



# Efficacy of *Mimosa Pudica* Ethanol Extract Cream in Monobenzene-Induced Vitiligo in Rats

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**Abstract** | Background: Vitiligo is a skin disorder characterized by well-defined depigmented macules caused by the progressive loss of melanocytes. *Mimosa pudica* L. (sensitive plant) leaves are rich in flavonoids and phenolic compounds, both known for their high antioxidant activity. This is the first study to investigate *Mimosa pudica* ethanol extract cream for vitiligo treatment on reducing vitiligo lesion size and promoting skin repigmentation in a black rat model. Methods: A total of 24 black rats were induced with vitiligo using 40% monobenzene for 50 days. The rats were then divided into four equal groups, consist of six rats per group. One group served as the positive control and received no treatment, while the other three groups were treated with *Mimosa pudica* L. ethanol extract cream at concentrations of 50%, 75% and 90%. The extract cream was applied three times per week for 30 days. Depigmentation and repigmentation areas were measured in all groups on Day 0, 14 and 30. Results: All concentration of *Mimosa pudica* L. ethanol extract cream had a positive impact on the black rat model of vitiligo ( $p < 0.001$ ). Yet, the group treated with 50% *Mimosa pudica* L. ethanol extract cream showed the greatest repigmentation and the smallest vitiligo area compared to all other groups ( $p < 0.05$ ). Conclusion: The 50% ethanol extract cream from *Mimosa pudica* L. leaves is an effective alternative treatment for vitiligo.

**Keywords** | *Mimosa pudica* L., Vitiligo, Antioxidants, Repigmentation, Rats

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## INTRODUCTION

Vitiligo is a skin disorder characterized by the destruction of melanocytes, resulting in the formation of depigmented macules. The predisposing factors for this condition are multifactorial, with common triggers including trauma, sunburn, stress and systemic illnesses. Vitiligo

affects approximately 1% of the global population, with a higher incidence among individuals under the age of 20, though it can manifest later in life. The disease affects both males and females equally (Taieb and Picardo, 2009).

Several theories have been proposed to explain the loss of epidermal melanocytes in vitiligo. The leading hy-

hypotheses regarding its pathogenesis include autoimmune mechanisms, cytotoxic processes, biochemical factors, oxidant-antioxidant imbalance and neural mechanisms. The convergent theory further suggests that factors such as stress, the accumulation of toxic substances, autoimmunity, genetic mutations, changes in the cellular environment and impaired melanocyte migration significantly contribute to the disease. Studies also highlight a genetic component in the development of vitiligo (Halder and Taliaferro, 2008; Nicolaidou *et al.*, 2012).

Monobenzyl ether of hydroquinone is commonly used to induce depigmentation in areas of the skin containing melanocytes. Monobenzene is a phenolic compound that causes the destruction of epidermal melanocytes and is available in cream formulations at concentrations ranging from 20 to 40%. However, its use can lead to skin irritation and allergic reactions (Felsten *et al.*, 2011).

The use of sunscreen is commonly recommended as part of the management of vitiligo to prevent sunburn. Topical corticosteroids are also frequently used, particularly for localized lesions and often the first-line treatment for children. Combining topical corticosteroids with ultraviolet A (UVA) phototherapy has been shown to accelerate repigmentation, achieving results three times faster than topical monotherapy alone (Gupta *et al.*, 2007; Tolaymat and Sluzevich, 2010; Nicolaidou *et al.*, 2012).

Current treatments for vitiligo often fail to deliver fully satisfactory results, leading to increased interests in alternative therapies, including herbal medicines derived from medicinal plants. Herbal remedies, widely used in regions such as Africa, Asia and Latin America, are increasingly seen as a complementary approach to conventional healthcare. Compared to modern pharmaceuticals, herbal treatments offer notable advantages, including fewer side effects when used properly, the synergistic effects of multiple active compounds and the potential for a single plant to provide a variety of therapeutic benefits (Sumantri, 2018).

One of the plants that is particularly interesting to study is *Mimosa pudica* L. This plant has long been used by various traditional communities to treat skin disorders (Rajan *et al.*, 2014; Kyaw *et al.*, 2021). It is known that this plant possesses a broad range of pharmacological properties, including antioxidant activity (Muhammad *et al.*, 2016). Its application to the skin is likely linked to the bioactive compounds found in its leaves (Adurosakin *et al.*, 2023). Previous reports indicate that the leaves of *Mimosa pudica* L. contain compounds that can treat acute toxicity (Limsuwan *et al.*, 2009; Aziz *et al.*, 2014), potentially manage hyperglycemia (Wahjuni *et al.*, 2023) and exhibit hepatoprotective effects (Kowsalya and Sangeetha, 2020). However,

experiments regarding the *Mimosa pudica* L. leaves ability to treat vitiligo still need further exploration. Therefore, this study aims to investigate the potential benefits of an ethanol extract cream from *Mimosa pudica* in improving the severity and extent of vitiligo.

## MATERIALS AND METHODS

This study employed a quasi-experimental laboratory design using black rats as a model for vitiligo. This design enabled researchers to assess the treatment effects (intervention) by comparing the experimental group with a control group. Treatment was administered simultaneously to all experimental groups, utilizing a pre- and post-test randomized controlled group design. The primary variables examined in this study were the degree and extent of depigmentation.

### PREPARATION OF *MIMOSA PUDICA* LEAVES EXTRACT

The *Mimosa pudica* leaf extract was prepared using the maceration method with 80% ethanol. The procedure is outlined as follows: 10 parts of dried plant material (simplicia) were placed in a dark-colored container. To this, 75 parts of the solvent were added and the container was sealed and kept in a dark place for 5 days, with frequent stirring to ensure proper extraction. The mixture was then filtered and the residue was pressed and washed with sufficient solvent to obtain a total of 100 parts. The filtrate was transferred to a sealed vessel and stored in a cool, dark place for 2 days. The clear liquid was carefully decanted to avoid disturbing the sediment. Finally, the macerate was evaporated using a rotary evaporator at approximately 40°C until a thick extract was obtained.

To determine the yield of the extract, the final weight of the evaporated extract was recorded. The yield was calculated as a percentage of the initial dried plant material. The concentration of the extract was standardized by determining the total content of active compounds, either through spectrophotometric analysis or high-performance liquid chromatography (HPLC), based on the specific bioactive compounds of interest in the study. The results of these analyses provided the necessary information regarding the concentration and potency of the extract.

### APPLICATION TECHNIQUE

**EVALUATION OF *MIMOSA PUDICA* LEAVES CREAM:** The cream prepared from *Mimosa pudica* leaves was evaluated for various properties to ensure its suitability for skin application. These properties include texture, color and spreadability.

**pH TEST:** The pH of the cream was determined by diluting the cream with distilled water (aquadest) and measuring

the pH using a calibrated pH meter. The pH meter was calibrated with standard buffer solutions of pH 4.0 and 7.0 before use. pH measurements were conducted at room temperature ( $25 \pm 2^\circ\text{C}$ ). To ensure accuracy, the pH meter electrode was thoroughly cleaned with distilled water and dried with tissue paper before each measurement. The pH measurement results showed that the ethanol extract cream formulation of *Mimosa pudica* leaves had a pH of  $5.8 \pm 0.2$ , which falls within the ideal pH range for skin application (pH 4.5-6.0) (Wasitaatmadja 1997, 2010). The measurements were conducted in triplicate to ensure the consistency of the results.

**EFFECTIVENESS TEST OF MIMOSA PUDICA LEAVES CREAM:** The effectiveness of *Mimosa pudica* leaf cream was evaluated to assess its potential in reducing the spread of vitiligo-affected areas. The cream was applied to the affected regions of the skin.

**CREAM APPLICATION:** The cream was applied to the skin three times per week for a duration of one month. The application was conducted in the morning, between 8 a.m. and 10 a.m., to ensure optimal exposure during these hours.

#### MONOBENZONE-INDUCED VITILIGO IN RATS

A total of 24 black rats were selected and marked on their backs. Monobenzene was applied at a concentration of 0.1 mg/cm for 50 consecutive days to induce vitiligo. The rats were closely monitored throughout the process and were provided with fresh food and water daily. Following the 50-day monobenzene application period, randomization was carried out using Systematic Random Sampling. In this method, all rats were listed in numerical order, ensuring each animal had an equal chance of being selected. The rats were subsequently divided into four treatment groups, with six rats per group. Over the next 30 days, the rats were treated with *Mimosa pudica* leaf cream, which was applied to the vitiligo-affected areas according to their treatment groups.

#### MEASUREMENT OF REPIGMENTATION AREA

The measurement of depigmentation area and degree was conducted over a 30-day period, with observations made at three intervals: Day 0, Day 14 and Day 30. The extent of vitiligo was determined by tracing the depigmented area onto a transparent plastic sheet. The traced areas were then photographed on graph paper with a millimeter scale for accuracy. The area of vitiligo was subsequently measured using the ImageJ software to assess repigmentation progress.

ImageJ is a widely used software tool for displaying, quantifying and analyzing the area, number and intensity of objects in scientific studies. It provides numerical values that are then analyzed for further interpretation. In this study,

the Macbiophotonics version of ImageJ was employed to assess the area of the observed regions.

#### INCLUSION CRITERIA

The inclusion criteria for this study are as follows:

- Male black rats
- Aged between 2.5 and 3 months
- Weighing between 145 and 220 grams
- Healthy rats (active and free from any deformities)

#### EXCLUSION CRITERIA

The exclusion criteria for this study are:

- Inactive male black rats
- Male black rats that die during the study period

After obtaining a homogeneous sample based on the inclusion and exclusion criteria outlined above, the samples were randomly allocated into groups. The study adhered to proper animal handling procedures in accordance with the principles of the 3Rs (Reduction, Replacement, Refinement) and the 5F principles (Freedom from Hunger and Thirst, Freedom from Discomfort, Freedom from Pain, Injury, or Disease, Freedom to Express Normal Behavior and Freedom from Fear and Distress). Additionally, a dropout criterion was implemented: if a subject became ill or died, during the study, preventing completion of the 30-day observation period, it was excluded from the study (Agarwal *et al.*, 2014; Sosa *et al.*, 2015).

#### SAMPLE SIZE

The sample size was determined using Federer's formula, as recommended by previous researcher (Ihwah *et al.*, 2018), that calculated as follows:  $(n - 1)(t - 1) \geq 15$ , where:  $n$  = the number of samples per group and  $t$  = the number of treatment groups (in this study,  $t = 4$ ). By substituting the values, the minimum sample size is six rats, resulting in a total sample size of 24 for four groups.

The groups rats were then divided assigned to groups as follows:

**POSITIVE CONTROL GROUP:** Received monobenzene only, to induce depigmentation (refer to Zhu *et al.*, 2013).






**TREATMENT GROUP 1 (P1):** Treated with *Mimosa pudica* leaves ethanol extract cream at a concentration of 50% for 30 days (Nasihah and Susila, 2019).

**TREATMENT GROUP 2 (P2):** Treated with *Mimosa pudica* leaves ethanol extract cream at a concentration of 75% for 30 days.

**TREATMENT GROUP 3 (P3):** Treated with *Mimosa pudica* leaves ethanol extract cream at a concentration of 90% for



30 days. The timeline and grouping of the experiment are outlined below:

Step 1: Induction of Vitiligo	Step 2: Groups Allocation	Step 3: Follow-up and Measurement
 male black rats n=24 40% monobenzene  50 days	 Control: no treatment (n = 6)	Observation Days: Day 0 Day 14 Day 30  Measurements: Depigmentation and repigmentation areas
	 P1: treatment 50% (n = 6)	
	 P2: treatment 75% (n = 6)	
	 P3: treatment 90% (n = 6)	
	30 days	

### CONTROL OF ANIMAL SUBJECTS (RATS)

To maintain control over the rat strain, this study used *Rattus norvegicus* of the same strain, all sourced from a certified breeding facility. Prior to the study, all rats underwent a health check and a 7-day acclimatization period. The rats were housed individually in standard-sized cages (30 x 20 x 12 cm), with bedding replaced every 2 days and cleaned regularly. The rats had ad libitum access to water and food and were fed according to a consistent schedule throughout the study.

### EXPERIMENTAL ENVIRONMENT

The experiment was conducted in a room maintained at a constant temperature of 22±2°C and a relative humidity of 55±10%. The lighting cycle was set to 12 hours of light followed by 12 hours of darkness. Ventilation was ensured with 15 to 20 air exchanges per hour.

### STATISTICAL TEST

The outcomes measured were repigmentation and the vitiligo area. Both variables were assessed for normality and homogeneity. To compare the percentage of repigmentation and vitiligo area between groups, one-way ANOVA was used followed by Tukey HSD post-hoc test, as the data were normally distributed. All statistical tests were performed at an alpha level of 0.05 or a 95% confidence level.

## RESULTS AND DISCUSSION

Following the application of *Mimosa pudica* L leaf extract cream, statistical testing showed that the data for both the repigmentation indicator and vitiligo. Table 1 presents the normality test results for both repigmentation and vitiligo area, establishing a 5% error margin or a 95% confidence

level. The p-values for all groups in both repigmentation and vitiligo area were found to be greater than 0.05, indicating that the data for all groups follow a normal distribution. This suggests that the assumption of normality is met for both variables. Table 2 presents the homogeneity test results for both repigmentation and vitiligo area, establishing a 5% error margin or a 95% confidence level (Table 2). The F-values for repigmentation (F = 9.84) and vitiligo Area (F = 10.23) are both significant with p-values less than 0.001. This indicates that the variances between the groups are not homogeneous. Thus, the assumption of homogeneity of variances is violated for both variables in the study.

Table 1: Normality test of repigmentation and vitiligo area.

Group	n	p	Description	n	P	Conclusion
			Repigmentation	Vitiligo Area		
Control	6	0.089	Normal	10	0.092	Normal
P1	6	0.542	Normal	10	0.534	Normal
P2	6	0.678	Normal	10	0.645	Normal
P3	6	0.723	Normal	10	0.712	Normal

Table 2: Homogeneity test of repigmentation and vitiligo area.

Variables	F	P	Conclusion
Repigmentation	9.84	<0.001	Not homogenous
Vitiligo Area	10.23	<0.001	Not homogenous

### REPIGMENTATION

Significant differences in the percentage of repigmentation were observed across the control, P1, P2 and P3 (Table 3).

Table 3: Comparison test of repigmentation.

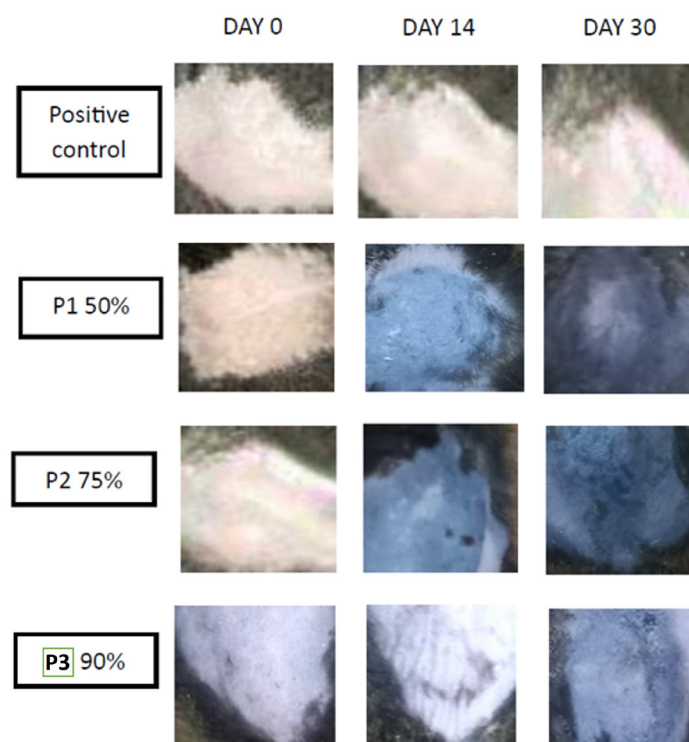
Subject Group	n	Mean Repigmentation	Std. Deviation	F	p
Control	6	7.25	2.21	75.32	<0.001
P1	6	59.33	41.76		
P2	6	47.40	36.39		
P3	6	39.40	41.72		

Table 3 presents the comparison test results for repigmentation. The analysis shows that the mean repigmentation values for the groups are significantly different from each other. The control group had a mean of 7.25 (SD = 2.21), while the experimental groups showed much higher means: P1 (mean = 59.33, SD = 41.76), P2 (mean = 47.40, SD = 36.39) and P3 (mean = 39.40, SD = 41.72). The F value of 75.32 with a p value of less than 0.001 indicates that there are significant differences between the groups. This suggests that the treatment groups (P1, P2 and P3) showed a much greater degree of repigmentation compared to the control group, highlighting the effectiveness of the treatments in promoting repigmentation. Group P1, treat-

ed with 50% *Mimosa pudica* ethanol extract cream, demonstrated the highest repigmentation percentage at  $59.33 \pm 41.76\%$ , while the control group, treated showed the lowest repigmentation. Post Hoc analysis (Table 4) further clarified that all treatment groups had significantly higher repigmentation compared to the control, with the largest mean difference of -52.08 observed between the control and P1. In addition, P1 showed significantly better repigmentation than P2 and P3, with mean difference of 11.93 and 19.93, respectively. Again, this suggests that a 50% concentration in P1 was more effective than the higher concentrations used in P2 and P3.

**Table 4: Post hoc test of repigmentation.**

Groups	Mean Difference	P	Conclusion
Control and P1	-52.08	<0.001	Significantly different
Control and P2	-40.15	<0.001	Significantly different
Control and P3	-32.15	<0.001	Significantly different
P1 and P2	11.93	<0.001	Significantly different
P1 and P3	19.93	<0.001	Significantly different
P2 and P3	8.00	<0.001	Significantly different



**Figure 1: Depiction of the extent of depigmentation and repigmentation areas.**

This repigmentation can be observed visually as shown in Figure 1. In Figure 1, it was observed that in the positive control group, which only received monobenzone, exhibited progressively whiter skin each week. In contrast, the rats treated with *Mimosa pudica* ethanol extract cream displayed various reactions, including mild redness, dry skin and raised skin texture. Notably, in treatment groups 1 (P1)

and 2 (P2), signs of repigmentation were evident, with progressively darkening areas of pigmentation (tanning). The application of *Mimosa pudica* ethanol extract cream at a 50% concentration produced the most significant repigmentation effect, as indicated by noticeable color changes in rats' skin from day 0 to day 30.

**Table 5: Comparison test of vitiligo area.**

Subject Group	n	Mean Vitiligo Area	Std. Deviation	F	p
Control	6	93.75	2.63	82.45	<0.001
P1	6	40.67	41.76		
P2	6	54.60	38.16		
P3	6	73.67	37.29		

**VITILIGO AREA**

The average vitiligo area score was evaluated through morphological observations and statistical analysis among the control and treatment groups. As shown in Table 5, the control group had the highest mean vitiligo area (93.75), while P1, treated with 50% *Mimosa pudica* ethanol extract, had the lowest (40.67). Groups P2 and P3 had mean vitiligo areas of 54.60 and 73.67, respectively. The F value of 82.45 with a p value of less than 0.001 indicates significant differences between the groups. This suggests that the treatment groups (P1, P2 and P3) were effective in reducing the vitiligo area compared to the control group ( $p < 0.001$ ).

Table 6 presents the Post Hoc test results, showing significant differences in vitiligo area between all groups ( $p < 0.001$ ). Notably, P1 demonstrated the greatest reduction in vitiligo area compared to P2 and P3, suggesting its superior effectiveness in minimizing depigmentation.

**Table 6: Post hoc test of vitiligo area.**

Groups	Mean Difference	P	Conclusion
Control and P1	53.08	<0.001	Significantly different
Control and P2	39.15	<0.001	Significantly different
Control and P3	20.08	<0.001	Significantly different
P1 and P2	-13.93	<0.001	Significantly different
P1 and P3	-33.00	<0.001	Significantly different
P2 and P3	-19.07	<0.001	Significantly different

The table and figure above illustrate the significant potential of *Mimosa pudica* L. leaves in treating vitiligo. The natural compounds found in *Mimosa pudica* leaves show great promise for the treatment of vitiligo due to their ability to promote natural melanin production, as evidenced by a previous study (Fadrijanto et al., 2023). Key activities of these natural compounds include their potential to scavenge free radicals, activate melanogenesis-associated pathways, increase the expression of tyrosinase genes, reduce the

expression of inflammatory chemokines and cytokines and prevent the migration of CD8<sup>+</sup> T cells. These mechanisms are crucial for mitigating the autoimmune and inflammatory processes involved in vitiligo, making *Mimosa pudica* an attractive candidate for further investigation in vitiligo therapy (Pang *et al.*, 2021). Phytochemical testing of *Mimosa pudica* leaves extract, as reported by Thoa *et al.* (2015), revealed the presence of flavonoids in the plant. Additionally, Lu *et al.* (2006) noted that gallic acid (GA), a natural phenolic antioxidant compound extracted from plants, is widely used in food, medicine and cosmetics.

The ethanol extract cream from *Mimosa pudica* leaves, applied to black rats in a monobenzone-induced vitiligo model, demonstrated an anti-depigmentation effect. *Mimosa pudica* exhibits high antioxidant activity, which can be attributed to its flavonoid and phenol content (Azmi *et al.*, 2011). Plant-derived phenolic compounds have the potential to act as antioxidants and antibacterials. These compounds, which typically include tocopherols, flavonoids, cinnamic acid derivatives and coumarins, are naturally present in plants and contribute antioxidant effects that by inhibiting polyphenol oxidase (PPO) activity (Janah *et al.*, 2018).

Vitexin (apigenin-8-C- $\beta$ -D-glucopyranoside) is a natural flavonoid found in various medicinal plants, including such as *Crataegus* L., *Vigna* Savi, *Passiflora cristalina* Vanderpl and Zappi and *Mimosa pudica* L. (He *et al.*, 2016). Vitexin exhibits numerous a range of pharmacological activities, including such as anti-inflammatory, antiviral, anticancer and antihypertensive properties (Chen *et al.*, 2021; Ding *et al.*, 2021). Additionally, vitexin is known for its antioxidant properties (Ozarowski and Karpiński, 2021). In a study using the immortalized human melanocyte cell line (PIG1) with melanocytes induced by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), vitexin was found to inhibit hydrogen peroxide-induced apoptosis and promote cell proliferation by activating the MAPK-Nrf2/ARE pathway. This effect included a reduction in the expression of IL-1 $\beta$ , IL-17A, Bax, caspase-3 and reactive oxygen species (ROS), while upregulating the expression of p53, Bcl-2, Nrf2, HO-1, NQO-1 and SOD, which are protein associated with cell survival and antioxidant defense mechanisms (Li *et al.*, 2020).

Table 3 supports the findings in Figure 1, showing that treatment with *Mimosa pudica* L. positively influences the repigmentation process in the skin of rats previously treated with monobenzone. The significant difference observed compared to the positive control group suggests that the compounds in *Mimosa pudica* leaves may offer a promising alternative for promoting repigmentation in vitiligo.

Flavonoids derived from *Mimosa pudica* ethanol extract are potent exogenous antioxidants. These compounds are

widely used in cosmetics and typically consist of a mixture containing lipophilic aglycones and glycosides. Such chemical structures enhance their antioxidant properties, allowing them to scavenge almost all types of free radicals. Flavonoids have a high affinity for singlet oxygen and reduce tocopheryl and tocotrienol anion radicals. Additionally, flavonoids inhibit various factors that contribute to the formation of reactive oxygen species (ROS), thereby preventing skin aging.

Quercetin, a member of the flavonol subclass of flavonoids, has demonstrated positive effects both in vivo and in vitro in the treatment of pigmentation disorders. Research shows that quercetin has the potential to protect melanocytes and keratinocytes from oxidative stress. Furthermore, the topical application of quercetin can prevent cellular damage caused by ultraviolet radiation. H<sub>2</sub>O<sub>2</sub>, as one of the ROS, can induce endoplasmic reticulum enlargement and inhibit the production of functional tyrosinase in melanocytes, contributing to the pathogenesis of vitiligo. Quercetin can mitigate oxidative reactions mediated by H<sub>2</sub>O<sub>2</sub>, ultimately reducing the incidence of vitiligo (Gianfaldoni *et al.*, 2018; Luo *et al.*, 2020).

The findings of this study suggest that the composition of the cream could be optimized. The extent of vitiligo in the P1 treatment group was found to be lower compared to the other groups (P2 and P3) (Table 5). The hydroxyl group attached to the phenyl ring plays a crucial role in stimulating melanogenesis. Acting as a hydrogen donor, the hydroxyl group neutralizes free radicals, thereby reducing oxidative stress, which is a key factor in the pathogenesis of vitiligo (Zeb, 2021). Additionally, this phenyl ring is believed to interact with tyrosinase, a key enzyme in the melanin synthesis process, enhancing its activity and promoting melanin production (Masum *et al.*, 2019; Ali Al-Mamary and Moussa, 2021). The presence of the hydroxyl group on the phenyl ring also improves the stability of flavonoid molecules, facilitating better penetration into the skin layers and providing a more optimal effect at lower concentrations (50%) compared to higher concentrations. Therefore, *Mimosa pudica* ethanol extract cream at a 50% concentration shows more effective repigmentation and reduces the extent of vitiligo more effectively than higher concentrations.

## CONCLUSION AND RECOMMENDATION

Our findings suggest that *Mimosa pudica* L. ethanol extract cream at a 50% concentration holds promising potential in reducing vitiligo lesion area and promoting repigmentation in the experimental model used. The results indicate that the plant-based cream could serve as a potential therapeutic option for managing vitiligo, a condition that significantly affects the quality of life of those affected. This study



provides important insights into the efficacy of *Mimosa pudica* in addressing vitiligo lesions, offering a new avenue for plant-based treatments in dermatology.

However, while the current findings are promising, further research is necessary to fully evaluate the therapeutic potential of *Mimosa pudica* as a treatment for vitiligo in humans. Future studies should not only replicate these results in clinical trials but also explore the long-term effects of *Mimosa pudica* cream. Investigating its safety, efficacy and potential side effects over extended periods of use will be essential in determining its viability as a sustainable treatment option. Moreover, clinical trials involving a larger, more diverse human population will help assess its broader applicability and effectiveness in different demographic groups.

In addition to evaluating the cream's clinical application, future research should focus on optimizing plant cultivation methods to ensure consistent quality control. Variability in the composition of plant extracts could affect their efficacy, so establishing standardized cultivation and extraction protocols will be crucial for the reproducibility and reliability of future studies. Overall, this study lays a solid foundation for future investigations and has significant implications for the development of natural, plant-based treatments for vitiligo and other skin disorders.

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## NOVELTY STATEMENT

The effects of ethanol extract cream from *Mimosa pudica* leaves on depigmentation and repigmentation in a monobenzene-induced vitiligo model in rats have not been extensively explored in published research. While there have been studies on the potential of plant extracts for treating skin conditions, the specific use of *Mimosa pudica* in this context, particularly in a monobenzene-induced vitiligo model, remains novel. This study is unique in its approach, offering new insights into the therapeutic potential of *Mimosa pudica* for vitiligo, a condition that severely affects individuals' quality of life. The findings present a promising direction for future therapeutic interventions and contribute to the growing body of knowledge in plant-based treatments for skin disorders.

## AUTHOR'S CONTRIBUTIONS

Defi: Conceptualization, Data Curation, Formal Analysis,

Funding Acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, review and editing and Project Administration.

Endy Juli Anto: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, review and editing and Project Administration.

Jekson M Siagaan: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, review and editing and Project Administration.

Hadyanto Lim: Formal Analysis, Investigation, Resources, Supervision, Validation, Writing - original draft, review and editing.

Fotarisman Zaluchu: Conceptualization, Data Curation, Formal Analysis, Methodology, Supervision, Writing - original draft, review and editing.

## LIMITATIONS

As this study was conducted in a laboratory setting, one limitation is the relatively small sample size, which may affect the generalizability of the findings. Future research should aim to include a larger sample to enhance the external validity of the results. Additionally, the study did not employ blinding in the treatment application, which could introduce bias in the assessment of treatment outcomes. Another limitation is the potential variability in the *Mimosa pudica* extract, as differences in plant cultivation methods and extraction processes could lead to inconsistent concentrations of active compounds. To address this, it is important to standardize the cultivation of *Mimosa pudica* leaves, ensuring they are grown under consistent conditions and to establish standardized extraction protocols. Furthermore, individual variation in response to monobenzene, despite efforts to control for it, could affect topical absorption rates among subjects, contributing to variability in the results. Addressing these limitations in future studies would help to improve the robustness and applicability of the findings.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in this study. The authors also declare that this study was not conducted using any funds or financial support from any individual or organization.

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