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Research Paper

A Phytotherapeutic Approach to Wound Healing: Evaluation of a Dual-Herb Gel Containing Euphorbia hirta and Tridax procumbens

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ABSTRACT

This study provides an emphasis on the formulation and evaluation of a polyherbal wound healing gel from Euphorbia hirta and Tridax procumbens. The plants are known to have been traditionally used in treating wounds and skin disorders due to their rich phytochemical profile. All tests were performed on gelling properties such as colour, appearance, pH, spreadability, homogeneity, viscosity, stability, and antibacterial activity. From among three developed formulations and completed with the total weight of three batches in which F3 showed the best features, which were a stable pH value of 6.9 and also showed an excellent spreadability with a batch free from microbial contamination. This present work shows a study on formulation and evaluation of a polyherbal wound healing gel with Euphorbia hirta and Tridax procumbens. Both plants are known for their traditional usefulness against wounds and skin disorders due to the high purport of several different phytochemical profile. The prepared gels were evaluated on parameters like colour, appearance, pH, spreadability, homogeneity, viscosity, stability, and antimicrobial activity. Out of the three formulations developed, batch F3 showed optimum characteristics, while stable pH with excellent spreadability made it free from microbial contamination. Phytochemical analysis corroborated the presence of bioactive constituents like flavonoids, alkaloids, tannins, and saponins. Molecular docking studies provided further support for wound healing potential by showing the strong interactions of Gallotannins and Quercetin with the active site residues of several key wound-related protein targets. These findings suggest that the formulated polyherbal gel can be deemed safe, stable, and efficacious for topical wound healing applications, thus paving the way for its further development for herbal therapeutics.

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1. INTRODUCTION

For long, herbal medicines have been associated with general features such as antimicrobial, anti-inflammatory, and wound healing [1]. *Euphorbia hirta* (Family: Euphorbiaceae) and *T. procumbens* (Family: Asteraceae) are the most celebrated plants of traditional medicine for wound healing process [2]. This plant has also been used in folk medicine as a healer of wounds based on the antibacterial, antioxidant, and anti-inflammatory properties of coat buttons [3]. The activity of these plants in wound healing has been reported to depend on high flavonoids, tannins, and terpenoids, which are important for collagen synthesis, wound contraction, and regeneration of tissues [4]. Both *Euphorbia hirta* and *Tridax procumbens* bear bioactive compounds, namely flavonoids, alkaloids, glycosides, and tannins [5]. The antimicrobial, anti-inflammatory, and antioxidant properties of the plants are conferred upon them by these compounds. In this way, by lowering the toxic level of reactive oxygen species (ROS), these phytochemicals stimulate cellular repair and, therefore, wound healing [6].

A polyherbal formulation- namely gel of *Euphorbia hirta* and *Tridax procumbens*- is presumed to provide advanced wound-healing activities such as acceleration of contraction, prevention of infections, and

regeneration of tissues [7]. Gel is indeed a suitable topical formulation owing to its stability and ease of application and enhancing penetration of active ingredients at the wound site [8]. But in research, it is proving difficult to ensure stability, shelf life, and efficacy for such herbal formulations [9]. The present study emphasizes the preparation, evaluation, and stability analysis studies of the polyherbal gel containing *Euphorbia hirta* and *Tridax procumbens* in varying proportions, which are assessed here for wound healing efficacy by a combination of pharmaceutical and biological assays [10].

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

Fresh plant leaves of *Euphorbia hirta* and *Tridax procumbens* were collected, washed very well with distilled water (not tap water or borehole water) to remove any impurities, and dried under shade to protect their bioactive compounds. The dried plant materials were coarsely powdered and transferred to airtight containers in order to prevent any moisture absorption [11].

For the extraction of phytochemicals from *Euphorbia hirta* and *Tridax procumbens* plant materials, extraction was done under a temperature range of 45-55 °C, using a Soxhlet apparatus with ethanol as the solvent [12]. Maximum yield of extract was

ensured during the extraction process until complete discoloration of the solvent occurred. The solvents were evaporated under reduced pressure in a rotary evaporator to obtain concentrates [13]. The concentrated extracts were collected and preserved in a vacuum dryer at controlled temperature in an airtight container for formulation development applications [14].

2.2 Formulation of Placebo Gel

In making herbal gel formulation, the first step was to disperse Carbopol 940 powder in distilled water and allow it to hydrate for one night [15]. The preservatives methylparaben, and propylparaben were introduced along with glycerin as humectant for retaining skin moisture [16].

Then the solvent of ethanolic extracts of *Euphorbia hirta* and *Tridax procumbens* was prepared by dissolving them in propylene glycol to enhance their solubility and permeability. Subsequently, this extract solution was added slowly to the hydrated polymer dispersion, stirring continuously [17].

Then the remaining amount of distilled water was added and the gel formulation was neutralized to pH 7 with triethanolamine under stirring for 10 minutes to ensure mixing [18].

The optimized placebo gel formulation was taken for further studies based on evaluation parameters of appearance, viscosity, pH, and spreadability [19].

Table No:1. Control batch

Extract	0.20g
Carbapol	1.8g
Propylene Glycol	12ml
Methyl Paraben	0.4g
Propyl Paraben	0.3g
Glycerine	2.5ml
Triethanolamine	Q.S
Water	Upto 100ml

2.3 Development of Formulation

Initially, the Carbopol 940 was dispersed in distilled water and allowed to hydrate overnight. Preservatives were then added: methyl paraben and propyl paraben, while

glycerin was used as a humectant to retain moisture [20].

The ethanolic extracts of *Euphorbia hirta* and *Tridax procumbens* were separately dissolved in pure propylene glycol in order to enhance solubility and bioavailability.

The extract solution was then added dropwise to the hydrated polymer dispersion under continuous stirring to ensure uniform distribution of the active agents [21].

After adding the remainder of the distilled water, the mixture was carefully adjusted to a pH 7 using triethanolamine, while stirring continuously for about 10 minutes, to yield a smooth and homogenous gel [22].

Table No:2. Formulation Batches

Ingredients	F1	F2	F3
Extract	0.5g	0.15g	0.20g
Carbopol	1.5g	1.7g	1.8g
Propylene Glycol	8ml	10ml	12ml
Methyl Paraben	0.6g	0.2g	0.4g
Propyl Paraben	0.6g	0.3g	0.3g
Glycerine	3ml	1.3ml	2.5ml
Triethanolamine	Q.S	Q.S	Q.S
Water	Upto 100ml	Upto 100ml	Upto 100ml

3. CHARACTERIZATION OF HERBAL GEL FORMULATION

3.1 Physical Evaluation: All required parameters have been recorded, such as the color of the specimens and their physical appearances.

3.2 Homogeneity: Homogeneity was determined visually for all formulations after being matured in the package, based on appearance and the presence of aggregates.

3.3 pH Determination of Gel Formulations

Measurements of the pH of each prepared system and assessment of compatibility with skin were done by taking up to 2.5 grams of each formulation into 25 mL

beaker that contained distilled water and storing the beaker for stabilization up to 2 hours. The pH of the samples was then recorded thrice consecutively for accuracy. Then, the mean value of the pH obtained was calculated. The measurement of pH was done using a calibrated pH meter to ensure its reliability.

3.4 Spreadability:

The methodology used in testing for spreadability is that which employs an apparatus that has a wooden block coupled to one end with a pulley. Such an apparatus defined spreadability as the qualities of slip and drag for gels. Put about 2g of gel under study on this ground slide, then sandwich it between this slide and another glass slide

having the dimension of fixed ground slide and provided with a hook. The top of the slide was weighted for 5 minutes with 1 kg at its end in order to expel air and precede appearance of a uniform film of the gel between the slides. They had only just removed from the edges any gel excess. The upper plate was then pulled using a 50 g weight. The slide is pulled with a string from the hook and the time (in seconds) is noted to take to travel a distance of 6.5 cm. The shorter the time it takes, the better the spreadability [23]. Spreadability thus measured using the formula: $S = M \times L / T$ Where: S = Spreadability, M = Weight in pan (tied to upper slide), L = Length moved by glass slide and T = Time (in sec) required for separating nozzle completely from each other.

3.5 Microbial growth:

Nutrient agar medium was used for the study of microbial growth. Basically, Petri plates were prepared blank and sample. The gel sample was then aseptically transferred onto the sample plates in a cross manner and under sterile condition. The plates were then incubated, with particular observation made for subsequent microbial growth to check for the antimicrobial property of the gel formulation.

3.6 Viscosity: The viscosity of the herbal gel was determined using a Brookfield rotational viscometer. The rpm used were 5, 10, 20, 30, and 50, with spindle number 62

being used for 5-20 rpm, and spindle number 63 for 30-50 rpm. Readings were taken after allowing the sample to equilibrate for two minutes. These viscosity readings were taken three times for each of the samples.

3.7 Anti-microbial Activity Study

For the evaluation of the antimicrobial activity of the prepared gel, the ditch-plate method, a classical method for demonstrating anti-inflammatory activity of semi-solid formulations, has been used. Agar media were prepared and sterilized according to standard procedure. A ditch was cut in the centre of the agar plate and filled with the test formulation. Then, freshly prepared inflammatory mediators or stimulated cell cultures were streaked across the surface of the agar perpendicular to the ditch, reaching the edge of the plate. The plates were kept for 18-24 hours at 25°C and then individually observed for zones of inhibition or decreased migration of inflammatory cells; this, therefore, confirmed the antimicrobial activity of the gel formulation.

3.8 Stability Test

The above-stated stability test has been performed as per ICH guidelines. The formulated gels were filled in airtight containers and kept there in a stability chamber at 40 °C and 75% RH for three months. Samples were withdrawn and analyzed monthly for pH, physical

appearance, texture, grittiness, extrudability to indicate any changes over spreadability, viscosity, odour, and time in the formulation.

4. RESULTS AND DISCUSSION

4.1 Characterization

Table No:3. Characteristics of Euphorbia Hirta

Test	Result
Colour	Green
Appearance	Powder

Table No:4. Characterization of Tridax Procumbens

Test	Result
Colour	Green
Appearance	Powder

4.2 Physico-chemical evaluation

Table No:5. Physico-chemical evaluation of Euphorbia Hirta Leaf

Sr.No	Test	Result
1	Description	Greenish-brown powder, aromatic odour
2	Determination of moisture	7.25%
3	Total Ash value	10.12%
4	Acid insoluble ash value	2.45%
5	Water soluble ash value	4.67%

Table No:6. Physico-chemical evaluation of Tridax Procumbens Leaf

Sr.No	Test	Result
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1	Description	Brownish-green powder, mild odour
2	Determination of moisture	6.85%
3	Total Ash value	9.78%
4	Acid insoluble ash value	2.13%
5	Water soluble ash value	5.21%

4.3 Preliminary Phyto-chemical screening:

Table No:7. Preliminary Phyto-chemical screening of Euphorbia Hirta

Sr.No	Test	Result
1	Test for carbohydrates	(+)ve
2	Test for steroids	(+)ve
3	Test for alkaloids	(+)ve
4	Test for saponins	(+)ve
5	Test for flavoids	(+)ve
6	Test for tannins and phenolic compound	(+)ve

Table No:8. Preliminary Phyto-chemical screening of Tridax Procumbens

Sr.No	Test	Result
1	Test for carbohydrates	(+)ve
2	Test for steroids	(+)ve
3	Test for alkaloids	(+)ve
4	Test for saponins	(+)ve
5	Test for flavoids	(+)ve
6	Test for tannins and phenolic compound	(+)ve

4.4 Evaluation of Topical Gel Formulation

4.4.1 Physical Evaluation

Table No:9. Physical parameters such as colour and appearance

Sr.No	Batch	Colour	Appearance
1.	F1	White	White, smooth gel
2.	F2	White	White, smooth gel
3.	F3	White	White, smooth gel

4.4.2 Homogeneity

Table No:10. Homogeneity of formulation

Sr.No	Batch	Homogenicity
1.	F1	Homogeneous
2.	F2	Homogeneous
3.	F3	Homogeneous

Homogeneity testing was carried out on different batches of the prepared formulations to assess uniform distribution of ingredients. The observations are as follows:

All three batches—F1, F2, and F3—were found to be homogeneous in nature. This

indicates that the formulations had a consistent texture, colour, and appearance throughout, with no signs of phase separation or clumping. Such uniformity is essential to ensure reproducibility, stability, and effectiveness of the final product.

4.4.3 pH

Table No:11. pH of the formulation

Sr.No	Batch	Ph
1.	F1	6.8
2.	F2	7.5
3.	F3	6.5

The digital pH meter recorded the pHs of certain gel formulations. "Accurately weigh 2.5g of gel and disperse in 25ml of distilled water, and then condition for two hours.

Mean values as presented in the table were measured for each formulation in triplicate (Table 11). The pH of the dispersions was measured using a pH meter."

4.4.4 Spreadability

Table No:12. Spreadability of formulation

Sr.No	Batch	Spreadability
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1.	F1	16.10
2.	F2	15.50
3.	F3	14.20

A formulation was prepared by placing it in between two glass slides, wherein the assembly was compressed with a weight of 100 gm on the upper glass slide for 5 min,

along with 50 gm of weight pan. Spreadability was evaluated as the time, in seconds, required to separate the two slides (Table 12).

4.4.5 Microbial growth

Table No:13. Microbial growth of formulation

Batch	Observation
F1	No microbial growth
F2	No microbial growth
F3	No microbial growth

The Nutrient agar media was adopted for studying microbial growth. In this experiment, the blank and the sample Petri plates were used. The gel samples were carried in a cross pattern aseptically onto

the Sample plates and monitored for microbial growth (Table 13). Antimicrobial assay was performed on microorganisms using E. coli and it was found to have profound antimicrobial activity.

4.4.6 Viscosity

Table No: 14. Viscosity value of herbal gel

F1	RPM	Cp
1.	5	3610
2.	10	3714
3.	15	3840
F2	RPM	Cp
1.	5	4318
2.	10	3990
3.	15	3830
F3	RPM	Cp
1.	5	5327
2.	10	2753

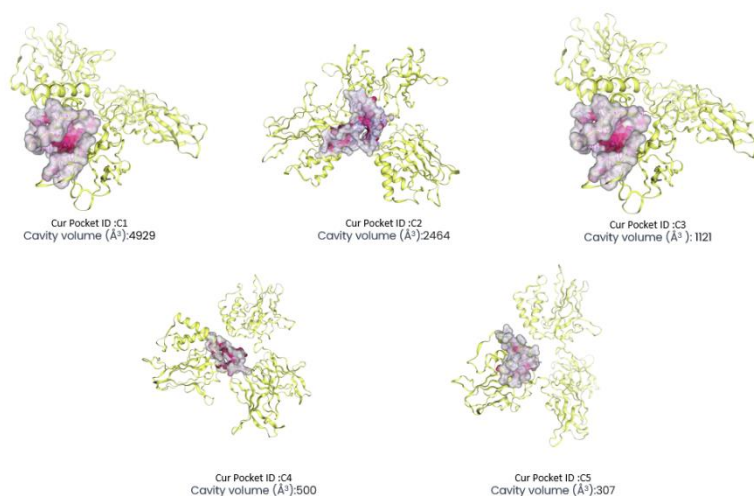
3.	15	1980
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The viscosity of gel was determined by the Brookfield rotational viscometer at 5, 10, and 15 revolutions per minute. A reading was taken after two minutes of equilibrium with scales set to zero. This procedure was

followed by taking three replicates for each speed (Table 14).

5. VIRTUAL SCREENING

5.1 Pockets:

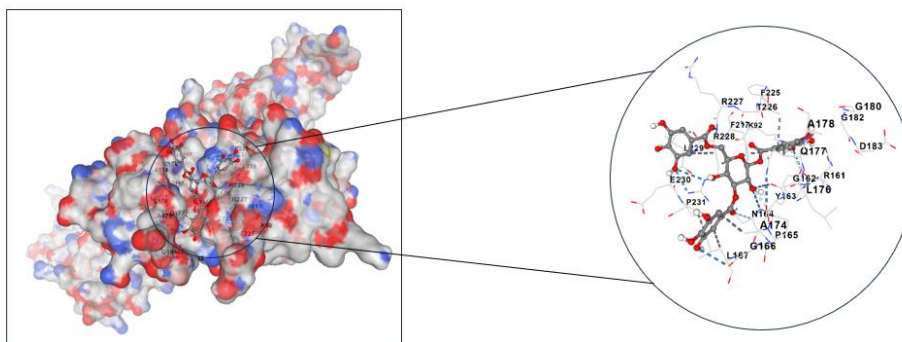


The image is a structural analysis of the protein in which probable binding pockets have been detected by a cavity detection tool most probably CB-Dock or any other similar software. Six different pockets have been visualized and labelled as Cur Pocket IDs:c1 to c6, with each having a specific cavity volume, measured in cubic angstroms (Å³). Among them, Pocket c1 stands with the biggest cavity volume of 4929 Å³, holding the value of being the biggest and most spacious site, and thus potentially acting as the main binding site for ligand interaction. Pocket c2 and c3 hold next positions with volumes of 2464

and 1121 Å³, respectively, for moderate binding ability. The other three pockets, c4, c5, and c6, possess volumes between 307 and 500 Å³, indicating the company's belongings and providing most opportunities for small ligands or fragment-based drug design. The cartoon ribbon model represents the protein structure, with important areas indicated and highlighted surface areas and cavities that help show the spatial arrangement and access of each pocket to the structure. Pokie analysis is one of the essential functions in structure-based drug design because the identification and characterization of the

appropriate binding site increase the accuracy of molecular docking and drug targeting efforts.

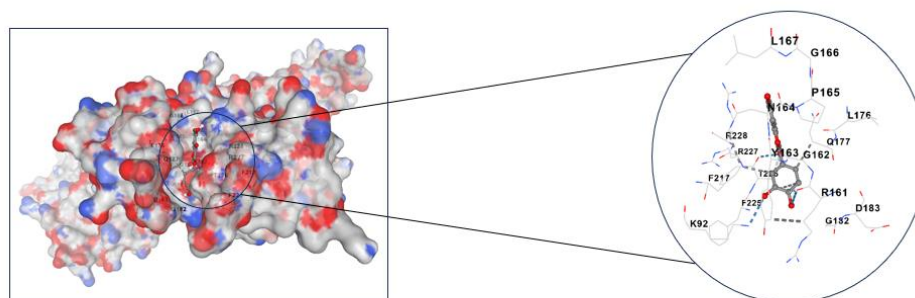
5.2 Docking Results of Gallotannins:



In the main image, a molecular snapshot of the protein-ligand complex is seen, with the main panel showing a 3D structure of the protein in a colour scheme of red to blue, likely representing structural or electrostatic properties, and features an encircled area focusing on the binding site of interest. In the inset, amino acid residues A178, G180, and R227 are shown to interact with the ligand, with lines present to suggest hydrogen-bonding interactions, hydrophobic interactions, or Van der Waals

interactions, indicating the tightly bound nature of this site and its possibly relevant binding pocket. This scenario is often through structural biology or drug design, where detailed assessment of residue-specific interactions (e.g., polar R227 or hydrophobic L167) of the ligand will serve to optimize its affinity and stability, possibly even for drug development, though there is no way without more content to guess what type of protein or ligand is being shown.

Docking Results of Quercetin:



A protein-ligand complex can be viewed as a molecular snapshot which shows the 3D structure of the protein painted in a red-to-blue colour gradient possibly indicating electrostatic or structural domains, with an important highlight for examining details of a circled binding site. By zooming into this inset image, we see an interaction of some ligand with particular amino acid residues (e.g., L167, G166, R227) brought into contact by means of lines that indicate hydrogen bonds, hydrophobic interactions, or Van der Waals force: with additional participation likely from residues R227 and D183 through polar interactions and L167 as regards hydrophobic stabilization. Specific interaction maps are rather typical of structural biology or drug design studies, where understanding specific residue-ligand contacts (e.g., P165, Y164) is key to optimizing respect as well as specificity in binding and uses such as therapeutics. However, precise interpretation will need more context to identify the particular protein or ligand involved.

6. CONCLUSION

The polyherbal wound healing gel made of *Euphorbia hirta* and *Tridax procumbens* showed promising results when tested for physicochemical properties, homogeneity, pH, spreadability, stability, and antimicrobial activity. Among the three formulations, F3 showed the best results.

The gel was smooth, non-irritant, with acceptable viscosity and pH, and showed no growth of microorganisms. Phytochemical screening confirmed the presence of very important bioactive compounds such as flavonoids, alkaloids, and saponins, which are recognized for their healing potential. Virtual screening and docking studies with gallotannins and quercetin also showed strong binding interactions with the target proteins, thus supporting their therapeutic potential. The polyherbal gel, therefore, becomes effective and safe to be considered for clinical prospects in the future.

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