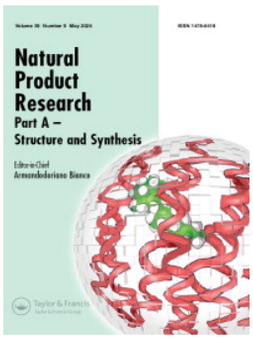

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Mahamanalactone A, a new triterpenoid from *Dysoxylum malabaricum* bark: a case study for rapid identification of new metabolites via LC-HRMS profiling and database mining strategy

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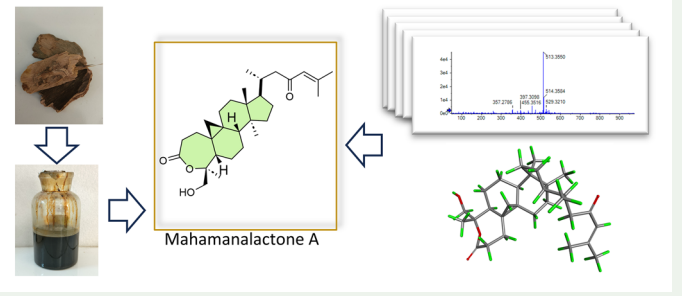
ABSTRACT

In this recent investigation, the focus centred on exploring the potential phytoconstituents within the bark of *Dysoxylum malabaricum*. A profiling strategy employing LC-HRMS (Liquid Chromatography-High Resolution Mass Spectrometry) was implemented for the rapid identification of compounds from the bark extract. The crude extract underwent fractionation, resulting in the isolation of four previously known compounds (**1–4**) and a novel cycloartane triterpenoid named Mahamanalactone A (**5**). Compound 5 represents a cycloartane triterpenoid with a modified ring-A, featuring £-caprolactone fusion at positions 4 and 5, distinguishing it from other reported compounds where £-caprolactone is typically fused at positions 3 and 4. Cytotoxicity assessment revealed that the newly identified compound **5** exhibited a moderate cytotoxic profile (IC_{50} 29 to 78 µM) against a panel of cancer cell lines.



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1. Introduction

Natural products are a critical source of drug leads and play an essential role in drug discovery (Newman and Cragg 2020). To explore the natural products, various strategies and protocols have been demonstrated. LC-HRMS-based metabolomics profiling of extracts is a common practice to overcome time-consuming isolation and structure elucidation procedures of the known compound. Usually, the mass spectrometric-derived information is used in the chemical database to identify the metabolites. In the natural products-based drug discovery field, the LC-HRMS profiling technique focuses on the early identification of known metabolites that overcome time-consuming isolation and structure elucidation procedures of the known compound and help target new metabolites (Lang et al. 2008). A comprehensive database exclusively for natural products is required to sort the metabolites based on the taxonomic classification, physicochemical and spectral properties. In this work, a dictionary of natural products (DNP) was used which constitutes the collection of more than 300,000 natural products and provides access to the biological source (taxonomic classification), chemical (molecular weight, molecular formula and chemical classification, etc.), physical (UV, melting point, boiling point, etc.) and complete or sub-structural information of natural products (Jain et al. 2013; Bodare et al. 2017). The mass data, in conjunction with mutually supportive data, such as retention time (t_R), UV/vis, H-NMR spectra and substructure search through database mining, aids in accurately identifying metabolites (Kushwaha et al. 2022). Here, a DNP-based strategic dereplication platform was established where the taxonomic, chemical classification and spectroscopic information, acquired by LC-HRMS 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 for isolation, structural elucidation and bioactivity. This study focuses on relatively less explored plant *Dysoxylum malabaricum* (Meliaceae) a tree species native to Malabar region of Western Ghat of India (Bodare et al. 2017). The genus possesses significant medicinal properties as revealed from the isolated alkaloid rohitukine from *D.binecteriferum* which inspired the discovery of clinical drug candidates, flavopiridol and riviciclib, showing CDKs inhibitory action against lymphocytic leukaemia (Jain et al. 2012). The isolated metabolites from this genus have demonstrated promising pharmacological activity (Hu et al. 2014, Jiang et al. 2015, Kashiwada et al. 1992, Yan et al. 2014, Zhou et al. 2015, Zou et al. 2017). Plant species *D. malabaricum* has not been explored phytochemically and pharmacologically. In one of the recent studies, the crude extract has shown significant cytotoxicity against the cancer cell lines. Further fractionation followed by repeated chromatography yielded four triterpenoids viz beddomeilactone (**1**) binecterlactone-A (**2**), dihydrobinecterlactone-A (**3**) and mahamanadiol (**4**) (Figure 1) (Bhardwaj et al. 2023). Considering the diverse phytochemical properties and the nature of relatively unexplored phytoconstituents present in bark, an LC-HRMS profiling study was conducted on the bark extract of *D. malabaricum* to detect novel compounds.

2. Results and discussion

The shade-dried bark of *D. malabaricum* was extracted by methanol-dichloromethane (1:1) at room temperature. The crude extract obtained after filtration was

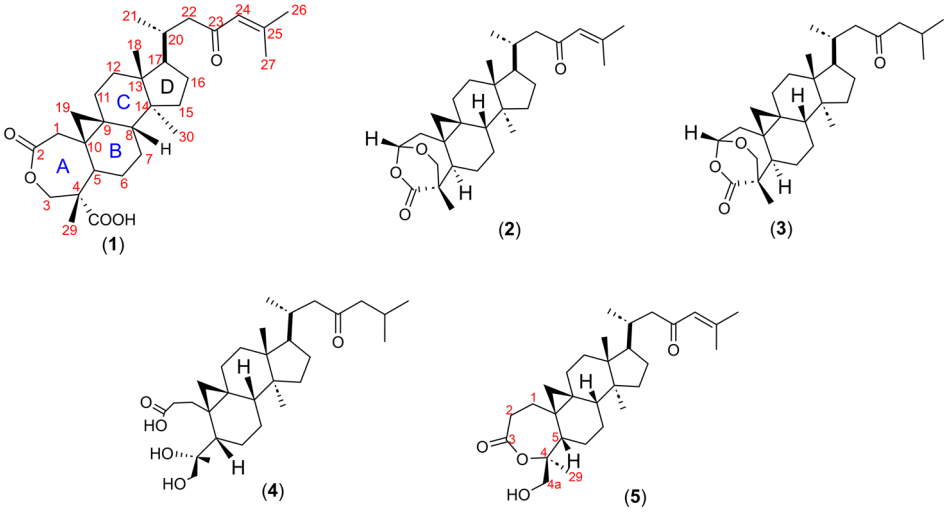

Figure 1. Compounds isolated from *D.malabaricum* (**1–5**).

concentrated. For the fractionation, the extract was suspended in distilled water and fractionated sequentially by hexane, ethylacetate and chloroform. The highest extractive content was enriched in the ethylacetate fraction. It was further loaded to silica gel column and fractionated into five sub-fractions using hexane-ethylacetate increasing polarity gradients of 100% hexane (F1), 15% ethylaceate-in hexane (F2), 30% ethylaceate-in hexane (F3), 60% ethylaceate-in hexane (F4) and 100% ethylaceate (F5). Active sub-fraction F2 was subjected to LC-HRMS profiling as shown in Table S.1 in S.I. In LC-HRMS spectra, the majority of individual mass peaks were in the range of 400–600 Da, suggested the presence of triterpenoids, further search for *Dysoxylum* genus in the DNP database confirms 184 (of total of 300) compounds belongs to triterpenoids class. Major ionized metabolites are summarised in Table S.3 in S.I.

To identify the new metabolite in the active subfraction F2, LC-HRMS-derived accurate mass were sorted within the acceptable range (± 5 ppm). Following eight major ions were noticed at different retention time; 9.48, 9.88, 10.04, 11.11, 11.29, 11.57, 12.09 and 12.96 min. Out of the eight identified peaks, four of them correspond to the previously reported compounds. The most abundant ion 485.3254 $(M+H)^+$ appeared at 12.09 min in the LC-HRMS profile correspond to molecular formula $C_{30}H_{44}O_5$+H^+ was characterized as beddomeilactone (**1**). Compound **2** showed mass *m/z* 469.3314 at 12.96 min, corresponding to $C_{30}H_{44}O_4$+H^+ and identified as binectarilactone-A (**2**). Similarly, compound **3** with *m/z* 471.3459 appeared at 11.57 corresponds to $C_{30}H_{46}O_4$+H^+ identified as dihydrobinectarilactone-A (**3**) and compound **4** with *m/z* 491.3611 at retention time of 9.48 min having molecular formula $C_{30}H_{48}O_4$+H^+ was characterized as mahamanadiol (**4**) (Bhardwaj et al. 2023). Remaining four compounds were identified as new metabolites and subjected to the purification using the HPLC. Only one metabolite (**5**) was isolated which appeared at 11.29 min in LC-HRMS spectra in sufficient amount for characterization. The concentration of other metabolites present in fraction was very less (<1 mg). However, a DNP search

revealed that these compounds could be new metabolites. Compound **5**, showed *m/z* 471.3487 for molecular formula $C_{30}H_{46}O_4$+H^+ and its acetonitrile adduct $[M + CH_3CN]^+$ at *m/z* 511.3378 in its HRESIMS spectra. The DNP search for D. *malabaricum* resulted in zero hits, indicating the new compound. Further, HPLC purification followed by ^{1}H-NMR found several spectral properties identical to compounds **1–4** demonstrating the presence of a fused cyclopropane ring. It was also assumed to be a new cycloartane, therefore subjected to detailed spectral characterization. The 1D and 2D NMR data of compound **5** suggested the existence of same carbon skeleton as compound **1** except differences in ring A. Only one carboxylic carbon and eleven methylene groups were observed in 13 C and DEPT-135 NMR spectra, respectively, as shown in Table S.4 in S.I. This infers the presence of CH_2OH instead of COOH in compound **5**. The methyl proton at 0.96 showed four major HMBC correlationswith carbon at 16.8 (CH_3), 40.1 (CH), 68.4 (CH_2OH) and 76.5 (C). The proton of CH (40.1) at 2.00 showed correlations with cyclopropane ring's CH2 (2.66 and 1.21) and quaternary carbon at 24.6 and 76.5. The junction quaternary carbon at 24.6 correlated with protons at 2.66 and 1.21 attached to C-1 (30.5) and CH_2 of cyclopropane ring proton at 0.62 and 0.47 attached to carbon at 29.2. From the COSY data, a spin system was observed among the methylene protons of the cyclopropane ring at 0.62 and 0.47. The coupling of protons of C-1 (2.66 and 1.26) and C2 (2.09) was also noticed. The protons of the ring B carbon C-5 (CH, 2.00), C-6 (CH_2, 2.11 and 1.88), C-7 (CH_2, 1.24) and C-8 (CH, 1.31) were fixed by COSY correlations (Figure S.14a in S.I). The structural feature of ring A of compound **5** is fused £-caprolactone fused at 4 and 5 positions, while in compound **1**; the £-caprolactone was fused at 3 and 4 positions. No cross-peaks were observed between H-4a/H-29 in the NOE spectrum showing them in different plane. However, H-4a/H-29, H-4a/H-5 and H-5/H-19 showed NOE coupling (Figure S.11 and Figure 14b in S.I). Compound **5** showed negative cotton effect as optical rotation decreases on decreasing wavelength and geometric maximum (peak) and minimum (troughs) were observed in the ECD spectra in Figure S.15 in S.I. The experimental and TD-DFT-simulated ECD spectra showed a good match and unveiled the stereochemistry of compound **5** and characterised it as (1S,5aR,6aS,8aR,9R,11aS,11bS,13aS)-1-(hydroxymethyl)-1,8a,11a-trimethyl-9-(6-methyl-4-oxohept-5-en-2-yl)tetradecahydro-3H,6H-cyclopenta[5,6]cyclopropa[1,8a]naphtho[2,1-c]oxepin-3-one and named as Mahamanalactone A.

The isolated compounds **1**–**5** were evaluated against seven human cancer cell lines viz MCF-7 (breast carcinoma), A549 (lung epithelial carcinoma), MDA-MB-231(breast adenocarcinoma), Hs578t (breast carcinoma), ZR-75-30, FaDU (human pharynx squamous cell carcinoma) and BT549 (breast carcinoma) for their cytotoxicity screening using MTT assay for 48 h to identify the most active compound. Compound **5** showed the highest cytotoxicity with IC_{50} 29 µM against the BT549 cell line. Compounds **1** and **2** also showed moderate cytotoxicity compared to others with IC_{50} range of 20–40 µM against MCF-7, MDA-MB-231, Hs578t and BT549 cells. While detail cytotoxicity study of compounds **3** and **4** against MCF-7 cell line have been reported in our previous study. The cytotoxicity results of the isolated compound are summarised in Table S.16 in Supporting information.

3. Conclusion

The study demonstrated the application of LC-HRMS to identify novel compounds by applying different spectral information and DNP chemical database. The LC-HRMS profiling effectively detected the new triterpenoids in the ethylacetate fraction. Further, the targeted compounds were subjected to isolation and the study resulted in the isolation of five cycloartane triterpenoid containing one new compound named Mahamanalactone A (**5**). Also, this compound showed notable cytotoxicity against BT549 breast cancer cells.

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