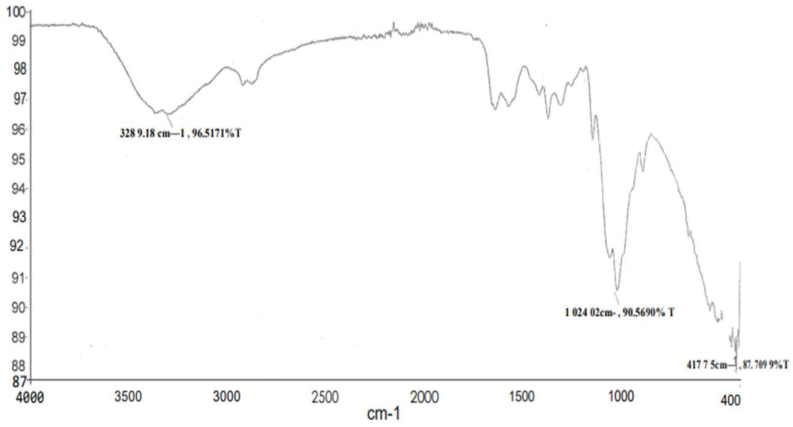


Fig 7: FTIR structure of Hydrogel

A band at 3379.05 (OH stretching) and 3436.91 (-NH2 stretching) can be seen in the badam gum spectrum. The absorption bands at 2923.88 (-CH2 stretching) and 1118.64 (asymmetric stretching of the C-O-C bridge). The chitosan spectrum's band at 1652.88 was identified as the C=N creation resulted from an imine reaction between the chitosan's amino group and glutaraldehyde.

pH Determination

Skin barrier renewal and the antimicrobial response are significantly influenced by the acidic nature of the skin [62, 63]. Numerous endogenous (such as sweat composition, sebum secretion intensity, sex, age), as well as exogenous (such as cosmetics, detergents, dermatological medicines), factors can affect the pH of the skin. On the other hand, preserving an acidic pH protects the stratum corneum's integrity and the lipid barrier while being advantageous to the physiology of the epidermis and skin microbiota [64]. The investigation found that the HF1, HF4, and combination of badam gum and tamarind-based formulations had the lowest pH values (4.36±0.02 and 5.13±0.03, respectively) and the highest pH values (6.84±0.04 for HF3, respectively) (Table 3). The pH values of the produced formulations ranged from 4.36±0.02 to 6.84±0.04, indicating that they can be applied to the skin without producing irritation. This pH range corresponds to the physiological pH of the skin.

Table 6: Characterization of pH and spreadability of

F.Code	pH	Spreadability (mm2)
HF1	4.36±0.02	271.03±0.23
HF2	5.69±0.01	286.94±1.35
HF3	6.84±0.04	316.52±2.46
HF4	5.13±0.03	294.86±1.03
HF5	5.47±0.01	256.34±0.49

Scanning electron microscopy (SEM) of T. Terrestris hydrogel

Scanning electron microscopy was used to analyze the hydrogel's morphology. The surface morphology of the *T. Terrestris* hydrogel at various magnifications was displayed in Fig. The majority of the hydrogel had a rough, wavy shape, as the SEM image demonstrated. The presence of *T. Terrestris* may be the cause of the hydrogels' rough surface.

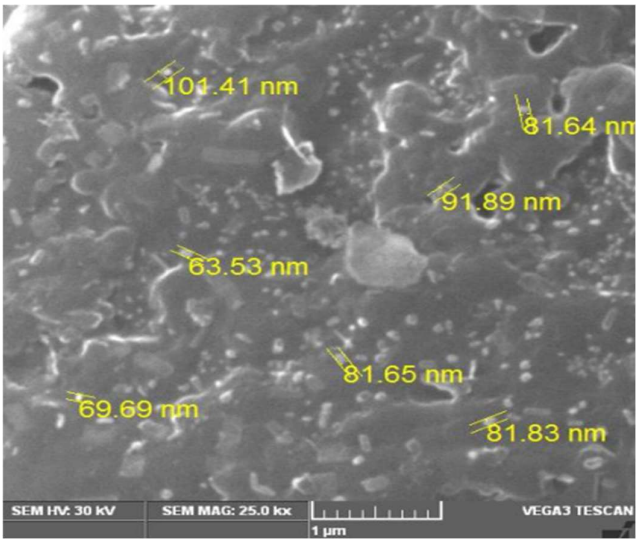


Fig 8: SEM image of optimized Hydrogel (HF3)

Particle size analyzer

The hydrogels were found to have a particle size of 461.3 µm and a PDI of 0.072. Zeta potential measurements were used to assess the relative charge of the hydrogels beyond their hydrodynamically stationary layer. Glutaraldehyde crosslinking resulted in a negative potential (-3.07) for formulation (F5). Similar to hydrogels made with a higher chitosan content, the hydrogels with higher potential values have a higher charge density of the amino groups on the surface. The chitosan amino groups undergo protonation in the acidic region, leading to comparatively high potential values.

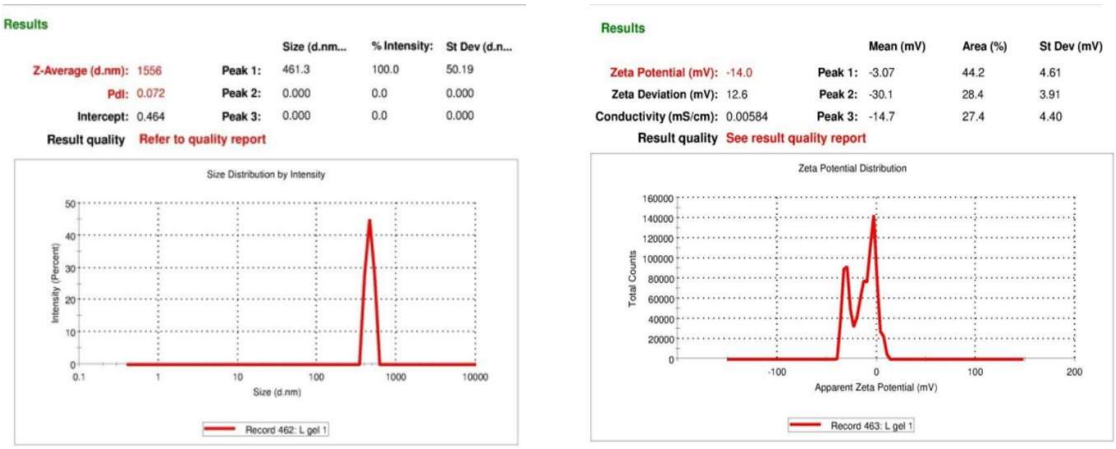


Fig 9: Size distribution & . Zeta potential of *T. Terrestris* loaded hydrogel.

Stability study

The optimal formulation of T. Terrestris loaded badam gum and tamarind hydrogel tablets underwent stability testing for three months at 25±2ºC/60±5% RH and 40±2ºC/75±5% RH. Table 14 displays the results of the analysis of the hydrogel's appearance, average weight, assay, and entrapment efficiency. The findings showed that there were no appreciable differences seen across all parameters.

Table 7: Stability study report of *T. Terrestris* loaded hydrogel

F.Code	Conditions	Time Interval (Month)	Average Wt (mg)±5	Colour	% EE	Drug content
HF3	25 °C±2 °C/75±5 % RH	0	350.05	Pale yellow	98.47	99.86
		3	346.52	Pale yellow	97.53	99.06
	40 °C±2 °C/75±5 % RH	0	351.24	Pale yellow	98.47	99.86
		3	342.15	Pale yellow	97.03	98.76

CONCLUSION

Every stage of the wound healing process was discovered to be affected by the sophisticated formulations containing tamarind seed polysaccharide and badam gum. At any point during the wound recovery process, it permits the healing process to proceed uninterrupted, efficiently, and in a planned manner. For this reason, antibacterial treatments combined with formulations containing polysaccharide from tamarind seeds and badam gum will undoubtedly prove to be effective wound healing agents. In vivo investigations clearly demonstrated that among the five formulations, HF3 had superior wound healing capacity. A unique topical gel formulation with promising wound healing activities made of natural components is the study's output.

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