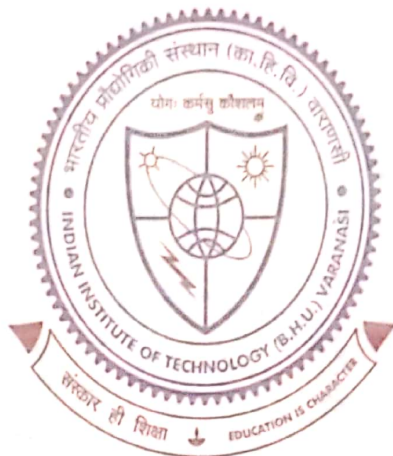


# Development and Evaluation of Formulations for Enhanced Antimicrobial & Antiphotaging Activities of Anacardic Acid



Thesis submitted in partial fulfillment for the  
Award of Degree

**Doctor of Philosophy**

By

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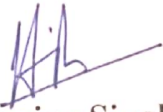
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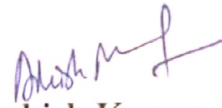
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I, *Md Meraj Anjum*, certify that the work embodied in this thesis is my own bona fide work and carried out by me under the supervision of *Prof. Sanjay Singh* and co-supervision of *Dr. Ashish Kumar Agrawal* from *July, 2015 to October, 2020* at the *Department of Pharmaceutical Engineering and Technology*, Indian Institute of Technology (BHU), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not willfully copied any other's work, paragraphs, text, data, results, *etc.*, reported in journals, books, magazines, reports dissertations, theses, *etc.*, or available at websites and have not included them in this thesis and have not cited as my own work.

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**Md Meraj Anjum**

## Table of Contents

List of Figures.....	xii
List of Tables.....	xviii
Abbreviations.....	xix
Preface.....	xxii
<b>1. Chapter 1: Introduction.....</b>	<b>1</b>
9.1 Background .....	1
9.2 Anacardic Acid activity against Biofilms .....	3
9.3 Anacardic Acid activity against Photoaging .....	8
<b>2. Chapter 2: Literature review .....</b>	<b>11</b>
2.1. Anacardic Acid.....	11
2.1.1 Pharmacological Activities of Anacardic Acid.....	12
2.1.2 Physicochemical properties of Anacardic Acid .....	14
2.1.3 Recent studies on Anacardic Acid .....	14
2.2. Microbial infection.....	16
2.3. Microbial resistance to antibiotics.....	17
2.4. Mechanisms of antibiotic resistance .....	17
2.5. Biofilm and biofilm mediated antimicrobial resistance .....	18
2.5.1 Stages in biofilm formation .....	19
2.5.2 Components of biofilm .....	21
2.5.3 Complications associated with biofilm mediated infections .....	23
2.5.4 Biofilm mediated resistance to antibiotics.....	23
2.5.5 Diseases associated with biofilm formation .....	25
2.5.6 Prevention and therapy of biofilm related complications .....	26
2.6. UV-B mediated skin photoaging.....	36
2.6.1 Aging.....	36
2.7. UV radiation and skin photoaging .....	38

2.8.	UV-B radiation and biochemical changes in skin .....	39
2.9.	Treatment approaches.....	41
2.9.1	Preventive approach.....	41
2.9.2	Topical retinoids.....	42
2.9.3	Alpha hydroxy acids.....	43
2.9.4	Antioxidants .....	43
2.10.	Role of Anacardic Acid in management of skin photoaging .....	45
2.11.	Cyclodextrin nanosponge for skin delivery .....	46
<b>3.</b>	<b>Chapter 3: Objectives.....</b>	<b>49</b>
<b>4.</b>	<b>Chapter 4: Plan of study .....</b>	<b>50</b>
<b>5.</b>	<b>Chapter 5: Extraction and isolation of Anacardic Acid.....</b>	<b>53</b>
5.1	Extraction of Cashew Nut Shell Liquid (CNSL), and group isolation of Anacardic Acid.....	53
5.1.1	Extraction of CNSL.....	53
5.1.2	Isolation of Anacardic Acid from CNSL .....	53
5.1.3	Isolation of C15:3 and C15:0 among the Anacardic Acid subtypes .....	54
5.2	HPLC analysis .....	54
5.3	Identification of the compounds using FT-IR and <sup>1</sup> H NMR .....	55
4.4.1	FT-IR.....	55
5.3.1	<sup>1</sup> H NMR.....	55
5.4	Results and Discussion.....	55
5.4.1	HPLC analysis.....	56
5.4.2	FT-IR.....	57
5.4.3	<sup>1</sup> H NMR.....	57
<b>6.</b>	<b>Chapter 6: Preparation and evaluation of Ana<sub>C15:3</sub>/HP-β-CD inclusion complex</b>	<b>59</b>
6.1	Materials .....	59
6.2	Methods .....	59

6.2.1	Analytical method.....	59
6.2.2	Preparation of Ana <sub>C15:3</sub> /HP-β-CD inclusion complex.....	60
6.2.3	Evaluation of Ana <sub>C15:3</sub> /HP-β-CD inclusion complex.....	60
6.2.4	Antimicrobial activity.....	63
6.3	Results and Discussion.....	65
6.3.1	Analytical method.....	65
6.3.2	UV-Visible spectroscopy.....	66
6.3.3	Preparation of Anacardic Acid/HP-β-CD inclusion complex.....	67
6.3.4	Phase solubility study.....	68
6.3.5	Fourier transform infrared spectroscopy (FT-IR).....	70
6.3.6	X-ray diffraction (XRD) study.....	72
6.3.7	Differential scanning calorimetry (DSC).....	73
6.3.8	Scanning Electron Microscopy.....	74
6.3.9	<sup>1</sup> H NMR (Proton) Spectroscopy.....	75
6.3.10	Antimicrobial Activity.....	77
7.	<b>Chapter 7: Development of Chitosan and DNase coated solid lipid nanoparticles encapsulating Anacardic Acid (Ana<sub>C15:3</sub>).....</b>	<b>82</b>
7.1	Materials.....	82
7.2	Methods.....	82
7.2.1	Preparation of Anacardic Acid loaded solid lipid nanoparticles.....	82
7.2.2	Chitosan and DNase coated SLNs.....	83
7.2.3	Characterization of Ana-loaded SLNs.....	83
7.2.4	Antimicrobial study.....	86
7.2.5	Statistical analysis.....	99
7.3	Results and Discussion.....	89
7.3.1	Characterization of Ana <sub>C15:3</sub> -SLNs.....	89
7.3.2	Antimicrobial study.....	96

<b>8. Chapter 8: Preparation and evaluation Ana<sub>C15:0</sub> nanosponge based gel</b> .....	<b>103</b>
8.1 Materials .....	103
8.2 Methods .....	103
8.2.1 Analytical method .....	103
8.2.2 Synthesis of $\beta$ -Cyclodextrin Nanosponge .....	104
8.2.3 Preparation of Ana <sub>C15:0</sub> loaded Nanosponge complex .....	104
8.2.4 Optimization of Ana <sub>C15:0</sub> -NS formulation using 3-level factorial design	105
8.2.5 Evaluation of Ana <sub>C15:0</sub> -Nanosponge.....	105
8.2.6 Preparation of Ana <sub>C15:0</sub> -NS enriched topical gel .....	107
8.2.7 Evaluation of Ana <sub>C15:0</sub> -NS topical gel.....	108
8.2.8 Animal Study.....	109
8.3 Results and Discussion .....	111
8.3.1 Analytical method .....	112
8.3.2 Formulation optimization.....	113
8.3.3 Solubility study .....	116
8.3.4 Fourier transform infrared spectroscopy .....	117
8.3.5 X-ray diffraction (XRD) study .....	118
8.3.6 Morphology evaluation of Ana <sub>C15:0</sub> encapsulated nanosponge .....	120
8.3.7 <i>In-vitro</i> release study .....	120
8.3.8 Evaluation of Ana <sub>C15:0</sub> -NS topical gel.....	121
8.3.9 Physical evaluation of skin photoaging.....	123
8.3.10 Histopathology study.....	124
8.3.11 Estimation of biochemical parameters .....	125
<b>9. Chapter 9: Summary &amp; conclusion.....</b>	<b>129</b>
9.1 Summary.....	129
9.2 Conclusion .....	133
<b>11. References.....</b>	<b>135</b>

## List of Figures

Figure No	Description	Page No
<b>Figure 1.1:</b>	Chemical structure of Anacardic Acid and its subtypes	1
<b>Figure 1.2:</b>	Photograph demonstrating various stages of <i>Staphylococcus aureus</i> biofilm formation and proposed formulation approaches to combat biofilm.	7
<b>Figure 1.3:</b>	Photograph illustrating skin photoaging pathway, Anacardic Acid (C <sub>15.0</sub> ) activity and formulation approach to improve photoaging therapy.	10
<b>Figure 2.1:</b>	Overview of different components mixture of solvent extracted CNSL	12
<b>Figure 2.2:</b>	Solar UV radiations and its penetration in skin layers	39
<b>Figure 2.3:</b>	Biochemical routes of UV-B mediated skin photoaging and course of Anacardic Acid action	46
<b>Figure 5.1:</b>	Representative HPLC profiles of Anacardic Acid (a), Ana <sub>C15.3</sub> (b), and Ana <sub>C15.0</sub> (c).	56
<b>Figure 5.2:</b>	Comparative FT-IR spectra of Ana <sub>C15.3</sub> (a) and Ana <sub>C15.0</sub> (b).	57
<b>Figure 5.3:</b>	<sup>1</sup> H NMR spectra of Ana <sub>C15.3</sub> (a) and Ana <sub>C15.0</sub> (b).	58
<b>Figure 6.1:</b>	Calibration curve of Ana <sub>C15.3</sub> in ethanol (A) and phosphate buffer pH 6.8 (B) for UV spectrophotometry.	66
<b>Figure 6.2:</b>	UV absorption spectra Ana <sub>C15.3</sub> in phosphate buffer pH 6.8 at different HP-β-CD concentrations: (1) 0.00 mM, (2) 10.00 mM, (3) 20.00 mM and (4) 25.00 mM	67
<b>Figure 6.3:</b>	Phase solubility diagram of Ana <sub>C15.3</sub> as a function of HP-β-CD concentrations in phosphate buffer pH 6.8 at different temperatures (A) 25°C, (B) 35°C and (C) 45°C.	69
<b>Figure 6.4:</b>	The FT-IR spectra of HP β-CD (A), Ana <sub>C15.3</sub> (B), and inclusion complex (C).	71
<b>Figure 6.5:</b>	X-ray diffraction patterns of HP-β-CD (A), Ana <sub>C15.3</sub> (B),	72

- and inclusion complex (C).
- Figure 6.6:** DSC thermograms of HP- $\beta$ -CD (A), Ana<sub>C15.3</sub> (B), and the inclusion complex (C). 74
- Figure 6.7:** Scanning electron microscopy image of HP- $\beta$ -CD (A) and Inclusion complex (B). 75
- Figure 6.8:** Comparative <sup>1</sup>H NMR spectrum of HP- $\beta$ -CD (A), Ana<sub>C15.3</sub> (B), and inclusion complex (C). 76
- Figure 6.9:** Disruption effect of free Ana<sub>C15.3</sub> and Ana<sub>C15.3</sub>-IC on mature biofilm grown in 96 well plates. The graph depicts the percentage biofilm residue in response to different concentration of Ana<sub>C15.3</sub> and Ana<sub>C15.3</sub>-IC. The study demonstrates a reduction of 48 h grown *S. aureus* biofilm on a single dose (Figure 6.9-A) and repeated dose for three recurrent days (Figure 6.9-B) of different Ana<sub>C15.3</sub> and Ana<sub>C15.3</sub>-IC concentrations. The data represent a mean  $\pm$  standard deviation (n=3). *ap* < 0.005 as compared to Ana<sub>C15.3</sub> (Two way ANOVA followed by Bonferroni test). *ns* indicates the results are not significant as compared between the two groups. The viable count for the control experiment without Ana<sub>C15.3</sub> were  $2.39 \times 10^9 \pm 1.19 \times 10^8$  CFU/mL. 79
- Figure 6.10:** CLSM image of *S. aureus* biofilm formed on the coverslip surface with media supplementation. The images demonstrate dense biofilms formed without treatment (A) or with Ana<sub>C15.3</sub> (B) and Ana<sub>C15.3</sub>-IC (C) treatment. The live cells are indicated by colored fluorescence while the dead cells appear as red colored cells. The control group demonstrates more green colored cells indicating live bacterial cells while the Ana<sub>C15.3</sub>-IC treated group exhibited maximum red colored cells indicating dead cells. 80
- Figure 7.1:** TEM image of Ana<sub>C15.3</sub>-SLNs-CH-DNase 91

<b>Figure 7.2:</b>	FT-IR spectra of Ana <sub>C153</sub> (A), Ana <sub>C153</sub> -SLNs (B), and Blank SLNs (SLNs without Ana <sub>C153</sub> ) (C) and Ana <sub>C153</sub> -SLNs-CH-DNase (D)	92
<b>Figure 7.3:</b>	XRD plots of Ana <sub>C153</sub> (A), Blank SLNs (SLNs without Ana <sub>C153</sub> ) (B) and Ana <sub>C153</sub> -SLNs (C) and Ana <sub>C153</sub> -SLNs-CH-DNase (D)	93
<b>Figure 7.4:</b>	Plot indicates the cumulative amount of Ana <sub>C153</sub> release on Y axis with respect to time on X axis from the Ana <sub>C153</sub> and Ana <sub>C153</sub> -SLNs-CH-DNase in phosphate buffer (PBS, pH 6.8) as release media containing 0.1% SLS to maintain the sink conditions.	94
<b>Figure 7.5:</b>	Bar diagrams representing the results of the in vitro toxicity study (MTT assay). Data are represented as mean±SD (n=3). No significant differences were observed among the different treatments (One-way ANOVA followed by Newman keul's post hoc test).	95
<b>Figure 7.6:</b>	Inhibition of <i>S. aureus</i> biofilm formation following the different treatments. Graph represents the percentage biofilm residue following the treatment with different concentration of Ana <sub>C153</sub> . The results are presented as the mean ± standard deviation of three replicates from three independent experiments. Two-way ANOVA was performed (where a represents p <0.05 in comparison with biofilms not receiving any treatment i.e. concentration of Ana <sub>C153</sub> = 0 µg/mL). The viable cell count in the control experiments without Ana was 2.41×10 <sup>9</sup> ±1.09×10 <sup>8</sup> CFU/mL.	97
<b>Figure 7.7:</b>	Disassembling of mature biofilm following the treatment with a single dose (A) and repeated dose (B). The data represents the percentage of biofilm residue that remained following the treatments. The values are expressed as mean	100

± standard deviation of three replicates from three independent experiments. Two-way ANOVA was performed (where a represents  $p < 0.05$  as compared with Ana<sub>C15.3</sub>-SLNs-CH-DNase). The viable cell count in the control experiments without Ana<sub>C15.3</sub> was  $2.91 \times 10^9 \pm 1.13 \times 10^8$  CFU/mL.

- Figure 7.8:** CLSM image showing *S. aureus* biofilm formation without any treatment (A) and following the treatment with Ana<sub>C15.3</sub> (B), Ana<sub>C15.3</sub>-SLNs (C), Ana<sub>C15.3</sub>-SLNs-CH (D), Ana<sub>C15.3</sub>+DNase (E), and Ana<sub>C15.3</sub>-SLNs-CH-DNase treatment. The cells of the biofilm were stained using the Live/Dead Backlight™ bacterial viability kit. CLSM images demonstrate the staining pattern of live cells (SYTO-9, green) and dead cells (propidium iodide, red). 102
- Figure 8.1:** Calibration curve of Ana<sub>C15.0</sub> in ethanol (A), and phosphate buffer pH 6.8 (B) by using UV spectrophotometry. The graph indicates the absorbance at Y axis in response to the different concentrations on X axis. 112
- Figure 8.2:** 3D plots indicating the effect of different formulation variables on PS (A) and EE% (B). 115
- Figure 8.3:** Comparative solubility profiles of Ana<sub>C15.0</sub> and different ratios of Ana<sub>C15.0</sub>:NS. All experiments were performed in triplicate and data represented as mean±S.D (n=3). a represents significant difference as compared to Ana<sub>C15.0</sub>, b represents significant difference compared to Ana<sub>C15.0</sub>:NS (1:2).  $P < 0.005$ , one-way ANOVA followed by Newman-Keuls multiple-comparison test. 117
- Figure 8.4:** FT-IR spectra of HP-β-CD (A), Ana<sub>C15.0</sub> (B), and Ana<sub>C15.0</sub>-Nanosponge (C) 118
- Figure 8.5:** XRD spectra of HP-β-CD (A), Ana<sub>C15.0</sub> (B) and Ana<sub>C15.0</sub>-Nanosponge (C). 119

<b>Figure 8.6:</b>	Photograph showing a TEM image of Ana <sub>C15.0</sub> -NS	120
<b>Figure 8.7:</b>	In-vitro drug release profiles of Ana <sub>C15.0</sub> and Ana <sub>C15.0</sub> -NS. Graph represents the cumulative amount of Ana <sub>C15.0</sub> with respect to time	121
<b>Figure 8.8:</b>	(A) Cumulative amount of drug permeated through skin with respect to time from Ana <sub>C15.0</sub> -gel and Ana <sub>C15.0</sub> -NS-gel. (B) Percentage drug retained in different compartments from Ana <sub>C15.0</sub> -gel and Ana <sub>C15.0</sub> -NS-gel. All data are presented as mean±SD (n=3) *represents significant difference compared to Ana <sub>C15.0</sub> -gel, P<0.001 two-way ANOVA followed by Bonferroni posttest.	123
<b>Figure 8.9:</b>	Photographs indicating the effect of UV-B radiation on the animals in different treatment groups. There was no visible lesion or redness in the control group (I), severe photoaging could be observed in the UV-B irradiated group (II), whereas the severity decreases on treatment with Ana <sub>C15.0</sub> -gel (IV) and Ana <sub>C15.0</sub> -NS gel (v)	124
<b>Figure 8.10:</b>	Histological examination of the H&E stained dorsal skin samples taken from different groups; control (a), UVB-irradiated (b), Blank gel treated (c), 0.1% Ana <sub>C15.0</sub> -gel treated, and 0.1% Ana <sub>C15.0</sub> -NS gel treated group.	125
<b>Figure 8.11:</b>	Effect of different treatment groups on ROS production. All data are presented as mean±SD (n=3) a represents significant difference as compared to UV-B irradiated group b represents significant difference compared to control c represents significant difference compared to Blank gel d represents significant difference compared to Ana <sub>C15.0</sub> -gel. P<0.005 one-way ANOVA followed by Newman-Keuls multiple-comparison test.	126
<b>Figure 8.12:</b>	Effect of different treatment groups on the expression of MMP-1 & HAT p300 enzymes (A). relative level of HAT	128

P300 among various groups (B), and relative MMP-1 level in different groups under study. All data are presented as mean $\pm$ SD (n=6) a represents significant difference as compared to UV-B irradiated group b represents significant difference as compared to control, c represents significant difference as compared to Blank gel d represents significant difference as compared to Ana<sub>C15:0</sub>-gel P<0.005 one-way ANOVA followed by Newman-Keuls multiple-comparison test.

## List of Tables

Table No:	Description	Page No:
<b>Table 2.1:</b>	Physicochemical properties of Anacardic Acid	13
<b>Table 2.2:</b>	Summary of various pharmacological activities of Anacardic Acid.	14
<b>Table 6.1:</b>	List of chemicals used in the preparation of inclusion complex	59
<b>Table 6.2:</b>	Various parameters for calculation of stability constant	70
<b>Table 6.3:</b>	Variation of the <sup>1</sup> H NMR chemical shifts (δ /ppm) of Ana <sub>C15.3</sub> and HP-β-CD in inclusion complex formation.	77
<b>Table 6.4:</b>	Biofilm parameters of S. aureus biofilms without treatment (control), Ana <sub>C15.3</sub> , and Ana <sub>C15.3</sub> -IC treatment.	81
<b>Table 7.1:</b>	List of chemicals used in the study	82
<b>Table 7.2:</b>	Data representing particle size, PDI, and zeta potential of different formulations.	90
<b>Table 7.3:</b>	Illustration of variation in particle size and encapsulation efficiency following the storage of SLNs at different temperature conditions.	95
<b>Table 7.4:</b>	Data displaying biomass and thickness of biofilm upon treatment with different formulations.	101
<b>Table 8.1:</b>	List of materials used in the study	103
<b>Table 8.2:</b>	Experimental design demonstrating PS and EE% of the different formulation batches	113
<b>Table 8.3:</b>	Identified independent variables with their levels used in 3-level factorial experimental design and the optimized levels of the variables	115
<b>Table 8.4:</b>	Predicted values of PS and EE% based on the optimized level and the experimental response obtained	116
<b>Table 8.5:</b>	Changes in mechanical properties of hydrogel upon the incorporation of Ana <sub>C15.0</sub> -NS	122

## List of Abbreviations and Symbols

Anacardic Acid	: Ana
Anacardic Acid C15:0	: Anac <sub>15:0</sub>
Anacardic Acid C15:3	: Anac <sub>15:3</sub>
Analysis of Variance	: ANOVA
Carbonyldiimidazole	: CDI
Chitosan	: CH
Centimeter	: cm
Colony Forming Unit	: CFU
Confocal Laser Scanning Microscopy	: CLSM
Cyclodextrin	: CD
$\beta$ -Cyclodextrin	: $\beta$ -CD
Deoxyribonuclease-I	: DNase
Differential Scanning Calorimetry	: DSC
Dimethyl Formamide	: DMF
Entrapment Efficiency	: EE
Extracellular DNA	: e-DNA
Extra Polymeric Substances	: EPS
Extra Polymeric Matrix	: EPM
Fourier Transform infrared	: FT-IR
Gram	: g
Heanotoxylin and Eosin	: H&E
Hour	: h
High Performance Liquid Chromatography	: HPLC
Histone Acetyl Transferase	: HAT
Hydroxy Propyl- $\beta$ -Cyclodextrin	: HP- $\beta$ -CD
Hydroxy Propyl Methyl Cellulose	: HPMC
Kilo Dalton	: KDa
Luria Bertani	: LB

Microgram	: $\mu\text{g}$
Micrometer	: $\mu\text{m}$
Milligram	: mg
Minute	: min
Milliliter	: ml
Millimole	: mM
Minimum Inhibitory Concentration	: MIC
Molecular Weight	: MW
Millivolt	: mV
MMP	: Matrix Metallo P <sub>n</sub>
Nanometer	: nm
Nanoparticles	: NPs
Nanosponge	: NS
Newton	: N
Poly Dispersity Index	: PDI
Particle Size	: PS
Phosphate Buffer Saline	: PBS
Response Surface Methodology	: RSM
Reactive Oxygen Species	: ROS
Correlation Coefficient	: $R^2$
Scanning Electron Microscopy	: SEM
Solid Lipid Nanoparticles	: SLNs
Sodium Lauryl Sulfate	: SLS
Standard Deviation	: SD
Texture Profile Analysis	: TPA
Transmission Electron Microscopy	: TEM
Ultra Violet	: UV
X-Ray Diffraction	: XRD
Water in Oil	: W/O
Percentage	: %

Degree Celsius	: °C
Wavelength Maxima	: $\lambda_{\max}$
Weight/Weight	: w/w
Weight/Volume	: w/v
Volume/Volume	: v/v
Zeta Potential	: ZP

## Preface

Cashew nutshell (CNS) is a by-product produced after processing of cashew nuts obtained from the plant *Anacardium occidentale* (family: *Anacardiaceae*). Cashew nutshell contains greenish-yellow liquid entrapped inside the soft honeycomb-like structure of the shell which is popularly known as cashew nut shell liquid (CNSL). CNSL is an extraordinary source of natural polyphenols constituting about 30-35% of the cashew nutshell weight. CNSL primarily contains three major constituents viz. Cardol (15-20%), Cardanol (10%) and Anacardic Acid (60-65%). Anacardic Acid has been proved for its wide pharmacological activities, including antimicrobial, antifungal, antimalarial, anticancer, antiphotaging, anti-inflammatory, and antioxidant activities. The antimicrobial activity of Anacardic acid is due to salicylic acid moiety whereas the alkyl side chain contributes to variation in the activities of different forms ( $C_{15.0}$  -  $C_{15.3}$ ). Maximum antimicrobial activity against *Staphylococcus aureus* has been reported for  $C_{15.3}$  while the activity is least with  $C_{15.0}$ . Anacardic Acid has also been proved to inhibit *Staphylococcus aureus* biofilms by inhibiting quorum sensing. Anacardic Acid ( $C_{15.0}$ ), has been reported to suppress the p300 HAT enzyme which plays a key role in UV-B radiation mediated skin photoaging. It has also been reported to inhibit MMP-1 gene transcription as well as histone modification in the human dermal fibroblasts. The antioxidant potential of Anacardic Acid may also play a role against photoaging by reducing the reactive oxygen species (ROS) production. Although Anacardic Acid has been found to have a diverse range of pharmacological activities yet, its therapeutic potential is limited due to physicochemical characteristics like poor aqueous solubility, vesicant nature, short half-life and thereby limited bioavailability.

The goal of the research work was to improve solubility, physicochemical characteristics of Anardic Acid and thereby enhance the pharmacological activity of Anacardic Acid. The study was designed to improve the anti-biofilm and anti-photoaging activities of Anacardic Acid. Two different formulation approaches were employed to improve anti-biofilm potential, the first approach was intended to develop an inclusion complex of Anacardic Acid and the second involved preparation of chitosan and DNase coated solid lipid nanoparticles (SLNs). Further, HP- $\beta$ -CDnanospongebased gel was developed to enhance the healing potential of photoaged skin. The formulation was developed successfully after the screening of excipients and optimization of various parameters. Finally, the formulation was evaluated for the respective pharmacological activities.

The overall purpose of the research was to provide exhaustive concepts to the readers about various pharmacological activities and physicochemical properties of Anacardic Acid. Further, the philosophy of the research was to provide insights into the Anacardic Acid encapsulated formulations and their potential in combating biofilms and skin photoaging.