HR-LC-MS based profiling of phytochemicals from methanol extracts of leaves and bark of *Myristica dactyloides* Gaertn. from Western Ghats of Karnataka, India

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ABSTRACT

Untargeted profiling of phytochemicals from plant extracts would aid in the exploration of various groups of compounds and assist in the identification of new bioactive compounds, reducing the redundancy in compound identification. The potential bioactive phytochemicals present in *Myristica dactyloides*, an endemic ethnomedicinal plant widely used in traditional treatment practices, were explored and cataloged in this study. The untargeted phytochemical profile of active methanolic leaves and bark extracts was assessed by Ultra high-performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry. Preliminary screening results highlighted the high efficiency of methanolic extract as an effective antioxidant and anti-inflammatory agent, along with a remarkable amount of total phenolics and flavonoid content. Thus, the methanolic extracts of leaves and bark samples were further subjected to catalog its chemical constituents through untargeted metabolite profiling. Analysis of high-resolution liquid chromatography-mass spectrometer spectra, exhibited 3,813 and 1,797 molecular features in the ESI+ mode with clean retention time-exact mass, resulted in identifying 35 major therapeutically important common compounds for the first time in both leaves and bark extracts of *M. dactyloides*, fitting to major groups like lignans, neolignans, phenylpropanoids, diarylhetranoid, flavonoids, and others. Our results prove the presence of Myoinositol, Malabaricone B, Malabaricone C, Malabaricone D, and 1-(2,6-dihydroxyphenyl)-9-(4-hydroxy-3-methoxyphenyl) nonan-1-one previously reported from *M. dactyloides*, along with promising chemical signatures like Monotropein, Austrobailignan 7, Fragransol B, Guaiacin, Myricanone, Nectandrin A, Argenteane, and Epicatechin to the phytochemical catalog of *M. dactyloides*. Furthermore, our research not only confirms the existence of previously known molecules and adds new phytochemicals, but it also sheds light on the wide application of *M. dactyloides* for possible bio-prospecting for a new chemical entity.

1. INTRODUCTION

The dependence of humans on plants to survive or to treat has been inevitable in the long history of humanity on this planet, as shown by comprehensive documentation. Also, now, plants play an important part in the healthcare system as a treasure for bioactive compounds. The scientific community’s dedication to conserving natural treasures has risen as never before, and the current trend is to create sustainable and reliable solutions
to prevent overexploitation by providing target-specific treatment/isolation of compounds. The concept of distinguishing chemical entities from plants spawned a modern branch of science known as ethnopharmacology, which seeks to separate possible lead drugs from medicinally important plants [1]. Initially, pharmacological researchers in search of new bioactive compounds faced significant technological difficulties in extracting, isolating, and characterizing the compounds. Despite significant obstacles, researchers have been able to address methodological difficulties in characterizing plant metabolites from chemically diverged complex crude mixtures by continuing to work on studying the complex chemistry in plants. This was made possible by researchers advocating for the use of liquid chromatography–mass spectrometry techniques for untargeted phytochemical profiling in recent years [2–5]. Because of its accuracy, sensitivity, speed, and specificity, the ability of liquid chromatography with tandem mass spectrometry to couple with other chromatographic techniques provides many advantages in studying and characterizing the phytoconstituents of medicinal plants. Furthermore, advances in computational bioinformatics techniques and the development of online metabolite databases have made detection easier to an extent but with its limitations [6,7]. Plant metabolite characterization using chromatographic methods has advanced in recent decades, contributing significantly to the cataloging of a large number of metabolites from pharmacologically relevant plants. However, there is still a need to use chromatographic-based chemical fingerprinting extensively for a number of medicinally significant plants that have remained unidentified due to traditional extraction and identification procedures.

The current research is one such approach to cataloging and validating the chemical constituents of *Myristica dactyloides* bioactive potentials. It is a prominent member of the Myristicaceae family, native to India and Sri Lanka, with 18–21 genera and nearly 300–520 species [8,9]. It is listed as vulnerable by the International Union for Conservation of Nature due to its widespread use and exploitation for its wide range of medicinal benefits. Coughs, bronchitis, fever, burning sensations, inflammation of joints, skin disorders, wounds, sleeplessness, indigestion, liver disorders, and worms are all treated with arils [10]. Bark and leaves are used in Ayurvedic preparations and decoctions to treat throat ailments [11]. Various researchers around the world have explored the chemistry and bioactive potentials of a similar species, *Myristica fragrans* [12]. Despite having the similar pharmacological potential to *M. fragrans*, *M. dactyloides* has remained unexplored except for a few early attempts [13–17]. In these reports, Myoinositol, Malabaricone A, B, C, D, Dactyloidin, Acylresorcinols, Arylalkanones, and Lignans were found in various parts of the *M. dactyloides* Gaertn.

With this background, the present investigation was carried out to catalog the chemical constituents of *M. dactyloides* using Ultra high-performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry (UHPLC-ESI-QTOF-MS) analysis and to validate their pharmacological significance through *in-vitro* assays in the context of *M. dactyloides* extracts anti-inflammatory potential. The study emphasizes the importance of early metabolite identification in crude extracts to prevent redundancy in the characterization of new bioactive compounds in drug discovery process.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

All the solvents used for the extraction of plant materials were of analytical grade and hydrochloric acid was procured from Sisco Research Laboratory (Mumbai, India). Reagents, enzymes, and positive controls such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri (2-115 pyridyl)-s-triazine, Quercetin, gallic acid, Ascorbic acid (AC), butylated hydroxytoluene, 15-Lipoxygenase (15-LOX) were purchased from Sigma-Aldrich (St. Louis, MO). Solvents used for high-resolution liquid chromatography-mass spectrometer (HR-LCMS) were of Spectroscopic grade obtained from SD Fine Chemicals Limited (SDFCL; Mumbai, India).

2.2. Collection of Plant Material

Naturally grown healthy leaf and bark samples of *M. dactyloides* were harvested and collected from the Kigga village (13°24′5.8″N 75°11′01.7″E) located at the Western Ghats region of Karnataka, India, during September month of the monsoon season. A sample specimen of the plant was deposited at the herbarium of the Department of Studies in Botany, University of Mysore, Mysore, India. Plant materials were collected in sterile polythene bags and processed within 12 hours at the laboratory.

2.2.1. Preparation of extracts

The leaf and bark samples were separated, washed under running tap water to reduce undesirable materials, followed by shade drying at room temperature for 5–6 days. The dried leaf and bark samples were ground to a coarse powder using the mechanical grinder and stored at 4°C until further use. The leaves and bark powders were sequentially extracted using 500 ml of solvents with increasing polarity (hexane < chloroform < methanol) by continuous hot percolation method using a Soxhlet apparatus (boiling point, 52°C–62°C) until the solvent became colorless. The solvent extracts were concentrated in a rotary flash evaporator (G1 Heidolph, Germany) under controlled pressure and stored at 4°C before further analysis.

2.3. Phytochemical Analysis

2.3.1. Estimation of total phenolic contents (TPC)

Estimation of the TPC in plant extracts gives an overview of the phenolic compounds which indirectly are responsible for the bioactivity. TPC estimation was carried using the Folin–Ciocalteu reagent method according to Ainsworth and Gillespie [18]. The TPC of samples was estimated based on the standard gallic acid calibration curve with concentrations ranging from 0 to 250 μg/ml. The results were expressed as mg gallic acid equivalents (mg GAEg⁻¹) per 100 g of the sample.
2.3.2. Estimation of total flavonoid contents (TFC)
The TFC were estimated by the aluminum chloride method [19]. Quercetin served as a positive standard and concentrations ranging from 0 to 500 μg/ml were prepared, and the standard calibration curve was developed using a linear fit curve. The results were expressed as mg quercetin equivalents (mg QEq⁻¹) per 100 g of the sample.

2.4. Antioxidant Activity

2.4.1. DPPH radical scavenging
Evaluation of free radical scavenging capacity of the plant extracts was carried out by DPPH method [20]. Briefly, in a 96 well microtiter plate, 10 μl of different solvent extracts and AC were individually added to 95 μl DPPH (300 μM) solution in methanol. The absorbance of the samples was measured at 517 nm (Spectra Max 340PC MultiMode plate reader) after the mixture was incubated for 30 minutes in dark at room temperature. The results were expressed as total antioxidant capacity and a dose-dependent curve was plotted to calculate the inhibitory concentration (IC₅₀) value and expressed as mean ± standard deviation (SD) of three independent experiments along with the standard AC. The activity is represented as % radical scavenging calculated with the equation:

\[
\% \text{ DPPH radical scavenging} = \frac{(Ac - As)}{(Ac)} \times 100
\]

2.4.2. Ferric ion reducing antioxidant power (FRAP) assay
The reducing abilities of different leaf and bark extracts were determined by the FRAP method for the electron-donating ability of antioxidants [21]. An aliquot of 30 μl sample was mixed with 90 μl of different solvent extracts and AC were individually added to 95 μl FeCl₃ (0.1 mM) solution in 0.5 M HCl and incubated for 30 minutes at room temperature. The absorbance was measured at 593 nm (Beckman Coulter, DU 730 Life Sciences). The calibration curve was generated using a linear fit curve. The results were expressed as mg quercetin equivalents (mg QEq⁻¹) per 100 g of the sample.

2.4.3. 15-LOX inhibition assay
Lipoxygenase with their products plays an important role as a mediator of inflammation with series of cellular pro-inflammatory and immune-modulatory responses. Inhibition of this enzyme would regulate the progression of inflammatory response. Evaluation of LOX inhibition was studied by a spectrophotometric assay with Soybean 15-LOX measuring the loss of soybean 15-LOX activity (5 μg) with 0.2 μM linoleic acid (Sigma) as the substrate prepared in a solubilized state in 0.2 M borate buffer (pH 9.0) [20]. Different concentrations of plant extracts were mixed with 15-LOX enzyme and incubated for 2 minutes at room temperature. The substrate was added to the mixture and the absorbance was measured at 243 nm using a UV-Vis spectrophotometer (Beckman Coulter, DU 730 Life Sciences). Values of hydroperoxide content and lipoxygenase activity were calculated from equation,

\[
\text{Specific activity (LOX)} = \frac{\Delta A}{V \cdot \varepsilon \cdot l \cdot c}
\]

where ΔA is the value of absorbance increase per minute, V is the volume of incubation mixture, ε is the extinction coefficient for linoleic acid (25 × 10⁻³ mol/l/cm), l is the length of the cuvette (1 cm), and c is the concentration of enzyme in mg (0.005).

2.5. High-Resolution Liquid Chromatography-Mass Spectroscopy (HR-LCMS)
Metabolomics analysis was performed using a HR-LCMS, with UHPLC-ESI-QTOF-MS (Agilent Technologies, Santa Clara, CA). MassHunter LC/MS Data Acquisition software (version B.06.01) was used for controlling the instrument and data acquisition. MassHunter Qualitative and Quantitative Analysis software (version B.07.00) was used for data evaluation. All samples were filtered with a 0.2 μm nylon membrane filter before injection.

For the chromatographic separation, Zorbax Eclipse C18, (2.1 × 150 mm 5-micron) column was used with gradient solvent system, (a) water with 0.1% formic acid and (b) acetonitrile with 10% water + 0.1% formic acid (2–20 minutes-A) 95% B 5%, 20–25 minutes (A) 5%, (B) 95%, and 26–30 minutes (A) 95%, (B) 5% with 0.2 ml minute flow rate with pressure maintained at 1,200 bar. The mass spectral data were acquired in electrospray in positive mode. The capillary voltage, source cone voltage, and extraction cone voltage were maintained at 3.25 kV, 30 V, and 4V, respectively, for positive mode. Nitrogen was applied as the desolvation gas at a flow rate of 900 l hours⁻¹. The source and desolvation temperatures were maintained at 120°C and 550°C, respectively. Mass spectra were acquired over the m/z range of 100–1,200 at a mass resolution of 22 000 FWHM (full-width half at maximum).

2.5.1. Data processing and identification
Raw data pre-treatment, including peak alignment, peak extraction, normalization, deconvolution, and compound identification, was carried out using Progenesis QI software (version 2.2, Waters, Milford, MA) with default settings. Untargeted data analysis with Progenesis QI exhibited 3813 and 1797 molecular features in the ESI⁺ mode with clean retention time-exact mass were obtained in each sample profile both in leaves and bark extract, respectively, which finally produced a matrix of features with the retention time, m/z, mass error, isotope similarity, and peak intensity. Each m/z value obtained both in the leaf and bark samples was searched against the in-house databases with different parameters set for putative identification based on the score with accurate mass matching, isotope similarity, and fragmentation score along with MS/MS data also included for the identification.

2.5.2. Building a custom in-house database
An in-house library of different metabolites was created through a literature search of previously reported metabolites from different species of the Myristicaceae family such as M. fragrans, Myristica malabarica, Myristica beddomei [12,22–24]. The structural and spectral information of metabolites were retrieved from different online metabolites databases like Metlin (https://metlin.scripps.edu/) PubChem (https://pubchem.ncbi.nlm.nih.gov/), HMDB (https://hmdb.ca/), ChemSpider (http://www.chemspider.com/), CHEMBL (https://www.ebi.ac.uk/chembl/) and ChEMBL (https://
www.ebi.ac.uk/chembl/) in “.sdf” and “.mol” file formats. These structural files were examined individually for correct information and were combined as one file in “.sdf” file format using Progenesis SDF Studio software (v1.0.5667/43006), for the identification of metabolites through Progenesis QI software. Similarly, other databases like bio-molecules provided by the Waters Corporation also was used for the identification.

2.6. Statistical Analysis
All the experiments were conducted in triplicates, and a statistically significant difference was calculated using a one-way analysis of variance at \( p \leq 0.001 \) followed by Tukey’s post hoc test with \( p \leq 0.05 \) using IBM Statistical Package for the Social Sciences (version 25) software. Results were represented as mean ± SD.

3. RESULTS AND DISCUSSION
3.1. Screening and Chemical Characterization of Phytochemical Extracts
Difficulties and challenges in conventional procedures for the identification of total chemical signatures in a complex system have been advocated for decades. The present investigation is an effort towards simplifying the critical and complex procedures using modern analytical tools to characterize and dereplicate pharmacological essentials present in the vulnerable medicinal plant \( M. dactyloides \). Initially, sequential extraction of metabolites from leaves and bark of \( M. dactyloides \) based on the polarity of the solvents using Soxhlet apparatus was achieved using non-polar (hexane), moderately polar (chloroform), and polar (methanol) solvents. Different solvent extracts of \( M. dactyloides \) leaves and bark were subjected for quantitative estimation of total phenol and TFCs and antioxidant activity along with 15-LOX inhibition of individual extracts for the selection of extract with a significant amount of bioactive chemical constituents. Results of quantitative analysis for TPC and TFC indicated that methanolic extract had the highest TPC and TFC content in both leaf and bark extracts (Table 1).

3.2. Radical Scavenging and Anti-Inflammatory Activities of Leaf and Bark Extracts of \( M. dactyloides \)
Efforts towards finding new anti-inflammatory and antioxidant molecules always remain a prime point in pharmacological research as they are very essential to combat inflammatory and oxidative stress-induced diseases [25–30]. As the antioxidant and anti-inflammatory potential of extracts substantially correlates with their biological significance [31], in the present study the leaves and bark solvent fractions of \( M. dactyloides \) were evaluated for their antioxidant efficiency via anti-radical (DPPH), reducing power (Ferric Reducing Antioxidant Power) assays and anti-inflammatory efficiency through LOX inhibitory assay model. Methanolic leaf and bark extracts have shown significant antioxidant activities with an \( IC_{50} \) value of 1.48 and 6.88 µg/ml when subjected to scavenge DPPH free radicals, respectively (Table 2). They also have a significant reducing ability at 217.46 mmol (FeII)/g and 263.68 mmol (FeII)/g, respectively. These results highlight a strong relationship between the total phenolic/flavonoid contents of the extracts and their antioxidant efficiency [31]. Results of anti-inflammatory potential of \( M. dactyloides \) indicated significant inhibitory effects on LOX when treated with methanolic leaf and bark extracts which scored lowest \( IC_{50} \) values of 2.4 and 10.4 µg/ml, respectively (Table 2). Since the methanolic leaf and bark extracts of \( M. dactyloides \) showed promising potential in neutralizing the free radicals and inhibiting

| Sl. No. | Sample          | TPC mg GAE/g (\( R^2 = 0.9095 \)) | TFC mg QE/g (\( R^2 = 0.9541 \)) |
|--------|-----------------|------------------------------------|----------------------------------|
| 1.     | Leaf-hexane     | –                                  | –                                |
| 2.     | Leaf-chloroform | 15.86                              | 92.02                            |
| 3.     | Leaf-methanol   | 53.13                              | 119.47                           |
| 4.     | Bark-hexane     | 18.92                              | 43.58                            |
| 5.     | Bark-chloroform | 13.50                              | 54.88                            |
| 6.     | Bark-methanol   | 32.54                              | 64.47                            |

GAE/g = gallic acid equivalents (mg GAE/g) per 100 g of the sample. QE/g = quercetin equivalents (mg QE/g) per 100 g of the sample.

| Sl. No. | Sample          | DPPH \( IC_{50} \) value µg/ml | FRAP (mmol (FeII)/g extract) | LOX \( IC_{50} \) value |
|--------|-----------------|---------------------------------|------------------------------|--------------------------|
| 1.     | Leaf-hexane     | 59.47                           | 111.50                       | 14.5                     |
| 2.     | Leaf-chloroform | 1.12                            | 117.93                       | 3.8                      |
| 3.     | Leaf-methanol   | 1.48                            | 217.46                       | 2.4                      |
| 4.     | Bark-hexane     | 31.93                           | 126.51                       | 15.1                     |
| 5.     | Bark-chloroform | 11.29                           | 223.86                       | 13.6                     |
| 6.     | Bark-methanol   | 6.88                            | 263.68                       | 10.4                     |
| 7.     | AC              | 1.61                            | 350.76                       | 9                        |

\( IC_{50} \) = inhibitory concentration at 50% sample.
LOX, these extracts were further subjected to metabolite profiling to catalog their important chemical constituents which may have potent bioactivities [32].

3.3. High-Resolution Liquid Chromatography Mass Spectrometry (HR-LC-ESI-MS/MS) Analysis of Bioactive Extract

Conventional methods of characterization of bioactive phytoconstituents involve series of steps that include extraction, evaluation, chromatographic separation, and spectroscopic characterization. However, due to the unavailability of suitable phytochemical standards, most of the researchers end up characterizing few known phytochemicals despite extensive effort and time. Hence unveiling the complex chemistry of bioactive crude extracts using high throughput and high-resolution techniques is a key to pinpoint the pharmacologically potent bio-actives and simplify the efforts to understand its action on the target. Among genus *Myristica*, there is overwhelming research on understanding the chemistry of *Myristica fragrans*, common name “nutmeg,” due to its innumerable medicinal and bioactive applications [12]. However, in spite of its usage as a replacement constituent for *M. fragrans*, the efforts towards understanding the chemistry of *M. dactyloides* have remained considerably low. Hence in the present study, HR-LC-ESI-MS/MS was used in order to characterize the chemical composition of methanolic crude extracts of leaves and bark from *M. dactyloides*. Untargeted data analysis with Progenesis QI exhibited 3,093 and 1,797 molecular features in the ESI+ mode with clean retention time-exact mass in the sample profile of leaves and bark extract, respectively. For the identification of compounds, an in-house database of previously reported metabolites from different species of the Myristicaceae family such as *M. fragrans*, *M. malabarica*, *M. beddomei* [12,22–24] used with a mass accuracy of 10 ppm. Similarly, other databases like bio-molecules provided by the Waters Corporation also was used for the identification.

The representative base peak chromatogram of *M. dactyloides* leaf and bark extracts is depicted in Figure 1A and B and the phytochemical identification data is presented in Table 3, which summarizes the tentative compounds characterized from these extracts including their retention time, experimental m/z, mass, proposed metabolites, molecular formula, and reported activity. These compounds mainly belong to lignans, neolignans, phenylpropanoids, diarylpropenoids, flavonoids, and others.

The LC chromatograms of both leaves and bark showed a varied concentration of metabolites present in each extract, with leaves showing higher metabolite content compared to bark due to the production of metabolites based on light-dependent pathways and the abundance profile of representative compounds (Fig. 2) both in leaf and bark also supports the leaves showing higher metabolite content. Mass Fragmentation trace of representative compounds like Malabaricone C, Malabaricone B, Guaiacin, Myricanone, and Epicatechin along with their and structures are given in Figure 3. In addition, there are considerable number of metabolites present both in leaves and bark extract as only a few metabolites have been focused in the present study.

The HR-LC-ESI-MS/MS data highlighted the increased concentration of lignans and neolignane derivatives in the leaf and bark extracts of *M. dactyloides*. Lignans and neolignans are the derivatives of phenylpropanoids generated through oxidative coupling and are among the major group of plant secondary metabolites found in the genus *Myristica* [12,33]. The peak at m/z 390 was proposed to be Myrifralignan A, a compound identified in *M. fragrans* and reported to have nitric oxide radical scavenging activity [12,34]. A peak at m/z 360 was identified as Austrobaillignan 7 (A7) the existence of which was also reported in *M. fragrans*, *Urbanodendron verrucosum*, and several other plant systems [35,36]. However, research efforts in unveiling the biological significance of this compound remained inconclusive compared to its analogs such as Austrobaillignan 1, Austrobaillignan 3, Austrobaillignan 5, and Austrobaillignan 6 which were extensively evaluated for their anti-inflammatory, antioxidant, anti-cancer, and anti-wrinkling activities [37–40]. Peak at 295, with the mass of 330.1467 was proposed as Fragransol B which was only identified in *M. fragrans* and its biological significance has remained largely unknown. Peak at m/z 309 is identified as Machilin A, previously reported in the members of genus *Machilus* and *Myristica* and is a well-known inhibitor of cytochrome P450 1A and 2B6 [41,42].

Similarly, several other lignans and neolignans like Argenteane, (peak at m/z 619), Nectadrin A (peak at m/z 376), Myristicanol B (peak at m/z 405), Myrifralignan E (peak at m/z 383), Fragransin D1 (peak at m/z 406), Sesamin (peak at m/z 337), Guaiacin (peak at m/z 329) detected in the methanolic extracts of leaf and bark sample of *M. dactyloides* in the present study strongly advocate the potential of these groups of compounds for biological activities [12,34,43–48]. Monotropein an iridoid glycoside tentatively annotated for the molecular ion at m/z 391.1238 (M + H) previously reported from the *Morinda officinalis* with well-established Antinociceptive and anti-inflammatory potential [49,50]. In addition, the present study also revealed the pharmacological richness in *M. dactyloides* wherein 25 bioactive chemical compounds other than lignans and neolignans were identified. These include alcoholic sugars, flavonoids, and steroids like Myoinositol, Methyleugenol, Monotropein, Parakmerin A, Eugenol, Anchirsinol, Syringic acid, Fragransol C, (+)-Epicatechin, Elemicin, Eugenol, Malabaricone D, (+)-Myristinin A, (+)-Myristinin D, 1-(2,6-Dihydroxyphenyl)-9-(4-Hydroxy-3-Methoxyphenyl), Dihydroguaiaretic acid, 4-Terpineol, Malabaricone C (Mal C), Malabaricone B (Mal B), Myricanone, Dodecanoylphloroglucinol, Saffrole, Isoeugenol, and Daucosterol.

Myoinositol, Malabaricone C, Malabaricone B, Malabaricone D, and 1-(2,6-Dihydroxyphenyl)-9-(4-Hydroxy-3-Methoxyphenyl) nonan-1-one which have been previously reported from the *M. dactyloides* have also been identified in the present study confirming their presence in the plant [13,14].

In addition to the above phytochemical constituents, the presence of compounds Mal C and Mal B, which are present in most of the members of genus *Myristica* potentially proves its consideration in developing a chemo-taxonomical library for the identification of this genus. Mal C is one among the extensively explored
chemical content predominantly present in genus *Myristica*, known for its possible therapeutic potential for treating cancer and inflammatory disorders [51,52], Alzheimer’s disease [53], and infectious diseases [54]. The present study also highlights the importance of exploring the bioactive potential of Mal B which is underutilized compared to Mal C. The presence of Mal C and Mal B in *M. dactyloides* may be responsible for the antioxidant and anti-inflammatory efficiency in the extracts recorded earlier in preliminary *in vitro* investigation.

The present investigation also added prominent chemical signatures like Monotropein Malabaricone B, Malabaricone C, Frangrason B, Guaiacin, Myricanone, and Epicatechin to the phytochemical catalog of *M. dactyloides*. Though the components like Malabaricone C, Monotropein have been studied extensively by researchers worldwide, the other chemical constitutes cataloged in this study are more promising for pharmacological industries facing challenges in the discovery of synthetic drugs which is known to be expensive and risky in terms of capital investment and side effects. The unexplored phytochemicals cataloged from *M. dactyloides* for their bioactive potentials represent novel natural interventions towards finding a solution for the industrial challenges.

Figure 1. LC chromatograms methanol extracts of (a) Bark and (b) Leaf of *Myristica dactyloides*.
Table 3. List of major chemical constituents identified from methanolic extracts of leaves and bark of *Myristica dactyloides* using HR-LC-ESIMS/MS.

| Retention time (minute) | m/z   | Neutral mass | Proposed metabolites | Formula | Adducts | Mass error (ppm) | Activity                                        | Reference |
|-------------------------|-------|--------------|----------------------|---------|---------|------------------|------------------------------------------------|-----------|
| Lignans and neolignans  |       |              |                      |         |         |                  |                                                |           |
| 1.2717                  | 390.1883 | 372.4116     | Myrifulignan A Neolignan | C_{21}H_{28}O_{5} | M+NH₄ | -7.5             | Anti-inflammatory                             | [12,34]  |
| 2.0896                  | 279.1354 | 296.1412     | Parakmerin A (Lignan) | C_{18}H_{22}O_{4} | M+H-H₂O | -8.3             |                                                | [55]      |
| 5.6954                  | 209.0825 | 208.0735     | Anthricinol (Lignan phenylpropanoid) | C_{11}H_{12}O_{4} | M+H | 8.2             | CC chemokine receptor 3 antagonist              | [56,57]  |
| 6.2463                  | 321.1506 | 356.1623     | Fragransol C (Neolignan) | C_{21}H_{24}O_{4} | M+H₂O | 6.03             |                                                | [35,58]  |
| 7.8989                  | 360.1792 | 342.1467     | (+)-Machilin F (Lignan) | C_{20}H_{22}O_{4} | M+NH₄ | -3.84            |                                                | [59]      |
| 9.201                   | 295.1317 | 330.1467     | Fragransol B (Lignan) | C_{20}H_{22}O_{4} | M+H₂O | -3.42            |                                                | [35]      |
| 10.3028                 | 309.1470 | 326.1518     | Machilin A (Lignan) | C_{20}H_{22}O_{4} | M+H-H₂O | -4.43            | Inhibits tumor growth, stimulates osteoblast differentiation | [59,60]  |
| 13.1406                 | 619.3038 | 654.3192     | Argenteane (Dilignan) | C_{40}H_{46}O_{8} | M+H₂O | -2.39            | Antioxidant                                   | [43]      |
| 13.992                  | 376.2147 | 358.1780     | Nectandin A (Lignan) | C_{21}H_{22}O_{4} | M+NH₄ | 8.17             | AMP-activated protein kinase (AMPK) activators   | [45,61]  |
| 14.8768                 | 348.2141 | 330.1831     | Dihydrologiaiaretic acid (Lignans, neolignans) | C_{20}H_{22}O_{4} | M+NH₄ | -8.50            | Antioxidant                                   | [62,63]  |
| 16.8967                 | 405.1947 | 404.1835     | Myristicanol B (Lignan) | C_{20}H_{22}O_{4} | M+H | 9.79             | Inhibit lipid peroxidation                     | [46,58]  |
| 17.264                  | 383.1499 | 418.1627     | Myrifulignan E (Neolignans) | C_{20}H_{22}O_{4} | M+H₂O | 2.45             | Inhibit nitric oxide production                | [12,34]  |
| 17.264                  | 406.2260 | 388.1885     | Fragransin D1 (Lignan) | C_{20}H_{22}O_{4} | M+NH₄ | 9.27             |                                                | [35]      |
| 17.3474                 | 337.1091 | 354.1103     | Sesamin (Lignan) | C_{20}H_{20}O_{5} | M+H₂O | 5.83             | Antioxidant, anti-inflammatory, anticancer      | [47]      |
| 19.5844                 | 365.1362 | 342.1467     | Austroballiignan 7 (Lignan) | C_{20}H_{22}O_{4} | M+Na | 0.91             |                                                | [35]      |
| 21.8714                 | 329.1752 | 328.1674     | Guaiacim (Saponin, Lignans, neolignans) | C_{20}H_{22}O_{4} | M+H | 1.43             | Neuroprotective/Anti-inflammatory               | [64,65]  |
| Phenylpropanoids        |       |              |                      |         |         |                  |                                                |           |
| 1.255                   | 201.0902 | 178.1010     | Methylisoeugenol (Phenylpropanoid) | C_{11}H_{14}O_{2} | M+Na | 9.2              | Antinociceptive & anti-inflammatory            | [12,66]  |
| 3.8925                  | 165.0919 | 164.0837     | Eugenol (Phenylpropanoid) | C_{10}H_{12}O_{2} | M+H | 5.6              | Anti-inflammatory                              | [67]      |
| 7.8822                  | 226.1422 | 208.1099     | Elemicin (Phenylpropanoid) | C_{12}H_{14}O_{2} | M+NH₄ | -7.07            |                                                | [12,68]  |
| 7.9323                  | 182.1188 | 164.0837     | Eugenol | C_{10}H_{12}O_{2} | M+NH₄ | 8.05             | Anti-inflammatory                              | [67]      |
| 21.6878                 | 180.1003 | 162.0680     | Saffrole Phenylpropanoids | C_{10}H_{14}O_{2} | M+NH₄ | -9.75            | Tyrosinase inhibitory                          | [69]      |
| 28.8827                 | 147.0816 | 164.0837     | Isoeugenol (Terpenoid) | C_{10}H_{12}O_{2} | M+H₂O | 7.23             | Antioxidant                                   | [22]      |
| Diarylmononanoids, diarylalkanone, and diarylethananoids |       |              |                      |         |         |                  |                                                |           |
| 8.4832                  | 353.1743 | 370.1780     | Malabaricone D (Diarylmononanoid) | C_{22}H_{25}O_{3} | M+H-H₂O | -1.02            |                                                | [70]      |
| 13.3076                 | 395.1810 | 372.1936     | 1,4-Dihydroxy-3-Methoxyphenyl) | C_{22}H_{25}O_{3} | M+Na | -5.02            | Antiproliferative                              | [8,24]    |
| 17.0637                 | 359.1885 | 358.1812     | Malabaricone C (Diarylmononanoid) | C_{21}H_{24}O_{3} | M+H | 9.01             | Anti-inflammatory                              | [70,71]  |
| 17.0804                 | 357.1714 | 356.1623     | Myricanone (Diarylethananoid) | C_{21}H_{24}O_{3} | M+H | 5.07             | Anticancer                                     | [72]      |
| 18.4826                 | 343.1933 | 342.1831     | Malabaricone B (Diarylmononanoid) | C_{21}H_{24}O_{3} | M+H | 8.54             | Antimicrobial, anti-inflammatory               | [70,73]  |
| 21.6377                 | 309.2067 | 308.1987     | Dodecanoylphloroglucinol | C_{18}H_{22}O_{4} | M+H | 2.18             | Antituberculosis & antiviral activity          | [74]      |

Continued
| Retention time (minute) | m/z     | Neutral mass | Proposed metabolites                  | Formula       | Adducts    | Mass error (ppm) | Activity                                | Reference |
|------------------------|---------|--------------|---------------------------------------|---------------|------------|-----------------|-----------------------------------------|-----------|
| 7.7988                 | 291.0891| 290.0818     | (+)-Epicatechin (Flavanol)            | C_{15}H_{14}O_{6} | M+H       | 9.6             | Antioxidant, anti-inflammatory          | [75]      |
| 9.5516                 | 571.2665| 548.2774     | (+)-Myristinin A (Flavan)             | C_{35}H_{40}O_{7} | M+Na      | −0.18           | COX-2 inhibitors and antifungal agent   | [76]      |
| 10.837                 | 565.2573| 582.2617     | (+)-Myristinin D (Flavan)             | C_{36}H_{38}O_{7} | M+H-H_2O  | −1.90           | COX-2 inhibitors and antifungal agent   | [76]      |
| 1.2049                 | 203.0545| 180.0653     | Myo-inositol (Carbocyclic sugar)      | C_{7}H_{8}N_{4}O_{2} | M+Na      | 3.4             | Anti-inflammatory                       | [13, 77] |
| 1.8225                 | 391.1238| 390.1162     | Monotropein (Iridoid glycoside)       | C_{16}H_{22}O_{11} | M+H      | 1.02            | Antinociceptive, anti-inflammatory      | [49, 50] |
| 5.7956                 | 199.0600| 198.0528     | Syringic acid (Phenolic)              | C_{9}H_{10}O_{5}  | M+H       | −0.068          | Antioxidant, anti-microbial, anti-inflammation, anti-cancer, anti-diabetic | [78]      |
| 15.1272                | 172.1706| 154.1357     | 4-Terpineol (Isoprenoids)             | C_{10}H_{18}O_{2}  | M+NH4     | 6.82            | Anticancer, antioxidant, anti-inflammatory | [79, 80] |
| 27.18                  | 541.4288| 576.4389     | Daucosterol (Terpenoid)               | C_{35}H_{60}O_{6} | M+H-2H_2O  | 6.36            | Immunoregulatory, anti-cancer           | [81, 82] |

Flavonoids and others

**Figure 2.** Abundance profile of representative compounds from leaf and bark extract.
4. CONCLUSION
The present study is a first report to the best of authors’ knowledge, on cataloging the chemicals constituents in leaves and bark methanolic extracts of *M. dactyloides*, and indicates chemical and bioactive resemblance between *M. dactyloides* and *M. fragrans* (Table 3). This evidently supports the current practice of using *M. dactyloides* as a viable alternative in pharmaceutical formulations of *M. fragrans*. The study also highlights the potential of modern analytical tools in cataloging chemical constituents within a genus of medicinally important plants, which may certainly aid in the development of a chemo-taxonomical database to authenticate and identify the plant species. The present investigation strongly advocates the importance of constructing a medicinal plant species-based chemical library which can be accessed by researchers for developing species-specific chemo-taxonomical tools in the future. This will in turn cut down the time taken for the characterization of bioactive compounds from natural resources.

5. AUTHORS' CONTRIBUTIONS
All the authors have made substantive intellectual contributions to the content of this manuscript in the following areas: Concept and design—KMM and KRK, Data acquisition and analysis—KMM, and BRN, Drafting manuscript—KMM, SCR, BRN, and KRK, Critical revision of Manuscript—SNB, KKS, and SS, Statistical analysis—KMM and BRN, and Supervision and Final approval—KRK.
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7. CONFLICT OF INTEREST
The authors have declared no conflict of interest.

8. ETHICAL APPROVALS
This study does not involve experiments on animals or human subjects.

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