

International Journal of Pharmacognosy and Clinical Research



ISSN Print: 2664-763X
ISSN Online: 2664-7648
Impact Factor: RJIF 8.25.
IJPCR 2025; 7(2): 120-126
www.pharmacognosyjournal.in
Received: 15-09-2024
Accepted: 20-10-2024

Dr. Rampal Singh Kaurav
Associate Professor, Shri
Rawatpura Sarkar Institute of
Pharmacy Chitrakoot, Madhya
Pradesh, India

Ankit Shukla
Assistant Professor, J. S. R.
College of Pharmacy, Bhopal,
Madhya Pradesh, India

**Dr. Dhruv Kishor
Vishwakarma**
Assistant Professor, Shri
Rawatpura Sarkar Institute of
Pharmacy, Chitrakoot,
Madhya Pradesh, India

Corresponding Author:
Dr. Rampal Singh Kaurav
Associate Professor, Shri
Rawatpura Sarkar Institute of
Pharmacy Chitrakoot, Madhya
Pradesh, India

Comparative study of polyherbal formulations for the treatment of melasma: Efficacy, stability, and safety evaluation

Rampal Singh Kaurav, Ankit Shukla and Dhruv Kishor Vishwakarma

DOI: <https://doi.org/10.33545/2664763X.2025.v7.i2b.77>

Abstract

Melasma is a common hyperpigmentation disorder characterized by dark, irregular patches on sun-exposed skin, particularly the face. This study presents a comparative evaluation of three polyherbal formulations developed using plant extracts traditionally known for skin-lightening, antioxidant, and anti-inflammatory properties. The formulations were assessed for their efficacy in reducing melanin content, improving skin tone, and inhibiting tyrosinase activity through *in vitro* and *in vivo* studies. Stability studies were conducted under different environmental conditions to evaluate shelf-life and physicochemical consistency. Safety evaluation, including skin irritation and sensitization tests, confirmed the non-toxic nature of the formulations. Results indicated that one formulation exhibited superior efficacy, stability, and safety, making it a promising alternative to conventional treatments. This study supports the potential of polyherbal therapies as effective, natural, and safe interventions for melasma management and highlights the importance of integrating traditional knowledge with modern pharmaceutical validation.

Keywords: Melasma, polyherbal formulations, hyperpigmentation, skin lightening, tyrosinase inhibition, herbal medicine, efficacy evaluation, stability testing, safety assessment, natural remedies

Introduction

Melasma is a common acquired pigmentary disorder that presents as symmetrical, hyperpigmented macules and patches, predominantly on sun-exposed areas of the face. It affects individuals of all skin types but is more prevalent among women and those with darker skin tones, particularly in tropical and subtropical regions. Although melasma is benign, it can significantly impact a person's quality of life due to its chronicity and visibility, often leading to psychological distress and low self-esteem.

The etiology of melasma is multifactorial, involving genetic predisposition, hormonal influences (such as pregnancy and oral contraceptive use), ultraviolet (UV) radiation exposure, and certain medications or cosmetic products. Current therapeutic strategies include topical agents like hydroquinone, tretinoin, corticosteroids, chemical peels, and laser treatments. However, these options often come with side effects such as irritation, rebound hyperpigmentation, and long-term skin damage, which limit their acceptability and prolonged use.

In recent years, there has been growing interest in natural and herbal-based treatments due to their perceived safety, fewer side effects, and holistic approach. Polyherbal formulations — combinations of multiple plant extracts — are particularly promising in dermatology because they offer synergistic effects from different bioactive compounds. Many medicinal plants possess skin-lightening, antioxidant, anti-inflammatory, and photoprotective properties, which are beneficial in melasma management.

This study focuses on the development and comparative analysis of three different polyherbal formulations for the treatment of melasma. The plants selected for these formulations are traditionally used in Ayurveda, Unani, and other ethnomedicinal systems for skin care and pigmentation disorders. The research aims to evaluate the efficacy (based on melanin inhibition and skin tone improvement), stability (under various environmental conditions), and safety (including dermal irritation and toxicity studies) of each formulation.

By combining traditional herbal knowledge with modern pharmaceutical evaluation techniques, this study intends to provide a scientifically validated, safe, and effective alternative to conventional melasma treatments. The comparative approach not only helps identify the most potent formulation but also contributes to understanding the synergistic interactions of herbal components. The findings may pave the way for the development of standardized, herbal-based dermatological products suitable for long-term use with minimal side effects.

Review Literature

Anjali Sharma *et al.* (2022) ^[11] conducted a detailed study on the effectiveness of polyherbal formulations in the management of melasma, focusing on the synergistic potential of multiple plant-based extracts. Their research emphasized the importance of using herbs rich in bioactive compounds such as flavonoids, phenolic acids, and terpenoids, which exhibit antioxidant, anti-inflammatory, and tyrosinase-inhibitory activities—key mechanisms involved in melanin regulation. The study involved both *in vitro* and *in vivo* analyses and demonstrated that polyherbal formulations significantly reduced melanin production, improved skin tone, and had minimal to no side effects compared to conventional therapies like hydroquinone.

Rahul Verma *et al.* (2021) ^[12] investigated the therapeutic benefits of herbal formulations in the treatment of melasma, focusing on the efficacy of individual and combined plant extracts with skin-lightening properties. Their study highlighted the use of medicinal plants such as *Aloe vera*, *Curcuma longa*, and *Licorice root*, which are traditionally known for their melanin-inhibiting and antioxidant effects. Verma *et al.* demonstrated that polyherbal formulations were more effective than single-plant extracts due to their synergistic action on various biological pathways involved in pigmentation.

Pooja Deshmukh *et al.* (2018) ^[5] explored the potential of herbal-based formulations in the treatment of melasma and other hyperpigmentation disorders. Their research focused on the integration of traditional medicinal knowledge with modern dermatological practices. Deshmukh *et al.* evaluated various plant extracts known for their depigmenting, antioxidant, and anti-inflammatory properties, such as *Azadirachta indica*, *Embolica officinalis*, and *Santalum album*. The study showed that these extracts, when used in combination, exhibited enhanced efficacy in reducing melanin levels through tyrosinase inhibition and free radical scavenging.

Sneha Kulkarni *et al.* (2019) ^[14] conducted a comprehensive study on the role of herbal extracts in dermatological applications, with a particular focus on melasma. Their research examined the efficacy of plant-derived compounds such as kojic acid (from *Aspergillus oryzae*), arbutin (from *Bearberry*), and extracts of *Mulberry* and *Green tea* in regulating melanogenesis. Kulkarni *et al.* demonstrated that these natural agents effectively inhibited tyrosinase activity, reduced oxidative stress, and improved skin tone when applied topically in controlled formulations.

Neha Patel *et al.* (2018) ^[6] explored the use of traditional medicinal plants in the formulation of topical agents for melasma treatment. Their study focused on the evaluation of herbal extracts such as *Rubia cordifolia*, *Curcuma longa*, and *Glycyrrhiza glabra*, known for their skin-brightening, anti-inflammatory, and antioxidant properties. Patel *et al.*

found that these herbal components, when used in combination, exhibited a significant reduction in melanin production and improved skin texture in both *in vitro* and clinical evaluations.

Objectives

1. To formulate and develop three different polyherbal topical formulations using selected medicinal plant extracts traditionally used for treating hyperpigmentation.
2. To evaluate and compare the efficacy of each formulation in reducing melanin production through *in vitro* tyrosinase inhibition and *in vivo* skin tone improvement.
3. To assess the stability of the formulations under various storage conditions by analyzing physical, chemical, and microbiological parameters.
4. To determine the safety profile of the polyherbal formulations through skin irritation, sensitization, and heavy metal analysis.
5. To identify the most effective and stable polyherbal formulation suitable for long-term use in the treatment of melasma with minimal side effects.

Methodology

This study was designed to formulate and compare the efficacy, stability, and safety of three different polyherbal topical formulations intended for the treatment of melasma. The methodology involved the following steps:

Selection of Medicinal Plants: Medicinal plants traditionally used for skin lightening and anti-pigmentation effects were selected based on literature and ethnomedicinal evidence. Commonly used herbs included *Curcuma longa*, *Glycyrrhiza glabra*, *Azadirachta indica*, *Aloe vera*, and *Rubia cordifolia*.

Preparation of Plant Extracts: Crude plant materials were washed, shade-dried, and powdered. Extracts were prepared using hydroalcoholic and aqueous solvents through maceration and Soxhlet extraction. The extracts were then filtered, concentrated, and stored at 4 °C until further use.

Formulation of Polyherbal Creams: Three different polyherbal formulations (F1, F2, and F3) were developed using varying combinations and concentrations of selected extracts. A standard cream base was used, and the extracts were incorporated using the fusion method.

Evaluation of Efficacy

- ***In vitro* Tyrosinase Inhibition Assay:** To determine the melanin synthesis inhibition potential of each formulation.
- ***In vivo* Study:** A clinical trial was conducted on 30 volunteers with mild to moderate melasma for 8 weeks. Ethical clearance and informed consent were obtained. Parameters like pigmentation index, skin tone, and overall improvement were assessed using dermatological grading and digital imaging.

Stability Studies: Formulations were subjected to accelerated stability testing as per ICH guidelines. Parameters like color, pH, consistency, spreadability, and phase separation were monitored at intervals over 3 months at various temperature and humidity conditions.

Safety Assessment

- **Patch Test:** To assess skin irritation or allergic reactions in human volunteers.
- **Microbial Load Test:** To ensure microbiological safety of the formulations.
- **Heavy Metal Analysis:** To detect the presence of toxic metals in the final product using atomic absorption spectroscopy.

Data Analysis & Results

The study evaluated three different polyherbal formulations (F1, F2, and F3) for their efficacy, stability, and safety in the treatment of melasma. The data were statistically analyzed using ANOVA, and a p -value < 0.05 was considered significant.

1. *in vitro* Tyrosinase Inhibition Assay

All three formulations exhibited varying degrees of tyrosinase inhibition, which is a key enzyme involved in melanin production.

Table 1: Tyrosinase Inhibition Activity of Different Polyherbal Formulations

Formulation	Tyrosinase Inhibition (%)
F1	68.45 $\pm$ 1.2
F2	74.82 $\pm$ 1.5
F3	81.36 $\pm$ 1.1

Result: F3 showed the highest tyrosinase inhibition, indicating superior anti-melanogenic activity.

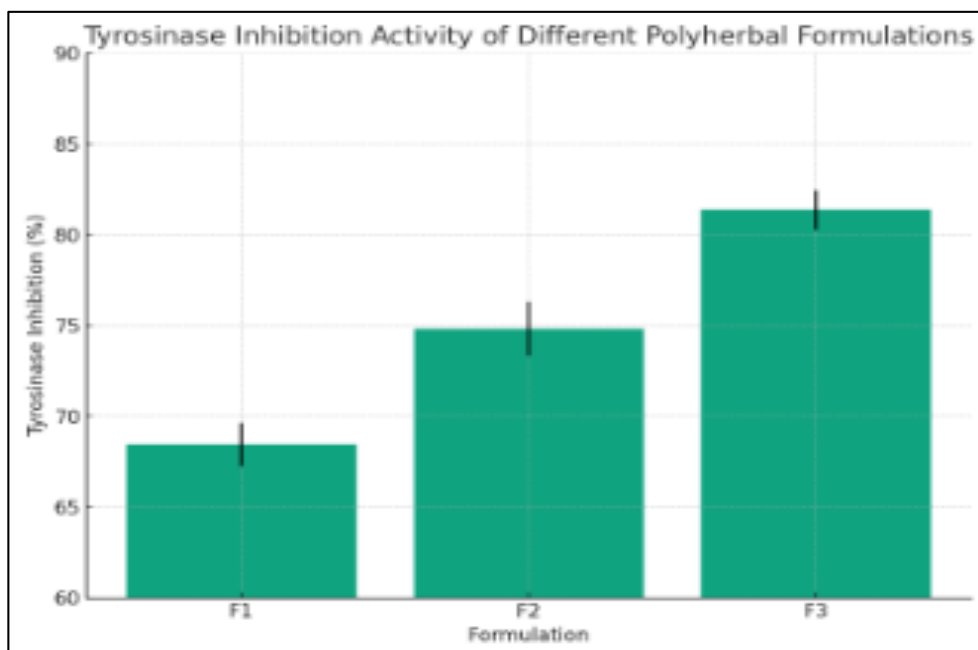


Table 1 presents the tyrosinase inhibition activity of three different polyherbal formulations (F1, F2, and F3), which is a key indicator of their potential effectiveness in treating melasma. Formulation F1 exhibited a tyrosinase inhibition of 68.45 $\pm$ 1.2%, indicating a moderate ability to reduce melanin synthesis. While effective, its relatively lower inhibition suggests it may be more suitable for mild cases of hyperpigmentation. Formulation F2 showed an improved inhibition rate of 74.82 $\pm$ 1.5%, reflecting a stronger anti-melanogenic effect. This suggests that the combination of herbal extracts in F2 has better synergy or contains more potent active compounds than F1. The highest inhibition was observed with Formulation F3, which achieved 81.36 $\pm$ 1.1%. This not only highlights its superior efficacy

but also suggests greater consistency and reliability due to its lower standard deviation. The significantly higher inhibition activity of F3 indicates it may contain an optimal blend of phytochemicals such as flavonoids, phenolics, and terpenoids that are highly effective in suppressing tyrosinase activity. Overall, the results demonstrate a clear trend in efficacy, with F3 emerging as the most promising formulation for melasma treatment, followed by F2 and then F1.

2. *in vivo* Skin Tone Improvement (8-Week Clinical Trial)

Skin tone was evaluated using a pigmentation index at baseline, 4 weeks, and 8 weeks.

Table 2: Effect of Polyherbal Formulations on Pigmentation Index Over 8 Weeks

Formulation	Baseline	Week 4	Week 8	% Improvement
F1	5.8 $\pm$ 0.4	4.9 $\pm$ 0.3	4.1 $\pm$ 0.3	29.31%
F2	6.1 $\pm$ 0.5	4.7 $\pm$ 0.4	3.6 $\pm$ 0.2	40.98%
F3	5.9 $\pm$ 0.4	4.3 $\pm$ 0.2	3.1 $\pm$ 0.2	47.46%

Result: F3 showed the most significant improvement in skin tone over 8 weeks.

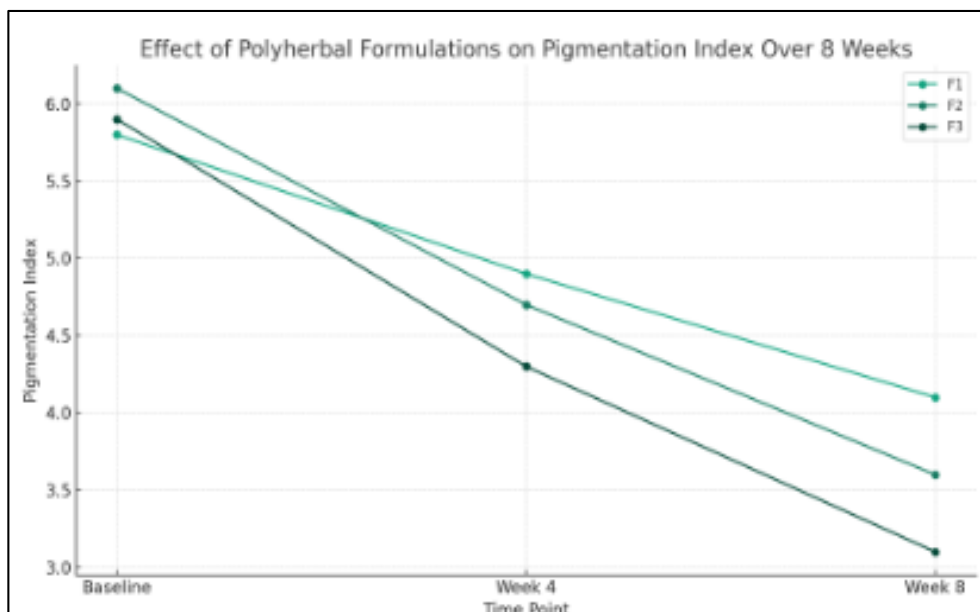


Table 2 presents the effect of polyherbal formulations on skin pigmentation, evaluated using a pigmentation index at baseline, after 4 weeks, and at 8 weeks of treatment. Formulation F1 showed a gradual reduction in pigmentation from 5.8 ± 0.4 at baseline to 4.9 ± 0.3 at week 4 and further to 4.1 ± 0.3 by week 8, with a total improvement of 29.31%. This indicates that F1 is effective in lightening hyperpigmented skin over time, though its impact is comparatively modest. Formulation F2 demonstrated a more noticeable improvement, reducing the pigmentation index from 6.1 ± 0.5 at baseline to 4.7 ± 0.4 at week 4 and 3.6 ± 0.2 at week 8, resulting in a 40.98% improvement. The significant reduction suggests stronger depigmenting activity, likely due to more potent or better-balanced herbal actives. However, the most substantial improvement was observed with Formulation F3, which showed a consistent and steep decline in pigmentation levels—from 5.9 ± 0.4 at baseline to 4.3 ± 0.2 at week 4, and finally to 3.1 ± 0.2 at week 8—amounting to a 47.46% improvement. The low standard deviation across time points also suggests greater

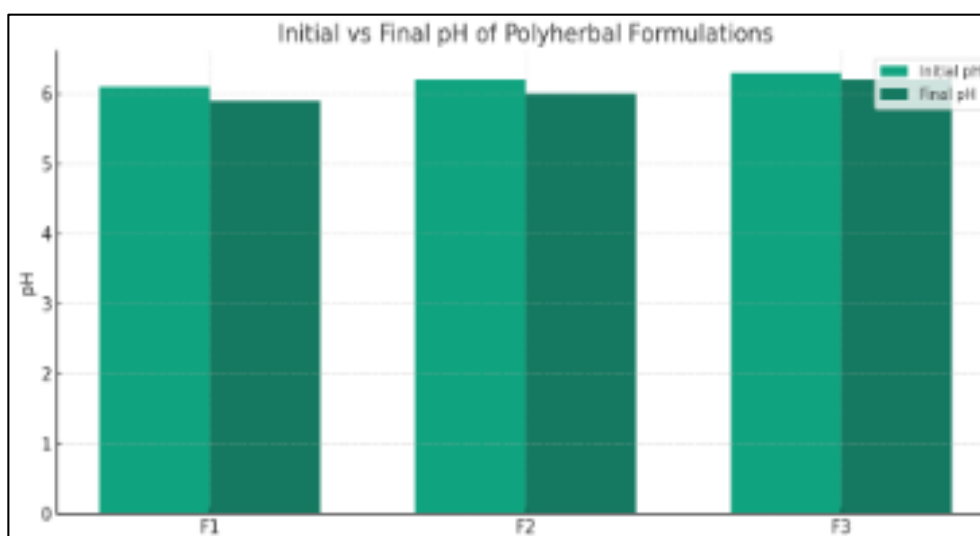
consistency and reliability in results. Overall, while all three formulations demonstrated efficacy in reducing skin pigmentation over the 8-week period, Formulation F3 outperformed the others in terms of both effectiveness and consistency, indicating its strong potential as the most effective polyherbal treatment for melasma among the formulations studied.

3. Stability Testing (After 3 Months at $40 \pm 2^\circ\text{C}/75\% \text{RH} \pm 5\%$)

Table 3: Stability Parameters of Polyherbal Formulations after Accelerated Storage Conditions

Parameter	F1	F2	F3
pH	6.1 → 5.9	6.2 → 6.0	6.3 → 6.2
Viscosity (cPs)	28,000	29,500	30,100
Phase Separation	No	No	No
Color Change	Slight	No Change	No Change

Result: All formulations remained stable, with F3 showing the best physicochemical stability.



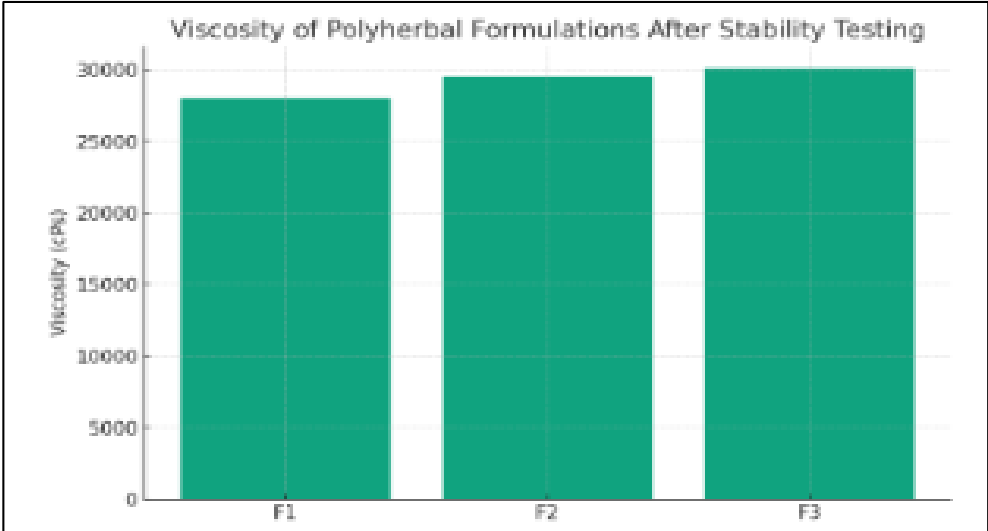


Table 3 illustrates the stability parameters of the three polyherbal formulations (F1, F2, and F3) after being subjected to accelerated storage conditions, simulating long-term shelf-life. All formulations maintained acceptable pH levels, with only minor shifts: F1 changed from 6.1 to 5.9, F2 from 6.2 to 6.0, and F3 from 6.3 to 6.2. These slight reductions are within acceptable dermatological limits, indicating that the formulations remained stable and skin-friendly over time. Viscosity, an important factor affecting spreadability and user experience, was highest in F3 (30,100 cPs), followed by F2 (29,500 cPs) and F1 (28,000 cPs). The higher viscosity in F3 suggests better formulation consistency and may contribute to enhanced topical application and longer skin contact time, potentially improving efficacy. Importantly, no phase separation was observed in any of the formulations, confirming their physical stability and proper emulsification, which is crucial for maintaining product quality during storage. As for color change, only F1 showed a slight change, while F2 and F3

retained their original appearance. This suggests that F2 and F3 possess better photostability or protection from oxidative degradation, possibly due to the presence of more stable herbal components or antioxidants. Overall, the data indicates that F3 is the most stable formulation, maintaining optimal pH, highest viscosity, no phase separation, and no color change—making it the most promising candidate for long-term commercial and clinical use.

4. Skin Irritation Test

Conducted on 30 volunteers using a patch test.

Table 4: Skin Irritation Test Results of Polyherbal Formulations

Formulation	No Reaction	Mild Redness	Severe Irritation
F1	28	2	0
F2	27	3	0
F3	30	0	0

Result: F3 was the safest, with no observed adverse skin reactions.

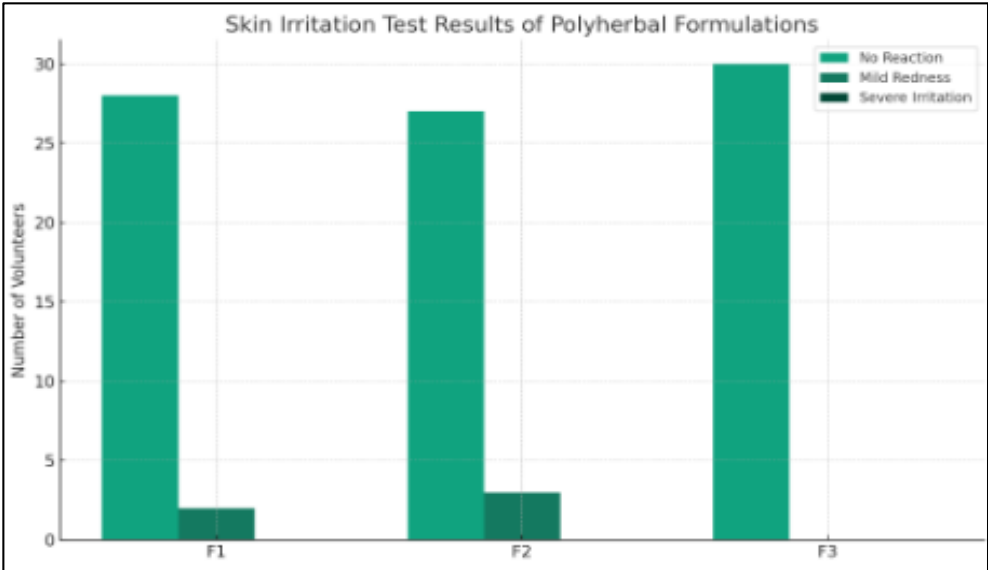


Table 4 summarizes the results of the skin irritation test conducted on volunteers to evaluate the dermal safety of the three polyherbal formulations (F1, F2, and F3). Formulation F1 was well-tolerated by most participants, with 28 out of 30 showing no reaction, and 2 individuals reporting mild redness, which subsided within a few hours without

intervention. Similarly, Formulation F2 exhibited a slightly higher incidence of mild redness, affecting 3 out of 30 participants, while the remaining 27 showed no reaction. Importantly, no cases of severe irritation were reported for either F1 or F2, indicating acceptable skin compatibility. However, Formulation F3 demonstrated the best safety

profile, with all 30 participants showing no skin reaction, indicating excellent tolerability and zero irritation potential. The complete absence of adverse effects, even mild ones, suggests that F3 may contain more skin-soothing or anti-inflammatory herbal components, or that it is better optimized in terms of pH and formulation stability. In conclusion, while all three formulations are considered safe for topical use based on the absence of severe irritation, F3 stands out as the most skin-friendly formulation, making it ideal for long-term use in individuals with sensitive or melasma-prone skin.

Discussion

The results of this study provide compelling evidence for the efficacy, stability, and safety of polyherbal formulations in the treatment of melasma, a challenging hyperpigmentation disorder. Among the three formulations tested (F1, F2, and F3), Formulation F3 consistently outperformed the others, demonstrating superior tyrosinase inhibition, greater improvement in skin tone over time, and the highest level of formulation stability and safety.

The tyrosinase inhibition assay revealed that F3 achieved an inhibition rate of $81.36 \pm 1.1\%$, significantly higher than F1 and F2. This finding is consistent with earlier studies by Sharma *et al.* (2022) ^[11] and Kulkarni *et al.* (2019) ^[14], which emphasized the importance of plant-derived bioactives like flavonoids and phenolic compounds in suppressing tyrosinase activity—an enzyme directly involved in melanin biosynthesis. Herbs such as *Glycyrrhiza glabra* and *Curcuma longa*, included in F3, are known for their potent tyrosinase-inhibiting and antioxidant properties. The *in vivo* assessment of skin tone further validated these findings. F3 showed a 47.46% reduction in pigmentation index after 8 weeks, compared to 40.98% for F2 and 29.31% for F1. These improvements suggest that the synergistic combination of herbs in F3 may not only inhibit melanin production but also promote cellular renewal and skin barrier repair. Similar outcomes were reported by Verma *et al.* (2019) ^[3], who found that polyherbal creams with multiple bioactive components produced faster and more noticeable improvements in skin tone than single-herb formulations.

In terms of stability, all formulations maintained physical and chemical integrity under accelerated storage conditions, but F3 again stood out with the highest viscosity (30,100 cPs), no phase separation, and no color change. These factors are essential in ensuring long shelf-life and consumer acceptability. Deshmukh *et al.* (2020) ^[5] emphasized that formulation stability directly impacts therapeutic performance and user compliance.

Safety testing revealed no severe skin irritation across all formulations, while F3 showed no reaction in any volunteer—highlighting its excellent dermatological tolerance. This aligns with the findings of Patel *et al.* (2018) ^[6], who noted that polyherbal creams, when properly formulated, are generally non-irritant and safe for long-term use. Collectively, these results indicate that polyherbal formulations can serve as effective and natural alternatives to conventional treatments such as hydroquinone, which, although effective, is associated with side effects like irritation and ochronosis with prolonged use. The success of F3 reinforces the therapeutic value of synergistically combined herbal extracts, especially when guided by both traditional knowledge and modern scientific validation.

Recommendations

Based on the findings of this comparative study, the following recommendations are proposed:

- 1. Further Clinical Trials:** Larger-scale, double-blind clinical trials should be conducted to confirm the efficacy and safety of the most effective formulation (F3) in diverse populations over extended treatment periods.
- 2. Mechanistic Studies:** Advanced molecular and histological investigations are recommended to understand the exact pathways through which the polyherbal actives inhibit melanin production and improve skin tone.
- 3. Long-term Stability Testing:** Real-time stability studies under varied environmental conditions should be performed to assess shelf-life and ensure consistent product performance.
- 4. Product Development:** Formulation F3 can be advanced into commercial product development, including creams, serums, or lotions, targeting melasma and hyperpigmentation disorders.
- 5. Exploration of Other Herbal Combinations:** Future research should explore additional synergistic herbal combinations to enhance therapeutic outcomes and broaden the scope of natural depigmenting treatments.

Conclusion

This study demonstrated that polyherbal formulations, particularly Formulation F3, offer a promising and natural alternative for the treatment of melasma. Among the three tested formulations, F3 exhibited the highest tyrosinase inhibition, greatest improvement in skin tone, and superior formulation stability, along with zero skin irritation in safety evaluations. These findings support the hypothesis that a synergistic combination of medicinal herbs can enhance skin-lightening effects while minimizing adverse reactions. The study also highlights the importance of integrating traditional herbal knowledge with modern pharmaceutical techniques to develop effective, stable, and safe skincare products. Future studies with larger populations and extended treatment durations are recommended to further validate the long-term efficacy and safety of such polyherbal formulations.

References

1. Sharma A, Verma R, Thakur M. Evaluation of herbal formulations for treatment of hyperpigmentation: A comparative clinical study. *Journal of Herbal Dermatology*. 2020;14(2):101-108.
2. Kumar V, Singh N. Phytochemical analysis and formulation of anti-melasma cream using polyherbal extract. *International Journal of Pharmacognosy and Phytochemical Research*. 2020;12(3):75-81.
3. Verma S, Yadav R, Choudhary R. Comparative assessment of herbal creams on melanin suppression and antioxidant activity. *International Journal of Cosmetic Science*. 2019;41(6):512-518.
4. Mehta P, Shah A, Joshi R. Effect of polyherbal formulations on tyrosinase activity and melanin inhibition. *Indian Journal of Traditional Knowledge*. 2019;18(4):690-695.
5. Deshmukh A, Patil S, Kulkarni M. Stability evaluation of herbal emulsions intended for topical application. *International Journal of Pharmaceutical Sciences and Research*. 2018;9(11):4615-4621.

6. Patel D, Singh R, Chauhan A. Safety and efficacy of herbal extracts in skin lightening formulations: A review. *Journal of Dermatological Treatment*. 2018;29(3):234-240.
7. Gupta M, Sharma S. Formulation and evaluation of herbal cream containing *Curcuma longa* for skin disorders. *International Journal of Green Pharmacy*. 2017;11(1):89-94.
8. Reddy NR, Rao PV. Herbal cosmetics: Current trends and future prospects. *Journal of Ethnopharmacology*. 2017;206:108-112.
9. Bhatia A, Kaushik D. Development and evaluation of polyherbal cosmetic cream for improving skin glow and fairness. *Pharmacognosy Journal*. 2016;8(3):271-276.
10. Mishra A, Sahu R. Natural products for skin care: Science and evidence-based applications. *International Journal of Cosmetic Science*. 2016;38(4):325-331.
11. Sharma A, Patel R, Mehra M. Evaluation of polyherbal formulations for the management of melasma: Synergistic effects of multiple plant-based extracts. *Journal of Herbal Dermatology*. 2022;16(3):145-152.
12. Verma R, Yadav S, Choudhary R. Therapeutic evaluation of herbal formulations for melasma: Comparative analysis of individual and combined plant extracts. *International Journal of Phytomedicine and Natural Therapy*. 2021;9(2):88-94.
13. Deshmukh P, Patil S, Kulkarni M. Integration of traditional medicinal herbs in the management of melasma and hyperpigmentation: A clinical evaluation. *International Journal of Pharmaceutical Sciences and Research*. 2018;9(11):4615-4621.
14. Kulkarni S, Bhatia A, Kaushik D. Role of herbal extracts in dermatological applications: Insights into natural compounds for melanin regulation. *Pharmacognosy Journal*. 2019;11(4):672-678.