

STUDY REPORT		 RADIANT RESEARCH
DEPARTMENT: BIOCHEMISTRY	STUDY No.: RR260155/BC/MT/02-26	

STUDY REPORT

Copy No. 1/2

Study Title

**DETERMINATION OF SKIN LIGHTENING ACTIVITY OF
UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION BY
MUSHROOM TYROSINASE INHIBITORY ASSAY**

Study Director

Mr. Dinesh K G

Test Facility

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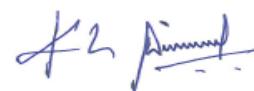
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COMPLIANCE STATEMENT

The Study Director hereby declares that the work was performed under his supervision and in accordance with the mutually agreed study plan and the in house procedures. It is assured that the reported results represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study. The Study Director accepts overall responsibility for the technical conduct of the study as well as the interpretation, documentation and reporting of the results.

Date: 24/03/2026



Study Director
Dinesh K G

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CERTIFICATE OF AFFIRMATION AND CONFIDENTIALITY

The Management hereby attests to the originality, accuracy and authenticity of the study to the best of their knowledge. This report contains confidential and proprietary information of **M/s. V Cosmetic Crafters Pvt Ltd., S R Towers (3rd floor) Shed No d-32/2, IDA Jeedimetla, Postal quarters Jeedimetla, Medchal-Malkjgiri (D), Hyderabad, Telangana.** which will not be disclosed to anyone without the expressed or written approval of authorized personnel.

Date: 24/03/2026



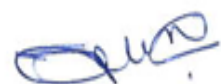
Management
Dr. Ashok G
C.E.O

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DECLARATION

The Study No: RR260155/BC/MT/02-26, entitled “**Determination of skin lightening activity of UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION by Mushroom Tyrosinase inhibitory assay**” has been inspected regularly according to the Standard Operating Procedure of the test facility’s Quality Assurance Unit. The report was audited against approved study plan and pertinent raw data and accurately reflects the raw data.

Date: 04/03/2026



Quality Manager
Gopi Mareedu

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ABBREVIATIONS USED

BC	: Biochemistry
DTL	: Drug Testing laboratory
OD	: Optical density
µg	: Micro gram
mg	: Milli gram
mL	: Milli litre
mM	: Milli Molar
nm	: Nanometer
DMSO	: Dimethyl sulfoxide
L-DOPA	: L-3, 4-dihydroxyphenylalanine
MT	: Mushroom tyrosinase
PBS	: Phosphate Buffered Saline
RR	: Radiant Research

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1. STUDY DETAILS

- 1.1. Study Title** : Determination of skin lightening activity of UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION by Mushroom Tyrosinase inhibitory assay
- 1.2. Study Number** : RR260155/BC/MT/02-26
- 1.3. Test Item** : UNDERATED 25% AHA + 2% BHA+5% PHA PEELING SOLUTION
- 1.4. Sponsor** : **M/s. V Cosmetic Crafters Pvt Ltd.,**
S R Towers (3rd floor) Shed No d-32/2,
IDA Jeedimetla, Postal quarters Jeedimetla,
Medchal-Malkjgiri District, Hyderabad, Telangana.
- 1.5. Test Facility** : **Radiant Research Services Pvt Ltd.,**
No: 99/A, 8th Main, 3rd Phase,
Peenya Industrial Area,
Bangalore – 560058, Karnataka, India.
- 1.6. Test Schedule**
- Study Initiation Date : 24/02/2026
- Experimental Start Date : 25/02/2026
- Experimental Completion Date : 03/03/2026
- Study Completion Date : 03/03/2026
- Draft Report Date : 04/03/2026
- Final Report Date : 24/03/2026
- 1.7. Study Responsibility**
- Study Director : Mr. Dinesh K G
- Study Scientist : Mr. Akash S V

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2. OBJECTIVE

To Determine the skin lightening activity of UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION by Mushroom Tyrosinase inhibitory assay.

3. SUMMARY

In vitro estimation of test item by mushroom tyrosinase inhibition was carried out using a biochemical assay. Test item (UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION) of different concentrations ranging from 1000µg/mL to 62.5µg/mL were tested to determine the percentage inhibition on mushroom tyrosinase enzyme. UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION exhibited Mushroom tyrosinase inhibition activity with an IC₅₀ value of **1285.78 µg/mL**. Kojic acid was used as Standard.

4. GUIDELINES/REFERENCE

- Uchida, R., Ishikawa, S. and Tomoda, H., 2014. Inhibition of tyrosinase activity and melanine pigmentation by 2-hydroxytyrosol. *Acta Pharmaceutica Sinica B*, 4(2), pp.141-145.

5. AMENDMENT AND DEVIATION PROCEDURE

No deviation has been observed during the conduct of the experiment.

6. MATERIALS

6.1 Test item information

SI. No.	RR number	Sample name	Batch No.	Mfg. Date	Physical appearance	Storage condition
1.	RR260155	UNDERATED 25% AHA + 2% BHA+5% PHA PEELING SOLUTION	250182	10/25	Liquid	RT

6.2 Reference Material/Chemicals

Chemicals	Batch / Lot No.	Manufacturer	Expiry Date
Kojic acid	3897380	SRL, India	Sep-2029
DMSO	4706576	SRL, India	Aug-2030
L-DOPA	4592196	SRL, India	Aug-2030
Mushroom tyrosinase	1022551	MCE, India	Feb-2028

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6.3 Equipments

SI. No.	Name of the Equipments	Make	Equipment ID
1.	Digital Weighing Balance	Saffron, India	RRS/INS/DTL/54
2.	Sonicator	Life-care, India	RRS/INS/DTL/33
3.	Cyclomixer	Remi, India	RRS/INS/DTL/15
4.	Incubator	Lab matrix, India	RRS/INS/CB/28
5.	Micropipettes 1000µL 100 µL	Gilson, USA Gilson, USA	RRS/INS/DTL/36 RRS/INS/DTL/37
6.	Automated micro plate reader	Biotek, USA	RRS/INS/MB/05

7. METHOD

7.1 Outline of the method

The test item - **UNDERATED 25% AHA + 2% BHA+5% PHA PEELING SOLUTION** was evaluated for *in vitro* mushroom tyrosinase inhibitory activity at different concentrations ranging from 1000 to 62.5µg/mL.

7.2 Mushroom Tyrosinase assay

7.2.1 Preparation of test item

10 mg of test item was weighed and dissolved in 1 mL DMSO to obtain 10 mg/mL concentration. The test item was serially diluted to obtain the lower concentrations (1000 to 62.5µg/mL).

7.2.2 Preparation of Standard

10 mg of Kojic acid was weighed and dissolved in 1 mL DMSO to obtain 10 mg/mL concentration. Further, for the study 1% Kojic acid was used.

7.2.3 Preparation of L- DOPA

0.9 mg of L- DOPA was dissolved in 1mL PBS to obtain the required concentration (9 µg/mL) of solution for the experimental use.

7.2.4 Preparation of Mushroom tyrosinase

1mg of tyrosinase was dissolved in 1mL buffer and this was used as stock (1mg/mL concentration). Then from the stock, 100 µL was taken and the volume was made up to 1mL using buffer.

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7.3 Inhibition assay

A volume of 10 μ L of test item was added to 96-well microplate and mixed with 60 μ L of 50 mM phosphate buffer (pH 6.8) on ice. Solvent (DMSO) was taken in place of test item for control. Then, 20 μ L of 0.9 mg/mL L-DOPA was added. Finally, mushroom tyrosinase was added to achieve the final volume of 100 μ L reaction mixture and the assay mixture was then incubated at 27 °C for 10 minutes. Following incubation, the amount of dopachrome production in the reaction mixture was determined spectrophotometrically at 405 nm in a microplate reader. From the absorbance values, the percentage inhibition by each test dose was determined and IC₅₀ value was determined by interpolation method. Each measurement was made in triplicate. The concentration for 50% inhibition (IC₅₀) was determined.

8. RESULTS

Table 1: The inhibitory activity of UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION for Mushroom Tyrosinase assay

Test item	Concentration (μ g/mL)	% Inhibition	IC ₅₀ (μ g/mL)
Standard (Kojic acid)	1000	73.52 $\pm$ 1.10	310.07 $\pm$ 1.02
	500	68.56 $\pm$ 0.55	
	250	58.45 $\pm$ 1.47	
	125	45.22 $\pm$ 1.83	
	62.5	27.02 $\pm$ 0.18	
UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION	1000	40.10 $\pm$ 0.27	1285.78 $\pm$ 0.59
	500	30.89 $\pm$ 0.81	
	250	25.74 $\pm$ 0.54	
	125	22.22 $\pm$ 0.81	
	62.5	14.90 $\pm$ 0.54	

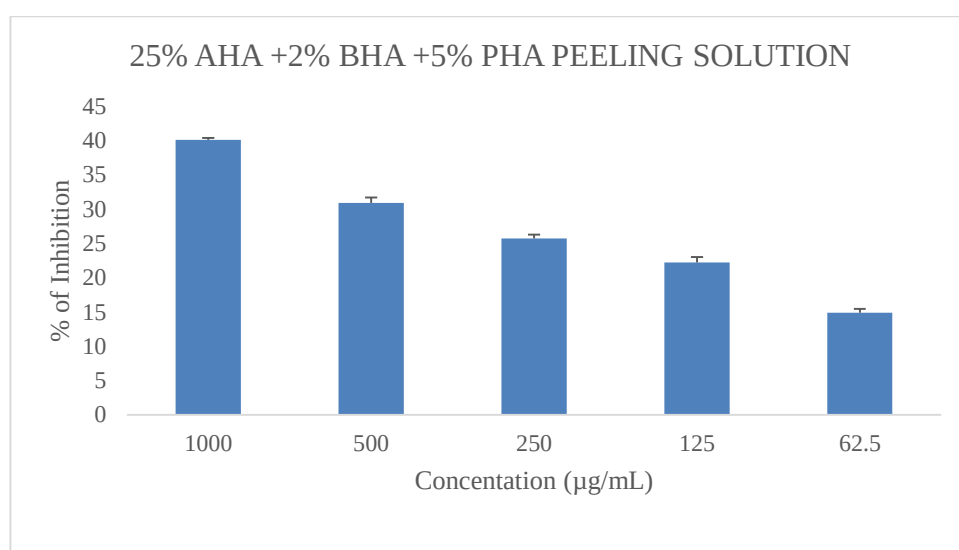
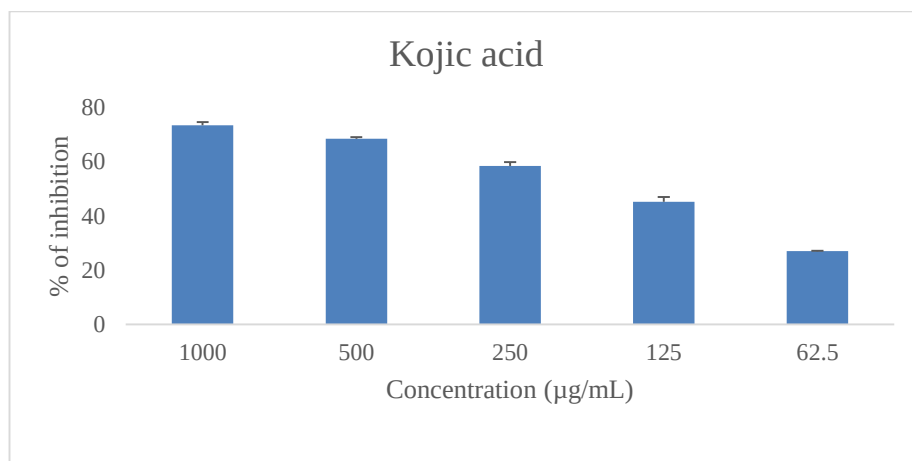


Figure 1: Graphical representation of inhibitory activity of Standard and UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION for Mushroom Tyrosinase assay

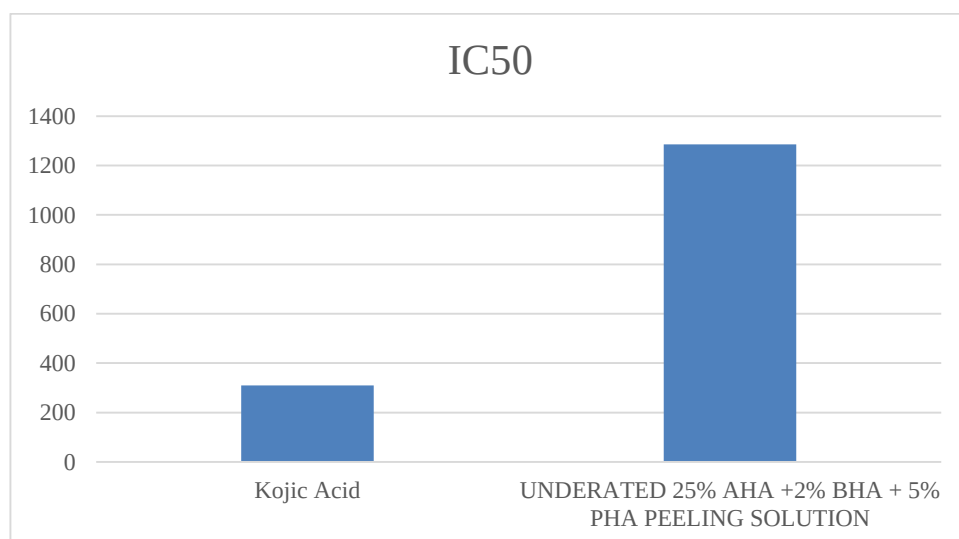


Figure 2: IC₅₀ value for Mushroom Tyrosinase enzyme inhibition

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9. DISCUSSION AND CONCLUSION

Mushroom tyrosinase is an enzyme primarily found in mushrooms that catalyzes the oxidation of phenolic compounds, playing a key role in melanin production. It is widely used in cosmetic formulations for skin whitening, in food processing to control browning and in scientific research to study pigmentation and enzyme activity. Its ability to catalyze important biochemical reactions makes it valuable in various industries, including cosmetics, food and pharmaceuticals, as well as in the study of pigmentation-related disorders.

In the present study, **UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION** was tested for different concentration range of 1000 - 62.5µg/mL. At 1000 µg/mL the inhibition was found to be **40.10 ± 0.27 µg/mL** and at 62.5µg/mL the inhibition was found to be **14.90 ± 0.54 µg/mL**. **UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION** exhibited Mushroom tyrosinase inhibition activity with an IC₅₀ value of **1285.78 µg/mL**. Standard Kojic acid exhibited inhibition activity with an IC₅₀ value of **310.07 ± 1.02 µg/mL**.

CONCLUSION: The test results concluded that, **UNDERATED 25% AHA +2% BHA + 5% PHA PEELING SOLUTION** showed Moderate tyrosinase inhibitory activity.

Disclaimer: Helps reduce the appearance of pigmentation

10. ARCHIVING

- Test Samples will be stored for 3 months after the final report submission.
- Raw data, documents, report will be archived for 3 years.

11. REPORT DISTRIBUTION

- Sponsor: One signed final report (Copy no. 1/2) in original.
- Archives: One signed final report (Copy no. 2/2) in original along with raw data file.

***** End of the report *****