4. Chapter 4: Plan of study

4.1 Extraction of Cashew Nut Shell Liquid (CNSL), and isolation of Anacardic Acid

i. Extraction of cashew nut shell liquid (CNSL)

ii. Isolation of Anacardic Acid from CNSL

iii. Isolation of C15:3 and C15:0 among the Anacardic Acid subtypes

iv. HPLC analysis

v. Identification of the compounds using FT-IR and ^{1}H NMR

4.2 Development of $Ana_{C15:3}$/HP-β-CD inclusion complex with enhanced solubility and antimicrobial activity.

i. Preparation of $Ana_{C15:3}$/HP-β-CD inclusion complex

ii. Evaluation of $Ana_{C15:3}$/HP-β-CD inclusion complex

a) UV–Vis spectroscopy

b) Solubility study

c) Phase solubility study

d) Fourier transform infrared spectroscopy (FT-IR)

e) X-ray diffraction (XRD) study

f) Differential scanning calorimetry (DSC)

g) Scanning electron microscopy (SEM)

h) ^{1}H NMR

iii. Antimicrobial activity

a) Effect of $Ana_{C15:3}$ and $Ana_{C15:3}$-inclusion complex on *Staphylococcus aureus* biofilm.

b) Microscopic study of biofilm

4.3 Development of Chitosan and DNase coated solid lipid nanoparticles encapsulating $Ana_{C15:3}$ form improving antimicrobial activity

i. Preparation of $Ana_{C15:3}$ loaded solid lipid nanoparticles

ii. Chitosan and DNase coated SLNs

iii. Characterization of $Ana_{C15:3}$-loaded SLNs

a) Dynamic light scattering (DLS) studies for particle size, polydispersity index, and zeta potential

b) Entrapment efficiency

c) Transmission electron microscopy (TEM)

d) FT-IR Spectroscopy

e) X-Ray Diffraction analysis (XRD)

f) *In-vitro* drug release study

iv. Antimicrobial study

a) Effect of SLNs on the formation of biofilm (Evaluation of biofilm inhibition potential)

b) Effect of SLNs on mature biofilm (Evaluation of biofilm eradication potential)

c) Microscopic study of biofilm

v. Statistical analysis

4.4 Evaluation of therapeutic potential of $Ana_{C15:0}$ encapsulated HP-β-CD nanosponge enriched topical gel against UV-B induced skin photoaging.

i. Synthesis of HP-β-Cyclodextrin nanosponge

ii. Preparation of $Ana_{C15:0}$ loaded nanosponge

iii. Optimization of $Ana_{C15:0}$-NS formulation using 3-level factorial design

iv. Evaluation of $Ana_{C15:0}$-nanosponge

a) Determination of particle size, polydispersity index and zeta potential

b) Determination of percentage entrapment efficiency (EE%)

c) Solubility study

d) Fourier transform infrared spectroscopy (FT-IR)

e) X-ray diffraction (XRD) study

f) Morphology evaluation of $Ana_{C15:0}$ encapsulated nanopsonge

g) *In-vitro* drug release study

v. Preparation of $Ana_{C15:0}$-NS enriched topical gel

vi. Evaluation of $Ana_{C15:0}$-NS topical gel

a) Texture profile Analysis

b) *Ex-vivo* skin permeation and retention study

vii. Animal Study

a) Determination of reactive oxygen species (ROS) level

b) Western blot analysis