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CERTIFICATE

It is certified that the work contained in the thesis titled **"Exploring the Cytotoxic Potential of Secondary Metabolites of *Araucaria cunninghamii* Mudie: LCMS-based Approach"** by **Mr. Bharat Sahu** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy and SOTA for the award of Ph.D Degree.

Date 10/10/24

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DECLARATION BY THE CANDIDATE

I, **Bharat Sahu**, certify that the work embodied in this PhD thesis is my own bonafide work and carried out by me under the supervision of **Dr. Shreyans K. Jain,** from **January 2018** to **January 2024**, at the **Department of Pharmaceutical Engineering & Technology**, **Indian Institute of Technology (Banaras Hindu University), Varanasi**. The matter embodied in this Ph.D. 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 work has been cited in my work in this thesis. I further declare that I have not wilfully copy any other's work, paragraphs, text, data, results, *etc*.,or reported in journals, books, magazines, reports dissertations, thesis, *etc*., or available at websites and have not included them in this thesis and have not cited as my own work.

Date: 10/10/24

Bharat Sahu

Bharat Sahu

Place: IIT (BHU), Varanasi

CERTIFICATE BY THE SUPERVISOR(S) AND HEAD OF THE DEPARMENT

It is certified that the above statement made by the student is correct to the best of my/our knowledge.

Dr. Shreyans Kumar Jain
(Supervisor)

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Bharatsahu

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LIST OF ABBREVIATIONS

Å	Angstrom
ACN	Acetonitrile
ADMET	Absorption, distribution, metabolism, excretion and toxicity
AO	Acridine orange
BIOS	Biology oriented synthesis
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
CD_3OD	Methanol –d4
$CHCl_3$	Chloroform
COSY	Correlation spectroscopy
DCM	Dichloromethane
DEPT	Distortion less Enhancement by Polaromatory Transfer
DMSO	Dimethylsulphoxide
DNP	Dictionary of natural product
DNA	Deoxyribonucleic acid
DTS	Diverted- total synthesis
ESI	Electron spray ionisation
EtoAc	Ethyl acetate
FDA	Food and drug administration
FOS	Function-oriented synthesis
g	Gram
h or hr	Hour
H-bond	Hydrogen bond
HCl	Hydrochloric acid
HMBC	Heteronuclear multiple bond correlation
HOBt	Hydroxybenzotriazole
HRMS	High resolution mass spectrometer
HSQC	Heteronuclear single quantum coherence spectroscopy
IR	Infrared spectroscopy
J	Coupling constant
kg	kilogram
LC	Liquid chromatography

LC-MS	Liquid chromatography-mass spectroscopy
m	Metre
MD	Molecular dynamic
MeOH	Methanol
MHz	Megahertz
min	Minutes
ml	Millilitre
mp	Melting point
MS	Mass spectrometer
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser effect spectroscopy
NP	Natural product
NO	Number
PDB	Protein data bank
Rf	Retention factor
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
SAR	Structure activity relationship
SD	Standard deviation
TBHP	Tert-butyl hydroperoxide
TLC	Thin-layer chromatography
TMS	Tetramethylesilane
v/v	Volume by volume
w/w	Weight by weight
uL	Microliter
µM	Micromole

PREFACE

Since antique, natural products mainly the plants, have been widely used to treat many ailments. There are documents showing clinical uses of many herbs by people from thousands of years before Christ. Medicinal plants and microorganisms were the major source of medicines over many centuries. We have many examples of plant-derived extracts and/or compounds isolated from plants that have been widely used in the treatment of many significant diseases.

This thesis work has planned for discovery of new cytotoxicity lead molecules, derived from the natural product. In this work *A. cunninghamii* was selected for the drug discovery. The gum resin, and leaves were taken for isolation and characterization of cytotoxic lead. *A. cunninghamii* belongs to family *araucariaceae* commonly known as hope pine.

The consists of six chapters: (1) Introduction (2) Aims and objectives (3) Review of the literature (4) Exploring the cytotoxic potential of *A. cunninghamii* Gum resin *by* LC-MS based dereplication and (5) Exploring the cytotoxicity of biflavones from *A. cunninghamii* leaves: By Combined LC-MS-metabolomics, Network Pharmacology and *in-vitro* cytotoxicity. (6) Conclusion and future prospects

Chapter 1: The role of natural product in drug discovery is discussed, along with the examples, advantage and disadvantage of natural products. Various approaches for exploring the natural sources for drug discovery have discussed briefly.

Chapter 2: The aim and objective along with plan of thesis work is incorporated here.

Chapter 3: In this chapter deals with the review of the literature mainly focused on the pharmacological and phytochemical aspects.

Chapter 4: Describes the first objective: Exploring the cytotoxic potential of *A. cunninghamii* gum resin by LC-MS based dereplication. The phytochemical investigation of gum resin is discussed. Fourteen compounds, along with one new compound was isolated from ethanol extract of *A. cunninghamii* gum resin. All the isolated compounds were characterised on the basis of detailed NMR and mass spectral techniques. New metabolites have shown potential cytotoxic activity.

Chapter 5: This chapter deals with exploring the cytotoxicity of biflavones from *A. cunninghamii* leaves: using the LC-MS-metabolomics, Network Pharmacology and *in-vitro* cytotoxicity. The leaves of *A. cunninghamii* were investigated for phytochemistry and 14 compounds were isolated including flavone. All the known compounds were characterized by extensive NMR studies and comparing with reported data. The compounds were elucidated based on comprehensive spectroscopic analysis, including 1D, 2D NMR and HRMS. Cytotoxic potential of biflavones were explained based on the network pharmacology and in-vitro cytotoxicity.

Chapter 6: Describes the conclusion and future prospects of the thesis. The study suggested that the compound might serve as an interesting lead for further *in-vivo* and preclinical studies and further detailed cytotoxic activity.

The reference has been used to conduct research after chapter 6. An appendix of additional supporting information, spectral data of the respective compounds and a list of publications during the course of Ph.D. are included.