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

Nilesh Lakshman Dahibhate and Kundan Kumar

Biological Sciences, Birla Institute of Technology and Science Pilani, K K Birla Goa Campus, Zuarinagar, Goa, India

**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.

**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 compatible 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 (N₂, 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 × 10⁶ 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.
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 applications.

| 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_{29}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.
and therapeutic functions (Casillas-Vargas et al., 2021). Essential oils rich in terpenes are like squalene, neophytadiene has been shown to possess 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, Jayaseelansenthinath & 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
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 | Class | Biological activities | References |
|---------------|---------------|-------------------|-----------------|-------------|---------------------|-------|-----------------------|------------|
| 1             | Tropine       | C_{6}H_{11}NO     | 141.11516       | 1.45        | 98.2                | Full match | Alkaloid              | Bradycardic, and respiratory-stimulant action | Mitra, Naskar & Chaudhuri (2021) |
| 2             | 4-Hydroxybenzaldehyde | C_{7}H_{10}O_{2}  | 122.0366        | 1.47        | 96                  | Full match | Aldehyde              | Anti-angiogenic and anti-nociceptive activities | Lim et al. (2008) |
| 3             | Esculetin      | C_{5}H_{8}O_{4}   | 178.02637       | 1.35        | 88.9                | Full match | Coumarin              | Anti-proliferative and pro-apoptotic activity | Jeon et al. (2015) |
| 4             | Syringic acid | C_{9}H_{10}O_{5}  | 198.05251       | 1.6         | 75.8                | Full match | Carboxylic acid       | Prevention of diabetes, neuro and hepatoprotective, | Tanase, Coșarca & Muntean (2019) |
| 5             | 4-Coumaric acid | C_{9}H_{8}O_{3}   | 164.04717       | 1.08        | 84.7                | Full match | Carboxylic acid       | Anti-virus, anti-inflammatory | Tanase, Coșarca & Muntean (2019) |
| 6             | 4-Hydroxybenzaldehyde | C_{7}H_{8}O_{4}   | 122.0366        | 1.47        | 96                  | Full match | Aldehyde              | Anti-angiogenic and anti-nociceptive activities | Lim et al. (2008) |
| 7             | Esculetin      | C_{5}H_{8}O_{4}   | 178.02637       | 1.35        | 88.9                | Full match | Coumarin              | Anti-proliferative and pro-apoptotic activity | Jeon et al. (2015) |
| 8             | Syringic acid | C_{9}H_{10}O_{5}  | 198.05251       | 1.6         | 75.8                | Full match | Carboxylic acid       | Prevention of diabetes, neuro and hepatoprotective, | Tanase, Coșarca & Muntean (2019) |
| 9             | 4-Coumaric acid | C_{9}H_{8}O_{3}   | 164.04717       | 1.08        | 84.7                | Full match | Carboxylic acid       | Anti-virus, anti-inflammatory | Tanase, Coșarca & Muntean (2019) |
| 10            | Dioctyl phthalate | C_{24}H_{38}O_{4} | 390.27626       | 1.91        | 90.7                | Full match | Phthalates            | – | – |
| 11            | Isororin       | C_{21}H_{20}O_{11} | 448.09972     | 1.88        | 95.1                | Full match | Flavones              | Anti-nociceptive, and gastroprotective activities | Panche, Diwan & Chandra (2016) and Kumar & Pandey (2013) |
| 12            | Orientin       | C_{21}H_{20}O_{11} | 448.09972     | 1.88        | 95.1                | Full match | Flavones              | Anti-nociceptive, and gastroprotective activities | Panche, Diwan & Chandra (2016) and Kumar & Pandey (2013) |
| 13            | 12-oxo Phytodienoic acid | C_{18}H_{30}O_{3} | 292.20324     | 1.17        | 77.5                | Full match | Carboxylic acid       | – | – |
| 14            | Caryophyllene oxide | C_{15}H_{24}O_{2} | 220.18247     | 1.13        | 80.6                | Full match | Sesquiterpenoid        | Antifungal, Analgesic and anti-inflammatory activity | Dahham et al. (2015) |
| 15            | Monobutyl phthalate | C_{12}H_{10}O_{4} | 222.8899     | 0.8         | 95.1                | Full match | Phthalates            | – | – |
| 16            | 9-Oxo-10(E),12(E)-octadecadienoic acid | C_{18}H_{35}O_{3} | 294.21898     | 1.75        | 96.5                | Full match | Fatty acid            | Inhibits triglyceride accumulation | Kim et al. (2011) |
| 17            | Ursolic acid  | C_{30}H_{48}O_{3} | 456.36079      | 0.98        | 98.1                | Full match | Triterpene            | Anticancer, anti-diabetic, antioxidant | Mlala et al. (2019) |
| 18            | Betulin       | C_{30}H_{48}O_{2} | 422.38072      | 0.81        | 85.6                | Full match | Triterpene            | Antitumor | Hordyjewska et al. (2019) |
| 19            | Hexadecanamide | C_{16}H_{32}NO   | 255.25882      | 1.56        | 99                  | Full match | Fatty acid amide      | – | – |
| 20            | Linoleic acid | C_{18}H_{32}O_{2} | 280.4052       | 1.04        | 94.6                | Full match | Fatty acid amide      | Affects peptidoglycan synthesis | Casillas-Vargas et al. (2021) |
| 21            | 9-Octadecanamide | 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            | Palmitic acid | C_{16}H_{32}O_{2} | 256.24049      | 1.02        | 96.5                | Full match | Fatty acid amide      | Anti-quorum sensing against Acinetobacter baumannii | Casillas-Vargas et al. (2021) |
| 23            | Oleic acid    | C_{18}H_{36}O_{2} | 282.25612      | 1.04        | 94.9                | Full match | Fatty acid amide      | Antioxidative effect | Wei et al. (2016) |
| 24            | Stearic acid  | C_{18}H_{36}O_{2} | 284.27174      | 0.74        | 95.9                | Full match | Fatty acid amide      | Antibacterial activity | Casillas-Vargas et al. (2021) |
| 25            | Docosanamide | C_{22}H_{44}NO   | 339.34947      | 1.89        | 81.8                | Full match | Fatty acid amide      | – | – |
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 \([\text{M+H}]^+\) 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, Coșarca & 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, palmitic 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 \(\alpha\) 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, hexadecanamide 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-Orbitrap-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**

**Funding**

This work was supported by the Council of Scientific and Industrial Research, India [38(1416)/16/EMR-II]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Grant Disclosures**

The following grant information was disclosed by the authors:
Council of Scientific and Industrial Research, India: 38(1416)/16/EMR-II.
Competing Interests
The authors declare that they have no competing interests.

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.

REFERENCES
Alvarez-Rivera G, Ballesteros-Vivas D, Parada-Alfonso F, Ibañez E, Cifuentes A. 2019. Recent applications of high resolution mass spectrometry for the characterization of plant natural products. TrAC Trends in Analytical Chemistry 112:87–101 DOI 10.1016/j.trac.2019.01.002.
Bao S, Ding Y, Deng Z, Proksch P, Lin W. 2007. Rhyncosides A-F, phenolic constituents from the Chinese mangrove plant Bruguiera sexangula var. rhynchopetala. Chemical and Pharmaceutical Bulletin 55(8):1175–1180 DOI 10.1248/cpb.55.1175.
Boger DL, Patterson JE, Guan X, Cravatt BF, Lerner RA, Gilula NB. 1998. Chemical requirements for inhibition of gap junction communication by the biologically active lipid oleamide. Proceedings of The National Academy of Sciences 95(9):4810–4815 DOI 10.1073/pnas.95.9.4810.
Casillas-Vargas G, Ocasio-Malave C, Medina S, Morales-Guzman C, Del Valle RG, Carballeira NM, Sanabria-Ríos DJ. 2021. Antibacterial fatty acids: an update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. Progress in Lipid Research 82(6):101093 DOI 10.1016/j.plipres.2021.101093.
Casuga FP, Castillo AL, Corpus MJ. 2016. GC-MS analysis of bioactive compounds present in different extracts of an endemic plant Broussonetia luzeonica (Blanco) (Moraceae) leaves. Asian Pacific Journal of Tropical Biomedicine 6(11):957–961 DOI 10.1016/j.ajptb.2016.08.015.
Cheng MC, Ker YB, Yu TH, Lin LY, Peng RY, Peng CH. 2010. Chemical synthesis of 9 (Z)-octadecenamide and its hypolipidemic effect: a bioactive agent found in the essential oil of mountain celery seeds. Journal of Agricultural and Food Chemistry 58(3):1502–1508 DOI 10.1021/jf903573g.
Cui J, Deng Z, Li J, Fu H, Proksch P, Lin W. 2005. Phragmalin-type limonoids from the mangrove plant Xylocarpus granatum. Phytochemistry 66(19):2334–2339 DOI 10.1016/j.phytochem.2005.06.020.
Cui L, Wang X, Lu J, Tian J, Wang L, Qu J, Liu Z, Wei J. 2021. Rapid identification of chemical constituents in Artemisia argyi by UPLC-Q-Exactive-MS/MS. Journal of Food Quality 2021(5):1–7 DOI 10.1155/2021/5597327.
Davila Pontes AL, Mesquita VC, de Oliveira Chaves F, da Silva AJR, Kaplan MAC, Fingolo CE. 2020. Phthalates in Avicennia schaueriana, a mangrove species, in the State Biological Reserve, Guaratiba, RJ Brazil. *Environmental Advances* 2(19):100015 DOI 10.1016/j.envadv.2020.100015.

Dahham SS, Tabana YM, Iqbal MA, Ahamed MB, Ezzat MO, Majid AS, Majid AM. 2015. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna. *Molecules* 20(7):11808–11829 DOI 10.3390/molecules200711808.

Dahham SS, Tabana YM, Iqbal MA, Ahamed MB, Ezzat MO, Majid AS, Majid AM. 2015. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna. *Molecules* 20(7):11808–11829 DOI 10.3390/molecules200711808.

Dahibhate NL, Kumar D, Kumar K. 2021. Determination of bioactive polyphenols in mangrove species and their in-vitro anti-Candida activities by ultra-high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS). *Analytical Letters* 54(4):608–624 DOI 10.1080/00032719.2020.1774600.

Dahibhate NL, Roy U, Kumar K. 2020. Phytochemical screening, antimicrobial and antioxidant activities of selected mangrove species. *Current Bioactive Compounds* 16(2):152–163 DOI 10.2174/1573407214666180808121118.

Dahibhate NL, Saddhe AA, Kumar K. 2019. Mangrove plants as a source of bioactive compounds: a review. *The Natural Products Journal* 9(2):86–97 DOI 10.2174/2210315508666180910125328.

Du SJ, Qin ZH, Wang MA, Zhu W, Han CR, Bi HP. 2007. GC-MS analysis of the essential oils from Xylocarpus granatum. *Journal of Hainan Normal University* 20:247–250.

Eldeen IMS, Ringe J, Ismail N. 2019. Inhibition of pro-inflammatory enzymes and growth of an induced rheumatoid arthritis synovial fibroblast by Bruguiera cylindrica. *International Journal of Pharmacology* 15(8):916–925 DOI 10.3923/ijp.2019.916.925.

Gomez J, Simirgiotis MJ, Lima B, Paredes JD, Villegas Gabutti CM, Gamarra-Luques C, Borquez J, Luna L, Wendel GH, Maria AO, Feresin GE. 2019. Antioxidant, gastroprotective, cytotoxic activities and UHPLC PDA-Q orbitrap mass spectrometry identification of metabolites in Baccharis grisebachii decoction. *Molecules* 24(6):1085 DOI 10.3390/molecules24061085.

Hordyjewska A, Ostapiuk A, Horecka A, Kurzepa J. 2019. Betulin and betulinic acid: triterpenoids derivatives with a powerful biological potential. *Phytochemistry Reviews* 18(3):929–951 DOI 10.1007/s11101-019-09623-1.

Huang ZR, Lin YK, Fang JY. 2009. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. *Molecules* 14(1):540–554 DOI 10.3390/molecules14010540.

Jeon YJ, Jang JY, Shim JH, Myung PK, Chae JI. 2015. Esculetin, a coumarin derivative, exhibits anti-proliferative and pro-apoptotic activity in G361 human malignant melanoma. *Journal of Cancer Prevention* 20(2):106–112 DOI 10.15430/JCP.2015.20.2.106.

Jun W, Qiang X, Jing X, Min YL, Jian YP, Mei-hua Y. 2008. Natural products from true mangrove flora: source, chemistry and bioactivities. *Natural Product Report* 25(5):955–981 DOI 10.1039/b807365a.

Kathiresan K, Rajendran N. 2005. Mangrove ecosystems of the Indian Ocean region. *Indian Journal of Geo-Marine Science* 34:104–113.

Kim YI, Hirai S, Takahashi H, Goto T, Ohyane C, Tsugane T, Konishi C, Fujii T, Inai S, Iijima Y, Aoki K. 2011. Octadecadienoic acid derived from tomato is a potent PPARα agonist to decrease triglyceride accumulation in mouse primary hepatocytes. *Molecular Nutrition & Food Research* 55(4):585–593 DOI 10.1002/mnfr.201000264.

Kosyakov DS, Yanovskii NV, Falev DI. 2014. Determination of triterpenoids from birch bark by liquid chromatography-tandem mass spectrometry. *Journal of Analytical Chemistry* 69(13):1264–1269 DOI 10.1134/S1061934814130061.
Krishnamoorthy M, Sasikumar JM, Shamna R, Pandiarajan C, Sofia P, Nagarajan B. 2011. Antioxidant activities of bark extract from mangroves, Bruguiera cylindrica (L) Blume and Ceriops decandra Perr. Indian Journal of Pharmacology 43:557 DOI 10.4103/0253-7613.84972.

Kumar S, Pandey AK. 2013. Chemistry and biological activities of flavonoids: An Overview. The Scientific World Journal 2013:162750 DOI 10.1155/2013/162750.

Kumar T, Ray S, Brahmachary RL, Ghose M. 2009. Preliminary GC-MS analysis of compounds present in the root exudates of three mangrove species. Acta Chromatographica 21(1):117–125 DOI 10.1556/AChrom.21.2009.1.10.

Li T, Zeng H, Zeng Y, Zhang X, Ren Y, Gao Y, Huang Q, Tan J. 2021. Characterization of the bioactive compounds with efficacy against gout in Guizhi Shaoyao Zhimu Decoction by UHPLC-Q-Orbitrap HRMS combined with network pharmacological analysis. Arabian Journal of Chemistry 14(6):103185 DOI 10.1016/j.arabjc.2021.103185.

Lim E-J, Kang H-J, Jung H-J, Kim K-H, Lim C-J, Park E-H. 2008. Anti-inflammatory, anti-angiogenic and anti-nociceptive activities of 4-hydroxybenzaldehyde. Biomolecules and Therapeutics 16:231–236 DOI 10.4062/biomolther.2008.16.3.231.

Liu R, Su C, Xu Y, Shang K, Sun K, Li C, Lu J. 2020. Identifying potential active components of walnut leaf that action diabetes mellitus through integration of UHPLC-Q-Orbitrap HRMS and network pharmacology analysis. Journal of Ethnopharmacology 253(3):112659 DOI 10.1016/j.jep.2020.112659.

Lopez-Lazaro M. 2009. Distribution and biological activities of the flavonoid luteolin. Mini Reviews in Medicinal Chemistry 9(1):31–59 DOI 10.2174/138955709787001712.

Matsumoto K, Yanagi R, Oe Y. 2018. Recent advances in the synthesis of carboxylic acid esters. In: Badea G-I, Radu GL, eds. Carboxylic Acid-Key Role in Life Sciences. Vol. 2. London: InTechOpen, 7–34.

Mitra S, Naskar N, Chaudhuri P. 2021. A review on potential bioactive phytochemicals for novel therapeutic applications with special emphasis on mangrove species. Phytomedicine Plus 1(4):p100107 DOI 10.1007/s13659-021-0012-0.

Mlala S, Oyedeji AO, Gondwe M, Oyedeji OO. 2019. Ursolic acid and its derivatives as bioactive agents. Molecules 24(15):2751 DOI 10.3390/molecules24152751.

Nabeelah Bibi S, Fawzi MM, Gokhan Z, Rajesh J, Nadeem R, Rengasamy Kannan RR, Albuquerque R DDG, Pandian SK. 2019. Ethnopharmacology, phytochemistry, and global distribution of mangroves—A comprehensive review. Marine Drugs 17:231 DOI 10.3390/md17040231.

Nebula M, Harisankar HS, Chandramohanakumar N. 2013. Metabolites and bioactivities of Rhizophoraceae mangroves. Natural Products and Bioprospecting 3(5):207–232 DOI 10.1007/s13659-013-0012-0.

Nithyamol KV, Bhattacharya D, Chakravarty S, Venkata UM. 2018. Isolation, synthesis and AChE inhibitory potential of some novel cinnamyl esters of taraxerol, the major metabolite of the mangrove Bruguiera cylindrica. Chemistry & Biodiversity 15(4):pe1800008 DOI 10.1002/cbdv.201800008.

Ossipov V, Koivuniemi A, Mizina P, Salminen JP. 2020. UPLC-PDA-Q exactive orbitrap-MS profiling of the lipophilic compounds product isolated from Eucalyptus viminalis plants. Heliyon 6(12):e05768 DOI 10.1016/j.heliyon.2020.e05768.

Panche AN, Diwan AD, Chandra SR. 2016. Flavonoids: an overview. Journal of Nutritional Science 5:e47 DOI 10.1017/jns.2016.41.
Pereira CA, Yariwake JH, McCullagh M. 2005. Distinction of the C-glycosylflavone isomer pairs orientin/isoorientin and vitexin/isovitexin using HPLC-MS exact mass measurement and in-source CID. *Phytochemical Analysis* **16**(5):295–301 DOI 10.1002/pca.820.

Premanathan M, Kathiresan K, Nakashima H. 1999. Mangrove halophytes: a source of antiviral substances. *South Pacific Study* **19**:49–57.

Revathi P, Jeyaseelansenthinath T, Thirumalaikolundhusubramaian P. 2015. Preliminary phytochemical screening and GC-MS analysis of ethanolic extract of mangrove plant *Bruguiera cylindrica* (Rhizho) L. *International Journal of Pharmacognosy and Phytochemical Research* **6**(4):729–740.

Romeh AA. 2013. Diethyl phthalate and dioctyl phthalate in *Plantago major* L. *African Journal of Agricultural Research* **8**:4360–4364 DOI 10.5897/AJAR2013.7242.

Saddhe AA, Jamdade RA, Kumar K. 2016. Assessment of mangroves from Goa, west coast India using DNA barcode. *SpringerPlus* **5**(1):1–10 DOI 10.1186/s40064-016-3191-4.

Sadhu SK, Ahmed F, Ohtsuki T, Ishibashi M. 2006. Flavonoids from *Sonneratia caseolaris*. *Journal of Natural Medicine* **60**(3):264–265 DOI 10.1007/s11418-006-0029-3.

Schött HF, Konings MC, Schrauwen-Hinderling VB, Mensink RP, Plat J. 2021. A validated method for quantification of fatty acids incorporated in human plasma phospholipids by gas chromatography-triple quadrupole mass spectrometry. *ACS Omega* **6**(2):1129–1137 DOI 10.1021/acsomega.0c03874.

Schulte BC, Wu W, Rosen T. 2015. Azelaic acid: evidence-based update on mechanism of action and clinical application. *Journal of Drugs in Dermatology* **14**:964–968.

Seppänen-Laakso T, Laakso I, Hiltunen R. 2002. Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition. *Analytica Chimica Acta* **465**(1–2):39–62 DOI 10.1016/S0003-2670(02)00397-5.

Sithranga BN, Kathiresan K. 2010. Anticancer drugs from marine flora: an overview. *Journal of Oncology* **2010**(1):1–18 DOI 10.1155/2010/214186.

Stenvers V, Chi X, Javidpour J. 2020. Seasonal variability of the fatty acid composition in *Aurelia aurita* (Cnidaria: Scyphozoa): implications for gelativerse food web studies. *Journal of Plankton Research* **42**(4):440–452 DOI 10.1093/plankt/fbaa026.

Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. 2017. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. *Evidence-Based Complementary and Alternative Medicine* **2017**(87):1–10 DOI 10.1155/2017/157683.

Tanase C, Coșnar S, Muntean DL. 2019. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. *Molecules* **24**(6):1182 DOI 10.3390/molecules24061182.

Tang B, Row KH. 2013. Development of gas chromatography analysis of fatty acids in marine organisms. *Journal of Chromatographic Science* **51**(7):599–607 DOI 10.1093/chromsci/bmt005.

Taniguchi K, Funasaki M, Kishida A, Sadhu SK, Ahmed F, Ishibashi M, Ohsaki A. 2018. Two new coumarins and a new xanthone from the leaves of *Rhizophora mucronata*. *Bioorganic and Medicinal Chemistry Letters* **28**(6):1063–1066 DOI 10.1016/j.bmcl.2018.02.022.

Thiemann T. 2021. Isolation of phthalates and terephthalates from plant material-natural products or contaminants? *Open Chemistry Journal* **8**(1):1–36 DOI 10.2174/1874842202108010001.
Vadlapudi V, Naidu KC. 2009. Bioactivity of mangrove plant *Bruguiera cylindrica* against selected phytopathogens. *Biosciences Biotechnology Research Asia* 6:843–846.

Wang X, Yu H, Zhang Y, Lu X, Wang B, Liu X. 2018. Bioactive pimarane-type diterpenes from marine organisms. *Chemistry and Biodiversity* 15(1):e1700276 DOI 10.1002/cbdv.201700276.

Wang Y, Xu L, Shen H, Wang J, Liu W, Zhu X, Wang R, Sun X, Liu L. 2015. Metabolomic analysis with GC-MS to reveal potential metabolites and biological pathways involved in Pb & Cd stress response of radish roots. *Scientific Reports* 5(1):1–13 DOI 10.1038/srep18296.

Wei CC, Yen PL, Chang ST, Cheng PL, Lo YC, Liao VHC. 2016. Antioxidative activities of both oleic acid and *Camellia tenuifolia* seed oil are regulated by the transcription factor DAF-16/FOXO in *Caenorhabditis elegans*. *PLOS ONE* 11(6):e0157195 DOI 10.1371/journal.pone.0157195.

Wu J, Xiao Q, Xu J, Li MY, Pan JY, Yang MH. 2008. Natural products from true mangrove flora: source, chemistry and bioactivities. *Natural Product Reports* 25(5):955–981 DOI 10.1039/b807365a.

Zhang H, Hua Y, Chen J, Li X, Bai X, Wang H. 2018. Organism-derived phthalate derivatives as bioactive natural products. *Journal of Environmental Science and Health, Part C* 36(3):25–144 DOI 10.1080/10590501.2018.1490512.