

STUDY REPORT		 RADIANT RESEARCH
DEPARTMENT: MICROBIOLOGY	STUDY No.: RR260156/MCR/MIC/02-26	

STUDY REPORT

Copy No. 1/2

Study Title

**IN-VITRO EVALUATION OF THE ANTI-ACNE ACTIVITY OF
UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM AGAINST
Cutibacterium acnes BY MICRO DILUTION METHOD**

Study Director

Ms. Nagarathna C

Test Facility

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COMPLIANCE STATEMENT

The Study Director hereby declares that the work was performed under her 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
Nagarathna C

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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., SR 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.: RR260156/MCR/MIC/02-26, entitled “***In-vitro* evaluation of the anti-acne activity of UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM against *Cutibacterium acnes* by Micro Dilution method**” has been inspected regularly according to the Standard Operating Procedures of the test facility’s Quality Assurance Unit. The report was audited against the approved study plan and pertinent raw data and accurately reflects the raw data.

Date: 24/03/2026



Quality Manager
Gopi Mareedu

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ABBREVIATIONS USED

MCR	: Microbiology
MTCC	: Microbial Type Culture Collection
%	: Percentage
mL	: Milliliter
MIC	: Minimum Inhibitory Concentration
BHIB	: Brain Heart Infusion Broth
BHIA	: Brain Heart Infusion Agar
°C	: Degree Celsius
μL	: Microliter
nm	: Nanometer
mg	: Milligram
CFU	: Colony Forming Unit
<i>C. acnes</i>	: <i>Cutibacterium acnes</i>
RT	: Room Temperature
RR	: Radiant Research

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1. STUDY DETAILS

- 1.1. Study Title** : *In-vitro* Evaluation of the Anti-acne activity of UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM against *Cutibacterium acnes* by Micro Dilution Method
- 1.2. Study Number** : RR260156/MCR/MIC/02-26
- 1.3. Test Item** : UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM
- 1.4. Sponsor** : **M/s. V Cosmetic Crafters Pvt Ltd.,**
SR Towers (3rd floor) Shed No. d-32/2,
IDA Jeedimetla, Postal quarters Jeedimetla
Medchal-Malkjgiri (D), Hyderabad, Telangana.
- 1.5. Test Facility** : **Radiant Research Services Pvt. Ltd.,**
No: 99/A, 8th Main Road, 3rd Phase,
Peenya Industrial Area,
Bangalore -560 058, Karnataka, India.
- 1.6. Test Schedule**
- Experimental Start Date : 20/02/2026
- Experimental Completion Date : 07/03/2026
- Draft Report Date : 07/03/2026
- Final Report Date : 24/03/2026
- 1.7. Study Responsibility**
- Study Director : Ms. Nagarathna C
- Study Coordinator : Ms. Yogalakshmi K

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2. OBJECTIVE

The purpose of this study is to evaluate the Anti-acne activity of UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM against *C. acnes* by Micro Dilution Method.

3. SUMMARY

The Test item: UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM was evaluated for Anti-acne activity against *Cutibacterium acnes* by Micro Dilution Method at different concentration ranges from 100% to 0.048%. The Test item shown MIC value at 12.5% against *Cutibacterium acnes*. Whereas, Standard Ciprofloxacin shown MIC value at 0.000048% against *Cutibacterium acnes*.

4. GUIDELINES/REFERENCE

1. CLSI 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition Approved standard M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
2. Satyajit D. Sarker, Lutfun Nahar, and Yashodharan Kumarasamy. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals, Methods. 2007 August; 42(4): 321–324.

5. AMENDMENT AND DEVIATION PROCEDURES

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

6. MATERIALS

6.1. Test item Information

RR Number	RR260156
Sample Name	UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM
Physical Appearance	Liquid
Storage Condition	RT
Batch Number	250161
Mfg. Date	09/25

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6.2. Microbial Strain

SI. No.	Tester Strain	Strain No.
1.	<i>Cutibacterium acnes</i>	MTCC 1951

6.3. References Materials/Chemicals

SI. No.	Chemicals	Batch / Lot No.	Manufacturer	Expiry date
1.	Sodium Chloride	0000680931	HiMedia, India	Dec - 2028
2.	Brain Heart Infusion Broth	0000584503	HiMedia, India	Mar - 2028
3.	Brain Heart Infusion Agar	0000703276	HiMedia, India	Apr - 2030
4.	Ciprofloxacin	0000716864	HiMedia, India	Aug - 2028
5.	Resazurin Dye	0000626000	HiMedia, India	Jan - 2028

6.4. Equipments

SI. No.	Name of the Equipments	Make	Equipment ID
1.	Weighing Balance	Anamatrix, India	RRS/INS/MCR/40
2.	Autoclave (Sterilization)	Ascension Innovation, India	RRS/INS/MCR/10
3.	Autoclave (Decontamination)	Ascension Innovation, India	RRS/INS/MCR/20
4.	Bacteriological Incubator	Ascension Innovation, India	RRS/INS/MCR/21
5.	Biological Safety Cabinet-II	Thermocon, India	RRS/INS/MCR/27
6.	Digital Photo colorimeter	Anamatrix, India	RRS/INS/MCR/45
7.	Micropipette (2-200 µL)	Gilson, India	RRS/INS/MCR/42
8.	Refrigerator	LG, India	RRS/INS/MCR/02

7. METHOD

7.1. Preparation and standardization of stock culture

A loopful culture of *Cutibacterium acnes* was grown on BHIA media and incubated at 37 °C for 48 hrs in an anaerobic condition. Total numbers of cells were adjusted to 10⁶ CFU/mL at 620nm in digital colorimeter.

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7.2. Preparation of Test item

100% of test item was taken for the testing.

7.3. Preparation of standard antibiotic solution

Ciprofloxacin was used as standard antibiotic and 0.1% concentration solution was prepared by adding 0.1g of Ciprofloxacin to 100mL of sterile distilled water.

7.4. Preparation of resazurin solution

2.7mg of resazurin was weighed and added to 5mL of sterile normal saline solution. 1mL of prepared stock solution was added to 4mL of sterile saline to get working solution and this was used for the test.

7.5. Determination of MIC by Micro dilution method

7.5.1. Experiment was performed in triplicates for test item and for standard solution under aseptic conditions.

7.5.2. 96 well microtitre plate was taken to which 100µL of BHIB broth was added to all the wells except first six (A1B1C1D1E1F1) column wells.

7.5.3. In first three wells (A1B1C1) of plate, 200µL of the test item was added and double diluted till A12B12C12 to get the desired concentration.

7.5.4. In D1E1F1 wells of micro dilution plate, 200µL of prepared standard solution (Ciprofloxacin) was added and double diluted till D12E12F12 to get the desired concentration.

7.5.5. To the wells containing both Test item and standard, 10µL of *Cutibacterium acnes* suspension of 10^6 CFU/mL was added separately.

7.5.6. A growth control (10µL of microbial culture + 100µL broth medium) from G1 to G12 and (only 100µL broth medium) from H1 to H12 was kept as negative control.

7.5.7. The plate containing *Cutibacterium acnes* was incubated at 37 °C for 48 hrs in an anaerobic condition.

7.5.8. After incubation, 20µL of working solution of resazurin was added to all wells.

7.5.9. The plate was wrapped with aluminum foil and incubated for 1 hr.

7.5.10. The color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive (growth).

7.5.11. The lowest concentration at which there is no color change occurred was taken as the MIC value.

8. OBSERVATION

Table 1. Anti-acne activity of UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM against *Cutibacterium acnes*

Test item	Concentration tested (mg/mL)	MIC (Result Observed)
UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM	100% to 0.048%	12.5%
Ciprofloxacin (Standard)	0.1% to 0.000048%	0.000048%

9. PICTURE



Figure 1: Anti-acne activity of UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM against *Cutibacterium acnes*

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10. RESULTS

The **UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM** was evaluated for Anti-acne activity against *Cutibacterium acnes* by Micro Dilution method at different concentration ranges from 100% to 0.048%.

UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM shown MIC value at 12.5% against *Cutibacterium acnes*.

Hence, the Test item - UNDERATED SALICYLIC ACID 2% BETAINE 3% SERUM have anti-acne activity against *Cutibacterium acnes* at the tested concentration.

11. ARCHIVING

- Test samples will be stored for 30 days after the final report submission.
- Raw data, documents, reports will be archived for 3 years.

12. REPORT DISTRIBUTION

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

*****End of the Report*****