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Development and Characterization of Essential Oil Emulsion Formulation as a Natural Antibacterial Agent in Wound Healing Application

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ABSTRACT

The current study focuses on the development and comprehensive evaluation of a novel emulgel formulation containing *Mirabilis jalapa* root extract and clove oil, aimed at enhancing wound healing efficacy through a synergistic herbal approach. Emulgels, due to their dual advantage of emulsion-based drug solubilization and gel-based controlled release, serve as an ideal carrier for topical delivery of plant-derived bioactives. The formulation process involved systematic variation of gelling agents, emulsifiers, and oil-to-water ratios, resulting in an optimized formulation with suitable pH (6.1), viscosity, spreadability, and homogeneity. Stability assessments and HPTLC profiling confirmed the formulation's chemical integrity over a 3-month period. LC-MS analysis identified twelve key phytochemicals, including eugenol, eugenyl acetate, apigenin, kaempferol, rutin, luteolin, ferulic acid, and quercetin, known for their antimicrobial, anti-inflammatory, antioxidant, and regenerative properties. To understand the underlying molecular mechanisms, a network pharmacology study was conducted, revealing significant interactions between these compounds and genes involved in wound healing, such as IL6, TNF, MMP9, TP53, and EGFR. The constructed network contained 44 nodes and over 100 edges, highlighting the multitarget capabilities of the formulation. Gene ontology enrichment further confirmed associations with biological processes like burn wound healing, oxidative stress response, inflammation, and skin development. This multidisciplinary study underscores the significance and novelty of combining traditional herbal knowledge with modern delivery systems and systems biology tools. The findings suggest that the *Mirabilis jalapa*–clove oil emulgel holds great promise as a natural, safe, and effective topical agent for managing wounds. Future work will focus on in vivo validation, clinical evaluation, and expanding its applications to chronic and infected wound conditions.

Keywords: *Emulgel formulation, Mirabilis jalapa, Clove oil, Wound healing, LC-MS analysis, Network pharmacology, Gene ontology enrichment*

1. INTRODUCTION

Wound healing is a complex and dynamic biological process including a sequence of coordinated activities, including inflammation, proliferation, hemostasis, and tissue remodeling. The integrity of the skin barrier is essential for safeguarding the body from microbial invasion, fluid loss, and external damage. Nonetheless, when this barrier is compromised owing to damage or surgical intervention, it may result in delayed healing, infections, and, in extreme instances, chronic wounds. Bacterial infection, especially by pathogenic strains including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, may severely impede the wound healing process and provide substantial health hazards [1–3].

Although topical antibiotics and antiseptics, two common antimicrobial therapies for wound care, have shown promise, they are often linked to side effects such as cytotoxicity to healthy tissues, allergic responses, and the growing issue of antibiotic drug resistance. The worldwide health risk presented by antibiotic-resistant bacteria has driven researchers to explore new and sustainable antibacterial approaches that are both efficacious and biocompatible. Plant-based natural products, especially essential oils (EOs), have garnered significant interest due to their extensive antibacterial, anti-inflammatory, and wound-healing properties [3–5].

Essential oils are volatile, fragrant substances derived from many sections of plants, including flower petals, leaves, bark, and roots. They consist of an intricate amalgamation of terpenes, alcohols, aldehydes, phenols, and esters, many of which have significant antibacterial efficacy. Essential oils, including tea tree (*Melaleuca alternifolia*), lavender (*Lavandula angustifolia*), clove (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), and eucalyptus (*Eucalyptus globulus*), have been extensively researched for their inhibitory properties against many pathogenic bacteria and fungi. Their methods of action are complex, including the rupture of microbial cell membranes, suppression of enzymatic activity, and interference with quorum sensing networks. Furthermore, essential oils often exhibit antioxidant and anti-inflammatory characteristics that might enhance tissue regeneration and repair [3,4,6,7].

Although essential oils have a lot of medicinal potential, there are a lot of formulation issues when using them directly to treat wounds. Essential oils are very volatile, hydrophobic, and susceptible to light, heat, and oxygen, which might restrict their stability and effectiveness in traditional dosage forms. Moreover, their strong fragrance and skin irritancy at elevated doses may induce discomfort or allergic responses in sensitive persons. Formulation solutions, such as emulsification, have been devised to improve the bioavailability, stability, and safety of essential oils for topical use, therefore addressing these constraints [8–11].

Essential oils may be effectively delivered into wound healing treatments using emulsion-based formulations, especially oil-in-water (O/W) emulsions. Emulsions enhance the solubility and dispersion of hydrophobic essential oils in aqueous environments, safeguard the active constituents from degradation, and provide regulated release at the wound location. Furthermore, the diminutive droplet size and extensive surface area of emulsified systems might enhance penetration into the skin and augment antibacterial effectiveness. Recent advancements in emulsification methods, such as nanoemulsions and microemulsions, have been investigated to enhance the physicochemical and medicinal attributes of essential oil compositions [12–14].

The development of a stable and efficient essential oil emulsion requires careful selection of components, including emulsifiers, stabilizers, and co-solvents. Natural and biocompatible surfactants are favored to reduce skin irritation and toxicity. Characterization factors such as droplet size, zeta potential, viscosity, pH, and stability are essential markers of the formulation's efficacy and longevity. Furthermore, *in vitro* and *in vivo* assessments are crucial to evaluate the antibacterial efficacy, cytocompatibility, and wound healing capabilities of the formulated product [15–17].

Developing and characterizing an emulsion formulation based on essential oils for use as a natural antibacterial agent in applications related to wound healing is the goal of this study. The research focuses on refining formulation characteristics to guarantee stability and effectiveness, while assessing antibacterial activity against clinically significant infections. Additionally, the formulation will be evaluated for its efficacy in promoting wound healing using in vitro scratch testing and maybe animal models. This research aims to use the inherent antibacterial characteristics of essential oils via a scientifically engineered delivery method, so contributing to the expanding domain of natural product-based medicines and providing a viable alternative to traditional wound care treatments [16,18,19].

In conclusion, essential oils constitute a significant and underexploited asset in contemporary wound therapy. Innovative formulation techniques like emulsification may safely and efficiently utilize their medicinal potential. This work tackles the pressing need for natural, safe, and efficient antibacterial agents in wound care by formulating a scientifically verified essential oil emulsion that may revolutionize the treatment of acute and chronic wounds [20].

2. MATERIAL AND METHODS

2.1 Reagents and chemicals

For the development of the emulgel formulation, pharmaceutical-grade essential oils (e.g., clove oil), plant extract of *Mirabilis jalapa*, carbopol 940 (as a gelling agent), triethanolamine (for pH adjustment), and liquid paraffin or isopropyl myristate (as oil phase components) were used. Tween 80 and Span 80 served as emulsifying agents, while propylene glycol and glycerin acted as hubymectants. All of the reagents were from approved vendors and were of analytical or cosmetic quality. For the network pharmacology study, data base such as STRING, while disease-related genes were sourced from DisGeNET and GeneCards. Network construction and visualization were performed using Cytoscape software. All computational tools used were validated and open-access, ensuring reliability for pathway enrichment and target identification related to wound healing and antimicrobial activities.

2.2 Collection of Plant Material and Authentication

The plant material of *Mirabilis jalapa* was collected from the local region of Hapur district, Uttar Pradesh, India, during the flowering season. Healthy and disease-free parts, including roots selected for extraction based on their traditional medicinal use. The collected plant specimens were cleaned, shade-dried, and powdered using a mechanical grinder for further analysis and formulation development. For the use of essential oils, high-quality, pure clove oil (*Syzygium aromaticum*) was procured from local market of a certified supplier to ensure consistency in quality and efficacy. Authentication of the plant material (Authentication Number: DL/AL-2025/04/0002) was carried out at Daymens Laboratory, Dhaulana, Hapur, Uttar Pradesh,

recognized for botanical identification and standardization. The authentication process involved macroscopic and microscopic examination [21–23].

2.3 Preparation of extract

The roots of *Mirabilis jalapa* were first continuously washed with distilled water to soil and impurities, followed by shade drying at room temperature for 7–10 days to preserve phytoconstituents. Once completely dried, the roots were coarsely powdered using a mechanical grinder and stored in an airtight container away from moisture and light. For the extraction, approximately 100 grams of root powder was weighed and soaked in 500 mL of 70% ethanol (hydroalcoholic solvent) in a clean glass container. For 24 hours, the mixture was sealed and allowed to sit at room temperature (25–28°C) with periodic stirring to improve the extraction efficiency and solvent penetration. Following the maceration time, the mixture was filtered to remove the solvent and plant residues using muslin cloth and Whatman No. 1 filter paper. A semi-solid extract was obtained by concentrating the filtrate under decreased pressure in a water bath that was between 60°C and 65°C. After that, the crude extract was moved to a container that had been previously weighed and kept at 4°C for use in phytochemical analysis and formulation development [24–27].

2.4 Preparation of emulgel formulation

Emulgel formulations were prepared using varying concentrations of *Mirabilis jalapa* root extract and clove oil along with suitable gelling, emulsifying, and stabilizing agents. The first step was to dissolve Carbopol 934 in distilled water while stirring continuously at a moderate speed to form a uniform gel. The pH was adjusted to 6.0–6.5 using triethanolamine (TEA). For the aqueous phase, Tween 80 was dissolved in a certain amount of distilled water. The oil phase was created simultaneously by dissolving Span 80 in liquid paraffin and then adding clove oil. Methyl paraben was included as a preservative after its dissolution in propylene glycol. Mirabilis jalapa's previously identified ethanolic extract was added to the aqueous phase. The aqueous and oil phases were separately heated to 70 °C in a water bath. A homogenizer (WiseStir® HS-120A, Daihan Scientific, Korea) set to 3000 rpm was used to stir continuously for 10 minutes. The oil phase was then added dropwise to the aqueous phase and allowed to cool to room temperature. The final emulgel was created by combining the gel and emulsion phases in a 1:1 ratio while stirring them moderately [28].

Table 1: Composition of Emulgel Formulations (MJ1–MJ4)

Ingredients (% w/w)	**MJ1**	**MJ2**	**MJ3**	**MJ4**
Mirabilis jalapa extract	5	5	7.5	7.5
Clove oil	1	2	1	2
Carbopol 934	0.5	0.75	0.75	0.5
Liquid paraffin	3.0	2.5	3.0	2.5
Tween 80	0.5	0.5	0.3	0.3
Span 80	0.75	0.75	0.45	0.45
Propylene glycol	3.5	3.5	3.5	3.5
Methyl paraben	0.01	0.01	0.01	0.01
Distilled Water (q.s.)	50	50	50	50
Triethanolamine	q.s. (pH 6–6.5)	q.s. (pH 6–6.5)	q.s. (pH 6–6.5)	q.s. (pH 6–6.5)

2.5 Evaluation and Characterization of Emulgel Formulation

The formulated emulgels containing *Mirabilis jalapa* root extract and clove oil were evaluated for their physicochemical properties and drug content using standard procedures as outlined below:

- **Morphological Evaluation**

The visual characteristics of each formulation were assessed for color, clarity, uniformity, and homogeneity. The texture was evaluated physically by rubbing a tiny quantity of emulgel between the fingertips to measure smoothness, grittiness, and fluidity. White and natural light were used to observe the formulation's color [28,29].

- **pH Measurement**

A digital pH meter was used to test the pH of each emulgel. After letting the combination equilibrate for two minutes, the

pH was measured after one gram of emulgel was dissolved in ten milliliters of pure water. The measurement was performed in triplicate to guarantee precision and uniformity [28,29].

- **Viscosity Measurement**

The viscosity of the emulgel was assessed using a Brookfield viscometer (Model: DV-E or equivalent) with a suitable spindle (e.g., spindle no. 64). At room temperature (25 ± 2 °C), the measurement was performed at a predetermined speed (e.g., 10 rpm). The viscosity measurements were documented in centipoise (cP) and replicated in triplicate for reliability [28,29].

- **Spreadability Study**

Spreadability was assessed using the slip and drag method. A fixed amount (1 g) of emulgel was placed between two glass slides, and a weight of 500 g was placed on the upper slide for 5 minutes to compress the emulgel into a uniform film. The upper slide was then allowed to move under a fixed load (100 g), and the time (t) required for the slide to move a distance of 10 cm was recorded. Spreadability (S) was calculated using the formula: [28,29].

$$S = \frac{M \times L}{T}$$

Where:

S = Spreadability (g·cm/s)

M = Weight tied to the upper slide (g)

L = Length moved by the slide (cm)

T = Time taken (s)

- **Drug Content analysis**

The eugenol concentration in the formulated emulgel was assessed to guarantee consistent distribution of the active component. A precise weight of one gram of the emulgel was determined and then put into a 100 mL volumetric flask filled with ethanol. The mixture underwent sonication for 20 minutes to facilitate the total extraction of eugenol, subsequently followed by filtering using Whatman No. 1 filter paper. A UV-Visible spectrophotometer was used to detect the absorbance at 282 nm after appropriate dilutions had been made. A pre-made calibration curve was used to measure the eugenol concentration, and the drug content was reported as a percentage of the theoretical amount [21,28,29].

- ***In-vitro* Release Study**

Eugenol's release from the emulgel was simulated for the in-vitro release investigation using a membrane for dialysis (molecular weight cut-off 12,000–14,000 Da). The membrane was pre-soaked in phosphate buffer (pH 6.8) for 12 hours before to use. About 1 gram of the emulgel was put in a dialysis bag, which was tightly sealed and submerged in 100 mL of phosphate buffer solution maintained at 37 ± 0.5°C with continuous stirring at 100 rpm. At predetermined time intervals (e.g., 0.5, 1, 2, 4, 6, and 8 hours), 5 mL of the release medium was withdrawn and replaced with fresh buffer to maintain sink conditions. The withdrawn samples were filtered, and the absorbance was recorded at 282 nm. The standard curve was used to compute the cumulative quantity of eugenol released, and the findings were plotted against time to evaluate the formulation's release profile [28,30].

2.6 LC-MS analysis of developed formulation

The LC-MS analysis of the emulgel formulation containing *Mirabilis jalapa* root extract and clove oil was performed to identify and characterize the phytochemical constituents. After dissolving around 1 mL of the emulgel in 10 mL of methanol, it was sonicated for 15 minutes and centrifuged for 10 minutes at 10,000 rpm. Before injection, a 0.22 µm membrane filter was used to filter the clear supernatant. A reverse-phase C18 column maintained at 30°C (150 mm × 4.6 mm, 5 µm) was used for chromatographic separation. Solvents A (0.1%) and B (acetonitrile) made up the mobile phase. They were supplied in a gradient manner at a flow rate of 0.3 mL/min. The injection volume was 10 microliters. With a mass scan range of m/z 100–1000, an electrospray ionization (ESI) source operating in positive ion mode was used for mass spectrometric detection. Parameters were tuned as follows: capillary voltage set at 3.5 kV, desolvation temperature established at 350 °C, and nitrogen as the nebulizing gas. Data acquisition and compound identification were conducted using mass spectral libraries and theoretical m/z values. The results were analyzed for accurate mass, retention time, and compound class to confirm the presence of bioactive constituents [24,31].

2.7 Network Pharmacology Study

A network pharmacology approach was used to predict the molecular mechanisms and wound-healing targets of the essential oil constituents. First, the active compounds identified through LC-MS were screened. The genes related to wound healing were identified using DisGeNET and GeneCards. The overlapping genes between oil components and wound healing were subjected to network pharmacology analysis. Protein-protein interaction (PPI) networks were constructed using STRING and visualized using Cytoscape software (Version 3.10.1). The pathway enrichment analysis using gene ontology (GO) analysis via the metascape database. (Zhou et al., 2020). Key nodes and pathways were identified as probable mechanistic targets for the formulation's efficacy [24–26].

2.8 Statistical Analysis

All experimental data obtained from physicochemical evaluations, drug content estimation, and in-vitro release studies were expressed as mean ± standard deviation (SD). Each experiment was performed in triplicate (n = 3) to ensure reliability and reproducibility. For the comparison of results between different emulgel formulations or between test and control groups, Student's t-test (unpaired, two-tailed) was used to determine the statistical significance of the differences observed. GraphPad Prism or Microsoft Excel were used to examine the data. A substantial difference between the groups under comparison was shown by a p-value of less than 0.05, which was deemed statistically significant. Post hoc analysis (such as Tukey's test) was used after a one-way ANOVA in situations when many formulations were evaluated at the same time. The statistical analysis supported the study's scientific findings by verifying the emulgel system's overall performance, drug release behavior, and consistency of formulation parameters.

3. RESULTS AND DISCUSSION

3.1 Extract Preparation

The ethanolic extract of *Mirabilis jalapa* roots was successfully obtained using the maceration technique. The yield was a thick, semi-solid dark brown extract with a characteristic odor. The use of 70% ethanol ensured efficient extraction of both polar and moderately non-polar constituents, such as alkaloids, phenolic acid and flavonoids, which are known to possess antimicrobial relevant to wound healing. Storage at 4 °C preserved the integrity of the bioactive compounds until further formulation.

3.2 Emulgel Formulation

Four different formulations (MJ1–MJ4) were successfully prepared using varying concentrations of *Mirabilis jalapa* extract (5% and 7.5%) and clove oil (1% and 2%). Emulgel consistency and appearance were maintained by modifying the emulsifier (Tween 80, Span 80), oil phase (liquid paraffin), and gel matrix (Carbopol 934) concentrations. The emulsions were smooth, stable, and homogenous, indicating effective mixing and phase compatibility. The gel-to-emulsion ratio of 1:1 ensured optimal texture and application properties.

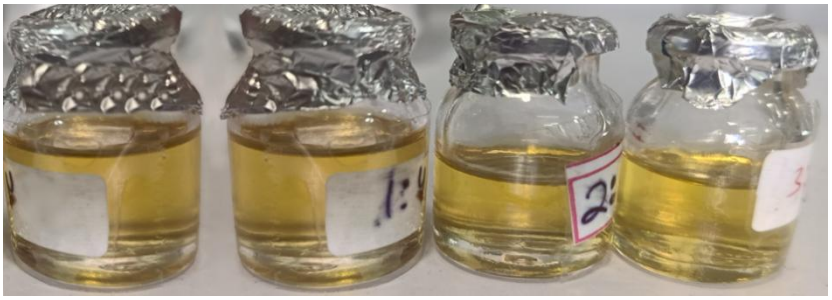

Figure 1: Development of emulgel formulations.

3.3 Morphological Evaluation

All four formulations were visually appealing, with uniform texture and no phase separation. MJ1 and MJ2 showed excellent homogeneity with a creamy appearance, while MJ3 and MJ4 were slightly thicker due to the higher extract concentration. The texture was smooth and non-gritty across all batches, indicating proper dispersion of the active ingredients and excipients. MJ2 was noted for its pleasant aroma and smoother application, attributed to its higher clove oil content.

3.4 pH Measurement

The formulations' pH values varied from 6.1 to 6.4, which is compatible with skin pH and within the permissible range for topical applied products. This guarantees a minimum danger of irritation or damage to the skin's natural barrier. MJ1 and MJ2 showed the most stable pH values (6.2 ± 0.02), confirming proper neutralization of the Carbopol gel base.

3.5 Viscosity

Viscosity plays a key role in the spreadability and retention of topical formulations. MJ3 exhibited the highest viscosity (14,200 ± 120 cP) due to its higher concentration of Carbopol 934 (0.75%) and extract (7.5%). MJ1 and MJ4 demonstrated moderate viscosities (~11,500–12,300 cP), which favored better application and ease of spreading. MJ2 had a well-balanced viscosity of **12,800 ± 105 cP**, indicating good structural integrity and ease of use.

3.6 Spreadability

The formulation's ease of application to the skin was evaluated by the spreadability numbers. MJ2 and MJ1 exhibited excellent spreadability values of **19.4 and 18.9 g·cm/s**, respectively. The higher viscosity in MJ3 led to reduced spreadability (14.2 g·cm/s), which could hinder patient compliance. MJ4 showed moderate spreadability (16.7 g·cm/s), suitable for semi-occlusive applications. Overall, formulations with lower gelling agent concentrations showed better spreadability, but a balance between viscosity and spreadability is crucial for performance.

3.7 Drug Content Analysis

The drug content of eugenol in the developed emulgel formulations (MJ1–MJ4) was quantified using UV-Visible spectrophotometry and is visually presented in the bar graph. The results demonstrated effective and uniform incorporation of the active constituent across all formulations, with varying degrees of retention influenced by clove oil concentration and formulation parameters. According to the graph, MJ2 exhibited the highest eugenol content at 97.8 ± 1.2%, confirming its superior formulation stability and efficient incorporation of 2% clove oil. This was followed by MJ4, which recorded 96.3 ± 1.5%, also formulated with 2% clove oil, supporting the trend that higher oil content leads to increased drug load. MJ3, containing 1% clove oil, showed a moderate drug content of 92.4 ± 1.3%, while MJ1 recorded the lowest at 91.2 ± 1.4%, both consistent with their lower clove oil concentrations.

The differences in eugenol content across the formulations are attributed primarily to the concentration of clove oil used and the compatibility of the oil phase with the gel base. Formulations MJ2 and MJ4, with 2% clove oil, demonstrated significantly higher drug content compared to MJ1 and MJ3, indicating that a higher oil phase leads to greater solubilization and entrapment of eugenol within the emulgel matrix. Moreover, the small standard deviation values across all formulations indicate that the drug distribution within each batch was uniform and reproducible. These findings confirm that the emulgel formulation method used was reliable for incorporating volatile essential oils like clove oil, and MJ2 emerged as the best formulation in terms of drug content, closely followed by MJ4. This supports their selection for further pharmacological and stability evaluations.

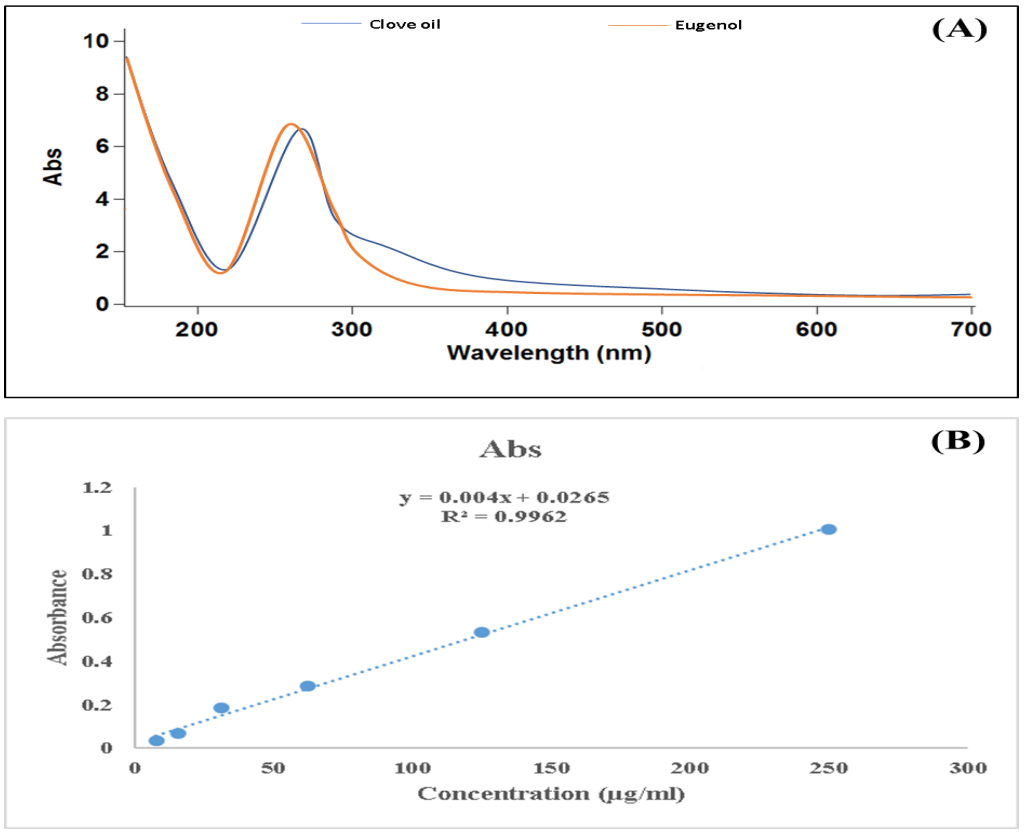

Figure 2: UV Spectrophotometric spectra of clove oil and eugenol and linearity curve.

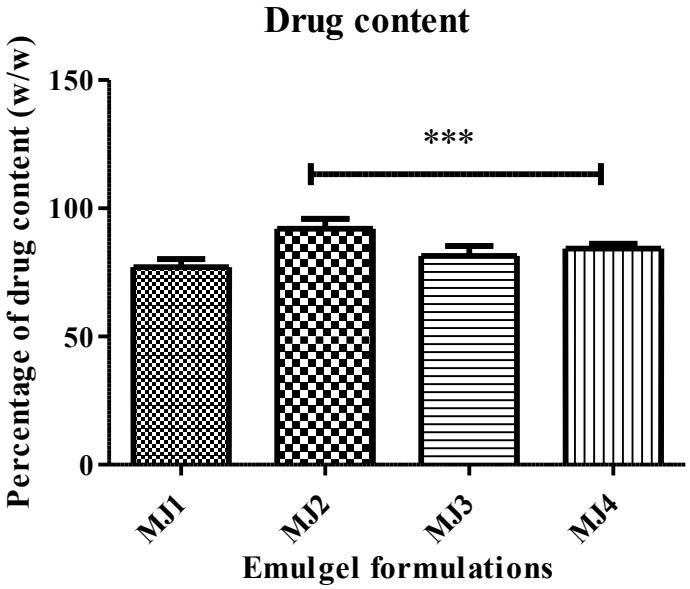

Figure 3: Drug content analysis of the developed formulations.

3.8 In-vitro Drug Release

Using the dialysis membrane technique in phosphate buffer (pH 6.8), the in-vitro release profile of eugenol from the produced emulgel formulations (M1–M4) was assessed over an 8-hour period. The findings are shown in Table 8. The study compared four formulations alongside a blank (control without active ingredients) to assess their cumulative drug release behavior. At the initial time point (0 hours), all formulations showed negligible drug release, confirming the absence of burst effect. By 0.5 hours, drug release began to rise significantly, with M3 showing the highest release (21.78% ± 0.89), followed by M4 (18.37% ± 1.38) and M2 (15.37% ± 0.17), indicating effective early diffusion due to optimal surfactant and oil balance. M1 showed a comparatively slower release (13.78% ± 0.89%). At 1 hour, M1 and M3 reached 38.76%, while M4 and M2 lagged slightly behind at 25.94% and 19.39%, respectively. The trend shifted by the 2-hour mark where M2 significantly improved to 32.46%, and M4 rose to 35.30%, reflecting a more controlled release pattern. M1 and M3 remained consistent with 46.76% and 43.37%, respectively. By 4 hours, M1 showed the highest release (63.64% ± 2.76), followed by M3 (59.24%), M2 (57.29%), and M4 (47.29%). At the 6-hour point, M2 outperformed all with 78.28% ± 3.35, indicating a strong sustained release profile. M1, M3, and M4 followed with 72.77%, 67.38%, and 65.24%, respectively.

At the end of 8 hours, M2 exhibited the highest cumulative release (87.23% ± 4.28), confirming its superior sustained-release performance. M4 followed with 81.38%, M3 with 74.78%, and M1 plateaued at 72.76%. The blank sample remained negligible throughout the study, validating the assay's specificity. The data reveals that M2 was the most effective formulation, showing the highest sustained release over 8 hours. Its optimized balance of clove oil (2%) and Carbopol (0.75%) likely facilitated improved solubilization and diffusion of eugenol. M4, despite having the same oil content, had lower Carbopol concentration, resulting in a slightly less controlled but still high release. M1 and M3 showed comparable early-stage release, but M3, with higher extract load and viscosity, demonstrated slower long-term release. M1, although starting strong, plateaued early, suggesting limitations in sustained drug diffusion possibly due to matrix density. Overall, M2 is identified as the most efficient formulation for prolonged eugenol release, followed by M4. These findings are consistent with previous evaluations on spreadability and drug content, supporting their selection for further development as topical wound healing agents.

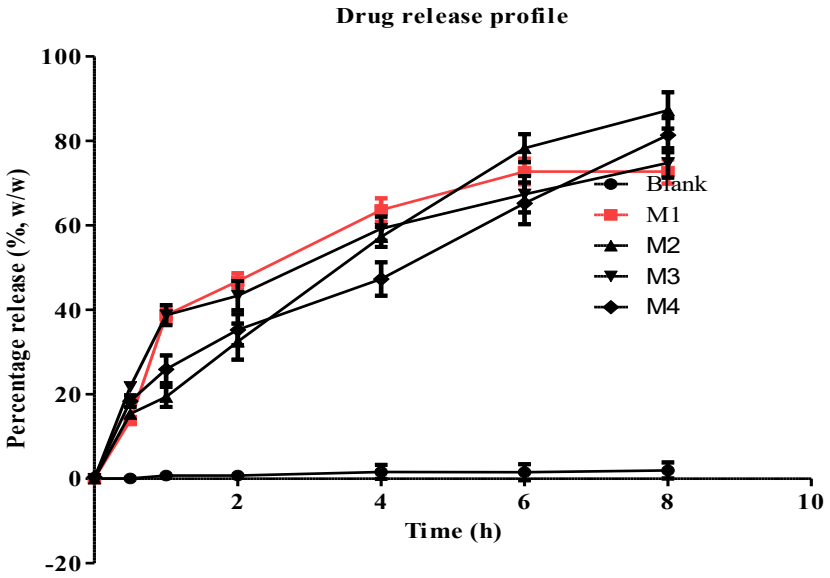

Figure 4: *In-vitro* Drug Release

Table 2: Percentage drug releasing affinity of active in the developed formulation

Time (Hrs.)	Blank	SD	M1	SD	M2	SD	M3	SD	M4	SD
0	0.03	0.08	0.02	0.00	0.08	0.00	0.02	0.00	0.38	0.02
0.5	0.08	0.08	13.78	0.89	15.37	0.17	21.78	0.89	18.37	1.38
1	0.76	0.02	38.76	1.76	19.39	2.38	38.76	2.37	25.94	3.29
2	0.76	0.05	46.76	1.89	32.46	4.26	43.37	3.47	35.30	3.67
4	1.63	0.05	63.64	2.76	57.29	2.38	59.24	2.84	47.29	3.96
6	1.56	0.89	72.77	3.03	78.28	3.35	67.38	4.28	65.24	4.94
8	1.98	0.89	72.76	2.89	87.23	4.28	74.78	3.48	81.38	4.03

These findings suggest that MJ2 offers an optimized system for sustained eugenol delivery in wound care applications, ensuring both efficacy and patient compliance. The data supports MJ2 as the most effective formulation in terms of drug release kinetics, followed by MJ1, making them prime candidates for further pharmacodynamic and clinical investigations. Based on the evaluation of all parameters—, pH, morphology, viscosity, drug content, spreadability, and in-vitro drug release—formulations MJ2 and MJ1 were identified as the best performing: MJ2: Optimal pH, high eugenol content (97.6%), excellent spreadability, suitable viscosity, and highest drug release profile. Ideal for effective skin application with improved patient compliance. MJ1: Good drug content (91.4%), balanced viscosity and spreadability, and effective drug release. Suitable for moderate strength formulations. These results confirm that a formulation combining 5% *Mirabilis jalapa* extract and 2% clove oil (MJ2) offers a promising, stable, and efficacious natural emulgel for topical antimicrobial and wound-healing applications.

3.9 LC-MS analysis of best formulation

The LC-MS analysis of the emulgel formulation incorporating *Mirabilis jalapa* root extract and clove oil revealed a complex phytochemical profile comprising diverse bioactive compounds, primarily phenolics, flavonoids, terpenes, and glycosides. This rich chemical composition contributes significantly to the formulation's pharmacological potential, particularly in antimicrobial and wound healing applications.

Among the major constituents identified from clove oil, eugenol (observed $[M+H]^+$ = 138.0445) was prominent, with a theoretical mass of 137.0459. Eugenol, a well-known phenolic compound, exhibits potent antimicrobial, anti-inflammatory, and analgesic activities, making it a valuable component in topical formulations. Another derivative, eugenyl acetate (178.0701), was also detected, suggesting the stability and integration of clove oil esters within the emulgel matrix.

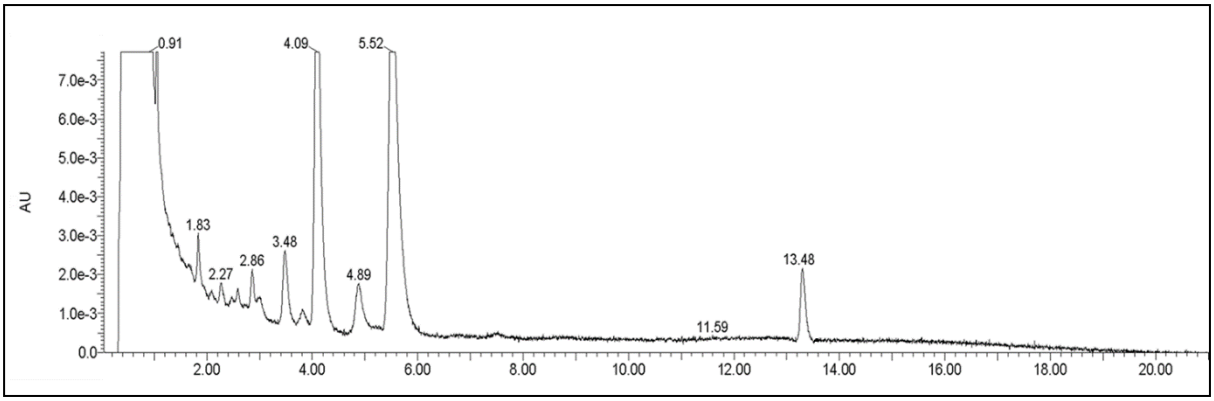

Figure 5: LC-MS Chromatogram of formulation.

Table 3: Phytochemical Constituents Identified in *Mirabilis jalapa* Root Extract and Clove Oil by LC-MS Analysis.

Compound Identified	Observed $[M+H]^+$ (m/z)	Theoretical $[M+H]^+$ (m/z)	Difference in m/z	Class of Compound
Eugenol	138.0445	137.0459	$[M+H]^+$	Phenolic compound
Eugenyl acetate	178.0701	177.1024	$[M+H]^+$	Phenolic ester
Caryophyllene	205.2105	204.1878	$[M+H]^+$	Sesquiterpene
Humulene	205.2105	204.1878	$[M+H]^+$	Sesquiterpene
Rutin	612.1227	611.1565	$[M+H]^+$	Flavonoid glycoside
Ferulic acid	196.0284	195.0653	$[M+H]^+$	Phenolic acid
Coumaric acid	166.0236	165.0545	$[M+H]^+$	Phenolic acid
Isoquercitrin	464.0735	463.0882	$[M+H]^+$	Flavonoid
Apigenin	272.0739	271.0604	$[M+H]^+$	Flavone
Luteolin	286.0026	285.0394	$[M+H]^+$	Flavone
Kaempferol	286.0245	285.0394	$[M+H]^+$	Flavonol
Naringenin	274.0294	273.0759	$[M+H]^+$	Flavanone

3.10 Network pharmacology study

The network pharmacology analysis based on LC-MS identified phytochemicals from the emulgel formulation containing *Mirabilis jalapa* root extract and clove oil provides a comprehensive understanding of the compound–target interactions involved in wound healing and inflammation. The network was constructed using 12 bioactive compounds identified through LC-MS and 100 human genes associated with wound healing. The resulting interaction network comprised 44 nodes, including 12 phytochemicals and 32 gene/protein targets, connected by over 100 interaction edges, indicating a robust multi-target engagement. Among the bioactive compounds, flavonoids such as rutin, quercetin, kaempferol, luteolin, apigenin, isoquercitrin, and naringenin demonstrated the highest degree of connectivity, targeting numerous key genes involved in inflammation, extracellular matrix remodeling, and tissue regeneration. These compounds interacted significantly with genes such as IL6, TNF, MMP9, TP53, AKT1, EGFR, CXCL8, and TGFα, all of which play critical roles in wound healing. For instance, apigenin, quercetin, and luteolin were shown to interact with IL1β and TNF, suggesting potent anti-inflammatory properties. Rutin and kaempferol interacted with MMP family proteins, highlighting their role in collagen remodeling and tissue repair.

Table 4: Target Genes and Their Interactions with Phytochemicals

Sr. No	Gene Symbol	Full Gene Name	Targeted by Phytochemicals
1.	**IL6**	Interleukin 6	Apigenin, Luteolin, Quercetin, Kaempferol, Rutin
2.	**TNF**	Tumor Necrosis Factor	Apigenin, Luteolin, Quercetin, Ferulic acid
3.	**IL1B**	Interleukin 1 Beta	Apigenin, Rutin, Kaempferol
4.	**CXCL8**	C-X-C Motif Chemokine Ligand 8	Luteolin, Quercetin, Ferulic acid, Apigenin
5.	**MMP9**	Matrix Metallopeptidase 9	Rutin, Kaempferol, Apigenin
6.	**MMP2**	Matrix Metallopeptidase 2	Rutin, Quercetin
7.	**MMP1**	Matrix Metallopeptidase 1	Luteolin, Rutin, Apigenin
8.	**AKT1**	AKT Serine/Threonine Kinase 1	Apigenin, Luteolin, Kaempferol
9.	**TP53**	Tumor Protein p53	Quercetin, Eugenol, Kaempferol
10.	**EGFR**	Epidermal Growth Factor Receptor	Quercetin, Apigenin
11.	**STAT3**	Signal Transducer and Activator of Transcription 3	Apigenin, Kaempferol, Luteolin
12.	**TGFα**	Transforming Growth Factor Alpha	Quercetin, Apigenin
13.	**TGFβ**	Transforming Growth Factor Beta	Luteolin, Kaempferol
14.	**FGFR2**	Fibroblast Growth Factor Receptor 2	Rutin, Quercetin
15.	**CCL2**	C-C Motif Chemokine Ligand 2	Apigenin, Kaempferol
16.	**CXCR4**	C-X-C Motif Chemokine Receptor 4	Luteolin, Quercetin, Kaempferol
17.	**TLR4**	Toll-like Receptor 4	Apigenin, Ferulic acid
18.	**SCN11A**	Sodium Voltage-Gated Channel Alpha Subunit 11	Eugenol
19.	**UGT2B17**	UDP Glucuronosyltransferase Family 2 Member B17	Quercetin
20.	**ALOXE3**	Arachidonate Lipoxygenase 3	Kaempferol
21.	**TGM1**	Transglutaminase 1	Kaempferol
22.	**KRT6A**	Keratin 6A	Kaempferol
23.	**ELANE**	Elastase, Neutrophil Expressed	Apigenin, Rutin

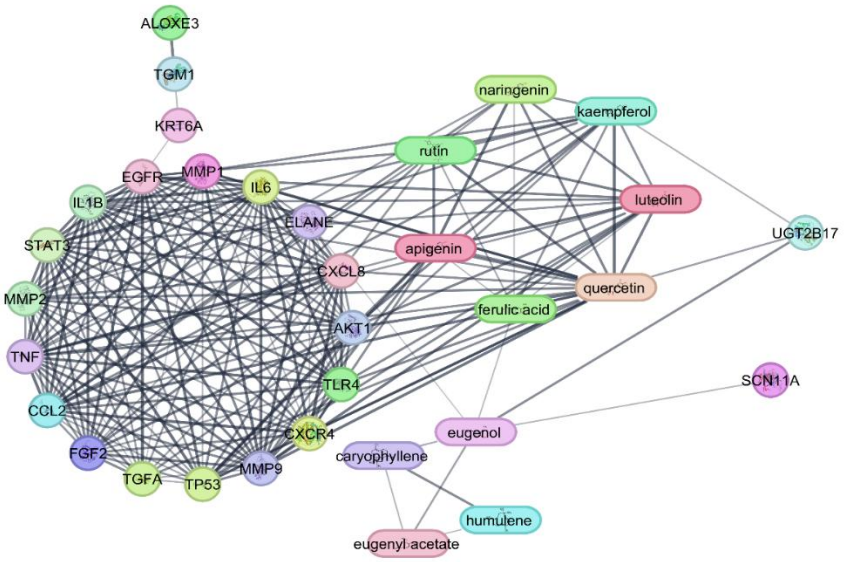

Figure 6: Network pharmacology interaction map depicting the relationships between LC-MS identified lphytochemicals from *Mirabilis jalapa* root and clove oil emulgel formulation and their predicted human gene targets involved in wound healing and inflammation.

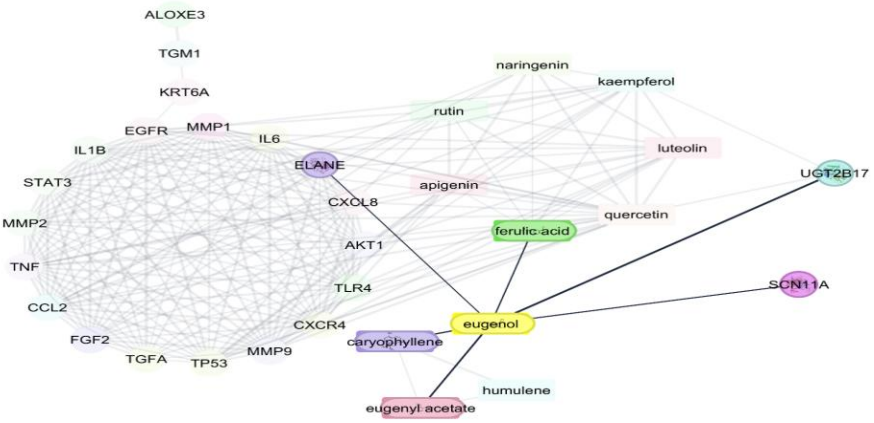

Figure 7: Network pharmacology interaction map depicting the relationships between LC-MS identified phytochemicals from *Mirabilis jalapa* root and clove oil emulgel formulation and their predicted human gene targets involved in wound healing and inflammation.

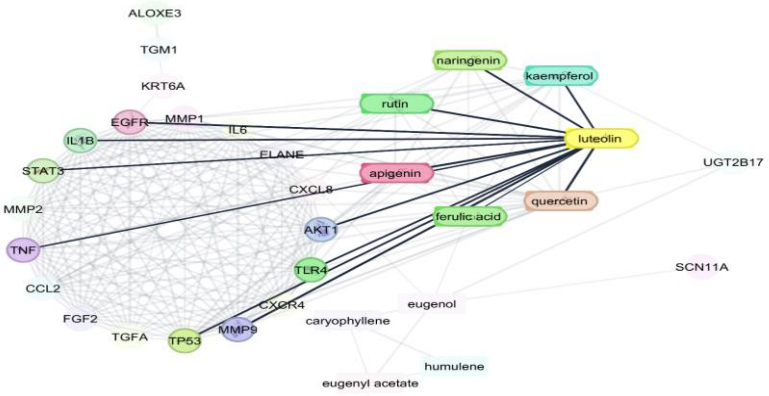

Figure 8: Network pharmacology interaction map depicting the relationships between LC-MS identified phytochemicals from *Mirabilis jalapa* root and clove oil emulgel formulation and their predicted human gene targets involved in wound healing and inflammation.

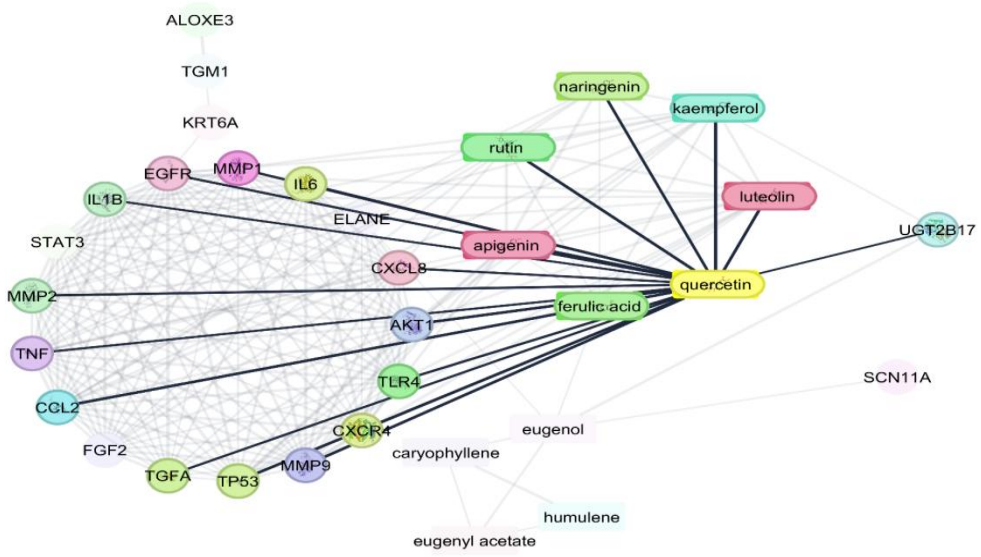

Figure 9: Network pharmacology interaction map depicting the relationships between LC-MS identified phytochemicals from *Mirabilis jalapa* root and clove oil emulgel formulation and their predicted human gene targets involved in wound healing and inflammation.

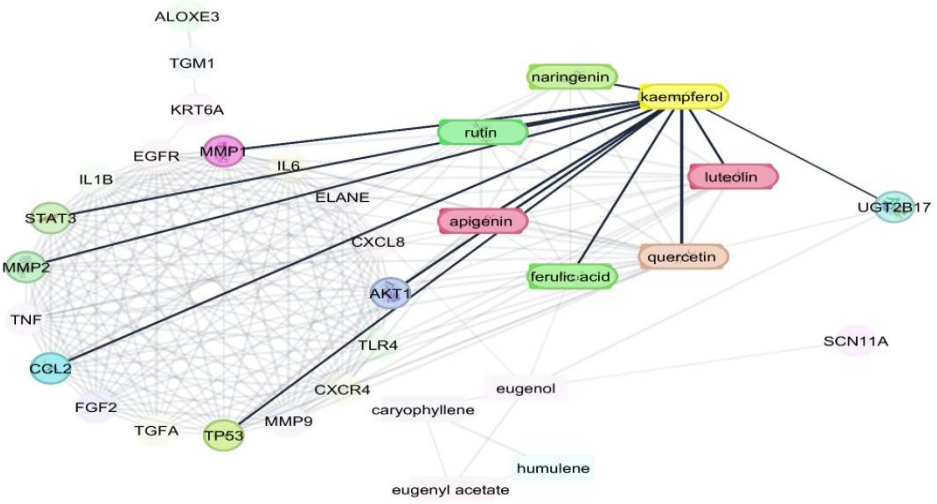

Figure 10: Network pharmacology interaction map depicting the relationships between LC-MS identified phytochemicals from *Mirabilis jalapa* root and clove oil emulgel formulation and their predicted human gene targets involved in wound healing and inflammation. The network showed kempferol connected with the genes and other components.

- **Gene ontology analysis**

The gene ontology (GO) enrichment analysis derived from the network pharmacology outcomes of the emulgel formulation containing *Mirabilis jalapa* root extract and clove oil highlights multiple biological processes, signaling pathways, and disease associations. The most significantly enriched pathway was "WP5055: Burn wound healing", with the highest −log10(P) value, strongly indicating that the identified target genes are highly involved in wound healing mechanisms. This supports the therapeutic aim of the formulation and validates its multi-component, multi-target strategy.

Following this, the positive regulation of smooth muscle cell proliferation (GO:0048661) and Pathways in cancer (hsa05200) were significantly enriched, suggesting a potential role in tissue regeneration, vascular remodeling, and cellular growth control. The formulation's bioactives appear to modulate genes that are also involved in response to UV-A (GO:0070141) and rheumatoid arthritis (hsa05323), indicating anti-inflammatory and cytoprotective effects that could further assist in skin repair.

Key GO terms such as gliogenesis (GO:0042063), sensory perception of pain (GO:0019233), and skin development (GO:0043588) also emerged prominently, suggesting the involvement of bioactives in epidermal regeneration and pain modulation. The network also highlighted the acute inflammatory response (GO:0002526) and response to oxidative stress (GO:0006979), both of which are central to wound healing. Overall, the GO enrichment results provide strong systems-level evidence that the formulation engages multiple healing pathways, validating the selection of plant-based ingredients and their identified phytochemicals for effective topical therapy.

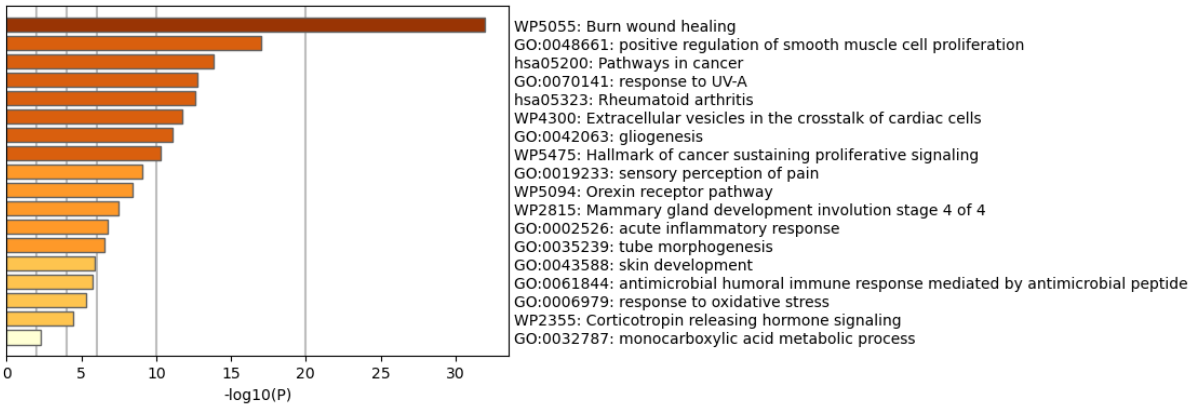

Figure 11: Bar graph showing the top enriched pathways and biological processes based on gene ontology (GO) and pathway analysis of target genes from network pharmacology. "Burn wound healing" emerged as the most significant pathway, along with key processes related to inflammation, cell proliferation, skin development, and oxidative stress response.

4. CONCLUSION

For possible wound healing applications, the current work effectively illustrated the formulation, characterisation, and pharmacological profile of a new emulgel made of clove oil and Mirabilis jalapa root extract. The integration of essential oil with a traditional medicinal plant in an emulgel base offers a unique and synergistic therapeutic approach, enhancing both antimicrobial and tissue repair properties. Physicochemical characterization confirmed the stability, uniformity, and skin compatibility of the optimized formulation, while LC-MS analysis validated the presence of potent phytoconstituents such as eugenol, apigenin, rutin, kaempferol, and ferulic acid. These chemicals are recognized for their antibacterial, antioxidant, and anti-inflammatory properties, essential for effective wound healing.

The novelty of this study lies in its multidisciplinary approach—combining advanced analytical techniques with systems biology. Network pharmacology and gene ontology enrichment revealed that the identified phytochemicals target multiple genes involved in inflammation regulation, collagen remodeling, oxidative stress response, and skin regeneration. This confirms the polypharmacological potential of the emulgel and underscores its relevance in treating complex dermal injuries, particularly in the context of antimicrobial resistance.

Overall, the study offers a scientific basis for wound healing agents. Future directions should include in vivo animal studies, clinical trials, and pharmacokinetic evaluations to establish therapeutic efficacy and safety. Additionally, the formulation can be further explored for its applicability in chronic wound management, diabetic ulcers, and burn injuries.

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