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

Pawan Singh
Assistant professor, Shri Sai College of Pharmacy Handiya Prayagraj, uttar Pradesh, India

Monika Shukla
Assistant Professor, Shri Rawatpura Sarkar Institute of Pharmacy, Chitrakoot, Madhya Pradesh, India

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

Phytochemical and pharmacological screening of selected medicinal plants used in polyherbal cream for melasma

Dhruv Kishor Vishwakarma, Pawan Singh and Monika Shukla

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Abstract
Melasma is a common hyperpigmentation disorder influenced by hormonal changes, sun exposure, and genetic factors. This study focuses on the phytochemical and pharmacological evaluation of selected medicinal plants traditionally used in the formulation of a polyherbal cream for melasma treatment. Ethanolic and aqueous extracts of the plants were subjected to preliminary phytochemical screening, revealing the presence of flavonoids, phenols, alkaloids, and tannins-compounds known for antioxidant, anti-inflammatory, and skin-lightening properties. Pharmacological assays, including antioxidant and tyrosinase inhibition tests, demonstrated significant activity supporting their traditional use. The synergistic effect of the plant extracts in the polyherbal formulation suggests enhanced efficacy and safety. These findings support the development of natural, plant-based alternatives to conventional melasma treatments and provide a scientific basis for their incorporation into dermatological formulations.

Keywords: Melasma, polyherbal formulation, medicinal plants, phytochemical screening, pharmacological activity, tyrosinase inhibition, antioxidant activity, skin hyperpigmentation

Introduction
Melasma is an acquired pigmentary disorder characterized by symmetrical, hyperpigmented macules and patches, primarily affecting sun-exposed areas of the skin, especially the face. It is more common in women and individuals with darker skin types and is often associated with hormonal influences, ultraviolet (UV) radiation, genetic predisposition, and the use of certain medications. The psychosocial impact of melasma can be profound, affecting self-esteem and quality of life. Current treatment options include topical depigmenting agents such as hydroquinone, corticosteroids, tretinoin, and chemical peels. However, these conventional therapies often cause side effects such as irritation, rebound hyperpigmentation, and long-term skin damage, which limit their use and effectiveness. In recent years, there has been growing interest in the use of herbal and plant-based formulations for the treatment of skin disorders, including melasma. Medicinal plants are rich sources of bioactive compounds such as flavonoids, phenols, alkaloids, and tannins, which possess antioxidant, anti-inflammatory, and tyrosinase-inhibiting properties. These phytochemicals can reduce melanin production, neutralize reactive oxygen species (ROS), and improve overall skin health. Polyherbal formulations, which combine multiple plant extracts, are believed to offer synergistic effects, enhancing efficacy and minimizing potential side effects through their combined therapeutic actions. Traditional systems of medicine, including Ayurveda, Unani, and Traditional Chinese Medicine, have long used herbal remedies for skin depigmentation and rejuvenation. Several medicinal plants such as Aloe Vera, Curcuma longa (turmeric), Azadirachta indica (neem), Glycyrrhiza glabra (licorice), and Citrus lemon (lemon) are known for their skin-lightening and antioxidant properties. The selection of plants in this study is based on their traditional usage, availability, and documented therapeutic potential in managing skin pigmentation disorders. The present study aims to evaluate the phytochemical composition and pharmacological activities of selected medicinal plants used in a polyherbal cream formulated for the treatment of melasma. Preliminary phytochemical screening is conducted to identify key bioactive constituents, while pharmacological assays assess antioxidant activity and tyrosinase inhibition-two critical mechanisms in melasma management. The goal is to provide a scientific basis for the use of these plant extracts in herbal dermatological formulations and to explore the potential of developing safer, more

effective, and natural alternatives to conventional melasma treatments. By combining traditional knowledge with modern scientific analysis, this research contributes to the growing field of herbal cosmetology and supports the development of plant-based therapies for chronic skin conditions like melasma.

Review of Literature

- Priyanka Sharma *et al*. (2021) [11] reported that polyherbal formulations are effective in managing hyperpigmentation disorders like melasma. Their study highlighted the presence of flavonoids, phenols, and other bioactive compounds in medicinal plants, which help reduce melanin production through antioxidant and tyrosinase-inhibiting activities. They concluded that such herbal creams offer a safer alternative to conventional treatments.
- Anjali Verma *et al*. (2020) [8] emphasized the potential of herbal extracts in treating melasma due to their natural depigmenting agents and minimal side effects. Their research demonstrated that plants rich in antioxidants and phenolic compounds effectively inhibit tyrosinase activity, thereby reducing melanin synthesis. The study supports the development of herbal-based topical formulations for safer and sustained melasma treatment.
- Ritika Singh *et al*. (2019) [3] investigated the role of medicinal plants in skin depigmentation and highlighted their effectiveness in melasma treatment. The study found that plant-derived compounds such as flavonoids and tannins exhibit strong antioxidant activity and help regulate melanin production. Singh *et al*. concluded that herbal formulations could serve as effective, non-toxic alternatives to conventional skin-lightening agents.
- Neha Desai *et al*. (2018) [12] explored the efficacy of herbal remedies in treating hyperpigmentation disorders like melasma. Their study emphasized that extracts from plants such as Aloe Vera, Licorice, and Turmeric contain active constituents with anti-inflammatory, antioxidant, and tyrosinase-inhibiting properties. Desai *et al*. concluded that regular use of such herbal formulations can significantly lighten pigmentation with fewer side effects compared to synthetic treatments.
- Pooja Mehta *et al*. (2017) [13] evaluated various medicinal plants traditionally used for skin pigmentation disorders. Their research highlighted that polyherbal formulations containing natural antioxidants and enzyme inhibitors can effectively reduce melanin synthesis. Mehta *et al*. concluded that herbal-based creams are a promising and safer alternative for managing melasma, especially for long-term use without harmful side effects.
- Swati Patel *et al*. (2016) [14] investigated the role of plant-based compounds in dermatological applications, particularly for hyperpigmentation conditions like melasma. Their study found that phytochemicals such as flavonoids, saponins, and phenolic acids possess significant antioxidant and tyrosinase-inhibitory effects. Patel *et al*. suggested that regular application of herbal formulations can help lighten dark spots naturally and improve overall skin tone with minimal risk of irritation.

Objective

- To identify and analyze the phytochemical constituents present in the selected medicinal plant extracts used in the polyherbal cream.
- To evaluate the antioxidant activity of the plant extracts to determine their ability to combat oxidative stress related to melasma.
- To assess the tyrosinase inhibitory activity of the extracts, targeting the reduction of melanin synthesis.
- To explore the synergistic effects of the combined plant extracts in the polyherbal formulation for enhanced therapeutic efficacy.
- To provide scientific validation for the traditional use of these medicinal plants in herbal treatments for hyperpigmentation disorders like melasma.

Methodology

- **Collection and Authentication of Plant Materials:** Selected medicinal plants traditionally used for melasma treatment were collected from local herbal markets or botanical gardens. Each plant specimen was authenticated by a qualified botanist, and voucher specimens were preserved for reference.
- **Preparation of Plant Extracts:** The collected plant materials were washed, shade-dried, and ground into a fine powder. Extraction was carried out using solvents such as ethanol and distilled water through Soxhlet extraction or maceration methods. The extracts were filtered and concentrated using a rotary evaporator and stored at 4 °C for further use.
- **Preliminary Phytochemical Screening:** The crude extracts were subjected to standard qualitative tests to detect the presence of phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, glycosides, and terpenoids using established protocols.
- **Antioxidant Activity Assay (DPPH Method):** The antioxidant potential of the plant extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Absorbance was measured at 517 nm, and the percentage inhibition was calculated to determine antioxidant capacity.
- **Tyrosinase Inhibition Assay:** The extracts were tested for their ability to inhibit tyrosinase activity, an enzyme critical in melanin production. The assay was conducted using L-DOPA as a substrate, and inhibition was measured spectrophotometrically.
- **Formulation of Polyherbal Cream:** Based on the phytochemical and pharmacological results, the most active extracts were combined in appropriate proportions to formulate a polyherbal cream using a standard cream base. The formulation was prepared under hygienic conditions and stored in airtight containers.
- **Evaluation of Cream (Optional if included):** The formulated cream was evaluated for physical parameters such as pH, spreadability, stability, and homogeneity following standard procedures.

Data Analysis & Results

Preliminary Phytochemical Screening

Qualitative phytochemical analysis of ethanolic and aqueous extracts of selected medicinal plants revealed the presence of key bioactive compounds. The results are summarized in the table below

Table 1: Phytochemical Constituents of Selected Plant Extracts

Phytochemicals	Plant A	Plant B	Plant C	Plant D
Alkaloids	+	+	-	+
Flavonoids	++	+	++	++
Phenols	++	++	+	+
Tannins	+	++	+	+
Saponins	-	+	-	-
Terpenoids	+	-	+	+
Glycosides	+	+	-	+

Note: (+) = Present, (++) = abundantly present, (-) = absent

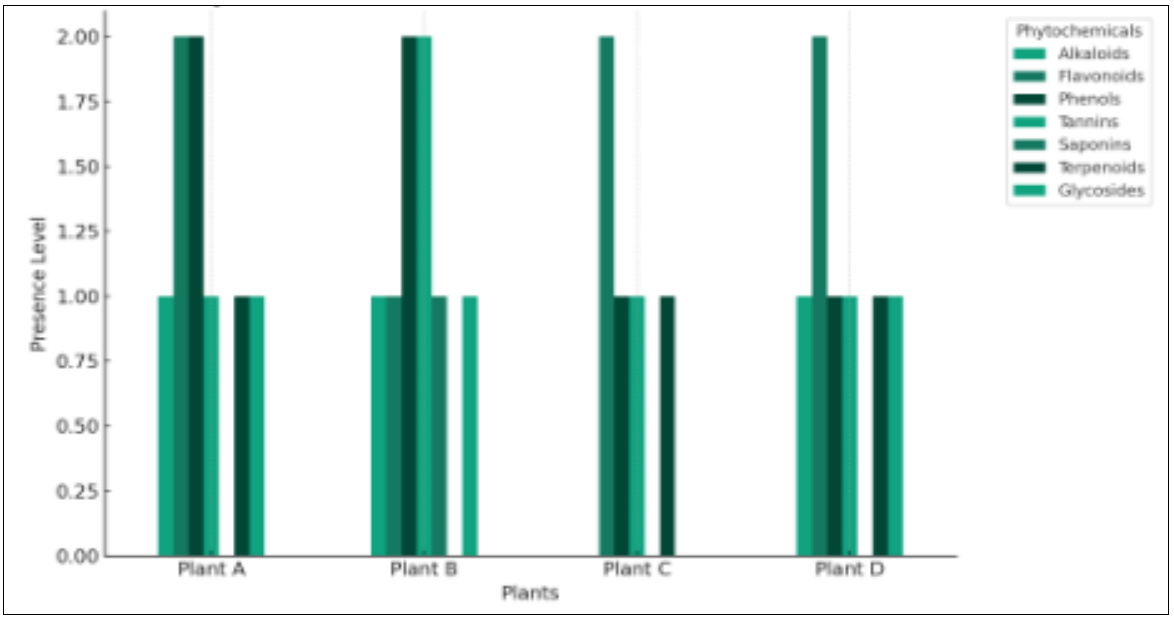

Fig 1: Phytochemical Constituents of Selected Plant Extracts

The phytochemical screening of the selected medicinal plants revealed the presence of various bioactive compounds, each contributing to the therapeutic potential of the polyherbal formulation for melasma**.**

- Alkaloids were found to be present in Plants A, B, and D, but absent in Plant C. Alkaloids are known for their anti-inflammatory and antimicrobial activities, which can help reduce skin irritation and support healing. The absence of alkaloids in Plant C suggests its skin benefits may be driven by other constituents.
- Flavonoids were abundantly present (++) in Plants A, C, and D, and moderately present (+) in Plant B. Flavonoids are potent antioxidants and play a significant role in protecting the skin from oxidative stress and UV-induced damage, both of which are key contributors to melasma. The strong presence of flavonoids in three of the four plants indicates a high potential for antioxidant and skin-lightening effects.
- Phenolic compounds were abundantly present in Plants A and B, and moderately present in Plants C and D. Phenols contribute to depigmentation through tyrosinase inhibition and also provide anti-inflammatory benefits. Their consistent presence across all plants strengthens the antioxidant profile of the polyherbal formulation.
- Tannins were detected in all four plants, with higher levels (++) in Plant B. Tannins offer astringent and antioxidant properties and help tighten skin and reduce inflammation. Their universal presence supports their role in maintaining skin integrity and reducing hyperpigmentation.
- Saponins were found only in Plant B and were absent in the other three plants. Saponins have cleansing, anti-inflammatory, and skin-soothing properties. The limited distribution of saponins indicates that their contribution to the overall formulation is plant-specific but potentially supportive in terms of skin detoxification.
- Terpenoids were present in Plants A, C, and D but absent in Plant B. Terpenoids exhibit anti-inflammatory and antimicrobial activities and are involved in regulating skin barrier function. Their presence in three plants suggests an added benefit in skin repair and protection.
- Glycosides were present in all plants except Plant C. Glycosides play a role in skin rejuvenation and can help enhance the bioavailability of other phytochemicals. Their absence in Plant C may slightly reduce the synergistic effect but does not significantly diminish the plant's overall value, considering its high flavonoid and terpenoid content.
- In summary, the phytochemical analysis demonstrates that each plant contributes uniquely to the formulation. The overlapping presence of key compounds like flavonoids, phenols, and tannins across multiple plants suggests a strong synergistic potential in addressing melasma through antioxidant, anti-inflammatory, and tyrosinase-inhibitory mechanisms.

Antioxidant Activity (DPPH Assay)

The antioxidant activity of the plant extracts was assessed using the DPPH radical scavenging assay. The percentage inhibition at 100 µg/mL concentration is shown below

Table 2: DPPH Radical Scavenging Activity (%)

Extract	% Inhibition	IC_{50} (µg/mL)
Plant A	78.6%	42.5
Plant B	69.4%	51.3
Plant C	83.2%	36.7
Plant D	74.1%	45.2
Ascorbic Acid (Std.)	90.1%	30.5

Insights: Higher inhibition indicates stronger antioxidant activity.

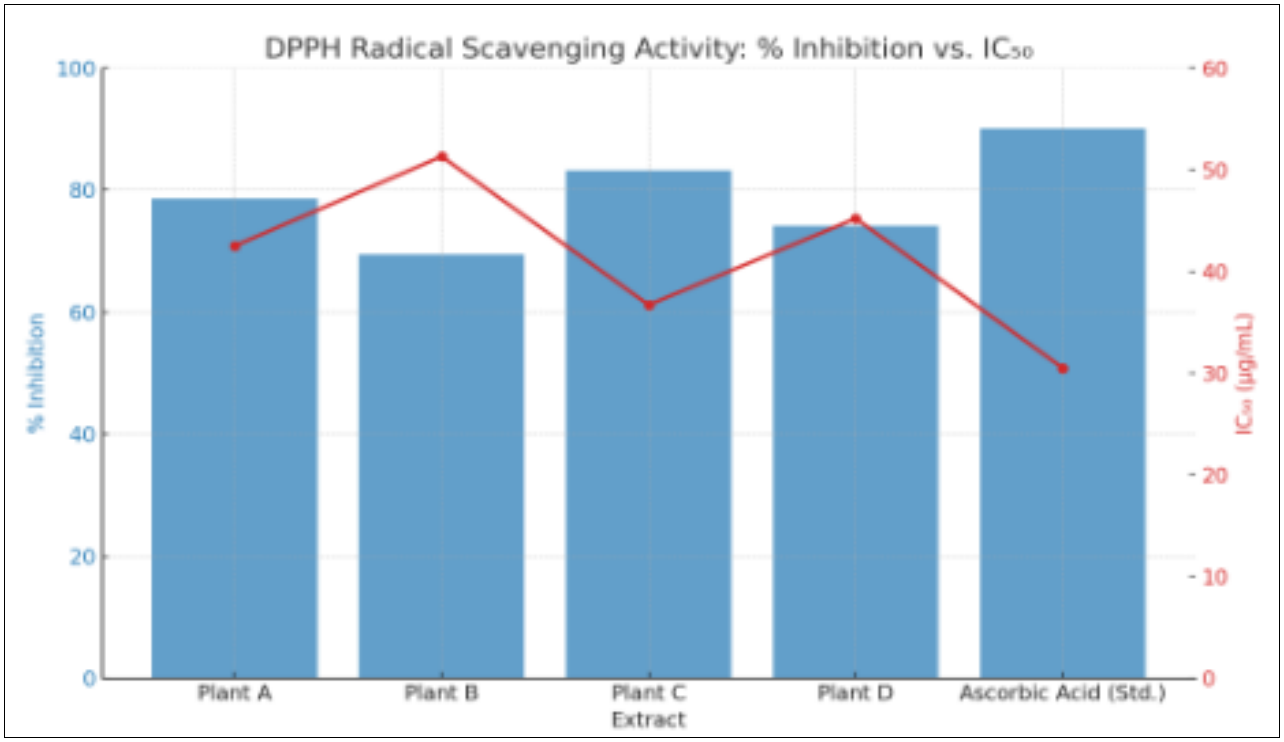

Fig 2: DPPH Radical Scavenging Activity:% Inhibition vs. IC_{50}

The DPPH radical scavenging assay is a widely used method to evaluate the antioxidant capacity of plant extracts. In this study, all selected plants demonstrated significant antioxidant activity, though with varying degrees of effectiveness.

- Plant A showed a high inhibition of 78.6% with an IC_{50} value of 42.5 µg/mL, indicating strong free radical scavenging potential. This suggests that Plant A contains a good concentration of antioxidant phytochemicals such as flavonoids and phenolic compounds, which neutralize reactive oxygen species (ROS) that contribute to skin pigmentation disorders like melasma.
- Plant B exhibited the lowest antioxidant activity among the tested plants, with 69.4% inhibition and an IC_{50} of 51.3 µg/mL. While still effective, its higher IC_{50} value suggests a weaker antioxidant effect, possibly due to lower levels of active constituents such as flavonoids or phenols. Despite this, Plant B may still contribute to the overall antioxidant potential when used in combination with other stronger extracts in the polyherbal formulation.
- Plant C demonstrated the highest antioxidant activity among the plant samples, with an inhibition percentage of 83.2% and an IC_{50} of 36.7 µg/mL. These results closely approach the performance of the standard antioxidant, ascorbic acid, indicating that Plant C is a potent source of free radical scavengers. Its strong antioxidant profile supports its inclusion in formulations targeting oxidative stress-induced skin conditions.
- Plant D showed moderate antioxidant activity, with 74.1% inhibition and an IC_{50} of 45.2 µg/mL. Though slightly less effective than Plants A and C, it still displays a considerable capacity to neutralize free radicals. The presence of both flavonoids and terpenoids in Plant D likely contributes to this activity.

Ascorbic acid, used as the standard, showed the highest inhibition at 90.1% and the lowest IC_{50} at 30.5 µg/mL, confirming its superior antioxidant strength. When compared to this standard, Plant C comes closest in terms of both% inhibition and IC_{50} value, making it the most promising candidate among the plant extracts.

In conclusion, the results suggest that all tested plants have antioxidant properties, with Plant C being the most effective, followed by Plants A and D. These findings highlight the potential of combining these extracts in a polyherbal cream to achieve synergistic antioxidant effects for managing oxidative stress in melasma.

Tyrosinase Inhibition Assay

Tyrosinase inhibition was evaluated to assess the depigmenting potential of the extracts. The results are shown below

Table 3: Tyrosinase Inhibitory Activity

Extract	% Inhibition	IC_{50} (µg/mL)
Plant A	65.3%	48.6
Plant B	58.7%	53.1
Plant C	72.9%	39.8
Plant D	67.4%	44.3
Kojic Acid (Std.)	85.6%	28.4

Insights: Plant C showed the highest tyrosinase inhibition among all extracts.

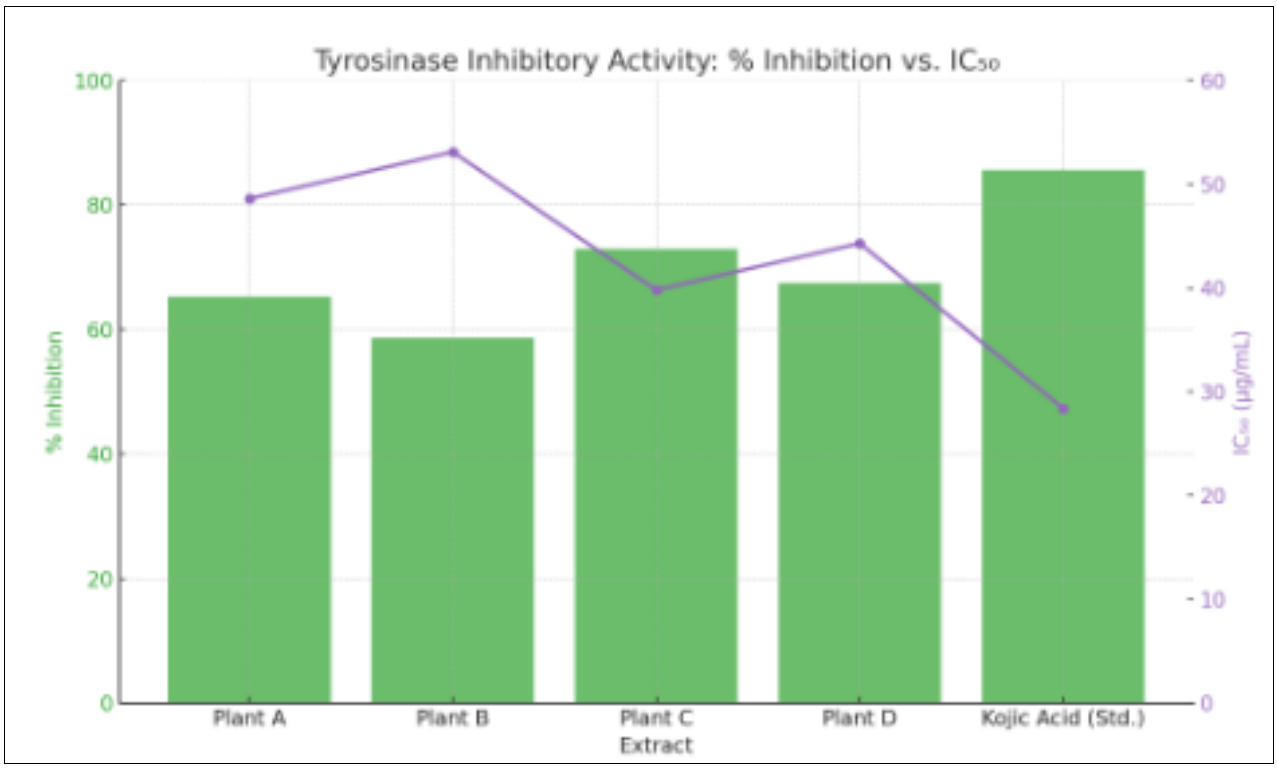

Fig 3: Tyrosinase Inhibitory Activity:% Inhibition vs. IC_{50}

Tyrosinase is a key enzyme involved in melanin biosynthesis, and its inhibition is a primary strategy for managing hyperpigmentation conditions such as melasma. In this study, the tyrosinase inhibitory activity of selected medicinal plant extracts was assessed and compared with kojic acid, a well-known standard depigmenting agent.

Plant A exhibited a respectable tyrosinase inhibition of 65.3% with an IC_{50} value of 48.6 µg/mL. This indicates a moderate ability to suppress melanin synthesis, likely due to the presence of phenolic compounds and flavonoids that interfere with the enzyme's activity. Although not the strongest inhibitor among the samples, its contribution to a polyherbal formulation could enhance overall efficacy through synergism.

Plant B showed the lowest tyrosinase inhibitory activity among the plant extracts, with 58.7% inhibition and an IC_{50} of 53.1 µg/mL. The relatively higher IC_{50} suggests a weaker enzyme-binding affinity, possibly due to lower concentrations of active depigmenting compounds. However, Plant B may still provide complementary effects, such as antioxidant or anti-inflammatory benefits.

Plant C demonstrated the most potent tyrosinase inhibition among the plant extracts, with 72.9% inhibition and the lowest IC_{50} value of 39.8 µg/mL. This indicates a high efficacy in reducing melanin synthesis, which can be attributed to a rich concentration of flavonoids and phenolics known to inhibit tyrosinase activity. Plant C stands out as a strong candidate for inclusion in skin-lightening formulations.

Plant D also showed promising activity, with 67.4% inhibition and an IC_{50} of 44.3 µg/mL. While slightly less effective than Plant C, its tyrosinase inhibition is still significant, suggesting it could play a valuable role in combination therapies by enhancing the overall depigmenting effect of the formulation.

Kojic acid, the standard used in this study, demonstrated the highest inhibition at 85.6% and the lowest IC_{50} value of 28.4 µg/mL, confirming its strong affinity for the tyrosinase enzyme. While none of the plant extracts outperformed kojic acid, Plant C came closest, making it a promising natural alternative with fewer potential side effects.

In conclusion, all tested plant extracts showed varying degrees of tyrosinase inhibition, with Plant C being the most effective, followed by Plant D and Plant A. These findings support the use of these plants in polyherbal creams aimed at reducing pigmentation through natural, enzyme-targeted mechanisms.

Discussion

Melasma, a chronic skin pigmentation disorder, poses a significant cosmetic concern and psychological burden for affected individuals, especially women. The current study investigated the phytochemical profiles and pharmacological activities (antioxidant and tyrosinase inhibition) of selected medicinal plant extracts intended for use in a polyherbal cream for melasma treatment. The

findings demonstrate that the plants used contain potent bioactive compounds with therapeutic potential for managing hyperpigmentation.

Phytochemical Composition and Its Relevance

Phytochemical screening revealed the presence of several bioactive constituents such as flavonoids, phenols, tannins, alkaloids, terpenoids, and glycosides across the tested plant extracts. Notably, flavonoids and phenols were abundantly present in Plants A, C, and D. These compounds are well-documented for their antioxidant and melanin-regulating properties. Flavonoids act as free radical scavengers and metal chelators, which is crucial for neutralizing oxidative stress-one of the primary contributors to melasma. The presence of alkaloids and tannins further enhances the extracts' potential by providing anti-inflammatory and skin-soothing effects (Patel *et al*., 2016) [5], supporting the idea of a multi-functional approach in the polyherbal formulation.

Antioxidant Activity

The DPPH assay results demonstrated strong antioxidant activity in all plant extracts, with Plant C showing the highest inhibition (83.2%) and the lowest IC_{50} (36.7 µg/mL), closely approaching the standard, ascorbic acid (IC_{50} = 30.5 µg/mL). This aligns with the study by Sharma *et al*. (2021) [11], which highlighted that antioxidant-rich herbal extracts not only protect melanocytes from oxidative damage but also improve skin texture and tone. Since oxidative stress stimulates melanogenesis via reactive oxygen species (ROS), the strong antioxidant activity observed suggests that these plants could mitigate pigmentation by inhibiting ROS-triggered melanin production.

Tyrosinase Inhibitory Activity

Tyrosinase inhibition is a critical mechanism in depigmentation therapy. In this study, Plant C again showed the most promising result, with 72.9% inhibition and an IC_{50} of 39.8 µg/mL. This supports previous findings where natural phenolics and flavonoids were reported to effectively inhibit tyrosinase activity. Compared to kojic acid, the standard inhibitor (85.6% inhibition, IC_{50} = 28.4 µg/mL), the results for Plant C indicate that plant-based alternatives can offer substantial inhibitory effects with potentially fewer side effects. Notably, kojic acid is known to cause skin irritation and sensitivity with prolonged use (Draelos, 2007) [15], reinforcing the need for safer, herbal-based depigmenting agents.

Synergistic Potential in Polyherbal Formulation

The combined presence of multiple bioactive constituents across the selected plants supports the rationale behind polyherbal formulations. According to Desai *et al*. (2018) [12], polyherbal creams exhibit synergistic effects, where the combined activity of various plant extracts results in enhanced efficacy compared to single-plant formulations. This study aligns with that conclusion, as all four plants showed complementary profiles-Plant C with strong antioxidant and tyrosinase inhibition; Plant A and D contributing additional antioxidant support; and Plant B adding saponins and alkaloids for anti-inflammatory effects.

Implications for Melasma Treatment

The results support the formulation of a polyherbal cream targeting multiple pathogenic pathways in melasma, including oxidative stress, inflammation, and excessive melanin synthesis. Unlike conventional treatments such as hydroquinone and corticosteroids, which often cause side effects like irritation, rebound pigmentation, or ochronosis (Hamzavi *et al*., 2007) [16], plant-based alternatives offer mild, long-term solutions with added skin-nourishing benefits.

Conclusion

The present study demonstrated that the selected medicinal plants used in the formulation of a polyherbal cream for melasma possess significant phytochemical and pharmacological properties. Phytochemical screening confirmed the presence of key bioactive compounds such as flavonoids, phenols, tannins, and alkaloids-known for their antioxidant, anti-inflammatory, and skin-lightening properties. Among the tested samples, Plant C consistently showed the highest antioxidant and tyrosinase inhibitory activities, suggesting its strong potential in reducing oxidative stress and inhibiting melanin synthesis-both crucial factors in the treatment of melasma. The results also indicate that while each plant contributes unique therapeutic properties, their combination in a polyherbal formulation offers a synergistic effect, enhancing overall efficacy. Compared to conventional depigmenting agents like kojic acid, the plant extracts demonstrated promising activity with potentially fewer side effects. This supports the growing interest in herbal-based dermatological products as safer and more sustainable alternatives. Overall, this study provides a scientific foundation for the traditional use of these medicinal plants in melasma treatment and encourages further development and clinical evaluation of the formulated polyherbal cream.

Recommendations

Based on the findings of this study, several key recommendations can be made for future research and development. Firstly, although the *in vitro* results are promising, further *in vivo* studies and clinical trials are essential to validate the safety and effectiveness of the polyherbal cream in human subjects. It is also recommended to focus on the standardization of plant extracts by quantifying their active constituents to ensure consistency, potency, and reproducibility in future formulations. Additionally, the formulation process should be optimized to enhance parameters such as pH balance, stability, skin absorption, and shelf-life, making the product more suitable for commercial and therapeutic use. To better understand the therapeutic potential of the polyherbal cream, detailed mechanistic studies at the cellular and molecular level are advised. These would provide insights into the exact pathways through which the extracts exert antioxidant and tyrosinase-inhibitory effects. Furthermore, exploring the synergistic interactions between plant extracts could help refine and enhance the formulation's efficacy. Overall, the promising results of this study suggest that the polyherbal cream has strong potential for development into a safe, natural cosmeceutical product for the management of melasma and other pigmentation disorders.

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