LCMS-Based Dereplication, Chemical Modification and Cytotoxic Evaluation of New Phytoconstituents Isolated from *Dysoxylum malabaricum* Bedd.

Thesis submitted in partial fulfilment for the award of degree

Doctor of Philosophy

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List of Abbreviations

2D NMR	Two-dimensional NMR
ACN	Acetonitrile
Calcd	Calculated
CD_3OD	Methanol-d4
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
COSY	Correlation spectroscopy
d, dd	Doublet, Doublet of doublets
DAPI	(4',6-Diamino-2-phenylindole)
DCM	Dichloromethane
DEPT	Distortionless enhancement by polarization transfer
TD-DFT	Time-dependent density functional theory
DMSO	Dimethylsulphoxide
DNP	Dictionary of Natural Products
ECD	Electronic circular dichroism
EDG	Electron donating group
Equiv	Equivalent
ESI	Electron spray ionization
EtOAc	Ethyl acetate
EWG	Electron withdrawing group
FDA	Food and drug administration
GC	Gas chromatography
g	Gram
mg	Miligram
h or hr	Hour
HMBC	Heteronuclear multiple bond correlation
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometer
HSQC	Heteronuclear single bond correlation

IR	Infrared spectroscopy
J	Coupling constant
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
MeOH	Methanol
MHz	Megahertz
Min	Minutes
mL	Mililitre
mmol	Milimol
mp	Melting point
MS	Mass spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Ni-NiO	Nickel-nickel oxide
NBS	N-bromo succinamide
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser effect spectroscopy
PBS	Perssian blue analogs
Rf	Retention factor
SD	Standard deviation
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilane
t_R	Retention time
v/v	Volume/volume
w/w	Weight/weight
µl or µl	Microlitre
µM	Micromolar

Preface

Nature has long been a valuable source of medicinal compounds, offering diverse chemical structures with potential therapeutic benefits. The exploration of bioactive compounds from natural sources often involves systematic screening plant extracts. Approximately 50 % of approved drugs directly or indirectly belong to natural products. Phytochemical investigations have led to the discovery of natural product-based leads for drug development, owing to their structural diversity and physicochemical properties. Overall, natural products remain a promising option for drug discovery due to their unique attributes and potential therapeutic value. However, the conventional extraction and isolation method of natural products is a time-consuming, expensive process and re-discovery of known compounds occurs. To address these challenges, natural product-based drug discovery programs have adopted multidisciplinary approaches and innovative technologies. These efforts have led to the identification of numerous natural product-based leads. Dereplication, in particular, has emerged as a key strategy for the early and efficient identification of compounds, helping to avoid rediscovery of known compounds and accelerating the discovery process. Hyphenated analytical techniques based on dereplication are one of the advanced approaches for speeding up the identification of natural products. Liquid chromatography (LC) coupled with mass spectrometry (MS) are commonly used hyphenated techniques for the phytochemical investigation of extract. LC-MS has been utilized since the early days of dereplication due to its ability to yield substantial structural information rapidly. This method facilitates online de novo structure determination of natural products when integrated with databases like Dictionary of Natural Products (DNP), Super Natural II, MassBank, MetLin, etc.

In the present study, considering the signification of these techniques, a DNP-based strategic dereplication platform was established where the taxonomic, chemical

classification and spectroscopic information acquired by LC-MS and NMR are applied for rapid identification of known metabolites so that a structure that is proposed to be a new one, can be targeted. This study focuses on the relatively unexplored plant *Dysoxylum malabaricum* Bedd. (Meliaceae), a tree species endemic to the Malabar region of India. The work embodied in this thesis has been presented in six chapters.

Chapter 1: The chapter introduces the impact of natural products on drug discovery, where the chemical diversity of natural products influenced by biodiversity has been discussed. Also, screening of biological extract *via* conventional and advanced approaches like hyphenated techniques-based dereplication strategy for isolation of natural products has been discussed.

Chapter 2: The objective and plan of the research work are incorporated here.

Chapter 3: The chapter deals with the background of the LC-MS-based dereplication approach for the identification and isolation of metabolites. It incorporates the objective of one of the present research works, where an LC-MS-based dereplication strategy was established to identify new compounds from a relatively unexplored plant, *Dysoxylum malabaricum*, using the DNP database. The mass ion peaks were targeted from the LC-MS profile of crude bark extract, followed by isolation, purification and structure elucidation. The isolated compounds were evaluated for cytotoxicity against a panel of cancer cell lines.

Chapter 4: The chapter constitutes the second objective of the research work describing chemical modifications in the isolated compound at the targeted reactive sites. During the chemical modification, two synthetic methodologies were developed. One of the methods deals with $ZnCl_2$-catalyzed amide synthesis using phenylhydrazine as an amine partner, and another methodology deals with Ni-NiO-catalyzed regioselective halogenation and

oxidative esterification in the presence of N-halosuccinamide. Further, the synthesized derivatives were evaluated for cytotoxicity.

Chapter 5: It outlines the summary and conclusion of the present research work.

The references used to conduct the research are present in the section after chapter 5. An appendix of additional supporting information, spectral data of representative compounds and a list of publications during the course of the Ph.D. are included.