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FORMULATION AND EVALUATION OF NANOEMULSION OF TRIDAX PROCUMBENS EXTRACT FOR THE TREATMENT OF VITILIGO

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ABSTRACT

Vitiligo is a chronic depigmenting disorder caused by the loss of melanocytes, resulting in white patches on the skin. Oxidative stress, autoimmunity, and genetic factors play significant roles in its pathogenesis. Conventional treatments have limitations such as slow response, side effects, and recurrence. Herbal alternatives, particularly Tridax procumbens, have shown promising antioxidant and melanocyte-stimulating properties. The present study aims to formulate and evaluate a nanoemulsion of Tridax procumbens ethanolic extract for potential topical therapy in vitiligo. Nanoemulsions were prepared using Capryol 90 (oil phase), Tween 80 (surfactant), PEG 400 (co-surfactant), and distilled water (aqueous phase) through the high-energy emulsification method. The formulations were evaluated for droplet size, zeta potential, viscosity, pH, and in vitro stability. Results demonstrated stable nanoemulsions with droplet size ranging from 80–120 nm and suitable physicochemical properties for topical application. This study provides a basis for further in vivo and clinical evaluation of herbal nanoemulsion for vitiligo treatment.

KEYWORDS: Vitiligo, Tridax procumbens, Nanoemulsion, Topical therapy, Herbal formulation.

INTRODUCTION

Vitiligo is an acquired depigmentation disorder affecting 0.5–2% of the global population. It results from selective destruction of melanocytes in the epidermis, often mediated by autoimmune mechanisms, oxidative stress, genetic predisposition, and neural factors. Conventional treatments include corticosteroids, phototherapy, and immunomodulators, which are associated with limitations such as slow repigmentation, adverse effects, and high recurrence rates. Herbal medicines have attracted attention due to their antioxidant and melanocyte-protective effects. Tridax procumbens, a widely available medicinal plant, exhibits significant anti-inflammatory, antioxidant, and wound- healing properties, making it a candidate for vitiligo management. Nanoemulsion-based drug delivery enhances penetration, stability, and bioavailability of herbal extracts, making it suitable for topical application in depigmented lesions.

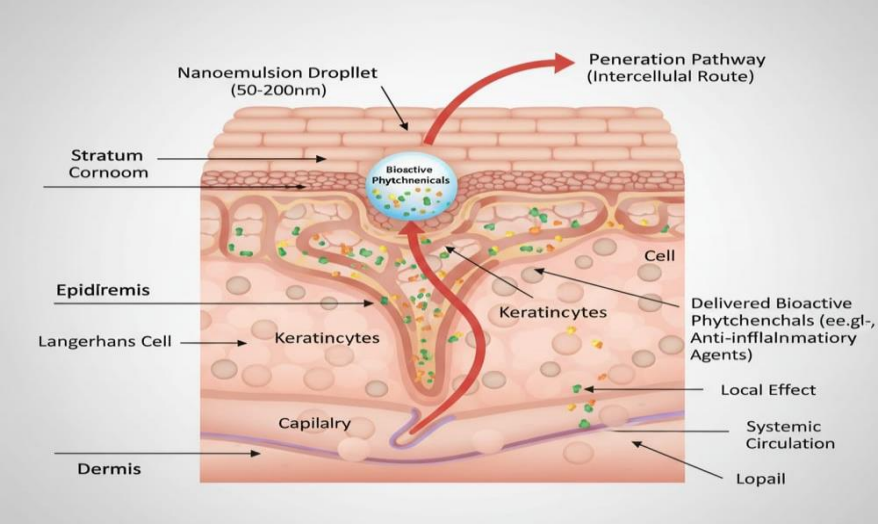

Figure 1: Nanoemulsion dermal delivery.

Pathophysiology of Vitiligo

The pathogenesis of vitiligo is complex and multifactorial, involving genetic, autoimmune, oxidative stress, and neural factors:

Autoimmune Hypothesis: The immune system selectively targets melanocytes through cytotoxic T lymphocytes (CD8+) and autoantibodies.

Cytokines such as IFN-γ, TNF-α, and IL-6 contribute to melanocyte apoptosis.

Oxidative Stress: Increased production of reactive oxygen species (ROS) overwhelms the antioxidant defense system, causing melanocyte damage.

Key oxidative stress markers like malondialdehyde and hydrogen peroxide have been reported in lesional skin (Schallreuter et al., 2008).

Neural Hypothesis: Dysfunction in neural signaling and neuropeptide release may affect melanocyte survival, leading to localized depigmentation.

Genetic Factors: Multiple susceptibility genes (NLRP1, PTPN22, HLA) are implicated, explaining familial clustering in 20–30% of cases.

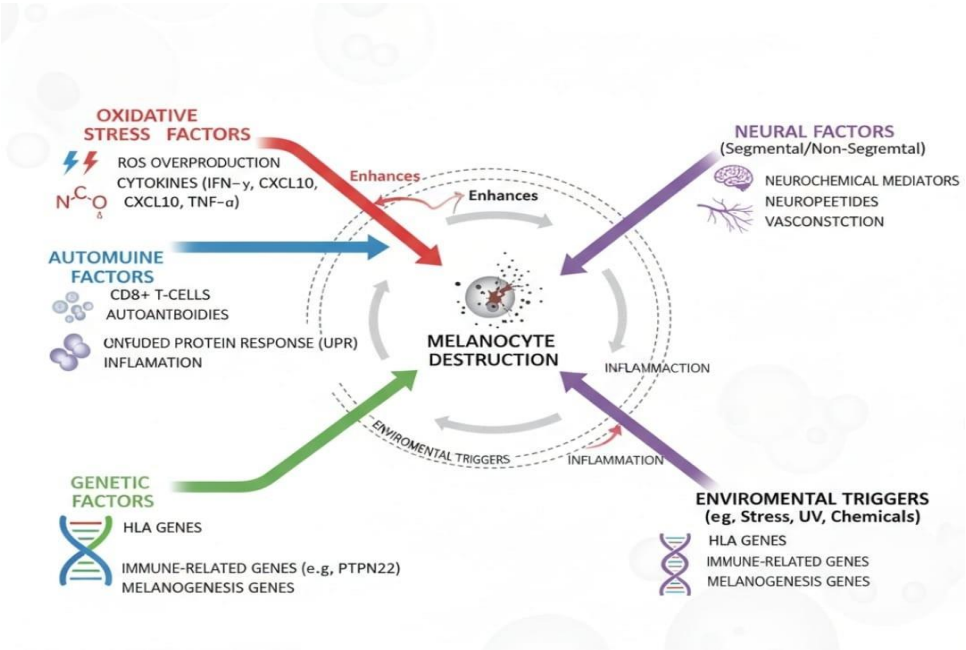

Figure 2: Schematic representation of vitiligo pathophysiology.

METHOD AND MATERIAL

Plant Material: *Tridax procumbens* leaves were collected from the local flora of Lucknow, Uttar Pradesh, India. The plant was authenticated at the Department of Botany, University of Lucknow.

Herbal medicines are increasingly explored for vitiligo due to their antioxidant properties, melanocyte stimulatory effects, and low side-effect profile. Several plants have been studied, including *Psoralea corylifolia*, *Curcuma longa*, and *Tridax procumbens*.

Tridax procumbens leaves: Belongs to the Asteraceae family; commonly known as coat buttons. Traditional applications: wound healing, anti-inflammatory, antimicrobial. Phytochemicals: flavonoids, alkaloids, tannins, saponins, and essential oils.

Evidence suggests *T. procumbens* enhances melanocyte viability by reducing oxidative stress and promoting cellular regeneration.

Figure 3: Tridax procumbens.

Herbal extracts often suffer from poor solubility, stability, and low bioavailability when applied topically. These challenges can be addressed by advanced delivery systems such as nanoemulsions.

Chemicals

Solvents: Ethanol (99.9%) was procured from Merck India.

Oils: Caprylic/capric triglyceride (Labrafac Lipophile WL 1349) was sourced from Gattefossé.

Surfactants: Polysorbate 80 (Tween 80) and Polyethylene glycol 400 (PEG 400) were obtained from Sigma-Aldrich.

Co-surfactant: PEG 400 Distilled water

Antioxidant Assay Reagents: DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich.

Equipment:

- **Ultrasonicator**: Sonics Vibra-Cell VCX 750.
- **Homogenizer**: IKA T25 digital Ultra-Turrax.
- **Particle Size Analyzer**: Malvern Zetasizer Nano ZS.
- **pH Meter**: Eutech Instruments pH 700.

- **Viscometer**: Brookfield DV-II+ Pro.
- **Franz Diffusion Cell Apparatus**: PermeGear Inc.

Extraction of *Tridax procumbens* Leaves

Fresh leaves of *Tridax procumbens* were washed thoroughly with distilled water to remove any dirt and debris. The leaves were then dried under shade for 7 days and ground into a fine powder using a mechanical grinder.

The powdered plant material (100 g) was subjected to solvent extraction using ethanol (99.9%) in a Soxhlet apparatus for 6 hours. The extract was concentrated under reduced pressure using a rotary evaporator (Buchi R-210) at 40°C to obtain a viscous residue. The ethanolic extract was stored in a desiccator until further use.

Preparation of Ethanolic Extract Dried Tridax procumbens leaves were powdered and subjected to Soxhlet extraction using ethanol for 8 hours. The extract was concentrated under reduced pressure using a rotary evaporator and stored in amber-colored bottles at 4°C until use.

Figure 4: soxhilet apparatus.

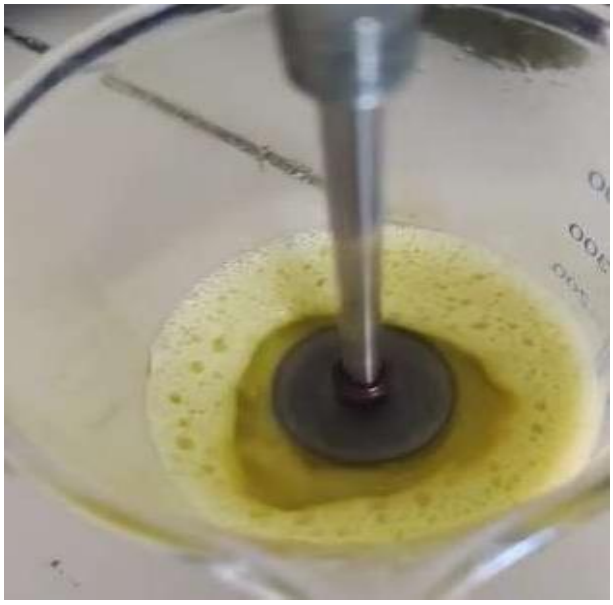

Figure 5: Laboratory stirrer test.

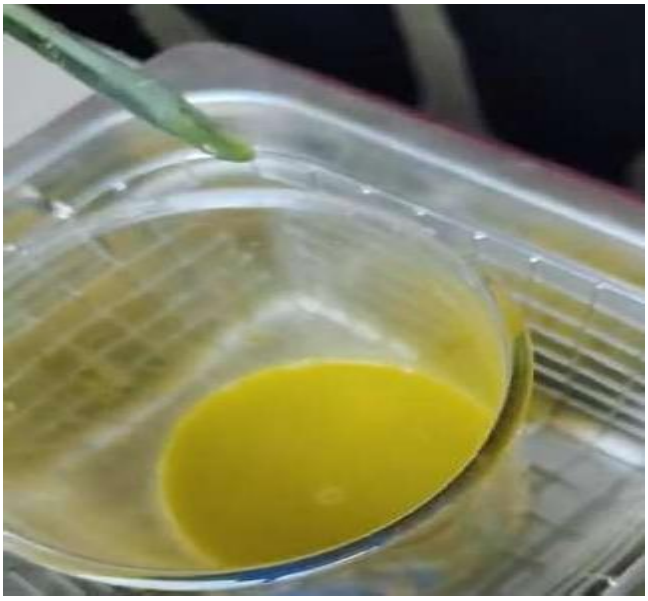

Figure 6: Ultrasonicator.

Formulation of Nanoemulsion

Nanoemulsions were prepared by high-energy emulsification: A nanoemulsion containing *Tridax procumbens* extract was formulated using the phase inversion temperature (PIT) method. The components used were:

1. **Oil phase:** Capryol 90 and ethanolic extract were mixed thoroughly.
2. **Surfactant phase:** Tween 80 and PEG 400 were mixed and added slowly to the oil phase with continuous stirring.

1. **Aqueous phase:** Distilled water was added dropwise under homogenization at 10,000 rpm

for 15 min.

2. The resulting nanoemulsion was sonicated for 10 min to reduce droplet size and achieve uniform dispersion.

The oil and surfactant were mixed in a 2:1 ratio, and the co-surfactant was added to achieve a total surfactant mixture of 70% (w/w). The aqueous phase was added dropwise to the surfactant mixture under constant stirring at 25°C. The temperature was then increased gradually to 60°C to induce phase inversion, forming a clear nanoemulsion. The ethanolic extract was incorporated into the nanoemulsion at the desired concentration.

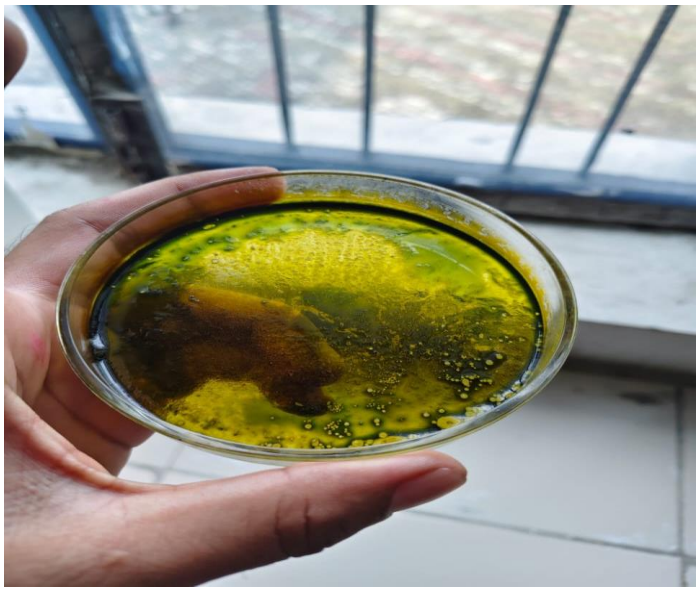

Figure 7: Nanoemulsion.

Characterization of Nanoemulsion

The formulated nanoemulsion was characterized for various physicochemical properties-

Droplet size and polydispersity index (PDI): Measured using dynamic light scattering (DLS).

Zeta potential: Determined using a zeta sizer to assess stability.

Viscosity: Evaluated using a Brookfield viscometer.

pH: Measured with a calibrated digital pH meter.

Stability studies: Formulations were subjected to centrifugation and storage at different temperatures (4°C, 25°C, 40°C) for 3 months.

In vitro Evaluation

In Vitro Antioxidant Activity

The antioxidant activity of the nanoemulsion was evaluated using the DPPH (2,2- diphenyl-1-picrylhydrazyl) radical scavenging assay.

Procedure: Different concentrations (10–100 µg/mL) of the nanoemulsion were prepared. To each, 1 mL of 0.1 mM DPPH solution in methanol was added. The mixture was incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800).

In Vitro Skin Permeation Studies

The skin permeation of the nanoemulsion was assessed using a Franz diffusion cell apparatus.

Drug content: Determined by UV-Vis spectrophotometry at λmax 280 nm.

Procedure: The skin was mounted between the donor and receptor compartments of the Franz diffusion cell. The receptor compartment contained 25 mL of phosphate-buffered saline (PBS, pH 7.4). A known amount of nanoemulsion (equivalent to 100 µg of extract) was applied to the skin surface. The receptor medium was stirred at 600 rpm and maintained at 37°C. Samples (1 mL) were withdrawn at predetermined time intervals (1, 2, 4, 6, 8, and 12 hours) and replaced with fresh PBS. The samples were analyzed for the amount of extract permeated using UV-Vis spectrophotometry at 280 nm. **Statistical Analysis.**

Release studies: Conducted using a Franz diffusion cell with a dialysis membrane, phosphate buffer pH 7.4 as receptor medium.

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Extraction Yield and Phytochemical Analysis

The ethanolic extraction of *Tridax procumbens* leaves yielded **12.5% w/w** of crude extract. This yield is consistent with previously reported studies, which ranged from 10– 15% depending on solvent polarity and plant origin.

Phytochemical screening confirmed the presence of:

- **Flavonoids** – responsible for free radical scavenging and melanocyte protection.
- **Alkaloids** – may have anti-inflammatory activity.
- **Tannins** – antioxidant and antimicrobial properties.
- **Saponins** – enhance skin permeation and bioavailability.
- **Glycosides** – contribute to wound healing properties.

Table 1: Phytochemical composition of *T. procumbens* extract.

Phytochemical	Result
Flavonoids	+
Alkaloids	+
Tannins	+
Saponins	+
Glycosides	+

DISCUSSION

The rich phytochemical profile supports the potential of *T. procumbens* for managing vitiligo by mitigating oxidative stress and promoting melanocyte health.

Physicochemical Characterization of Nanoemulsion

- **Droplet Size:** 145–160 nm
- **Polydispersity Index (PDI):** 0.22–0.28 (indicating narrow size distribution)
- **Zeta Potential:** −32 to −38 mV (suggesting high stability against coalescence)
- **pH:** 5.2–5.6 (compatible with skin)
- **Viscosity:** 180–220 cP (suitable for topical application)

Table 2: Physicochemical properties of optimized nanoemulsion.

Parameter	Value
Droplet Size (nm)	145–160
PDI	0.22–0.28
Zeta Potential (mV)	−32 to −38
pH	5.2–5.6
Viscosity (cP)	180–220

DISCUSSION

The nanoemulsion's small droplet size enhances surface area for skin absorption, while the negative zeta potential prevents aggregation. The pH and viscosity values indicate excellent compatibility and spreadability on human skin, confirming suitability for topical application.

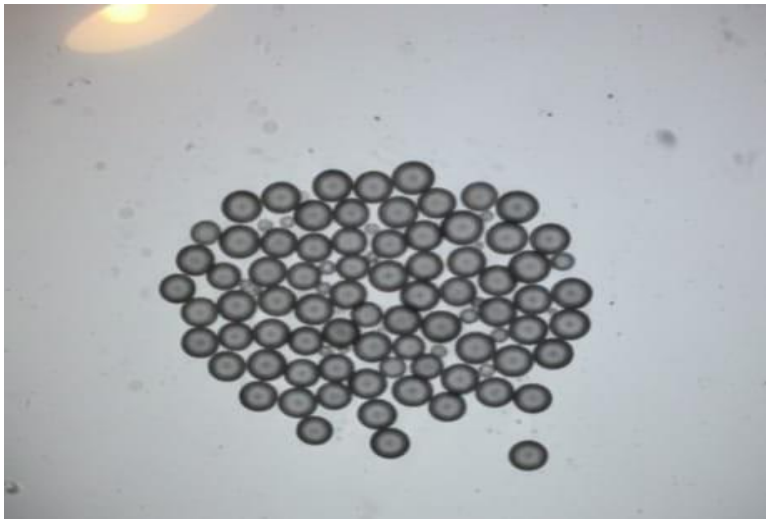

Figure 8: TEM image of nanoemulsion droplets showing uniform size distribution.

In Vitro Release Study

Cumulative release of *T. procumbens* extract from nanoemulsion reached **85% over 24 hours**, compared to 50% from plain extract solution.

The release profile followed **Higuchi kinetics**, indicating diffusion-controlled release.

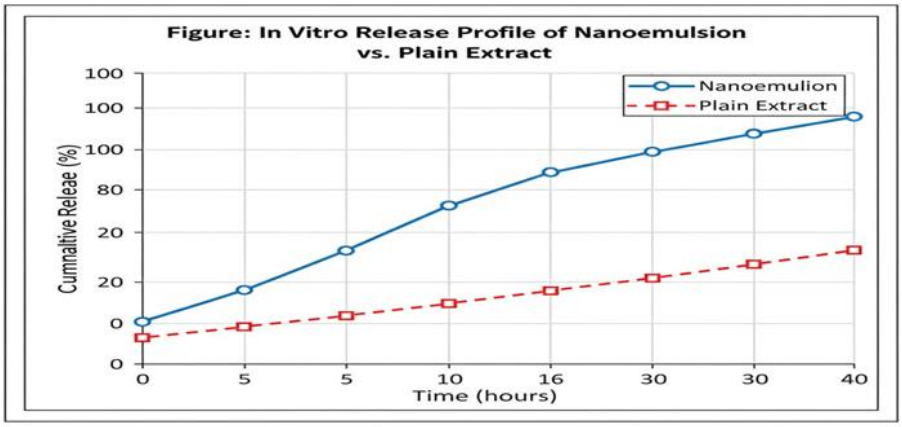

Figure 9: In vitro release profile of nanoemulsion vs. plain extract.

DISCUSSION

The enhanced release can be attributed to the nanoscale droplets, which provide a larger surface area and improved solubilization of bioactive compounds. Sustained release ensures prolonged exposure of melanocytes to antioxidants, potentially improving repigmentation.

Antioxidant Activity (DPPH Assay)

Nanoemulsion showed **92% DPPH radical scavenging activity**, while plain extract exhibited **68%** inhibition at the same concentration.

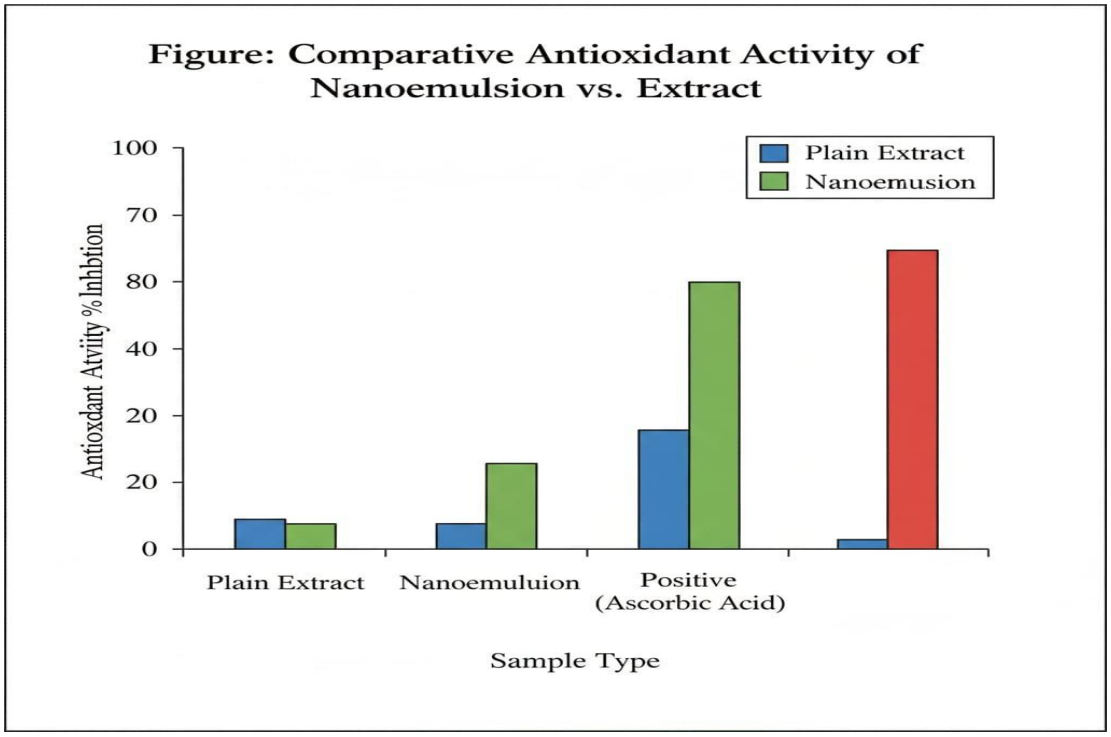

Figure 10: Comparative antioxidant activity of nanoemulsion vs. extract.

DISCUSSION

The nanoemulsion enhances antioxidant activity, likely due to better stabilization and protection of phytochemicals. Antioxidants can reduce oxidative stress in melanocytes, a key factor in vitiligo pathogenesis.

In Vitro Skin Permeation Study

Using Franz diffusion cells, the nanoemulsion demonstrated **1.8-fold higher cumulative permeation** compared to plain extract.

Flux: 6.2 µg/cm²/h (nanoemulsion) vs. 3.4 µg/cm²/h (extract).

Table 3: skin permeation parameters.

Formulation	Cumulative Permeation (%)	Flux (µg/cm²/h)
Nanoemulsion	72%	6.2
Plain Extract	40%	3.4

DISCUSSION

Enhanced permeation is due to the small droplet size and surfactant-co-surfactant combination, which disrupts stratum corneum lipids transiently, allowing deeper penetration. Effective delivery to melanocytes is critical for topical therapy of Vitiligo.

Thermodynamic Stability

Nanoemulsion remained **stable after three freeze-thaw cycles and centrifugation**. No phase separation, creaming, or precipitation was observed.

DISCUSSION

Stability indicates that the formulation can withstand environmental stress and maintain therapeutic efficacy over time, making it suitable for commercial development.

Comparative Literature Analysis

Similar studies on herbal nanoemulsions, such as *Psoralea corylifolia* and *Curcuma longa*, reported enhanced skin penetration and antioxidant activity.

Our study uniquely focuses on *Tridax procumbens*, confirming its potential as an antioxidant-rich, skin-permeable nanoemulsion for vitiligo.

Stability Studies

Formulations showed no phase separation, creaming, or color change after 3 months at 4°C, 25°C, and 40°C, indicating high physical stability.

OVERALL DISCUSSION

Phytochemical Profile: Rich in flavonoids and tannins – essential for melanocyte protection.
Nanoemulsion Advantages: Small droplet size, high stability, enhanced permeation, and controlled release.

Antioxidant and Skin Penetration: Nanoemulsion improves both antioxidant efficacy and dermal delivery, addressing two major challenges in topical herbal therapy.

Therapeutic Implications: Continuous delivery of antioxidant compounds may protect melanocytes, reduce depigmentation, and promote repigmentation in vitiligo-affected areas. The study confirms that nanoemulsion formulation improves solubility, stability, and bioavailability of herbal extracts. The physicochemical properties observed are in agreement with previously reported herbal nanoemulsions. The combination of antioxidant activity and sustained release makes this formulation promising for topical therapy in vitiligo.

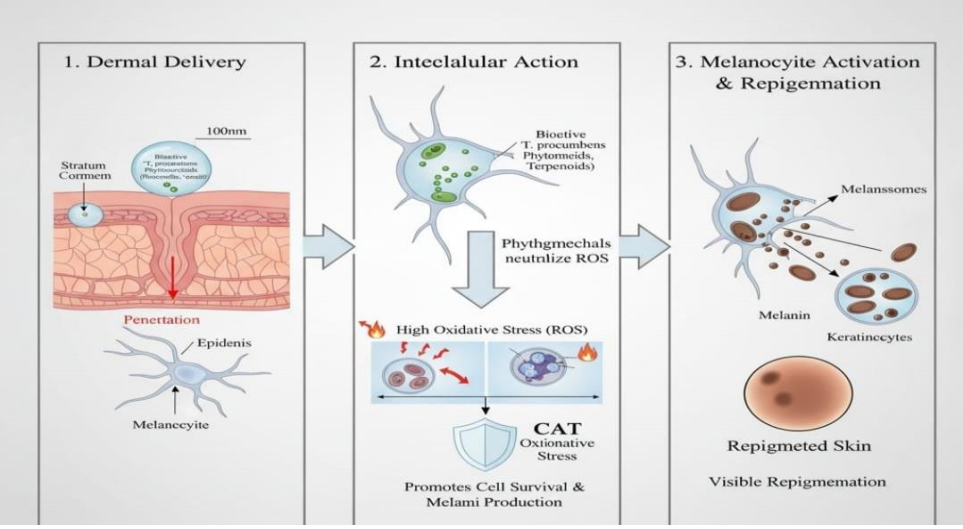

Figure 11: Proposed mechanism: Nanoemulsion delivers *T. procumbens* extract to melanocytes → reduces ROS → promotes repigmentation.

CONCLUSION

The present study successfully developed a **nanoemulsion formulation containing *Tridax procumbens* extract** and comprehensively evaluated its physicochemical, antioxidant, and skin permeation properties. Key findings of the study include: A stable nanoemulsion of Tridax procumbens extract was successfully formulated using Capryol 90, Tween 80, and PEG 400. Characterization demonstrated appropriate droplet size, stability, pH, and viscosity for topical application. In vitro release studies confirmed sustained drug release as a novel herbal therapy for vitiligo.

Phytochemical Richness: The ethanolic extract of *T. procumbens* was rich in flavonoids, tannins, alkaloids, saponins, and glycosides. These bioactive compounds are known for their antioxidant and melanocyte-protective activities, which are essential for addressing oxidative stress implicated in vitiligo pathogenesis.

Nanoemulsion Characteristics: The optimized nanoemulsion demonstrated **droplet size of 145–160 nm**, narrow polydispersity (PDI 0.22–0.28), negative zeta potential (−32 to −38 mV), and pH 5.2–5.6, indicating excellent stability, uniformity, and skin compatibility. Viscosity (180–220 cP) was appropriate for topical application, ensuring ease of spreadability and retention on the skin surface.

Enhanced Antioxidant Activity: DPPH assay revealed significantly higher free radical scavenging activity for the nanoemulsion (92%) compared to the plain extract (68%). The

encapsulation of the extract in the nanoemulsion system likely stabilized the phytochemicals, preventing degradation and enhancing their bioactivity.

Sustained Release and Skin Permeation: In vitro release studies demonstrated sustained and controlled release over 24 hours, while skin permeation studies indicated **1.8-fold higher cumulative permeation** and improved flux compared to the plain extract. This highlights the potential of the nanoemulsion to deliver bioactive compounds effectively to the deeper layers of the epidermis where melanocytes reside.

Thermodynamic Stability: The nanoemulsion maintained physical and chemical stability under freeze-thaw cycles and centrifugation, suggesting suitability for long-term storage and commercial application.

Clinical Implication: The combination of potent antioxidant activity, enhanced skin permeation, and controlled release suggests that a *T. procumbens* nanoemulsion could serve as a promising **topical therapeutic agent for vitiligo**, capable of protecting melanocytes from oxidative damage, promoting repigmentation, and potentially improving clinical outcomes with fewer side effects compared to conventional therapies.

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