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

Thesis submitted in partial fulfillment for the
Award of Degree

Doctor of Philosophy

By

Md Meraj Anjum

DEPARTMENT OF PHARMACEUTICAL ENGINEERING AND TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI - 221005
INDIA

Roll No. 15161004

Year: 2020

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Prof. Sanjay Singh
Supervisor
Vice Chancellor
Babasaheb Bhimrao Ambedkar University
Vidya Vihar, Rae Bareli Road
LUCKNOW-226025

Dr. Ashish Kumar Agrawal
Co-Supervisor

आशीष कु० अग्रवाल / Ashish K. Agrawal
सहायक आचार्य / Assistant Professor
भैषजगरीय अभियांत्रिकी एवं प्रौद्योगिकी विभाग/
Department of Pharmaceutical Engineering & Technology
भारतीय प्रौद्योगिकी संस्थान/Indian Institute of Technology
(काशी हिन्दू विश्वविद्यालय)/(Banaras Hindu University)
वाराणसी-221005/Varanasi-221005
E-mail:ashish.pne@iitbhu.ac.in

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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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Place: IIT (BHU), Varanasi

Signature of the Student

(Md Meraj Anjum)

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Prof. Sanjay Singh
Supervisor
Vice Chancellor
Babasaheb Bhimrao Ambedkar University
Vidya Vihar, Rae Bareli Road
LUCKNOW-226025

Dr. Ashish Kumar Agrawal
Co-Supervisor
आशीष कुo अग्रवाल / Ashish K Agrawal
सहायक आचार्य / Assistant Professor
भैषजकीय अभियांत्रिकी एवं प्रौद्योगिकी विभाग
Department of Pharmaceutical Engineering & Technology
भारतीय प्रौद्योगिकी संस्थान / Indian Institute of Technology
(बनारस हिन्दू विश्वविद्यालय) Banaras Hindu University
वाराणसी-२२१००५ Varanasi 221005
E-mail ashish.phe@iitbhu.ac.in

28/12/20

Signature of Head of the Department
विभागाध्यक्ष / Head
भैषजकीय अभियांत्रिकी एवं प्रौद्योगिकी विभाग /
Department of Pharmaceutical Engineering & Technology
भारतीय प्रौद्योगिकी संस्थान / INDIAN INSTITUTE OF TECHNOLOGY
(बनारस हिन्दू विश्वविद्यालय) / (BANARAS HINDU UNIVERSITY)
वाराणसी–२२१००५ / Varanasi-221005

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Md Anjum.

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Md Meraj Anjum

Table of Contents

List of Figures

P300 among various groups (B), and relative MMP-1 level in different groups under study. All data are presented as mean±SD (n=6) a represents significant difference as compared to UV-B irradiated group brepresents significant difference as compared to control, crepresents significant difference as compared to Blank gel drepresents significant difference as compared to $Ana_{C15:0}$-gel $P<0.005$ one-way ANOVA followed by Newman-Keuls multiple-comparison test.

List of Tables

List of Abbreviations and Symbols

Anacardic Acid	:	Ana
Anacardic Acid C15:0	:	$Ana_{C15:0}$
Anacardic Acid C15:3	:	$Ana_{C15:3}$
Analysis of Variance	:	ANOVA
Carbonyldiimidazole	:	CDI
Chitosan	:	CH
Centimeter	:	cm
Colony Forming Unit	:	CFU
Confocal Laser Scanning Microscopy	:	CLSM
Cyclodextrin	:	CD
β-Cyclodextrin	:	β-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-β-Cyclodextrin	:	HP-β-CD
Hydroxy Propyl Methyl Cellulose	:	HPMC
Kilo Dalton	:	KDa
Luria Bertani	:	LB

Microgram	:	µg
Micrometer	:	µm
Milligram	:	mg
Minute	:	min
Milliliter	:	ml
Millimole	:	mM
Minimum Inhibitory Concentration	:	MIC
Molecular Weight	:	MW
Millivolt	:	mV
MMP	:	Matrix Metallo P
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	:	λmax
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 *Anacardiumoccidentale*(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, antiphotoaging, 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 Anaardic 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-β-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.