FORMULATION AND EVALUATION OF *IN- VITRO* POLYHERBAL WOUND HEALING CREAM

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ABSTRACT:

Wound healing is a complex and dynamic biological process involving sequential phases such as hae- mostasis, inflammation, proliferation, and tissue remodelling. Herbal medicines have gained considerable attention in recent years due to their therapeutic efficacy, safety, and minimal side effects. The present study aimed to formulate and evaluate a polyherbal wound healing cream using leaf extracts of *carica papaya*, *vitex negundo*, *Psidium guajava*, which are well known for their antimicrobial, antioxidant, and anti-inflammatory properties. The plant materials were collected, authenticated, shade-dried, and extracted using a hydroalcoholic solvent system (ethanol, 70:30) by maceration. The extracts were subjected to preliminary phytochemical screening, which confirmed the presence of bioactive constituents such as flavonoids, tannins, phenolic, glycosides, and terpenoids. The polyherbal cream was formulated using the fusion method and evaluated for various physicochemical parameters including colour, odour, PH, viscosity, spreadability, homogeneity, washability, skin irritancy, and stability. The formulation exhibited satisfactory physiochemical properties with a PH of 5.67 ± 0.65, appropriate viscosity, and good spreadability, making it suitable for topical application. Antibacterial activity was assessed using the agar well diffusion method against Escherichia coli, and the formulation showed concentration-dependent inhibition with a maximum zone of inhibition of 7.06 ± 1.17 mm, comparable to the standard drug. Stability studies indicated no significant changes over four weeks. In the formulated polyherbal wound healing cream demonstrated promising antibacterial activity and desirable formulation characteristic, suggesting its potential as a safe and effective topical agent for wound healing. Further in vivo and clinical studies are recommended to validate its therapeutic efficacy.

KEYWORDS :

Polyherbal cream, Wound healing, phytoconstituents, antibacterial activity, Topical drug delivery

INTRODUCTION :

Medicinal plants have shaped human healthcare for centuries, and they still hold a central place in treatment worldwide. According to the World Health Organization, about 80% of people across the globe rely on herbal medicines as their primary healthcare option. These plants pack a variety of bioactive phytochemicals such as alkaloids, flavonoids, terpenoids, glycosides, tannins, saponins that drive their broad pharmacological effects such as antioxidant, antimicrobial, anti-inflammatory, and antidiabetic, to name a few. Ancient medical traditions like Ayurveda, Siddha, and Unani catalogued these uses extensively, laying the groundwork for modern pharmacognosy and drug discovery. *Carica papaya*, or papaya it's a tropical species from the Caricaceae family. Everywhere you look on the plant leaves, fruit, seeds, latex traditional medicine finds value. The leaves alone are loaded with flavonoids, alkaloids, phenolic compounds, plus powerful enzymes like papain and chymopapain. These phytoconstituents are behind the plant's anti-inflammatory, antimicrobial, antioxidant, antidiabetic, hepatoprotective, and wound-healing effects. Papaya leaf extract has drawn special notice for its ability to boost platelet counts in cases of dengue fever.

Figure: 01 *Carica Papaya*

Vitex negundo, known as Nochchi or Chinese chaste tree. This medicinal shrub thrives in the tropics, and its leaves are a staple in traditional remedies. People use them for pain relief, fever, inflammation, and infections. Flavonoids, iridoid glycosides, terpenoids, tannins, and essential oils make up its bio- active arsenal and explain why it's so effective for fever, arthritis, respiratory issues, and infectious diseases.

Figure: 02 *Vitex negundo*

Psidium guajava or guava is another heavy hitter in the world of medicinal plants, part of the Myrtaceae family. Guava leaves are packed with flavonoids, tannins, saponins, and phenolic acids. For generations, these leaves have helped treat diarrhea, diabetes, infections, and inflammation. Modern research backs up these claims, proving their antioxidant, antimicrobial, antidiabetic, and hepatoprotective power.

Figure:03 *Psidium guajava*

With their impressive phytochemical profiles and range of medicinal actions, these plants have sparked growing interest in pharmacognostic and phytochemical studies. This study aims to screen the phyto- chemicals in selected medicinal leaves and gauge their therapeutic potential.

MATERIALS AND METHODS :

1) Pharmacognostical Studies

a) Collection and Authentication of Plant Material

We collected Carica papaya leaves from villages near Karaikal, Puducherry, while Vitex negundo and Psidium guajava leaves came from villages in Nagapattinam district, Tamil Nadu. S. S. Hameed, Sci- entist 'R' and Head of Office at the Botanical Survey of India, TNAU Campus, Coimbatore, verified and authenticated our plant materials. We stored voucher specimens in the Department of Pharmacog- nosy for future reference.

b) Macroscopic and Microscopic Evaluation

For the macroscopic assessment, we examined the color, odor, and texture of the leaves. To study the microscopic structure, we washed fresh leaves with distilled water and cut thin transverse sections using a sharp blade. After staining these sections with safranin and mounting them in glycerin, we examined their anatomical features under a compound microscope.

c) Powder Microscopy

We shade-dried the leaves, powdered them, and then analyzed the samples under the microscope using several staining agents. Treating the powder with 1% phloroglucinol in ethanol and concentrated HCl stained the lignified cells pink, helping us identify important anatomical markers.

2) Physicochemical Studies

Following World Health Organization guidelines, we evaluated physicochemical parameters of the powdered leaves like total ash, water-soluble ash, loss on drying, and moisture content to determine the purity and quality of our crude drugs.

3) Phytochemical Studies

a) Preparation of Extract

Collected leaves were shade-dried at room temperature for a period of two weeks until a constant weight was achieved. The dried leaves were then pulverized into a fine powder using a mechanical grinder. The powdered material was subjected to extraction using a hydroalcoholic solvent system consisting of ethanol and water in a 70:30 ratio (v/v). The extraction was carried out by maceration for 48 hours at room temperature with intermittent shaking to ensure efficient solvent penetration. Following extraction, the mixture was filtered using Whatman No. 1 filter paper to remove plant debris. The filtrate was subsequently concentrated under reduced pressure (or at controlled temperature) to obtain a thick, semisolid crude extract.

b) Preliminary Phytochemical Screening

We performed standard qualitative tests on the extracts to identify key phytoconstituents: alkaloids, phenols, glycosides, tannins, saponins, flavonoids, carbohydrates, proteins, fats and oils, and terpenoids.

c) Chromatographic Analysis

We applied Thin Layer Chromatography (TLC) to separate phytoconstituents. We used silica gel plates for the stationary phase and selected suitable solvent systems as the mobile phase. The separated spots were identified by measuring their Rf values.

4) Pharmacological Studies

a) Antibacterial Activity

We evaluated the extracts for antibacterial activity using the agar well diffusion method. After preparing and sterilizing nutrient agar, we inoculated the plates with a 24-hour Escherichia coli culture from the Institute of Microbial Technology, Chandigarh. We added extract samples of 50 µL, 100 µL, and 150 µL to the wells, with chloramphenicol as the standard. After incubating at 37°C for 24 hours, we measured the resulting inhibition zones in millimeters using ImageJ software.

b) Formulation of Polyherbal Cream

We formulated a polyherbal wound healing cream with extracts of Carica papaya, Vitex negundo, and Psidium guajava, blending them with excipients such as stearic acid, cetyl alcohol, liquid paraffin, glycerin, triethanolamine, methyl paraben, and propyl paraben. We heated the oil and aqueous phases separately to 70–75°C, mixed them while stirring to form an emulsion, then added the plant extracts after cooling to get a uniform cream.

c) Evaluation of Formulation

We evaluated the cream for organoleptic properties like its colour, odour, texture, and appearance. We tested physicochemical parameters like pH, viscosity with a Brookfield viscometer, and spreadability. Homogeneity, washability, skin irritancy, and stability were also assessed under storage at 25±2°C and 40±2°C over four weeks.

d) Phytochemical and Antibacterial Studies of Formulation

Finally, we screened the cream for bioactive constituents and tested its antibacterial effectiveness using the agar well diffusion method against Escherichia coli, recording the zones of inhibition.

RESULT AND DISCUSSION :

1) Pharmacognostical Studies

a) Organoleptic Characters

Carica papaya leaves are simple, palmately lobed, and dark green. They have a slight, distinct smell and taste bitter. Vitex negundo leaves stand out as palmately compound, with lance-shaped leaflets, an aromatic scent, and a similarly bitter flavor. Psidium guajava leaves are simple, elliptical to oblong, and dark green, giving off a mild scent and astringent taste.

b) Macroscopic and Microscopic Studies

Under the microscope, each leaf shows clear distinguishing features. Carica papaya's cross-section reveals a single-layered epidermis with paracytic stomata, plus strong palisade and spongy parenchyma. Vitex negundo features multicellular trichomes, diacytic stomata, and a dorsiventral mesophyll. Psidium guajava is marked by paracytic stomata, oil glands, calcium oxalate crystals, and pronounced vascular bundles. These characters verify the plants' identities.

c) Powder Microscopy

Powdered samples display pieces of epidermis, stomata, trichomes, parenchyma, calcium oxalate crystals, and vascular elements. All three plants show these elements under the microscope, which help confirm the crude drug's identity.

2) Physicochemical Studies

Analysis of the leaf powders showed total ash at 6.2% w/w, water-soluble ash at 3% w/w, loss on drying at 5.2% w/w, and moisture at 6.5% w/w—all within limits for good crude drug quality and storage.

3) Phytochemical Studies

a) Extraction

Hydro-alcoholic extraction of the leaves, done by maceration, yielded extracts with distinct color and odor, a sign of phytochemicals present.

b) Preliminary Phytochemical Screening

Screening confirmed important bioactive groups alkaloids, flavonoids, tannins, saponins, glycosides, phenols, carbohydrates, proteins, fats and oils, and terpenoids across the formulation . These contribute antimicrobial, antioxidant, and anti-inflammatory actions.

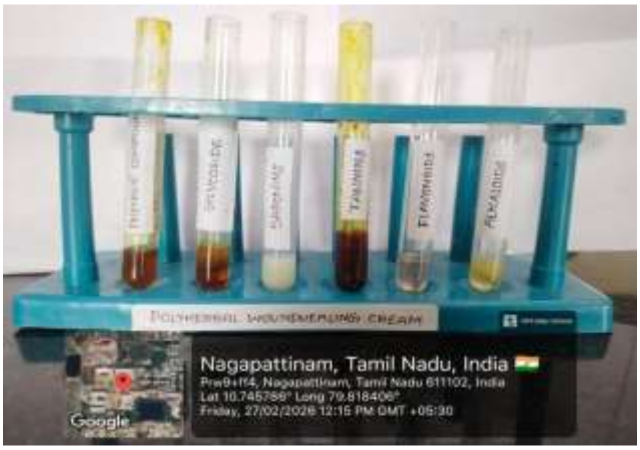

Figure 04: Preliminary Phytochemical Screening of formulation

a) Thin Layer Chromatography

TLC results revealed multiple spots with different Rf values, confirming the presence of flavonoids, phenolics, and tannins. These likely play a key role in the plants' biological activities.

4) Pharmacological Studies

a) Antibacterial Activity of Extracts

Testing against Escherichia coli by agar well diffusion showed all extracts worked in a concentration-dependent way, *with Psidium guajava* leading in activity, followed by *Carica papaya* and *Vitex negundo*. When combined in a polyherbal mixture, the extracts outperformed the individuals, suggesting a synergistic effect. Chloramphenicol, used as the standard, still showed the largest inhibition zones.

Samples	Zone of Inhibition (mm in diameter)			
	Escherichia coli			
	50µl	100µl	150µl	Standard* (30µl)
Vitex negundo	0.32±0.23	1.77±0.66	3.66±1.64	8.65±1.43
Carica papaya	0.75±0.42	2.65±0.69	4.63±1.57	8.73±0.77
Psidium guajava	1.09±0.20	3.18±0.26	5.51±1.52	8.68±0.75

Table 01: Antibacterial activity of the leaf extract against bacterial strain

***Figure 05 : E.coli in Psidium guajava* extract**

***Figure 06 : E.coli in Vitex negundo* extract**

Figure 07: E.coli in *Carica papaya* extract

Values expressed as Mean ± SD; Standard*- Chloramphenicol

Anti Bacterial Activity Of Formulation

Here the Agar wall diffusion method is used. The polyherbal formulation was lower than that of the standard antibiotic Chloramphenicol, which showed inhibition zones ranging from 8.65 ± 1.43 mm to 8.88 ± 1.48 mm, the results demonstrate that the tested plant extracts possess significant antibacterial potential against *Escherichia coli*. Overall, the findings suggest that the polyherbal formulation is more effective than individual plant extracts, supporting the concept that combining medicinal plants can enhance antimicrobial activity through synergistic effects.

Samples	**Zone of Inhibition (mm in diameter)**			
	Escherichia coli			
	50µl	**100µl**	**150µl**	**Standard* (30µl)**
Polyherbal cream	2.78±0.03	4.29±0.65	7.06±1.17	8.88±1.48

Table 02: Antibacterial activity of samples against bacterial strain

Values expressed as Mean ± SD; Standard*- Chloramphenicol

Figure 08 :E.coli in Polyherbal Cream

b) Evaluation of Polyherbal Cream

A cream containing extracts from all three species was prepared using a suitable base. The result: a smooth, uniform cream with no separation.

Figure 09 : *In vitro* polyherbal wound healing cream

Figure 10: Formulation placed on china dish

c) Physicochemical Evaluation

The cream measured a pH of 5.67 ± 0.65—ideal for skin. Viscosity hit 12,500 cP, which gives a pleasing, thick but spreadable consistency. It spread easily, at 4.8 g·cm/sec.

d) Homogeneity, Washability, and Irritancy

The cream felt consistent to the touch and washed off easily with water. It caused no irritation—no redness or itching making it safe for topical use.

e) Stability Studies

Kept under different conditions for four weeks, the cream stayed stable: color, odor, pH, feel, and spreadability all remained unchanged. The formulation held up well from start to finish.

SUMMARY AND CONCLUSION :

a) Summary

This study set out to develop and test a polyherbal wound healing cream, blending leaf extracts from Carica papaya, Vitex negundo, and Psidium guajava. These plants aren't chosen at random—they've long played a role in traditional medicine and they're known for antimicrobial, anti-inflammatory, an- tioxidant, and wound healing powers. First, the team collected the leaves, dried them in the shade, turned them into powder, and used solvent extraction to pull out the active phytochemicals. Tests showed the extracts are rich in flavonoids, tannins, phenolic compounds, glycosides, and terpenoids. These are the heavy hitters you want in a wound healing cream since they help fight infection, calm inflammation, and support tissue repair. To make the cream, researchers blended the extracts into a base by the fusion method, then checked its physical and chemical features such as colour, smell, consistency, pH, spreadability, viscosity, washability, and stability. The results were that the cream scored well across the board. It was smooth, spread easily, held the right pH for skin use, and stayed stable over time. Taken together, these findings open the door for this polyherbal blend to stand out as a viable option for topical wound healing.

b) Conclusion

When it came to formulating and evaluating a polyherbal wound care cream, the investigation hit its mark. Extracts from *Carica papaya, Vitex negundo, and Psidium guajava* three plants with reputations backed by science for antimicrobial and anti-inflammatory effects formed the base. The resulting cream measured up in every key area: it kept a pH range safe for skin, spread smoothly, and showed solid viscosity and stability. Phytochemical screening revealed the presence of core bioactive compounds like flavonoids, tannins, phenolics, glycosides, and terpenoids each one known to support healing. These results support the idea that this cream could be a safe, effective, and affordable route for wound care, especially compared to some synthetic alternatives that carry a higher risk of side effects. The journey shouldn't stop here. Advanced pharmacological studies, both in vitro and in vivo, alongside toxicity assessments and clinical trials, are needed to nail down its real-world potential and safety. With deeper research and standardization, this polyherbal cream could find its way onto pharmacy and cosmeceutical shelves as a go-to product for natural wound healing.

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