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

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Doctor of Philosophy

By

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Chapter 5

Summary and Conclusion

5. Summary and conclusion

The present study was focused on a DNP-based strategic dereplication platform where the taxonomic, chemical classification and spectroscopic information acquired by LC-MS and NMR were applied for the rapid identification of known metabolites so that a structure that is proposed to be a new could be targeted. The strategy was applied to the relatively unexplored plant *Dysoxylum malabaricum* (Meliaceae), a species endemic to the Malabar region of India. The genus possesses significant medicinal properties, as revealed by the metabolites from this genus, which have demonstrated promising anti-inflammatory, anti-viral, anticancer and antarthritics. However, *D. malabaricum* has not been much explored phytochemically. LCMS-DNP-based dereplication followed by bioassay-guided fractionation enabled targeted identification of the new bioactive compounds. The shade-dried bark of *D. malabaricum* was extracted, fractionated and subjected to LC-MS profiling followed by cytotoxicity screening. The fractions were evaluated for cytotoxicity using MTT assay against various human cancer cell lines *viz* lung, breast, pharynx, and embryonic kidney cell lines. The ethyl acetate fraction was found to be most cytotoxic against breast cancer cells. The active fraction was subjected to bioassay-guided fractionation *via* column chromatography and HPLC. The mass peaks in the range of 400-600 Da were targeted, isolated and purified. The isolated compounds were characterized *via* extensive spectral analysis like NMR, HRMS experiments and their absolute configurations were determined by ECD calculations. A total of fourteen triterpenoids were isolated for the first time from this species, out of which eight were new triterpenoids containing cycloartane nucleus. The new triterpenoids were named as dihydrobeddomeilactone, hydroxybeddomeilactone, mahamanalactone A, dihydromahamanalactone A, dihydrobinectarilactone, mahamanadiol, mahamanalactone C, dehydromahamanadiol. These triterpenoids were evaluated against MCF-7, A549

MDA-MB-231, Hs578t, ZR-75-30, FaDU, BT549, MCF-7, MDAMB-231and T-47D, breast cancer cell line and induced apoptotic cell death. Dihydrobinectarilactone was found to be most cytotoxic against MCF-7 breast cancer cells. This compound induced cell death *via* cell cycle arrest through the downregulation of CDC20 and CDC25 proteins. It was found that *Dysoxylum malabaricum* represents a repository of novel bioactive compounds that could possibly find potent cytotoxic compounds inhibiting various cancer cell growth, especially breast cancer.

Chemical modifications were performed on the isolated compound obtained in high amounts to enhance the potency and investigate the reactive site present in the molecule. During the chemical modification of beddomeilactone in order to add indole moiety at the carbonyl carbon of the aliphatic side chain, a new methodology was developed for amide synthesis. The reaction of beddomeilactone with phenylhydrazine in the presence of zinc chloride resulted in amidation. Further, this amidation reaction protocol of carboxylic acid and hydrazine was optimized for long-chain fatty acids, amino acids, and complex natural products. The reaction offers a clean protocol for amide synthesis in which ammonia is only a by-product. This is the first and unique report of direct amide synthesis using carboxylic acid with hydrazine as an amine partner. Later, the halogenation of beddomeilacton was proposed to synthesize halogenated products using N-bromosuccinamide to introduce further functionalization at the reactive site. The halogenation resulted in the formation of unusual ester derivatives along with brominated products. Further, the reaction protocol was optimized using Ni-NiO nanoparticles as catalyst, resulting in selective beddomeilactone halogenation. This reaction protocol has scope for further modification towards the environment-friendly process as it tolerates the semi-aqueous reaction medium. The reaction was also optimized for halogenation and esterification of other substrates. The Ni-NiO nanoparticle catalyst was optimized for

regioselective halogenation and esterification on aromatic carbonyl compounds and phenolic substrates, respectively. A remarkable para-selective halogenation was achieved for phenolic substrates under semi-aqueous conditions. Interestingly, the deactivated ring system favors oxidation *via* nucleophilic addition*, viz.* oxidative esterification, acidification, acetalization, ketalization, thioacetalization, and thioketalization. The semisynthetic derivatives of beddomeilactone obtained using both methodologies were evaluated for their cytotoxic evaluation against breast cancer cell lines and found to be more cytotoxic than beddomeilactone.