Metabolite profiling of *Bruguiera cylindrica* reveals presence of potential bioactive compounds

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ABSTRACT

Bruguiera cylindrica parts are commonly used in Chinese and Indian traditional medicine to treat diarrhea, fever, and many ailments. The present study aims non targeted analysis of key secondary metabolites of *B. cylindrica* by gas chromatography mass spectrometry (GC-MS) and ultra-high performance liquid chromatography hybrid quadrupole-Exactive-Orbitrap high resolution mass spectrometry (UHPLC-Q-Exactive Orbitrap HRMS). GC-MS and UHPLC-Q-Exactive Orbitrap HRMS were utilized for metabolic profiling of ethyl acetate extract of *B. cylindrica* leaves. Key metabolites in the extract were identified and predicted based on chemical similarity using online databases such as ChemSpider and mzCloud. Thirty-six compounds belonging to different classes of secondary metabolites *viz.* flavonoids, fatty acids, fatty acid amides, carboxylic acids, and alkaloids were identified in the extract. Pentacyclic triterpenes like betulin, ursolic acid and a tropine, an alkaloid with potential pharmacological and therapeutic activities such as anticancer properties, neuromuscular blockers and antioxidants, were also identified. This study combined GC-MS and UHPLC-Q-Exactive Orbitrap HRMS with available online database for effective and rapid identification of bioactive metabolites in the ethyl acetate extract of mangrove without individual standard application. This is the first report on the HRMS based secondary metabolic profiling of *B. cylindrica*, with comprehensive map of its biologically important metabolites.

Subjects Analytical Chemistry (other), Mass Spectrometry
Keywords Mangrove, *Bruguiera cylindrica*, GC-MS, UHPLC-Q-Exactive Orbitrap HRMS

INTRODUCTION

Mangroves are a unique ecosystem that found along the world's sheltered intertidal coastlines as well as in tropical and subtropical regions (*Kathiresan & Rajendran, 2005*; *Saddhe, Jamdade & Kumar, 2017*). The mangrove forest ecosystem is extremely productive and evolutionary adapted to extreme environmental stress (abiotic and biotic stress) and produces structurally diverse metabolites in response to it. Mangroves are rich source of metabolites belonging to class of fatty acids, limonoids, phenolics, coumarins, lignans, terpenoids, and alkaloids (*Du et al., 2007*; *Cui et al., 2005*; *Sadhu et al., 2006*; *Bao et al., 2007*; *Taniguchi et al., 2018*; *Wu et al., 2008*) which shows that they can be taken in an account for the search of novel bioactive metabolites. Furthermore, metabolites produced

by mangrove species such as, *Avicennia marina, Acanthus ilicifolius, Xylocarpus granatum, Excoecaria agallocha, Kandelia candel* and *Rhizophora mucronata* that may offer disease preventing or medicinal properties as antitumor, antifungal, antimicrobial, antiviral, anti-inflammatory, and insecticidal effect (*Wu et al., 2008*; *Dahibhate, Saddhe & Kumar, 2019*; *Nabeelah Bibi et al., 2019*; *Mitra, Naskar & Chaudhuri, 2021*).

Bruguiera cylindrica L. is an evergreen mangrove from Rhizophoraceae family widely distributed in Asia pacific region, Southeast Asia and Western part of India (*Saddhe, Jamdade & Kumar, 2017*). *B. cylindrica* has been used by the Thai people in folk medicine to treat diarrhea and wound healing. The different parts of *B. cylindrica* extract are used for the treatment of hemorrhage, ulcers (*Nithyamol et al., 2018*), antimicrobial (*Vadlapudi & Naidu, 2009*; *Dahibhate, Saddhe & Kumar, 2019*; *Dahibhate, Roy & Kumar, 2020*), antioxidant (*Krishnamoorthy et al., 2011*), antiviral (*Premanathan, Kathiresan & Nakashima, 1999*), thrombolytic (*Vadlapudi & Naidu, 2009*) and anti-inflammatory (*Eldeen, Ringe & Ismail, 2019*). Research on *B. cylindrica* and other *Bruguiera* species are mainly concentrated on qualitative phytochemical screening whereas, gas chromatographic studies of different solvent extracts have shown that *B. cylindrica* is the valuable source of fatty acids, flavonoids, tannins, terpenes, alkaloids, glycosides and so on (*Revathi, Jeyaseelansenthinath & Thirumalaikolundhusubramaian, 2015*; *Dahibhate, Roy & Kumar, 2020*). Additionally, sulfur containing compounds and pharmacologically active triterpenoids are identified from fruits and, leaves of various other *Bruguiera* genus. These terpenoids mainly comprise beyerane, pimarane, lupane, ursane, oleanane, and dammarane (*Wang et al., 2018*; *Jun et al., 2008*; *Nebula, Harisankar & Chandramohanakumar, 2013*). These terpenes and other metabolites are focused due to structural diversity, and a wide range of bioactivities with cytotoxic effect against lung cancer and sarcoma cells, *in vitro* (*Sithranga & Kathiresan, 2010*). Therefore, in order to enhance the quality check of medicinal properties of plant and its effective utilization, an accurate and reliable analysis approach for monitoring the plant's metabolite profile, which determines medicinal value, is required.

Metabolite profiling is one of the most recent approaches to the 'omics' revolution, which can provide a system-wide view of metabolite involved. It can elucidate complex processes by analyzing a large number of metabolites involved in numerous biochemical processes and across a wide range of biological systems. In plants, metabolite profiling is mainly performed by analytical platforms such as gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE) coupled to mass spectrometry (MS) and nuclear magnetic resonance (NMR) (*Wang et al., 2015*). It is challenging and time consuming to separate and identify every single metabolite in natural medicinal plants due to their complicated compositions. GC-MS is the most frequently used technology for plant metabolite, especially for identifying and quantifying metabolites involved in primary metabolism's core pathways, such as sugars, amino acids, and organic acids. Several studies have reported the use of GC-MS technique for fatty acids determination in animals, marine organisms, plants, flax seeds, dietary fatty acid, and quality analysis of edible oils (*Seppänen-Laakso, Laakso & Hiltunen, 2002*; *Tang & Row, 2013*; *Stenvers, Chi & Javidpour, 2020*; *Schött et al., 2021*). Due to the lower boiling point than other metabolites

fatty acids are easier to identify with GC-MS (*Seppänen-Laakso, Laakso & Hiltunen, 2002*). On the other hand, Ultra high performance liquid chromatography-Q-Exactive Orbitrap tandem mass spectrometry (UHPLC-Q-Exactive Orbitrap HRMS) technology combines the separation capabilities of chromatography with the qualitative functions of mass spectrometry. It has a high separation, scanning speed, resolution sensitivity, and compatibile with available natural product databases. UHPLC-Q-Exactive Orbitrap HRMS is the most widely used technique for analyzing fatty acids, polyphenolics, alkaloids, and terpenes category secondary metabolite from complex system like plants (*Li et al., 2021*; *Ossipov et al., 2020*; *Liu et al., 2020*). Additionally, this technique is especially suitable for the qualitative identification of the natural products and structural analysis of the novel compounds lacking the standard reference metabolite.

On the basis of our literature survey, no metabolic profiling studies have been reported from *B. cylindrica* of West coast India. However, metabolite profiling has not been done so far on *B. cylindrica* leaves by ultra-high performance liquid chromatography hybrid quadrupole-Orbitrap high resolution mass spectrometry (UHPLC-Q-Exactive Orbitrap HRMS). In fact, the leaves are a renewable and long-term source of secondary metabolites which are important for long term survival and defense against biotic stress. In the present study, to explore the major secondary metabolites of *B. cylindrica* leaves having various pharmacological activities, we used the GC-MS and UHPLC-Q-Exactive Orbitrap HRMS (Thermo Scientific, Waltham, MA, USA) coupled to online database (NIST, ChemSpider and mzCloud) to assess the metabolite composition of ethyl acetate extract from *B. cylindrica* leaves.

MATERIALS AND METHODS

Chemicals and reagents

Methanol, acetonitrile, formic acid, of LCMS grade (Thermo Fisher Scientific, Waltham, MA, USA), ethyl acetate, and water from Milli-Q purification unit (Merck, Merck, Germany) were obtained.

Plant collection and treatment

In present study, leaf samples of *B. cylindrica* were collected from Goa, located on India's west coast at geographical latitude of 15.5256°N and a longitude of 73.8753°E. *B. cylindrica* was identified based on morphological keys (*Saddhe, Jamdade & Kumar, 2016*, *2017*) and voucher specimens were maintained. The leaves were collected from the single sampling location in the month of August 2021. Leaves were washed, dried under shed at 30 °C and crushed using electric grinder. A grounded sample weighed (100 gm), packed in cellulose thimble and extracted with ethyl acetate (3×150 mL) using Soxhlet apparatus operated at 50 °C for 24 h. Extract was filtered using cellulose filter paper (125 mm; GE Healthcare, Chicago, IL, USA), concentrated using rotary evaporation (Medica Instruments, Mumbai, India) and kept at −20 °C until further analysis.

Sample preparation

Prior to analysis, parent stock was prepared by dissolving 10 mg of sample in one mL of ethyl acetate and heated in water bath (50 °C) to ensure its complete dissolution and then centrifuged (10,000 rpm, 8 min). For GC-MS analysis, sample was prepared by adding a volume of 10 μL from parent stock to 990 μL of ethyl acetate (v/v). For UHPLC-Q-Exactive Orbitrap HRMS analysis, final working stock of 1 mg/mL was prepared in an equal volume of ethyl acetate: methanol (v/v) and filtered through 0.22 μm nylon membrane filter (GE Healthcare, Chicago, IL, USA) before injection. The analysis was performed in three independent technical replicates.

Gas chromatography mass spectrometry (GC-MS) analysis

The ethyl acetate extract of *B. cylindrica* was analyzed using GC-MS system of Shimadzu (GC-2030) series equipped with headspace (HS-20) & triple quadrupole mass spectrometer GC-TQ8040NX. The fused silica column, SH-Rxi-5 (0.25 mm × 30 m, 0.25 μm) was used. Carrier gas (Helium) was used with a flow rate of one mL/min. The column temperature was maintained at 50 °C for 3 min and increased to 130 °C at the rate of 7 °C for 4 min, followed by increased to 250 °C at the rate of 7 °C for 3 min, through split ratio (50:50) mode. Injector, ion source, and interface temperature were set to 260, 220 and 260 °C respectively. For analysis 1 μL of the sample was injected with a constant temperature of 260 °C through an autosampler injector. The ionization energy was 70 eV and mass range of 40–500 amu. The system management, mass spectrometry, parameter settings, data receipt and processing were performed using Shimadzu real time analysis. Identification of metabolite constituents was made on the basis of retention, MS library search in National Institute of Standards and Technology (NIST 17) and comparing with literature data.

UHPLC-Q-Exactive Orbitrap HRMS analysis

Qualitative analysis of ethyl acetate extract of *B. cylindrica* was acquired using UHPLC system (Thermo Scientific, Chicago, IL, USA) coupled to Q-Exactive Orbitrap MS system equipped with electrospray ionization. Hypersil gold (100 × 2.1 mm, 3.0 μm) column was used for the analysis. The column temperature was maintained at 28 °C and the injection volume was 5 μL. Mobile phase consists of water (0.1% formic acid) as phase A and acetonitrile as phase B with 0.3 mL/min of flow rate. Linear gradient elution parameters were set as follows: 0–2 min 5% B and increased from 5% to 10% B in 1 min. Further in 20 min, the gradient was linearly increased to 95% B and returned to 5% B in 6 min and re-equilibrated for 4 min at 5% B. The blank injection was employed after each sample to avoid the carryover.

Mass spectrometry parameters were as follows; full scan data was acquired in both positive and negative mode at resolving power of 70,000 with 100–1,500 scanning range. Ion source parameter were: auxiliary gas 9 (N_2, 95%), sheath gas 37, spray voltage set to 3 kV and capillary temperature and auxiliary gas heater were at 300 °C and 350 °C respectively. The S-lens RF level was set at 55. The automatic gain control was set at 1×10^6 and injection time was set at 60 ms. Scan rate was 1 scans/s. The product ion spectra were

obtained in the range of 25–40 eV of collision energy. Mass tolerance was set to 5 ppm. Data acquisition was performed using the Thermo Scientific Xcalibur software (version 4.2.48.14). Compound Discoverer software 2.1 SP1 (Thermo Fisher Scientific, Chicago, IL, USA), a qualitative data-processing application that uses accurate mass data and mass spectral library searches for the small molecule identification, was used for data processing. Both spectral libraries and compound databases like mzCloud and ChemSpider were used for metabolite identification. The only those identified metabolites which are consistently appeared over three injections are reported.
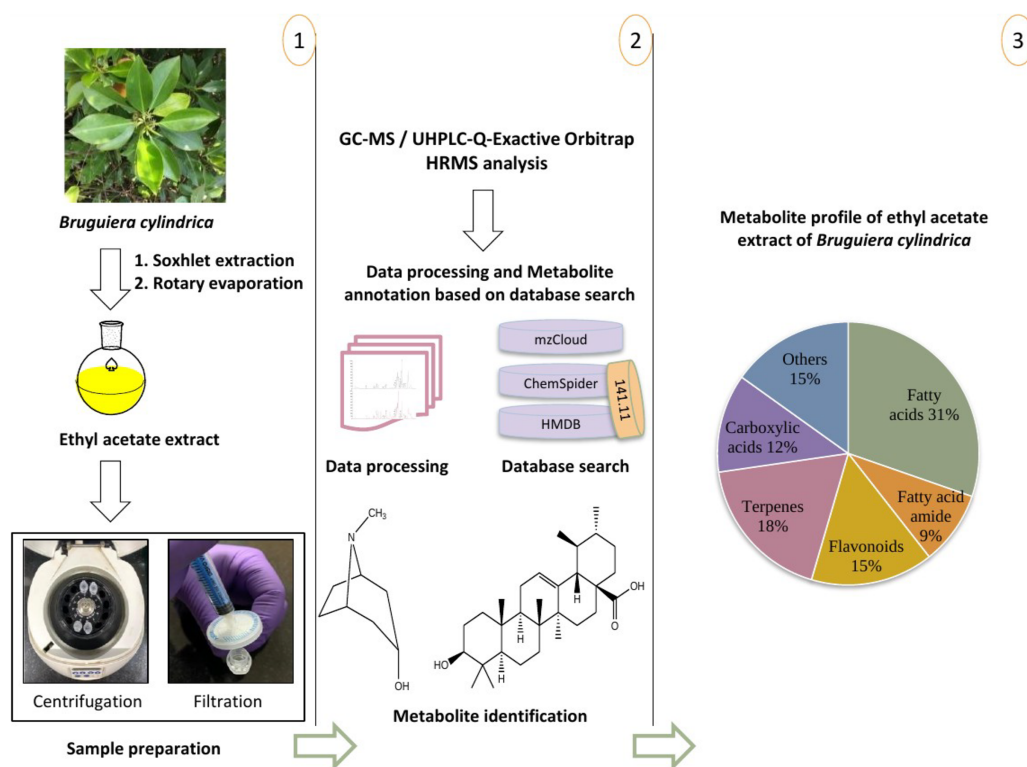
RESULTS AND DISCUSSIONS

Extraction of bioactive compound

In fact, the leaves are a renewable and crucial source of plant secondary metabolites which are important for long term survival and defense against biotic stress, therefore can be selected for screening of active metabolites. The Soxhlet extraction, also known as the hot continuous extraction process and its fundamental advantages are simplicity, allows thorough extraction with the least amount of solvent at high temperature, which ultimately improves the process kinetics. The disadvantage of this approach is that it is not ideal for the extraction of thermo-labile metabolites since prolonged heating may cause the compounds to degrade. In our previous research, we have employed Soxhlet apparatus using ethyl acetate for the extraction of biologically important metabolites and metabolic profiling studies (*Dahibhate, Roy & Kumar, 2020*; *Dahibhate, Kumar & Kumar, 2021*). Additionally, as a suitable solvent, ethyl acetate was selected because of its chemical and biological characteristics including medium polarity and minimum toxicity. Ethyl acetate biphasic nature enables to extract both polar and non polar biological compounds. Therefore, we selected ethyl acetate as extraction solvent and the extract was processed for the metabolic profiling using GC-MS and UHPLC-Q-Exactive Orbitrap HRMS technique. Figure 1 shows typical metabolite profiling workflow including experimental design and preparation of samples to metabolite annotation.

GC-MS profiling revealed presence of 11 metabolites

GC-MS profiling of the extracts showed presence of compounds from different classes with key biological properties. The compounds were identified by relating their retention time, retention index, peak area (%) and mass spectral fragmentation pattern with known metabolites library of NIST. From all the peaks observed in the ethyl acetate extract, only 11 compounds were identified with more than 89% similarity index, which is listed in Table 1. The GC-MS analysis of ethyl acetate of *B. cylindrica* has revealed the presence of lauric acid, myristic acid, neophytadiene, phytol, palmitic acid, 10(E),12(Z)-octadecadienoic acid, oleic acid, stearic acid, nonacosane, tetracontane and squalene. The squalene, a triterpene (22.69%) was determined as a major component of extract followed by palmitic acid (15.54%), oleic acid (12.71%), and neophytadiene, a diterpene (5.17%). Besides, straight chain hydrocarbon such as, tetracontane, nonacosane content was higher than those of saturated fatty acids such as lauric acid, stearic acid and myristic acid.

Figure 1 Metabolite profiling workflow including experimental design, preparation of samples and metabolite annotation. Full-size ⬜ DOI: 10.7717/peerj-achem.16/fig-1

Table 1 Chemical composition of *B. cylindrica* ethyl acetate extract by GC-MS.

Rt (min)	Compound name	Molecular formula	Molecular weight	Base m/z	Similarity (%)	Retention index	Area (%)	Class
24.947	Lauric acid	$C_{12}H_{24}O_2$	200	73	94	1,570	1.28	Fatty acid
28.825	Myristic acid	$C_{14}H_{28}O_2$	228	73	96	1,769	0.82	Fatty acid
29.996	Neophytadiene	$C_{20}H_{38}$	278	68	96	1,774	5.17	Diterpene
30.683	Phytol	$C_{20}H_{40}O$	296	81.05	93	2,045	1.95	Diterpene
32.048	Palmitic acid	$C_{16}H_{32}O_2$	256	73	96	1,968	15.54	Fatty acid
34.426	10(E),12(Z)-octadecadienoic acid	$C_{18}H_{32}O_2$	280	67	94	2,183	3.94	Fatty acid
34.514	Oleic acid	$C_{18}H_{34}O_2$	282	55	92	2,175	12.71	Fatty acid
34.798	Stearic acid	$C_{18}H_{36}O_2$	284	73	89	2,167	4.56	Fatty acid
36.023	Nonacosane	$C_{29}H_{60}$	408	57.05	95	2,904	4.4	Hydrocarbon
39.384	Tetracontane	$C_{40}H_{82}$	562	57	96	3,997	5.41	Hydrocarbon
39.727	Squalene	$C_{30}H_{50}$	410	69.05	97	2,914	22.69	Triterpene

Note:
Retention index derived from the NIST 17 database match.

Our results are in correlation with previous studies on *B. cylindrica*, in which palmitic and stearic acid was reported as dominant metabolites of *B. cylindrica* (*Revathi, Jeyaseelansenthinath & Thirumalaikolundhusubramaian, 2015*). Some of the compounds included in Table 1 have previously been reported for their role in various pharmaceutical

and therapeutic functions (*Casillas-Vargas et al., 2021*). Essential oils rich in terpenes are like squalene, neophytadiene has been shown to posses ability to reduce free radical damage of skin (*Huang, Lin & Fang, 2009*), antimicrobial activity, analgesic and anti-inflammatory (*Swamy et al., 2017*). The dissimilarity with previous report (*Revathi, Jeyaseelansenthinath & Thirumalaikolundhusubramaian, 2015*) were observed in current study which may be due to seasonal variation, difference in sample collection, or experimental method employed for analysis.

UHPLC-Q-Exactive Orbitrap HRMS profiling of *B. cylindrica* revealed presence of 25 metabolites

In the present study, a high-throughput and sensitive method of UHPLC coupled with Q-Exactive Orbitrap HRMS was used to identify the chemical constituents in *B. cylindrica*. By comparing with available secondary metabolite database such as chemSpider, mzCloud and available literature, 25 metabolites were confirmed from *B. cylindrica*, which have previously been shown to have various bioactive properties. Also, the identified metabolites were confirmed based on the accurate mass and theoretical fragmentation pattern matching criteria using the HMDB structural database (https://hmdb.ca/) and mass bank database (https://massbank.eu/MassBank/). All the identified metabolite information is summarized in Table 2. The base peak chromatogram of ethyl acetate extract obtained in the positive and negative mode under the optimized UHPLC-Q-Exactive Orbitrap HRMS conditions is shown in Fig. 2. Total 25 secondary metabolites were identified such as five flavonoids, three fatty acid amide, three terpenes, one alkaloid, four carboxylic acids, five fatty acids and four other components. Among these components flavonoids and fatty acids was found to be the major component of *B. cylindrica*.

Flavonoids are ubiquitously distributed among plants in flavone or flavonol form and considered as an essential component in nutraceutical, medicinal, cosmetic application (*Panche, Diwan & Chandra, 2016*), and have anti-oxidant, anti-inflammatory, and anti-tumor properties (*Lopez-Lazaro, 2009*). In UHPLC-Q-Exactive Orbitrap HRMS analysis of *B. cylindrica* four flavone *viz.* two C-glycosidic flavone of luteolin (isoorientin, orientin), and two aglycone form (luteolin, and apigenin) were detected. Isoorientin and orientin were identified with precursor ion at m/z 449.1078. Differences in relative abundances of products ions m/z $[M+H-60]^+$ and $[M+H-120]^+$ were demonstrated as characteristic ions of C-glycosides, which could be used for identification (*Pereira, Yariwake & McCullagh, 2005*). Distinction of isomer pair of isoorientin and orientin was performed according to the relative abundance of product ion fragments such as, m/z 329.0653 $[M+H-60]^+$ and m/z 299.0548 $[M+H-120]^+$. The bioactive flavonols such as kaempferol showed precursor ion at m/z 286.04720.

Carboxylic acids are compounds occurring naturally in different stages of plants. They have a beneficial effect on microbial growth by acting as vitamin for microbial nutrition (*e.g.* nicotinic acid, or p-aminobenzoic acid). There are several studies reported about the significance of medicinal effects of carboxylic acid containing metabolites in treatment of pain and disease (*Matsumoto, Yanagi & Oe, 2018*). In this study, carboxylic

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Table 2 Phytochemical composition of *B. cylindrica* ethyl acetate extract by UHPLC-Q-Exactive Orbitrap HRMS.

Peak	Rt (min.)	Compound name	Molecular formula	Calculated mass	Error (ppm)	mzCloud match score	ChemSpider match	Class	Biological activities	References
1	1.015	Tropine	$C_8H_{15}NO$	141.11516	−1.45	98.2	Full match	Alkaloid	Bradycardic, and respiratory-stimulant action	*Mitra, Naskar & Chaudhuri (2021)*
2	6.499	4-Hydroxybenzaldehyde	$C_7H_6O_2$	122.0366	−1.47	96	Full match	Aldehyde	Anti-angiogenic and anti-nociceptive activities	*Lim et al. (2008)*
3	7.546	Esculetin	$C_9H_6O_4$	178.02637	−1.35	88.9	Full match	Coumarin	Anti-proliferative and pro-apoptotic activity	*Jeon et al. (2015)*
4	9.654	Syringic acid	$C_9H_{10}O_5$	198.05251	−1.6	75.8	Full match	Carboxylic acid	Prevention of diabetes, neuro and hepatoprotective,	*Tanase, Coşarca & Muntean (2019)*
5	10.015	4-Coumaric acid	$C_9H_8O_3$	164.04717	−1.08	84.7	Full match	Carboxylic acid	Antivirus, anti-inflammatory	*Tanase, Coşarca & Muntean (2019)*
6	10.17	Dioctyl phthalate	$C_{24}H_{38}O_4$	390.27626	−1.91	90.7	Full match	Phthalates	–	–
7	11.159	Isoorientin	$C_{21}H_{20}O_{11}$	448.09972	−1.88	98.2	Full match	Flavones	Anti-nociceptive, and gastroprotective activities	*Panche, Diwan & Chandra (2016)* and *Kumar & Pandey (2013)*
8	11.437	Orientin	$C_{21}H_{20}O_{11}$	448.09972	−1.88	93.5	Full match	Flavones	Anti-nociceptive, and gastroprotective activities	*Panche, Diwan & Chandra (2016)* and *Kumar & Pandey (2013)*
9	12.921	Azelaic acid	$C_9H_{16}O_4$	188.10501	0.8	95.1	Full match	Carboxylic acid	Anti-infective and anti-inflammatory action	*Schulte, Wu & Rosen (2015)*
10	14.774	Luteolin	$C_{15}H_{10}O_6$	286.0472	−1.89	98.5	Full match	Flavones	Inhibit angiogenesis, induce apoptosis.	*Lopez-Lazaro (2009)*
11	15.194	Kaempferol	$C_{15}H_{10}O_6$	286.0472	−1.69	90.6	Full match	Flavonols	Antimicrobial, cardioprotective, neuroprotective,	*Panche, Diwan & Chandra (2016)*
12	15.628	Apigenin	$C_{15}H_{10}O_5$	270.05226	−2.07	96.5	Full match	Flavones	Block cell cycle and decrease prostate cancer cells	*Panche, Diwan & Chandra (2016)*
13	20.336	12-oxo Phytodienoic acid	$C_{18}H_{28}O_3$	292.20324	−1.17	77.5	Full match	Carboxylic acid	–	–
14	20.38	Caryophyllene oxide	$C_{15}H_{24}O$	220.18247	−1.13	80.6	Full match	Sesquiterpenoid	Antifungal, Analgesic and anti-inflammatory activity	*Dahham et al. (2015)*
15	20.51	Monobutyl phthalate	$C_{12}H_{14}O_4$	222.8899	−0.98	86.2	Full match	Phthalates	–	–
16	20.772	9-Oxo-10(E),12(E)-octadecadienoic acid	$C_{18}H_{30}O_3$	294.21898	−1.75	96.5	Full match	Fatty acid	Inhibits triglyceride accumulation	*Kim et al. (2011)*
17	21.709	Ursolic acid	$C_{30}H_{48}O_3$	456.36079	0.98	98.1	Full match	Triterpene	Anticancer, antidiabetic, antioxidant	*Mlala et al. (2019)*
18	22.316	Betulin	$C_{30}H_{50}O_2$	442.38072	−0.81	85.6	Full match	Triterpene	Antitumor	*Hordyjewska et al. (2019)*
19	22.747	Hexadecanamide	$C_{16}H_{33}NO$	255.25582	−1.56	99	Full match	Fatty acid amide	–	–
20	22.732	Linoleic acid	$C_{18}H_{32}O_2$	280.24052	1.04	94.6	Full match	Fatty acid	Affects peptidoglycan synthesis	*Casillas-Vargas et al. (2021)*
21	22.956	9-Octadecenamide	$C_{18}H_{35}NO$	281.27147	−1.4	75.6	Full match	Fatty acid amide	Increase cholinesterase and cholinesterase trasferase activity	*Boger et al. (1998)*
22	23.048	Palmitic acid	$C_{16}H_{32}O_2$	256.24049	1.02	96.5	Full match	Fatty acid	Anti-quorum sensing against *Acinetobacter baumannii*	*Casillas-Vargas et al. (2021)*
23	23.229	Oleic acid	$C_{18}H_{34}O_2$	282.25612	0.84	94.9	Full match	Fatty acid	Antioxidative effect	*Wei et al. (2016)*
24	23.743	Stearic acid	$C_{18}H_{36}O_2$	284.27174	0.74	95.9	Full match	Fatty acid	Antibacterial activity	*Casillas-Vargas et al. (2021)*
25	24.969	Docosanamide	$C_{22}H_{45}NO$	339.34947	−1.89	81.8	Full match	Fatty acid amide	–	–
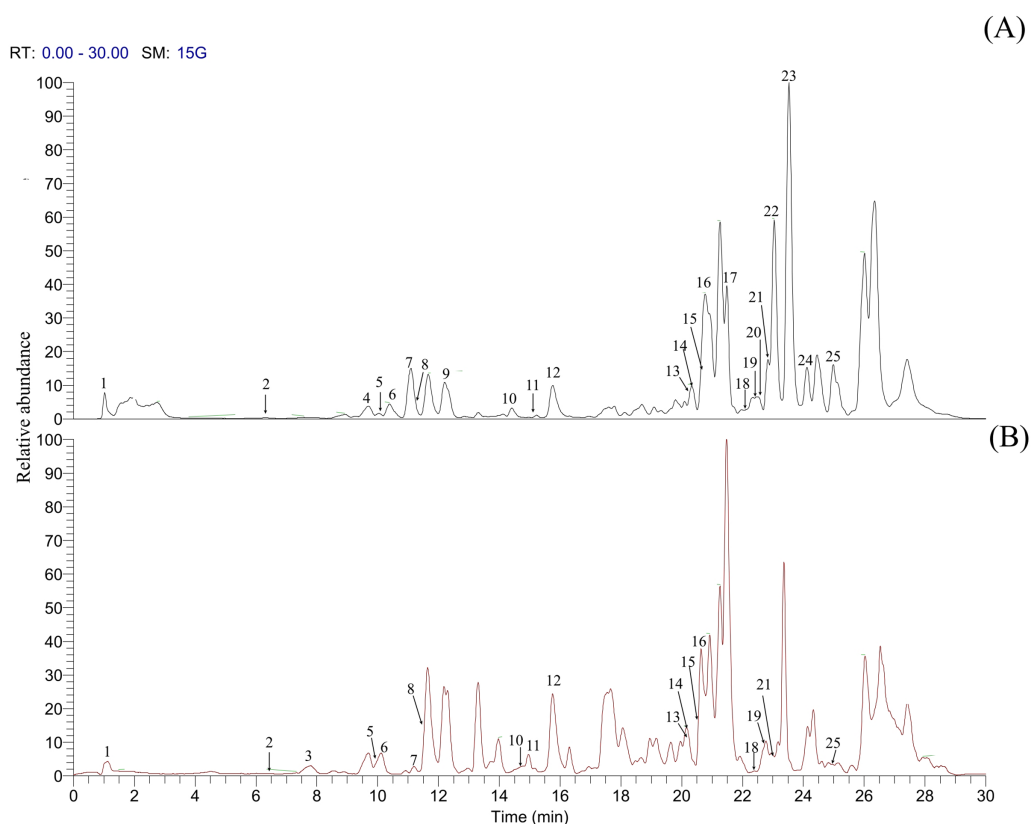
Figure 2 Base peak chromatogram of the metabolites detected using UHPLC-Q-Exactive Orbitrap HRMS. (A) positive mode and (B) negative mode. Full-size ☑ DOI: 10.7717/peerj-achem.16/fig-2

acids like azelaic acid, 12-oxo-phytodienoic acid, syringic acid, 4-coumaric acid were detected. The 12-oxo-phytodienoic acid, a precursor of jasmonic acid in plants showed protonated parent ion $[M+H]^+$ at m/z 293.2136. Further fragmentation resulted into product ions at m/z 275.200, 247.2051, 229.1947, 163.1115, and 149.0231. There is currently no literature available on the mass fragmentation of 12-oxo-phytodienoic acid available, hence we experimentally confirmed the fragments formed with perfect match using predicted MS/MS spectra from The Human Metabolome Database. Additionally, syringic acid, azelaic acid and 4-coumaric acids showed precursor ion at m/z 199.5301, 189.11021 and 165.04877 respectively. Moreover, it has been reported that syringic acid and 4-coumaric acids are found to be most effective as providing anti-oxidant, anti-virus, gastroprotective activities (*Tanase, Coşarca & Muntean, 2019*; *Gomez et al., 2019*) but current knowledge regarding the biological activities of 12-oxo-phytodienoic acid is still far from complete. Our findings showed presence of fatty acid such as, linoleic acid, plamitic acid, oleic acid, stearic acid and 9-oxo-10(E),12(E)-octadecadienoic acid in *B. cylindrica*. The recent study from tomato plant reported the effect 9-oxo-10(E), 12(E)-octadecadienoic acid on triglyceride accumulation by acting agonist of peroxisome proliferator-activated receptor α on mouse primary hepatocytes (*Kim et al., 2011*).

The published data shows that *Bruguiera* genus has been well known for the presence of different types of di- and triterpenoids like beyerane, pimarane, lupane, ursane, oleanane,

and dammarane (*Wang et al., 2018*; *Jun et al., 2008*). *Nithyamol et al. (2018)* reported a pentacyclic triterpene, a taraxerol is the main metabolite from various solvent fractions of *Bruguiera cylindrica*. In our work we searched for the taraxerol in analyzed samples but unfortunately we do not found it in single ion monitoring mode and through selective extraction of ion chromatogram as well. This variation in the metabolite produced which may be due to change in the geographical locations, as both the sampling studies are conducted from different part of India. Additionally, our study confirms the presence of one sesquiterpene, caryophyllene oxide and two pentacyclic triterpene, a ursane type ursolic acid (m/z 457.3702) and lupane type betulin (m/z 443.3883) using accurate mass measurement and fragmentations. The presence of an intense signal of product ions with m/z 191, 217, 335 were in correlation with reported literature for betulin (*Kosyakov, Yanovskii & Falev, 2014*). It has been studied extensively because of its remarkable biological properties like anti-melanoma and anti-HIV activities (*Mitra, Naskar & Chaudhuri, 2021*; *Hordyjewska et al., 2019*). Other compound belongs to nitrogenous bicyclic organic compound category such as tropine, which shown peak at m/z 142.1225. The tropine was characterized in *B. exaristata* and reported to have respiratory-stimulant action (*Nebula, Harisankar & Chandramohanakumar, 2013*; *Mitra, Naskar & Chaudhuri, 2021*). Furthermore, esculatin (m/z 179.0376) and 4-hydroxybenzaldehyde (m/z 123.0421) were detected as well.

Fatty acid amides (FAAs) are family of structurally diverse molecules formed from a fatty acid, and an amine is well studied in relevance to human than to other organisms. In this study, we have detected three different FAAs, namely 9-octadecenamide (m/z 282.3279), hexadecanamide (m/z 256.3282), and docosanamide (m/z 340.3988). 9-octadecenamide is well studied molecule among FAAs, which is derived from fatty acid oleic acid. It has a role as plant metabolite as well as human metabolite known for inhibiting gap junction communication in the glial cells (*Boger et al., 1998*). Previously, *Cryptotaenia japonica* was reported to have higher content of 9-octadecenamide, which function is attributed to superoxide scavenging and hypolipidemic bioactive property (*Cheng et al., 2010*). Whereas oleamide, hexadecamide along with other FAAs like stearamide, erukamide has been recently reported in *Artemisia argyi* (*Cui et al., 2021*). These compounds have been reported first time in *B. cylindrica* by UHPLC-Q-Exactive Orbitrap HRMS.

Phthalates or esters of phthalic acid are widely used to make the plastic softer and emitted into the environment during manufacturing, use, and disposal (*Thiemann, 2021*). With an exception to our main objective of the study, UHPLC-Q-Exactive Orbitrap HRMS analysis of *B. cylindrica* also reveals the presence of two monobutyl (m/z 223.0984) and dioctyl phthalates (m/z 391.3102), which might be associated with contamination of mangrove water bodies. In addition to this, *da Silva Pontes et al. (2020)* has recently carried systematic GC-MS based study to examine the accumulation of phthalate in mangrove *Avicennia schaueriana* from Sundarban. Surprisingly, bis-isobutyl phthalate, bis-tridecyl phthalate, bis-2-ethylhexyl phthalate were the major metabolites detected. On the other hand, phthalates have continued to be reported as endogenous product from plants, and microorganisms (*Romeh, 2013*; *Zhang et al., 2018*). Contrarily, it remains as puzzle,

whether the phthalates should be considered as natural product or contaminants of environmental pollution (*Thiemann, 2021*).

In comparison to high-performance liquid chromatography (HPLC), it is advantageous to use UPLC by considering short time of analysis, improved sensitivity with triple quadrupole. UPLC is often hyphenated with different sources of ionization and mass detectors has made it a convenient technique for analysis of complex mixtures, and also proved its prime role in exploring the constituents of traditional medicinal plants (*Alvarez-Rivera et al., 2019*). UPLC-Q-Orbitrap-MS/MS and GC-MS are currently commonly used for the metabolite profiling of different plants. In contrast to the traditional LC-ESI-MS, UPLC-Q-Orbirap-HRMS has higher resolution, selectivity and eliminate sample matrix interference. Moreover, it was reported that the secondary metabolites identified by GC-MS and UHPLC-Q-Exactive Orbitrap HRMS in this study possess group of bioactivities of therapeutic interest (*Kumar et al., 2009*; *Casuga, Castillo & Corpuz, 2016*). The chemical constituents reported in this study were relatively inclusive, and first report on UHPLC-Q-Exactive Orbitrap HRMS based analysis of *B. cylindrica*. As there are not enough research articles about GC-MS and UHPLC-Q-Exactive Orbitrap HRMS based analysis of *B. cylindrica* to correlate the current finding of this report, hence this work can be considered as foundation for further studies on *B. cylindrica*.

CONCLUSIONS

Current study discussed about the identification and characterization of secondary metabolites in ethyl acetate extracts from *B. cylindrica* leaves. In total, 11 compounds were identified by GC-MS and 25 chemical constituents of *B. cylindrica* were identified first time by UHPLC-Q-Exactive Orbitrap HRMS technology. Moreover, these HRMS, GC-MS platform could be used for the evaluation of the similarities between medicinal plant extracts and their commercial products. Previously, qualitative and *in vitro* studies on *B. cylindrica* have shown primarily phenols, sterols, diterpenoid, triterpenoids, and fatty acids. Our data confirmed the presence of flavones, fatty acids, terpenes, and carboxylic acids. This study can be considered as foundational work about metabolite analysis of *B. cylindrica*, which may provide the substructure for metabolite profiling, characterization and bioactive properties of *B. cylindrica* and other species of *Bruguiera* genus.

ADDITIONAL INFORMATION AND DECLARATIONS

Author Contributions

- Nilesh Lakshman Dahibhate performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Kundan Kumar conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw data are available in the Supplemental File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj-achem.16#supplemental-information.

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