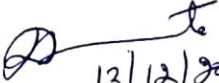


## CERTIFICATE

I certify that the thesis entitled “**Comparative Evaluation of Liposome Encapsulated *Hygrophila auriculata* (Schumach.) Heine Root and Betulin on Wound Healing Activity through *In silico*, *In vitro* and *In vivo* approaches**” submitted for the degree of Doctor of Philosophy (Ph.D.) by **Miss. Deepika E** is the record of research work carried out by her during the period from 2020 to 2023 under my guidance and supervision, and that this work has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other Titles in this Institute or any other University or institution of Higher Learning.

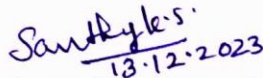
K. Maniavelan  
Signature of the HoD  
13.12.2023

Santosh K. S.  
13.12.2023  
Signature of the Supervisor

  
13/12/2023  
Signature of the Dean

## DECLARATION

I declare that the thesis entitled “**Comparative Evaluation of Liposome Encapsulated *Hygrophila auriculata* (Schumach.) Heine Root and Betulin on Wound Healing Activity through *In silico*, *In vitro* and *In vivo* approaches**” submitted by me for the award of Doctor of Philosophy (Ph.D.) is the record of the work carried out by me during the period from 2020 to 2023 under the guidance of **Dr. K.S. Santhy** and has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, Titles in this Institute or any other University or other similar Institution of Higher Learning.

  
Signature of the Supervisor

  
Signature of the Research Scholar

## ACKNOWLEDGEMENT

I am thankful to the almighty God for the countless blessings that have been showered on me from the beginning of my academic journey.

I extend my sincere thanks to **Dr. T.S.K. Meenakshisundaram** M.A., M.Phil., Ph.D., Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women for providing me with all the facilities for conducting my research work.

I would like to express my sincere gratitude to **Dr. (Mrs.) V. Bharathi Harishankar**, Ph.D., FRSA., Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women for providing me with all the resources for the completion of my research work.

I am extremely grateful to **Dr. (Mrs.) S. Kowsalya**, M.Sc., M.Phil., Ph.D., Registrar, Avinashilingam Institute for Home Science and Higher Education for Women for providing me with all the facilities for my research.

I owe my special thanks to **Dr. (Mrs.) K. Manimozhi**, M.Sc., B.Ed., M.Phil., Ph.D., Controller of Examinations, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for extending all possible help towards the submission of this work.

I would like to express my sincere gratitude to **Dr. (Mrs.) A. Vijayalakshmi**, M.Sc., M.Phil., Ph.D., former Dean, School of Biosciences of Avinashilingam Institute for Home Science and Higher Education for Women for the generous support during my research.

I record my gratitude and heartfelt thanks to **Dr. (Mrs.) Anitha Subash**, M.Sc., M.Phil., Ph.D., Professor of Biochemistry and Dean, School of Biosciences, for her constant encouragement and support.

I would like to express my gratitude to **Dr. (Mrs.) P. Lalitha**, M.Sc., M.Phil., Ph.D., Director, Research and Development (i/c), Avinashilingam Institute for Home Science and Higher Education for Women for all the encouragement that was given to me during my research period.

I extend my sincere thanks to **Dr. (Ms.) K. Manimegalai**, M.Sc., M.Phil., Ph.D, Head of the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women for extending the support for the submission of my thesis.

I would like to show my sincere and profound gratitude to my supervisor **Dr. (Mrs.) K. S. Santhy**, M.Sc., M.Phil., Ph. D., M.C.A., PGDBI, Professor, Department of Zoology and Deputy Dean, School of Biosciences, Avinashilingam Institute for Home Science and Higher Education for Women for all her guidance, direction, advice and patience throughout the period of my research. I owe the entire credit of my work to her, without her dynamic guidance, valuable suggestions, untiring help, this study would never have been the light of the day.

I would like to thank **all the teaching and non-teaching staff members of Department of Zoology** for all the words of encouragement and support extended during my research.

I extend my thanks to **Dr. P.U. Mahalingam**, M.Sc., M.Phil., Ph.D., Professor, Department of Biology, The Gandhigram Rural Institute, Gandhigram - 624 302, my doctoral committee expert for the valuable suggestions and support throughout my research.

I would also like to extend my thanks to my fellow scholars **Nithya R, Gunavathi V, Amrisha Pavithra E, Aparna G Kumar, Revathi unni K and Roobanayaki S** for all their help during my research work.

I am grateful to my family members for the enduring patience, support and stability during the study period.

## LIST OF TABLES

Table No	Title	Page No.
I	Total metabolites and cytological contents of <i>H. auriculata</i>	28
II	Phytochemicals of <i>H. auriculata</i>	29
III	Physicochemical parameters of <i>H. auriculata</i> leaves and roots	53
IV	Qualitative analysis of the primary and secondary metabolites of <i>H. auriculata</i>	54
V	Enzymatic and Non-enzymic antioxidant activity of <i>H. auriculata</i> leaves and roots	57
VI	The phytochemicals identified using LC-MS analysis	58
VII	Ligands chosen for docking	60
VIII	Compliance of compounds to computational parameters of drug likeness (Lipinski's rule of five)	61
IX	ADMET properties	62
X	Bioactivity score of the selected ligands	63
XI	Target wound pathogenic proteins with their characteristics and Structure	64
XII	Binding energy scores of docked complexes	65
XIII	Docking interaction between betulin and elastase	66
XIV	Docking interaction between betulin and collagenase	67
XV	Docking interaction between chlorogenic acid and GSK3 $\beta$	68
XVI	Docking interaction between gelatinase and kaempferol-7-O-Glucoside	69
XVII	Antimicrobial efficacy of liposomes synthesized using <i>H. auriculata</i> root extract and betulin without and with liposomal encapsulation	76
XVIII	Wound contraction percentage	83
XIX	Levels of Wound healing biomarkers	85
XX	Haematological parameters	87

## LIST OF FIGURES

Figure No.	Title	Page No.
1	Structure of skin	11
2	Stages of Wound Healing	13
3	Types of liposome	25
4	Schematic drawing of the percutaneous absorption mechanism of liposomes with different sizes in drug delivery	26
5	Chemical structures of some phytochemicals identified in <i>H. auriculata</i>	30
6	<i>Hygrophila auriculata</i> in its habitat	42
7	DPPH assay of leaves and roots of <i>H. auriculata</i>	55
8	ABTS assay of leaves and roots of <i>H. auriculata</i>	55
9	FRAP assay of leaves and roots of <i>H. auriculata</i>	56
10	LC-MS spectrum of <i>H. auriculata</i> root	59
11	2D plot of betulin complexed with elastase	66
12	2D plot of betulin complexed with collagenase	67
13	2D plot of chlorogenic acid complexed with GSK3 $\beta$	68
14	2D plot of kaempferol-7-O-Glucoside complexed with gelatinase	69
15	FTIR profile of ethanol extract of <i>H. auriculata</i> root	
16	FTIR profile of the liposomes loaded with ethanol extract of <i>H. auriculata</i>	71
17	FTIR profile of the liposomes loaded with betulin	71
18	XRD spectrum pattern of liposomes encapsulated with <i>H. auriculata</i>	73
19	XRD spectrum pattern of liposomes encapsulated with betulin	73

<b>Figure No.</b>	<b>Title</b>	<b>Page No.</b>
20	TEM analysis of liposomes synthesized using <i>H. auriculata</i> root	74
21	TEM analysis of liposomes synthesized the compound betulin	74
22	ZETA potential analysis of liposomes synthesized using <i>H. auriculata</i> root extract	75
23	ZETA potential analysis of liposomes synthesized using Betulin	75
24	Cytotoxicity activity of liposomes synthesized using <i>H. auriculata</i> root extract (LHA) and betulin (LB)	77
25	The cell growth by liposomes synthesized using <i>H. auriculata</i> root extract (LHA) and Betulin (LB)	78
26	Microscopic view keratinocytes cellular gap closure	79
27	Graph of percent cell migration versus time	80
28	Collagen 1 expression in HACAT cells by the liposomes	81
29	Wound area at regular intervals namely, 4th, 8th, 12th and 16th day	82
30	Histological analysis of the healed wound on treating with liposome showed normal appearing epidermis and derm	84

## LIST OF APPENDICES

Appendix No.	Title
I.	Determination of physicochemical parameters
II.	Phytochemical analysis
III.	Preparation of liposomes
IV.	Encapsulation efficiency of the eeha (ethanol extract of <i>H. auriculata</i> ) and betulin loaded liposomes
V.	Plant authentication certificate
VI.	IAEC certificate

## ABBREVIATIONS

ABTS	:	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
ACE	:	Angiotensin Converting Enzyme
ADMET	:	Absorption, Distribution, Metabolism, Excretion, and Toxicity
ANOVA	:	Analysis of Variance
bFGF	:	Basic Fibroblast Growth Factor
CAS	:	Chemical Abstract Service
CAT	:	Catalase
CRP	:	C-Reactive Protein
DMBA	:	Dimethylbenz(a)anthracene
DPBS	:	Dulbecco's Phosphate Buffered Saline
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
ECM	:	Extracellular Matrix
EDTA	:	Ethylene Diamine Tetra Acetic acid
EGF	:	Epidermal Growth Factor
EPR	:	Enhanced Permeation and Retention
ESR	:	Erythrocyte Sedimentation Rate
FRAP	:	Ferric Reducing Power Assay
FTIR	:	Fourier Transform Infrared
GPCR	:	G-Protein Coupled Receptor
GPx	:	Glutathione Peroxidase
GSK-3 $\beta$	:	Glycogen Synthase Kinase-3 $\beta$
GST	:	Glutathione S Transferase
HACAT	:	Human Keratinocyte cell line
HBA	:	Hydrogen Bond Acceptor
HBD	:	Hydrogen Bond Donor
hEGF	:	Human Epidermal Growth Factor
HepG2	:	Hepatoblastoma cell line
hERG	:	Human Ether-a-go-go Related Gene
HMGB1	:	High-Mobility Group Box 1
IGF-1	:	Insulin-like Growth Factor 1
IL-1 $\beta$	:	Interleukin-1 Beta
LCMS	:	Liquid Chromatography–Mass Spectrometry

MAPK	:	Mitogen-Activated Protein Kinases
MIP-1 $\alpha$	:	Macrophage Inflammatory Protein-1 Alpha
MMPs	:	Metalloproteinases
MRSA	:	Methicillin-Resistant <i>Staphylococcus aureus</i>
MTT	:	Phosphate-Buffered Saline
NBT	:	Nitro Blue Tetrazolium
NF $\kappa$ B	:	Nuclear Factor kappa B
NOS	:	Nitric Oxide Synthases
Nrf2	:	Nuclear factor erythroid 2-related factor 2
PBS	:	Phosphate Buffered Saline
PDB	:	Protein Data Bank
PDGF	:	Platelet Derived Growth Factor
PHMB	:	Poly Hexamethylene Biguanide
POD	:	Peroxidase
PPAR- $\gamma$	:	Peroxisome Proliferator-Activated Receptor gamma
RANTES	:	Regulated upon Activation Normal T Cell Expressed and Presumably Secreted
ROS	:	Reactive Oxygen Species
SOD	:	Superoxide Dismutase
SSTIs	:	Skin and Soft Tissue Infections
STAT3	:	Signal Transducer and Activator of Transcription 3
TEM	:	Transmission Electron Microscopy
TGF- $\beta$	:	Transforming Growth Factor- $\beta$
TGF- $\beta$	:	Transforming Growth Factor-beta
TNF- $\alpha$	:	Tumor Necrosis Factor alpha
UV-Vis	:	UltraViolet Visible spectrometry
VEGF	:	Vascular Endothelial Growth Factor
XRD	:	X-Ray Diffraction