Scope
Volume 14 Number 04 December 2024

Analysing the Anti-Tyrosinase Activity of Mung Bean Extract in Zebrafish Embryos- An Invitro Study

[1]Dr K. S. Janani Priya, [2]Dr R. Renuka Devi, [3]Dr H. Esther Nalini, [4]Dr G. Kokila, [5]Dr S. Tamil Selvi, [6]Dr C. Nitya Kala

[1]Postgraduate,[2]HOD &Professor,[3]Professor,[4]Professor, [5]Professor, [6]Reader

[1-6]Department of Periodontology,KSR Institute of Dental Science and Research, Tiruchengode, Tamil Nadu, India.

Corresponding Author: Dr R. Renuka Devi

Abstract

Background: Tyrosinase is a key enzyme in the melanin biosynthesis pathway, and its overactivity can lead to hyperpigmentation disorders. Natural compounds with anti-tyrosinase activity are of great interest in developing safe and effective treatments for these conditions. Mung bean (Vigna radiata) has been reported to possess various bioactive compounds with potential health benefits, including anti-tyrosinase activity. This study aims to evaluate the anti-tyrosinase activity of mung bean extract using zebrafish embryos as an in vitro model. **Methods:** Mung bean extract was prepared by the hot extraction method, and inoculated on zebrafish embryos in 12 well microtiter plates. The embryos were assessed at various developmental stages for changes in pigmentation on the surface of zebrafish embryos using visual observation and imaging techniques. The extract was tested at 1%, 5% and 10% concentrations. A control group using vitamin C was included to evaluate the efficacy of mung bean extract. **Results:** The results have shown reduced pigmentation in zebrafish embryos in various concentrations of mung bean (1%, 5%,10%), where significant results were obtained for 5%, and 10% of mung bean which was equivalent to vitamin C. **Conclusion:** Mung bean extract exhibits promising anti-tyrosinase activity, and reduced melanin pigmentation in zebrafish embryos. These findings suggest that mung bean extract could be a potential natural alternative for managing hyperpigmentation of gingiva.

Keywords: Mung bean, Tyrosinase activity, Zebrafish embryos, Melanin, Hyperpigmentation.

Introduction

A captivating grin is frequently seen as a representation of well-being, attractiveness, and self-assurance. The gingiva's appearance and elements such as tooth colour, alignment, and form greatly influence an individual's smile. The appearance of black patches or flecks on the gums, known as gingival pigmentation, can have a negative effect on the aesthetics of smiles and self-consciousness. Melanin is responsible for skin colour and oral mucosa, and it is a non-haemoglobin pigment.[4] Melanogenesis is the process through which melanocytes, dendritic cells of neuroectodermal origin located in the basal and pilose layers of the gingival epithelium, produce melanin.Tyrosinase, a multipurpose enzyme containing copper and also known as polyphenolic oxidase, plays a crucial role in melanogenesis.[2] Melanin deposition is the most prevalent cause of gingival pigmentation, however, foreign factors including dental materials and other pigments like hemosiderin can also induce discolouration.[1] Several variables, including genetic predisposition, ethnic background, hormone fluctuations, smoking, and certain drugs like oral contraceptives, minocycline, clozapine, chlorpromazine, cyclophosphamide, and chloroquine regulate the multifaceted phenomena of gingival pigmentation. There is no denying the aesthetic impact of gingival pigmentation, even though it is usually innocuous and does not provide a health concern. Many people want to know how to get rid of or minimize gingival pigmentation so they may have a more uniform and radiant smile. As a result, several treatment modalities have been established, ranging from non-invasive methods including topical medications such as chemical depigmenting agents like vitamin C and surgical methods like gingivectomy and gingival depigmentation procedures including scalpel scraping, bur abrasion, electrosurgery, cryotherapy and lasers.[1]

In recent years, there has been a growing interest in natural medicines and botanical agents because of their perceived safety, effectiveness, and lack of adverse effects compared to conventional treatments. Plant-based phytochemicals have demonstrated promise as depigmenting agents through the inhibition of melanin synthesis or by increasing the rate of melanin breakdown. This is a viable option for those looking for natural and non-invasive ways to improve the appearance of their smiles.

The mung bean (Vigna radiata L.), one of the most important edible legume crops, is grown on over 6 million hectares worldwide (about 8.5% of the world's pulse area) and consumed by most Asian households.[3]The mung bean extract have demonstrated good health implications thus far, including anti-melanogenesis, antihypertensive, anticancer and immunomodulatory properties as well as hypoglycemic and hypolipidemic effects.[4]

To assess anti-tyrosinase processes, researchers have widely used a variety of in vivo models. But while some of these models provide difficulties from a physiological or clinical perspective, others have practical limits. Consequently, scientists have

resorted to emerging models like as zebrafish (Danio rerio). This model has many benefits, such as its tiny size, ease of care and handling, high medication penetration effectiveness through the skin and gills, and quick reproduction rate. Furthermore, the genome of zebrafish has been well characterised, and the functional domains of several important proteins are almost exactly the same as those of humans.[8] Notably, because the pigmentation process can be seen on the surface of zebrafish embryos without the need for intricate experimental protocols, zebrafish analysis has been linked to the presence or lack of melanin.

This study seeks to explore the most recent scientific findings on melanogenesis inhibitors found in Mung bean, utilizing zebrafish as an experimental model. A key aspect of the study involves elucidating the zebrafish depigmentation system and assessing its similarity to the human system.

Materials & methods

Preparation of extract from Mung bean

The commercially available edible mung bean seeds were sun-dried for 2 days and powdered (Figure 1a,b). The prepared powder weighed 0.5mg, 2.5mg, and 5mg quantity was added to a clean, dry blender jar and 50mL distilled water was poured into the blender. The ingredients were mixed vigorously until a homogenous slurry was achieved. Later, the slurry was transferred into a glass beaker and heated in a heating mantle to a temperature of 60-80°C (Figure 1c). The temperature was maintained for 1-2 hours with occasional stirring and allowed to cool at room temperature. Using filter paper the extract was filtered (Figure 1d). The collected filtrate was stored in a sealed container at a refrigeration temperature (4°C). Vitamin C was also obtained commercially mixed with distilled water and stored.

Figure 1: a) Sun-dried mung bean; b)Powdered mung bean; c) extract in heating mantle; d)filtration.

a) b) c) d)

Anti-tyrosinase activity

A 96-well microtiter plate containing L-DOPA as the substrate was used to measure the tyrosinase inhibitory activities using a slightly modified Zengin et al. technique. The enzyme solution (40 µL), extract (1%, 5%, and 10%), and 100 µL of phosphate buffer (20 mmol/L, pH 6.8) were combined and preincubated at 37 °C for 15 minutes

prior to the addition of the substrate, which started the reaction. Following preincubation, 40 µL of L-DOPA was added, and the mixture was then incubated for 10 minutes at 37 °C. At 492 nm, absorbance was measured with a UV-visible spectrophotometer. The tyrosinase inhibitory was computed and represented as a percentage.

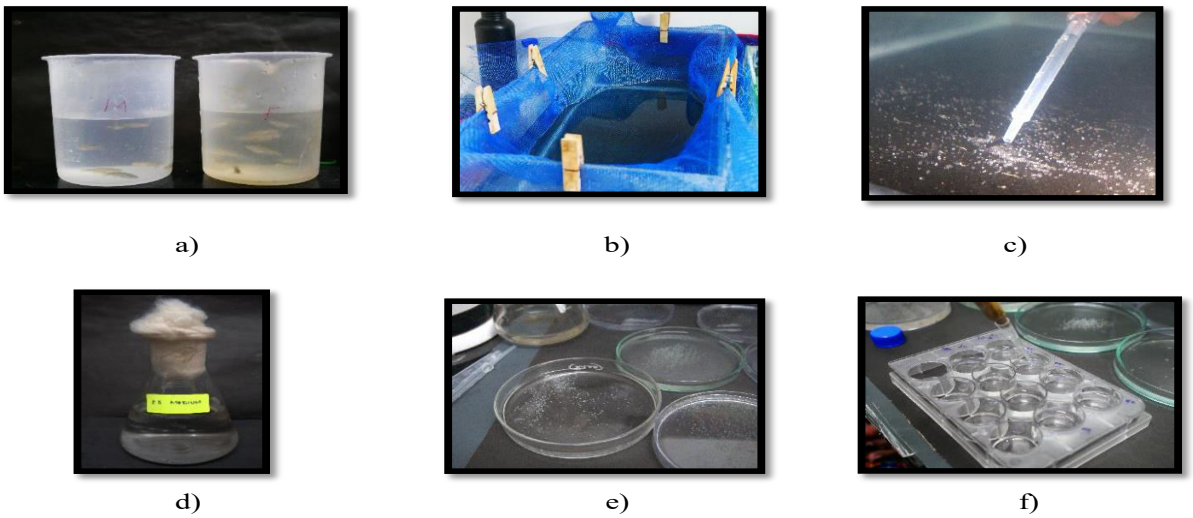
$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Absorbance of Control}}{\text{Absorbance of sample}}\right) \times 100$$

Experiment on Zebrafish

In a breeding tank, a polypropylene mesh was placed with 10 male and 15 female zebrafishes which were kept under laboratory conditions and allowed to reproduce (Figure 2a,b). The purpose of the mesh was to allow the eggs to escape through the holes and settle at the bottom of the tank. After 24 hours of fertilization, zebrafish embryos were collected and transferred to Petri dishes with E3 medium (Figure 2c,d). E3 (Embryonic) medium consists of sodium chloride, potassium chloride, magnesium chloride, and calcium chloride as this allows the permeability of the plant extract into the embryos. Later, in a 12-well microtiter plate, the groups were marked, and each group received 6 embryos along with E 3 embryo medium (Figure 2e,f). The wells of the test group were treated with 1%,5%, and 10% of mung bean extract, whereas control wells were treated with vitamin C. After the incubation period the embryos of zebrafish were examined under a microscope.

Different concentrations of mung bean extract (1%,5%, and 10%) were subjected to zebrafish embryos and evaluated at 24 hours post fertilization (hpf), 48 hpf, and 72 hpf, and checked for depigmentation. The evaluation of embryos was carried out using an imaging microscope.

Figure 2: a) Male & Female fishes; b) Breeding tank; c) Embryo harvesting; d) Embryonic medium; e) Petri dishes; f) 12 well-microtitre plates.



a) b) c)

d) e) f)

Results

Visual Observation :

On day 1 (24 hpf) – Zygote stage, the embryos didn't show any difference in pigmentation in all the concentrations of mung bean (Figure 3).On day 2 (48 hpf) – In the hatching stage, the embryos were hatched and no change in pigmentation (Figure 4). Later, on day 3 (72 hpf) – Early larvae stage, the embryos became larvae and upon visual inspection, it was shown that the maximum differences were observed in embryos treated with 10% mung bean extract concentrations had decreased pigmentation on the surface of the embryo than in 1%, 5% mung bean extract and in control (Figure 5).

Figure 3: Day 1 (24 hours post fertilization (hpf)) Zygote stage

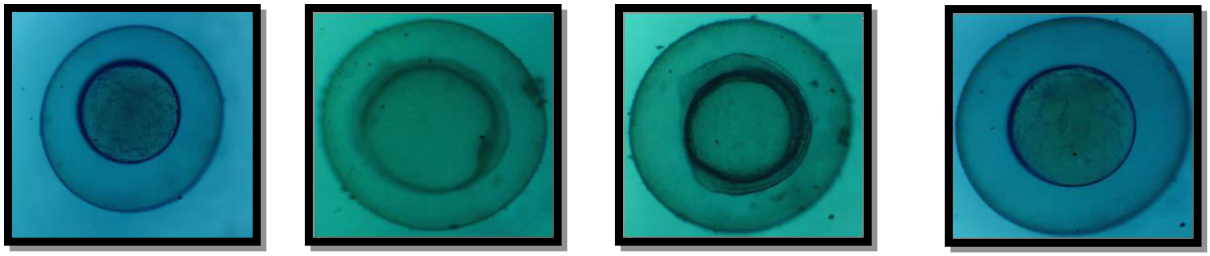


Figure 4: Day 2 (48 hpf) Hatching stage

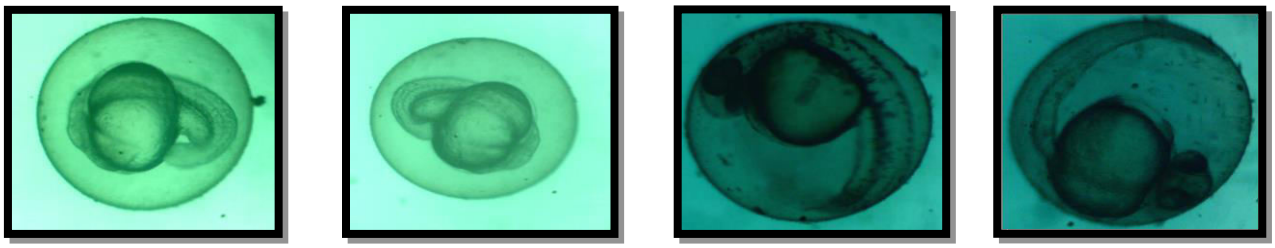


Figure 5: Day 3 (72 hpf) Early larvae

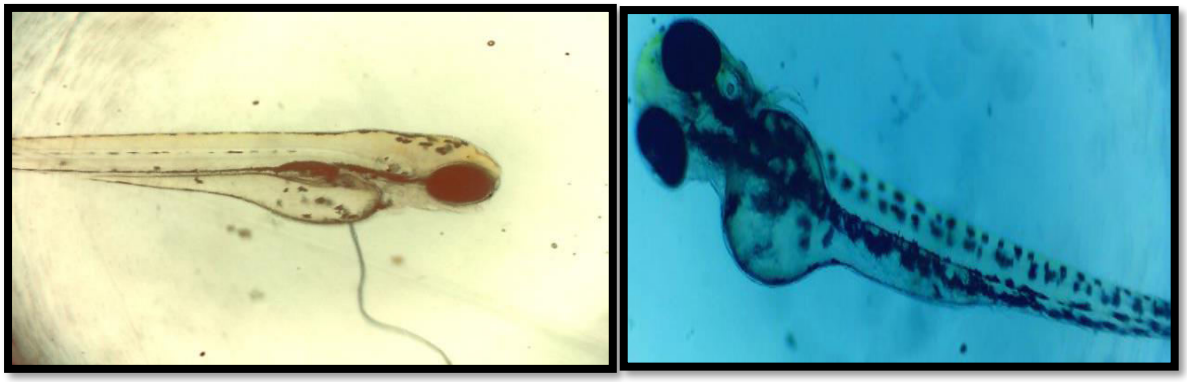


Table 1: Anti-tyrosinase activity using UV spectrometry

Control	1.214
Mung seed (1%)	0.724
Mung seed (5%)	0.699
Mung seed (10%)	0.683
Vitamin C	0.669

The decreasing values for mung seed concentrations indicate a potential inhibitory efficacy on tyrosinase activity, with higher concentrations showing stronger inhibition (Table 1). The results suggest that Mung seed extract exhibits notable anti-tyrosinase activity, especially at higher concentrations (5% and 10%). Vitamin C, often known for its antioxidant properties, also shows a comparable inhibitory effect.

The graph illustrates the percentage of tyrosinase inhibition observed at various concentrations of the extract. (Figure 6)

At lower concentrations, the inhibition percentage is relatively modest, indicating limited tyrosinase inhibition. As the concentration increases, the inhibition percentage rises, reaching a peak at the highest concentration tested. This peak represents the maximum inhibitory efficacy of mung bean extract on tyrosinase activity.

Overall, the graph provides a clear visualization of the effectiveness of mung bean extract in inhibiting tyrosinase activity across a range of concentrations, highlighting its potential as a natural tyrosinase inhibitor.

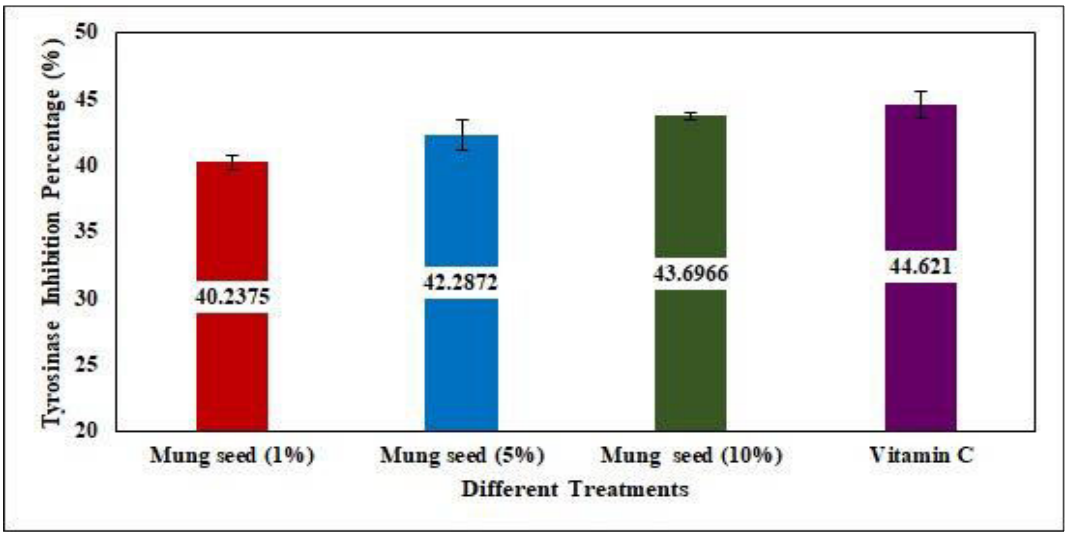


Figure 6: Graph showing the percentage of tyrosinase inhibition using different concentrations of Mung bean extract

The tyrosinase inhibition activity of mung bean extract and vitamin C was evaluated using Duncan's multiple range test. The results are presented below,

Table 2: Duncan's Multiple Range Test For Tyrosinase Inhibition Activity Homogeneous Subsets

Inhibition

Subset for alpha = 0.05

Duncan[a]	Treatments	N	1	2
	Mung seed (1%)	3	40.2375	
	Mung seed (5%)	3	42.2872	42.2872
	Mung seed (10%)	3		43.6966
	Vitamin C	3		44.6210
	Sig.		.117	.091

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Duncan test is a statistical method used to compare the means of multiple groups and identify subsets that do not differ significantly from each other. The treatments are divided into homogeneous subsets based on their inhibitory effects. Mung seed at 5% concentration shows the highest inhibition (42.2872), and it forms a subset with itself because it doesn't significantly differ from the other subsets at the 0.05 significance level. Mung seeds at 1% and 10% concentrations, as well as Vitamin C, are not significantly different from each other, forming another subset. The significance levels (.117 and .091) indicate the p-values associated with the differences between subsets, with higher p-values suggesting weaker evidence against the null hypothesis of no difference(Table 2).

The harmonic mean sample size of 3.000 is mentioned, which is a statistical measure used to account for varying sample sizes among groups when calculating means or averages. Overall, the interpretation reveals the grouping of treatments based on their inhibition levels, with subsets indicating where treatments show similar inhibitory effects without statistically significant differences.

Table 3: ANOVA Table for Assessing Treatment Differences in Inhibition Levels

Anova

Inhibition

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	32.752	3	10.917	5.356	.026
Within Groups	16.305	8	2.038		
Total	49.057	11			

Between Groups:

The Sum of Squares (SS) represents the variability between the group means. The Degrees of Freedom (df) indicates the number of independent comparisons among the group means. The Mean Square (MS) is the SS divided by df, representing the average variance between groups. The F-value is the ratio of MS between groups to MS within groups, used to test if there are significant differences among group means. The Significance (Sig.) value (.026) indicates that the differences among group means are statistically significant at the 0.05 significance level.

Interpretation:

- The F-value of 5.356 suggests significant differences in inhibition levels among the treatments.
- The Significance value of .026 indicates that these differences are statistically significant at the 0.05 level.
- The between-groups variance (32.752) is larger than the within-groups variance (16.305), suggesting that the differences among treatment group mean contribute significantly to the overall variance in inhibition levels (Table 3).

The ANOVA analysis indicates that the treatments have a significant impact on inhibition levels, with differences between treatment group means being statistically significant.

Table 4: Multiple Comparisons of Treatment Groups on Inhibition Levels

Multiple Comparisons							
Dependent Variable: Inhibition							
	(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Mung seed (1%)	Mung seed (5%)	-2.04977	1.16567	.117	-4.7378	.6383
		Mung seed (10%)	-3.45909*	1.16567	.018	-6.1471	-.7710
		Vitamin C	-4.38349*	1.16567	.006	-7.0715	-1.6955
	Mung seed (5%)	Mung seed (1%)	2.04977	1.16567	.117	-.6383	4.7378
		Mung seed (10%)	-1.40932	1.16567	.261	-4.0974	1.2787
		Vitamin C	-2.33372	1.16567	.080	-5.0218	.3543
	Mung seed (10%)	Mung seed (1%)	3.45909*	1.16567	.018	.7710	6.1471
		Mung seed (5%)	1.40932	1.16567	.261	-1.2787	4.0974
		Vitamin C	-.92440	1.16567	.451	-3.6124	1.7636
	Vitamin C	Mung seed (1%)	4.38349*	1.16567	.006	1.6955	7.0715
		Mung seed (5%)	2.33372	1.16567	.080	-.3543	5.0218
		Mung seed (10%)	.92440	1.16567	.451	-1.7636	3.6124

*The mean difference is significant at the 0.05 level.

The multiple comparisons table provides insights into the specific differences in inhibition levels among different treatments (Table 4).

Mung seed (1%) vs. Mung seed (10%):

- The mean difference is -3.45909 with a significant p-value (.018), suggesting a significant difference in inhibition levels between these two concentrations of Mung seed.

Mung seed (1%) vs. Vitamin C:

- The mean difference is -4.38349 with a significant p-value (.006), indicating a significant difference in inhibition levels between Mung seed (1%) and Vitamin C. The mean difference with other groups suggests no significant difference in inhibition levels.

Discussion

This study investigated the potential anti-tyrosinase activity of mung bean extract using zebrafish embryos as a model system. The ethyl acetate extract fractions of mung bean exhibited the highest inhibitory activity on mushroom tyrosinase when L-3,4-dihydroxyphenylalanine was used as the substrate, surpassing the inhibitory effects of the dichloromethane extract, n-butanol extract, and residual extract fractions.[6] Likewise, our study assessed the anti-tyrosinase activity of mung bean extract using L-DOPA as a substrate, following a method described by Zengin et al. The results showed increased tyrosinase inhibitory activity with mung bean extract concentrations of 5%, 10%, and vitamin C (0.699, 0.683,0.669).

Ascorbic acid (AA) has been shown to act as both a water-soluble antioxidant and a crucial nutrient for collagen synthesis. It contributes to immunomodulation and aids in reducing hyperpigmented spots by interacting with copper ions within the tyrosinase active site. This interaction inhibits tyrosinase activity, leading to reduced melanin production.[7]In this study, vitamin C served as the control to assess the effectiveness of mung bean in inhibiting tyrosinase activity in zebrafish embryos. The results demonstrated that both vitamin C and 10% mung bean extract significantly reduced pigmentation in zebrafish embryos at 72 hours post-fertilization, indicating comparable efficacy in tyrosinase inhibition and pigmentation reduction.

A study by Kavitha G. Singh (2019) demonstrated the effective conversion of L-Tyrosine to p-coumaric acid by the action of Tyrosine Ammonia Lyase (TAL) from Trigonella Foenum-graecum. They observed a clear indication of depigmentation in zebrafish embryos, suggesting that the enzymatic activity of TAL and the resultant production of p-coumaric acid play a significant role in this process. The depigmentation observed can be attributed to the inhibition of melanin synthesis, as p-coumaric acid is known to act as a competitive inhibitor of tyrosinase, the key enzyme involved in melanin production.[9]

Our study represents the pioneering effort to investigate the depigmentation potential of mung bean (Vigna radiata) extract using zebrafish (Danio rerio) embryos as a model organism. The findings demonstrate a significant reduction in melanin production in zebrafish embryos treated with mung bean extract, suggesting its potent anti-tyrosinase activity. However, this is the first instance of its application in a depigmentation study, marking a crucial advancement in the field of research.

It's important to acknowledge the limitations of this study, such as the focus on in-vitro assays using zebrafish embryos as a model system. Translating these findings to

human gingiva requires clinical studies to validate the efficacy and safety of mung bean extract-based interventions.

Conclusion

In summary, our research aimed to assess the anti-tyrosinase properties of mung bean extract and its ability to reduce pigmentation using zebrafish embryos as a model. Our findings revealed a significant decrease in pigmentation across varying concentrations of mung bean extract (1%, 5%, and 10%), with particularly noteworthy results at 5% and 10% concentrations. Notably, the efficacy of mung bean extract equaled that of vitamin C, a recognized tyrosinase inhibitor. These results suggest mung bean extract holds promise as a potential treatment for depigmentation issues, including gingival hyperpigmentation. However, further investigations, including clinical trials, are necessary to validate these findings and ascertain the safety and efficacy of mung bean extract in humans. Nevertheless, our study contributes valuable insights into the development of innovative therapies for hyperpigmentation disorders, offering potential advancements in dermatology and cosmetic dentistry.

References

1. Mostafa D Sr, A Alaizari N, M AlOtaibi S, Ahmed Aldosari N, Rabie Al-Anazi J, S Alsughayer R, M AlFayir H, S AlHarthi M, H AlAnazi M (2023). Gingival Depigmentation Using Microneedling Technique With Topical Vitamin C: A Prospective Case Series. Cureus.Feb 23;15(2):e35345.
2. Gupta R, Saxena R, Patidar A, Chourasiya Y, Malviya N (2020). Review on Antityrosinase Activity of Some Indian Medicinal Plants and their Phytoconstituents, Journal of Drug Delivery and Therapeutic. 10(5-s):199-204
3. Hou D, Yousaf L, Xue Y, Hu J, Wu J, Hu X, Feng N, Shen Q (2019). Mung Bean (*Vigna radiata* L.Bioactive Polyphenols, Polysaccharides, Peptides, and Health Benefits. Nutrients. May 31;11(6):1238.
4. Yao, Yang & Cheng, Xuzhen & Lixia, Wang & Wang, Suhua & Ren, Guixing. (2011). Mushroom tyrosinase inhibitors from mung bean (Vigna radiatae L.) extracts. International journal of food sciences and nutrition. 63. 358-61.
5. Eid, Hossam. (2012). Impact of melanin pigmentation on gingival inflammation-histopathological and biochemical analysis. Egyptian Dental Journal. Vol. 58. 1289:1294.
6. Yao, Yang, Xuzhen Cheng, Lixia Wang, Suhua Wang, and Guixing Ren (2012). "Mushroom Tyrosinase Inhibitors from Mung Bean (Vigna Radiatae L.) Extracts." International Journal of Food Sciences and Nutrition 63, no. 3: 358–61.
7. Mostafa D, Alotaibi SM (2022). A Successful Esthetic Approach of Gingival Depigmentation Using Microneedling Technique and Ascorbic Acid (Vitamin C). Case Rep Dent.Apr 25;2022:3655543.

8. Ferreira AM, de Souza AA, Koga RCR, Sena IDS, Matos MJS, Tomazi R, Ferreira IM, Carvalho JCT (2023). Anti-Melanogenic Potential of Natural and Synthetic Substances: Application in Zebrafish Model. Molecules.Jan 20;28(3):1053.
9. Singh, Kavitha &Umaima,. (2019). In vivo study of Depigmentation Using Tyrosine Ammonia Lyase from Trigonella Foenum-graecum on Zebra Fish Embryos. International Journal of Current Research and Review. 11. 10.31782/IJCRR.2019.1316.