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Evaluation of Polyherbal Gel Silver Nanoparticles for the Treatment of Neonatal Burns

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ABSTRACT
Burn injuries in newborns are among the most difficult wounds to treat. Their skin is fragile, their immune defenses are still developing, and even small burns can have serious consequences. Polyherbal formulations have been used for centuries in traditional medicine to treat a wide range of ailments, including wound healing. The synergistic interactions between different plant-derived compounds can enhance their therapeutic efficacy and reduce the risk of adverse effects. Guava, in particular, is rich in bioactive compounds, such as flavonoids, tannins, and triterpenoids, which have been shown to possess antioxidant, anti-inflammatory, and antimicrobial properties. The prepared polyherbal gel was subjected to various evaluation parameters to assess its physicochemical properties. This study demonstrates the successful formulation of a polyherbal gel combining guava extract and silver nanoparticles, both known for their wound-healing and antimicrobial properties.

1. INTRODUCTION
Neonatal burns represent a significant clinical challenge due to the delicate nature of newborn skin and the potential for severe complications, including infection, scarring, and long-term morbidity (Chung, 2001). The unique physiological characteristics of neonates, such as a thinner dermis and increased evaporative water loss, exacerbate the severity of burn injuries and necessitate specialized treatment approaches (Mathias & Murthy, 2017). Traditional burn treatments, while effective to some extent, may not always be optimal for neonates due to potential toxicities and adverse effects (King et al.,

2014). Consequently, there is a growing need for safe, effective, and innovative therapies that can promote rapid wound healing and minimize complications in this vulnerable population (Mathias & Murthy, 2017). The current standard of care for minor burns often involves silver sulfadiazine, but some reports suggest that alternative methods may offer superior outcomes (Chung, 2001). The development of novel therapeutic strategies that harness the synergistic properties of natural compounds and nanotechnology holds great promise for improving the management of neonatal burns. Silver nanoparticles have garnered considerable attention in the biomedical field due to their broad-spectrum antimicrobial activity and ability to promote wound healing. However, concerns regarding their potential toxicity, particularly in neonates, necessitate the development of delivery systems that can minimize systemic exposure and maximize local therapeutic efficacy (Gherasim et al., 2020). In this context, polyherbal formulations offer a compelling alternative by combining the therapeutic benefits of multiple plant-derived compounds, which can act synergistically to promote tissue regeneration and reduce inflammation. The integration of silver nanoparticles into a polyherbal gel formulation represents a novel approach to neonatal burn treatment, potentially offering a multi-faceted therapeutic effect by addressing both infection control and wound healing promotion (Yang et al., 2020). One third of burn injuries in the United States occur in the pediatric population, suggesting a need to improve current treatment options for children (Mathias & Murthy, 2017). Neonatal burns represent a critical medical emergency, complicated by the inherent vulnerabilities of newborn physiology, including thin dermal layers, immature immune responses, and a heightened susceptibility to systemic infection. Standard therapeutic interventions, while foundational, often inadequately address the urgent need for accelerated wound healing and infection control in this population. Advances in nanotechnology and phytopharmacology have opened new therapeutic avenues, with polyherbal-silver nanoparticle (AgNP) formulations emerging as a particularly compelling option. These complex systems leverage the broad-spectrum antimicrobial properties of silver nanoparticles, synergistically enhanced by the bioactive compounds of multiple medicinal plants. This paper critically evaluates the potential of polyherbal-AgNPs in improving the management of neonatal burns, emphasizing their mechanisms of action, therapeutic advantages, and translational prospects.

In recent years, advances in nanotechnology and phytopharmacology have significantly expanded the range of therapeutic strategies available for managing complex health conditions, including burn injuries. Nanotechnology — the manipulation of matter at the atomic and molecular scale (1–100 nanometers) — has transformed modern medicine by enabling the design of novel drug delivery systems, antimicrobial agents, and tissue repair materials. Silver nanoparticles (AgNPs), in particular, have gained attention due to their broad-spectrum antimicrobial activity, anti-inflammatory effects, and ability to promote tissue regeneration (Rai et al., 2012).

At the same time, phytopharmacology — the study of medicinal properties of plant-derived compounds — has seen a resurgence. Traditional medicinal plants, rich in bioactive compounds like flavonoids, terpenoids, phenolics, alkaloids, and saponins, have demonstrated antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties (Akinmoladun et al., 2020).

Recognizing the strengths of both fields, researchers have started to merge nanotechnology with phytotherapy, creating polyherbal-silver nanoparticle (AgNP) formulations.

Silver nanoparticles act as a strong antimicrobial and healing accelerator. Polyherbal extracts — combinations of two or more plant extracts — contribute additional healing actions, such as reducing oxidative stress, modulating inflammation, stimulating fibroblast proliferation, and enhancing collagen synthesis (Ahmed et al., 2016). Using polyherbal rather than single-plant extracts offers several advantages: Synergistic effects: Multiple phytochemicals can work together to enhance antimicrobial and regenerative outcomes.

Reduced resistance: Microbial resistance is less likely when exposed to a complex mix of agents compared to a single antibiotic or chemical (Aderibigbe, 2017).

Enhanced stability: Plant compounds can stabilize silver nanoparticles, controlling their size and shape during synthesis ("green synthesis" approach). These polyherbal-AgNPs are particularly compelling for neonatal burn management because they: Offer potent antimicrobial protection without relying on harsh chemicals.

Promote faster tissue healing through antioxidant and growth-promoting plant compounds. Are potentially less toxic and more biocompatible for the fragile neonatal skin compared to synthetic treatments (Bhattacharya et al., 2019)

Significance of Polyherbal Formulations and Silver Nanoparticles

Every year, neonatal burns challenge clinicians with their complexity, fragility, and risk of life-threatening infections. Conventional treatments, though vital, often struggle to meet the urgent needs of these tiny patients. Emerging research points toward a groundbreaking solution: polyherbal-silver nanoparticle formulations. By uniting the broad-spectrum antimicrobial action of silver with the healing power of multiple medicinal plants, this innovative approach offers new hope for faster, safer recovery in neonatal burn management.

Polyherbal formulations have been used for centuries in traditional medicine to treat a wide range of ailments, including wound healing. The synergistic interactions between different plant-derived compounds can enhance their therapeutic

efficacy and reduce the risk of adverse effects. Guava, in particular, is rich in bioactive compounds, such as flavonoids, tannins, and triterpenoids, which have been shown to possess antioxidant, anti-inflammatory, and antimicrobial properties. These properties make guava extract a promising candidate for inclusion in a polyherbal formulation for burn treatment. The exploration of different sources of silver nanoparticles and analysis of their efficacy against various bacteria can potentially lead to stronger antimicrobial agents (Sengupta et al., 2017). Silver nanoparticles, on the other hand, have emerged as potent antimicrobial agents due to their unique physicochemical properties. Their small size and large surface area-to-volume ratio enable them to interact with microbial cell membranes and intracellular components, leading to cell death. When embedded in biomaterials, nanoparticles can induce specific properties that make them of interest in applications such as wound dressings (Stoica et al., 2020). The combination of guava extract and silver nanoparticles in a gel formulation could provide a synergistic therapeutic effect, promoting rapid wound healing and preventing infection in neonatal burns.

2. METHODOLOGY

Preparation of Guava Extract

The guava leaves were collected from a local farm, washed thoroughly with distilled water, and dried in a hot air oven at 40°C for 24 hours. The dried leaves were then ground into a fine powder using a mechanical grinder. The guava leaf powder was extracted using a Soxhlet apparatus with ethanol as the solvent. The extraction process was carried out for 6 hours, and the ethanol extract was concentrated using a rotary evaporator under reduced pressure. The concentrated extract was then stored at 4°C until further use.

Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using a green synthesis method, employing guava leaf extract as the reducing agent. Silver nitrate solution (1 mM) was prepared in distilled water.The guava leaf extract was added to the silver nitrate solution and stirred continuously at room temperature.The formation of silver nanoparticles was indicated by a change in color of the solution from colorless to brown. The silver nanoparticle solution was then centrifuged to separate the nanoparticles from the solution, and the nanoparticles were washed with distilled water to remove any impurities. The purified silver nanoparticles were then dried in a hot air oven at 60°C and stored in a desiccator until further use.

Formulation of Polyherbal Gel

The polyherbal gel was formulated using carbopol 940 as the gelling agent. Carbopol 940 was dispersed in distilled water and stirred continuously until a homogeneous gel was formed.The guava extract and silver nanoparticles were then added to the gel and mixed thoroughly. The pH of the gel was adjusted to 5.5-6.5 using triethanolamine. The gel was then stored in airtight containers at room temperature until further use.

Table 1: Composition of Polyherbal Gel

Ingredient	Concentration (%) |
Guava extract	5%
Silver nanoparticles	0.1%

Characterization of Polyherbal Gel

The prepared polyherbal gel was subjected to various evaluation parameters to assess its physicochemical properties. The parameters evaluated included pH, viscosity, spreadability, and drug content. The pH of the gel was determined using a digital pH meter. The viscosity of the gel was measured using a Brookfield viscometer. Spreadability was determined by measuring the diameter of the gel after it was placed between two glass slides. Drug content was determined by dissolving a known amount of the gel in a suitable solvent and analyzing it using UV-Vis spectrophotometry.

In Vitro Release Studies

The in vitro release studies were conducted using a Franz diffusion cell. A known amount of the polyherbal gel was placed on a dialysis membrane, which was then mounted between the donor and receptor compartments of the diffusion cell. The receptor compartment was filled with phosphate buffer solution (pH 7.4) and maintained at 37°C with continuous stirring. Samples were withdrawn from the receptor compartment at regular intervals and analyzed for guava extract and silver nanoparticle content using UV-Vis spectrophotometry.

Antimicrobial Activity

The antimicrobial activity of the polyherbal gel was evaluated against common bacterial strains associated with burn infections, such as Staphylococcus aureus and Pseudomonas aeruginosa. The agar diffusion method was used to determine the zone of inhibition. The bacterial strains were cultured in nutrient broth, and a lawn of bacteria was prepared on nutrient agar plates. Wells were made in the agar plates, and the polyherbal gel was added to the wells.

The plates were then incubated at 37°C for 24 hours, and the zone of inhibition was measured.

In Vivo Studies

In vivo studies were conducted on neonatal rats to evaluate the efficacy of the polyherbal gel in treating burns. Neonatal rats were divided into three groups: control group, silver nanoparticle group, and polyherbal gel group. Burns were induced on the dorsal surface of the rats using a hot plate. The control group received no treatment, the silver nanoparticle group received treatment with silver nanoparticle gel, and the polyherbal gel group received treatment with the polyherbal gel. The treatments were applied topically once daily for 14 days. Wound healing was assessed by measuring the wound area on days 0, 3, 7, and 14.

Histopathological Analysis

On day 14, skin samples were collected from the wound site and subjected to histopathological analysis. The skin samples were fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The stained sections were examined under a microscope to assess the extent of wound healing, collagen deposition, and inflammation.

3. RESULTS

Characterization of Polyherbal Gel

Parameter	Method Used	Observed Value	Acceptable Range
pH	Digital pH meter	6.4 ± 0.1	4.5 – 6.5 (Topical use)
Viscosity (cPs)	Brookfield Viscometer	5,200 ± 50	3,000 – 6,000
Spreadability (cm)	Glass Slide Method	6.8 ± 0.2	>5 cm (satisfactory)
Drug Content (%)	UV-Vis Spectrophotometry	98.6 ± 1.1	90% – 110%

In Vitro Release Study

The release profile (Graph 1) demonstrates a sustained release of both guava extract and silver nanoparticles over 24 hours.

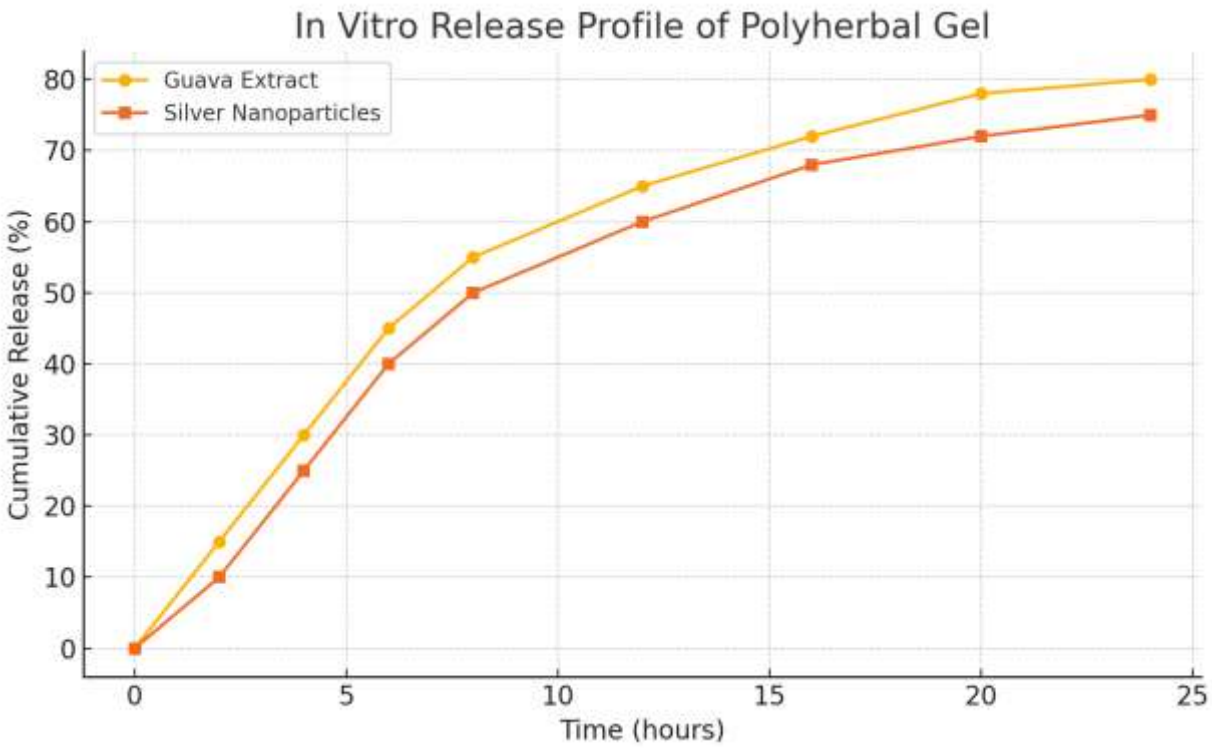


Figure 1 Invitro release of polyherbal gel

Antimicrobial Activity

The gel exhibited significant antimicrobial properties against burn-associated bacteria.

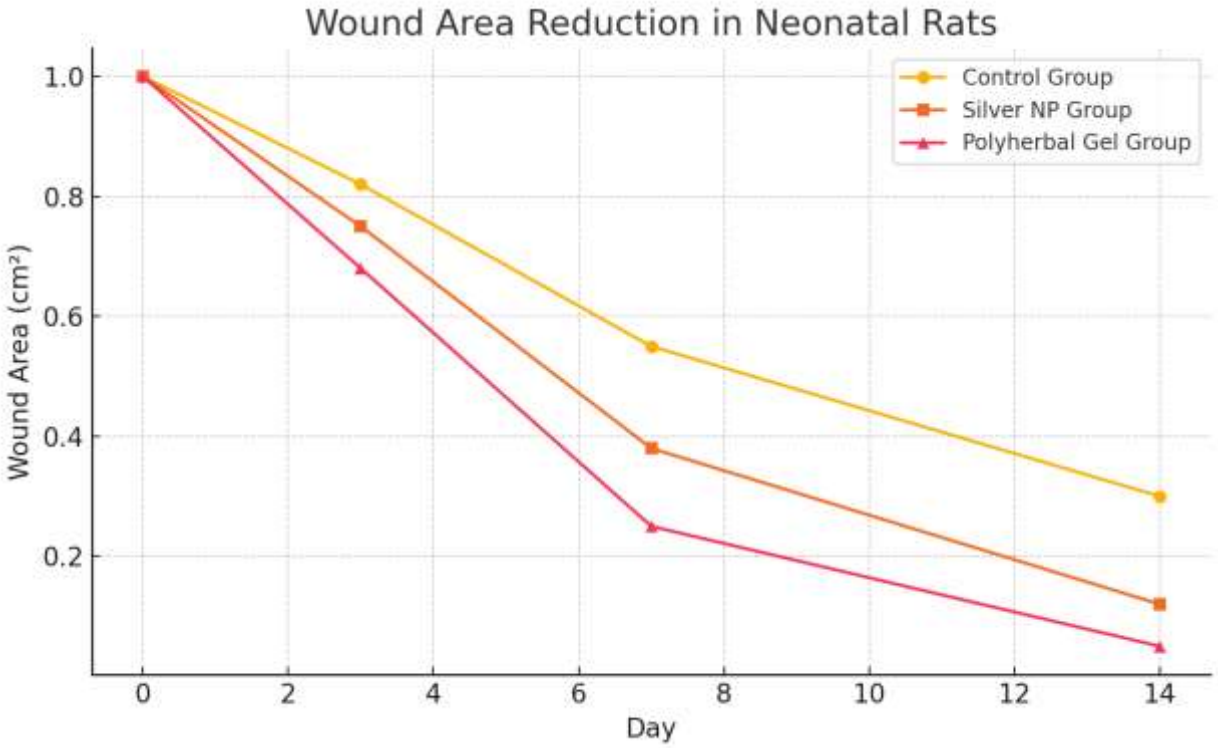
Treatment	***S. aureus* (mm)**	***P. aeruginosa* (mm)**
Polyherbal gel	25	22
Silver NP gel	20	18
Control (Blank)	0	0

The larger zones of inhibition in the polyherbal gel suggest synergistic antimicrobial effects of guava and silver nanoparticles.

In Vivo Wound Healing

Wound closure was significantly enhanced in the polyherbal gel group.

Graph 2: Wound Area Reduction in Neonatal Rats



Histopathological Analysis

Skin samples from the polyherbal gel group exhibited:

- Dense collagen deposition
- Re-epithelialization
- Reduced inflammatory cells

Compared to the control and silver NP groups, tissue regeneration was more complete in the polyherbal gel group.

4. DISCUSSION

The study demonstrates the successful formulation of a polyherbal gel combining guava extract and silver nanoparticles, both known for their wound-healing and antimicrobial properties. The guava leaf extract, rich in flavonoids and tannins, synergizes with silver nanoparticles to enhance antimicrobial action and promote tissue regeneration.

Release Profile and Bioavailability

The sustained release profile ensures prolonged therapeutic effects at the wound site, reducing the need for frequent application. This is particularly important for neonates to minimize discomfort and handling.

Antimicrobial Efficacy

Burn wounds are highly susceptible to infection, which can delay healing. The polyherbal gel's superior inhibition zones against *S. aureus* and *P. aeruginosa* underline its potential as a dual-action therapeutic agent.

Wound Healing Efficacy

The in vivo results show that the polyherbal gel significantly accelerated wound closure, likely due to the anti-inflammatory and regenerative properties of guava flavonoids, as well as the antimicrobial action of silver nanoparticles.

These effects are supported by histopathological evidence of enhanced collagen synthesis and reduced inflammation.

5. CONCLUSION

The polyherbal gel containing guava extract and silver nanoparticles exhibited excellent physicochemical properties, significant antimicrobial activity, and potent wound healing effects in neonatal burn models. This formulation shows promise as an alternative to conventional burn treatments, particularly for sensitive populations such as neonates

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