Biological activities of Indonesian mangroves obtained by subcritical water extraction

Ratih Pangestuti1,4, Evi Amelia Siahaan2, Febriana Untari1 and Byung Soo Chun3

1Research Center for Oceanography, Indonesian Institute of Sciences (LIPI), Jakarta 14430, Republic of Indonesia
2Research and Development Division of Marine Bio-Industry, Indonesian Institute of Sciences (LIPI), West Nusa Tenggara 83552, Republic of Indonesia;
3Department of Food Science and Technology, Pukyong National University, 45 Yongso-ro, Nam-Gu, Busan 48513, Republic of Korea
4Corresponding author email: pangestuti.ratih@gmail.com

Abstract. Mangroves are still considered as underexploited marine resources in Indonesia, and functional materials from Indonesian mangroves are not characterized yet. In this study, different part of mangroves (Sonneratia alba leaves - SAL, Sonneratia alba roots - SAR, Sonneratia alba fruit - SAF, Rhizophora mcrcronata - RMF, Rhizophora lamarkii - RLF) were hydrolyzed using the subcritical water extraction (SCWE) system at 120 °C. Mangroves hydrolysates (SAL, SAR, SAF, RMF and RLF) were further analyzed for total protein, sugar, reducing sugar, saponin and flavonoid content. The antioxidant capacity and functional material contents including total sugar, reducing sugar, protein, flavonoid and saponin contents. The highest flavonoid and saponin contents were obtained from RLF with the value of 20.13±0.17 mg/g and 31.83±0.18 mg/g, respectively. The antioxidant capacity and antibacterial properties of mangroves hydrolysates varied significantly based on the sample materials, with RLF showing the highest total antioxidant activity and antibacterial activity. The results of our study suggest that tropical mangroves especially R. lamarckii could be valorized efficiently, as a source of bioactive material using subcritical water extraction

1. Introduction
Mangroves are salt tolerant plant that can live in tropical and subtropical areas [1, 2]. Mangroves term is a combination of the Portuguese word “mangue” and English word “grove”. Mangrove ecosystem are among the most productive and important ecosystems because they provide goods and services to environment, other organisms and human society [3]. These salt tolerant plants have been used in traditional medicine practices for treatment of several diseases. Mangroves are recognized as rich sources of biologically active materials such as saponin, phenols, flavonoid, alkaloid and terpenoid. These bioactive materials can be obtained from the roots, leaves, barks, fruits and flowers of mangrove and can be used for treatment of many diseases [4].

In recent years, many studies reported that mangroves-derived bioactive compounds showed various pharmacological activities. As an example, Pcytotoxicity and antioxidant activity of Phoenix paludosa extracts have been reported [5]. Anti-inflammatory, anti-oxidant, anti-arthritic as well
as anti-cholinesterase activities of *Rhizophora mucronata* have also demonstrated [6, 7]. However, most of those studies are using organic solvent extraction technique to obtain bioactive substances from mangroves [8]. The massive, wide-scale use of organic solvents by a diverse range of global industries represents a serious threat to the environment and human health. In order to minimizing solvent consumption, it is important to develop environmental friendly technology. Subcritical water extraction (SCW) has become an increasing alternative technology in the extraction of bioactive compounds from natural resources. SCW provides alternative to conventional extraction methods due to the reduced extraction time, efficient, lower extraction cost, and most importantly environmental friendly extraction technique.

In the present study we used different parts of mangroves i.e leaves, fruit and roots and analyze the effectiveness of green SCW technique to obtain bioactive materials from mangroves. Chemical characteristics of mangrove hydrolysates such as total protein, sugar, reducing sugar, saponin and flavonoid content of various mangroves hydrolysates were investigated. In addition, antioxidant capacity and antibacterial properties of mangroves hydrolysates were also demonstrated.

2. Materials and methods

2.1. Materials

Mangroves parts including *Sonneratia alba* leaves (SAL), *Sonneratia alba* roots (SAR), *Sonneratia alba* fruit (SAF), *Rhizophora mucronata* (RMF), *Rhizophora x lamarckii* (RLF) were collected from Kendari, Southeast Sulawesi-Indonesia in 2017. All the chemicals used in this study were of analytical grade.

2.2. Sample preparation and subcritical water extraction

All mangroves samples (SAL, SAR, SAF, RMF, and RLF) were grind until become a fine powder. Mangroves powder were then transferred into the SCW reactor and mixed with distilled water (pH 7.2) at S/L ratios 1:50. The reactor was closed and heated up to 120 °C. Each hydrolysis process was run for 600 sec. Mangroves hydrolysates were then collected, filtered under a slight vacuum through a Buchi vacuum pump V100 with F1113 grade filter paper, pooled, and lyophilized using freeze dryer, hydrolysates were stored at −20 °C until further analysis.

2.3. Yield, pH and colour characteristics of mangroves hydrolysates

The yield of mangroves hydrolysates was determined. The initial weight of mangroves (\(W_{mi}\)) was obtained from the sample weight before loading into the reactor. The final weight of mangroves hydrolysates was obtained from the final dry weight of hydrolysates after freeze dried (\(W_{mf}\)). The following formula was used for the calculation of yield:

\[
\text{Yield: } \frac{W_{mf}}{W_{mi}} \times 100 \% \quad \text{Eq 1}
\]

Following hydrolysis process, pH of mangroves hydrolysates were measured using pH meter. The color characteristics of mangroves hydrolysates were determined using a reflectance tintometer (Lovibond RT Series, UK). The color values of HMH were expressed as lightness (L* value); green (−) to red (+) (a* value); and blue (−) to yellow (+) (b* value).

2.4. Total flavonoid and saponin contents

Total flavonoid contents (TFC) and total saponin contents (TSC) were measured following the method described in our previous study [9].

2.5. Total protein, sugar and reducing sugar

Protein concentrations of mangroves hydrolysates were measured based on Lowry’s methods. The samples were mixed with 5 mL of alkaline copper sulphate solutions (1% CuSO₄.5H₂O, 2% tartrate (KNaC₄H₄O₆), 2% Na₂CO₃) at 1:100 ratio, and added with 0.5 mL of 1 N Folin-Ciocalteu reagent. Absorbance was measured at 660 nm, total content was expressed as mg BSA per gram dried sample.
The total sugar content (g glucose/100 g dried mass) of mangrove hydrolysates was determined using phenol sulfuric acid method. The reducing sugar content (mg glucose/g dried mass) of mangrove hydrolysate was measured using 3,5-dinitrosalicylic (DNS) colorimetric assay [9].

2.6. Total antioxidant activity
Mangroves hydrolysates (100 μL) was mixed with 3 mL of radical solution (0.6 M H2SO4, 28 mM Na3PO4, and 4 mM (NH4)6Mo7O24). The hydrolysates and radical solution mixture was incubated at 95 °C for 1.5 h. After incubation, the mixture was loaded into 96-well plates and the absorbance was measured at 695 nm using multimode microplate readers. All the measurements were made in triplicate, and methanol was used as the negative control. Ascorbic acid was used as the reference standard, and a standard curve was constructed. The results were expressed as mg ascorbic acid equivalent per g dry weight (mg AAE g⁻¹ DW).

2.7. Anti-bacterial activity
Antibacterial activity of mangroves hydrolysates were tested against gram positive bacteria (Bacillus subtilis) and gram negative bacteria (Escherichia coli) by agar diffusion method [10]. Twenty μL of mangroves hydrolysates (500 μL) were poured into antibiotic disk in agar plate containing bacteria. The agar plates were incubated at 37 ºC for 24 h. The anti-bacterial activity was expressed as clearing zone around antibiotic disk (mm).

3. Results and discussion
3.1 Absorption spectra and MRPs of mangroves extracts
Mangroves extracts obtained from SCW process were analyzed for the UV-absorption spectra. As shown in Figure 1, mangroves extract obtained from SCW process showed high absorbance value ranging from 220 to 270 nm. These peaks might correlate to the absorption spectra of some aromatic compounds. Many studies have reported the presence of aromatic compounds in mangroves. As an example, Li et al. have reported five new aromatic compounds from Bruguiera gymnorrhiza [11]. Mangroves represent a unique marine ecosystem and have to adapt with drastic environmental conditions (especially salt, temperature and nutrient supply) due to the tidal changes. To adapt these conditions, mangroves synthesize various secondary metabolites and therefore being a rich source for natural products.

![Figure 1. Absorption spectra of mangrove hydrolysates obtained by SCWE from 200-500 nm](image)

It has been demonstrated that SCW process produced caramelized sugars and other functional materials from amino acids and reducing sugar by the Maillard reaction. The MRP of mangroves hydrolysates were analyzed by the absorbance at 290 and 420 nm. These wavelengths were used as indicator for the intermediate and final stages of MRPs. The mean values of the absorbance ratios
were ranged from 6.69±0.07 to 17.11±0.52, with the highest value obtained from *S. alba* roots followed by *R. lamarckii* fruit. MRPs also determines flavour and aroma during cooking process; and it is used to make food tastier [12]. In addition, MRPs also reported for their antioxidant properties and their ability to retard lipid oxidation.

**Table 1.** The MRPs of mangrove hydrolysates

|       | 294        | 420        | 294/420     |
|-------|------------|------------|-------------|
| SAL   | 1.65±0.05e | 0.25±0.00b | 6.69±0.07d  |
| SAR   | 1.41±0.06d | 0.08±0.00f | 17.11±0.52a |
| SAF   | 2.11±0.06a | 0.28±0.00a | 7.46±0.14c  |
| RMF   | 1.74±0.00b | 0.20±0.00f | 8.63±0.08b  |
| RLF   | 1.70±0.04bc| 0.19±0.00d | 8.76±0.18b  |

3.2. Total sugar, reducing sugar, and protein contents of mangroves extracts

In this study, total sugar content of mangroves obtained by SCW process (Figure 2) were ranged from 119.67±10.36 to 396.93±1.45 mg/g glucose equivalent (GE). During SCW process, different parts of mangroves showed different sugar content. Compared to other samples, RLF showed highest sugar content. Total sugar in Indonesian mangroves found in this study was comparable with the previous studies reported by Analuddin et al. (2019) and Basak et al. (2016) [13, 14]. Reducing sugar content was found highest in SAR (126.29±11.95 mg/g) followed by RLF (121.40±8.80 mg/g) and RMF (98.79±5.10 mg/g). Low amount of reduced sugar observed in mangroves extracts may be due to the degradation of sugar into other products, including aldehydes and ketones, from which organic acids could be produced [9].

![Figure 2. Total sugar contents (A) and reducing sugar (B) of mangrove hydrolysates. Values correspond to mean ± SD from three independent experiments. a–d letter in each sample are different significantly by Duncan’s multiple range test (p<0.05).](image)

The comparative evaluation of protein contents from SAL, SAR, SAF, RMF and RLF were carried out. The highest protein content was found in RLF (119.97±0.34 mg/g) whereas the lowest was obtained from SAL (33.10±0.20 mg/g) (Figure 3). Protein content of *R. lamarckii* fruit was up to two times as compared to *S. alba*. The results in the present study revealed that the protein content in fruits
of *R. lamarckii* was higher than *Xylocarpus granatum* fruits (4.49±0.17 mg/g), *Bruguiera gymnorrhiza* (1.09±0.72 mg/g), and *S. alba* (0.93±0.02 mg/g) collected from Rawa Aopa Watumohai National Park, Southeast Sulawesi Sites [14]. These results indicated that SCW is a suitable and potential method to obtain protein, peptides, and amino acids from mangroves.

![Figure 3](image.png)

**Figure 3.** Total protein contents of mangrove hydrolysates. Values correspond to mean ± SD from three independent experiments. a–e letter in each sample are different significantly by Duncan’s multiple range test (*p*<0.05)

### 3.3. Total flavonoid and saponin contents of mangroves extracts

Phytochemical constituents in the various parts of the mangroves vary significantly. The fruit of *R. lamarckii* and *R. mucronata* are rich source phenols and flavonoid. The flavonoids and saponin content of the various mangroves extracts are shown in Table 2 and following this order: RLF> RMF> SAF> SAR> SAL. The flavonoid contents found in this study were higher compared to the previous study carried by Syahidah et al. (2019). In their study, they extracted *Rhizophora* sp with acetone and methanol and reported that flavonoid contents were 2.641 mg/g, 1.998 mg/g, respectively [15]. In flavonoid and saponin extraction, solvent and temperature were the most important factors for extraction. At elevated temperature, the solubility of flavonoid and saponin increases because of the breaking of chemical bonds, mass transfer rates, and molecular diffusion was enhanced which indicated that SCW enabled the recovery of flavonoid and saponin from mangroves. Previous studies reported that, flavonoids and saponin show diverse biological activities including anti-cancer, antioxidant, anti-allergic, anti-inflammatory, and anti-microbial activity. In addition, flavonoids have play an important role in protecting DNA from damage, protecting product from degradation; therefore, flavonoids have been used in the production of cosmetics, pharmaceutical and also food products [16].
Table 2. Total Flavonoid and Saponin contents of of mangrove hydrolysates

|     | TFC (mg/g)          | TSC (mg/g)          |
|-----|---------------------|---------------------|
| SAL | 1.70 ± 0.12e        | 1.91 ± 0.05e        |
| SAR | 2.77 ± 0.03d        | 6.04 ± 0.10e        |
| SAF | 3.32 ± 0.00c        | 3.90 ± 0.10d        |
| RMF | 16.93 ± 0.06b       | 24.19 ± 0.29b       |
| RLF | 20.13 ± 0.17a       | 31.83 ± 0.18a       |

3.4 Antioxidant and antibacterial activity of mangroves extracts

The antioxidant activities of mangroves extracts were determined using total antioxidant assay. The total antioxidant results were represented as mg ascorbic acid equivalent per g dry weight (mg AAE g⁻¹ DW). As shown in Table 3, all mangrove species exhibited promising antioxidant activity. The highest antioxidant content was obtained from RLF (3.35 ±0.11 (AAE mg/g) followed by RMF (3.18±0.08 AAE mg/g) and SAF (2.60±0.22 g AAE/g). Natural antioxidants are presented in various part of plant, such as leaves, fruit, bark, flowers, and seeds. Interestingly, all mangrove fruit extract showed more potent antioxidant activity as compared to leaves and roots.

Table 3. Antibacterial and antioxidant activity of mangrove hydrolysates

|     | Inhibitory zone (mm) | Total Antioxidant Activity (AAE mg/g) |
|-----|----------------------|--------------------------------------|
|     | B. subtilis          | E. coli                              |
| SAL | -                    | 6.05 ± 0.00c                         | 1.21 ± 0.01d                           |
| SAR | -                    | 6.70 ± 0.00bc                        | 2.21 ± 0.04e                           |
| SAF | -                    | 7.68 ± 2.30ab                        | 2.60 ± 0.22b                           |
| RMF | -                    | 7.85 ± 0.21ab                        | 3.18 ± 0.08a                           |
| RLF | 8.40 ± 1.84a         | 8.80 ± 0.00d                         | 3.35 ± 0.11a                           |

The results of antibacterial activity of mangroves extract are presented in Table 2. All mangroves extract showed antibacterial activity in Escherichia coli but only R. lamarckii fruit extract showed antibacterial activity against Bacillus subtilis. The highest inhibition zone against E. coli was observed in RLF (8.80±0.00 mm) followed by RMF (7.85±0.21 mm) and SAF (7.68±2.30 mm). Mangroves has been known as treasure house of therapeutic compounds which affected by many factors such as the mangroves species, mangrove parts, habitat and the season of sample collection, different growth stages of plant and experimental methods [17]. In addition, the biological (antioxidant and antibacterial) activity of mangroves showed in this study was positively correlated with total flavonoid and saponin contents.

4. Conclusions

The findings of this study demonstrate that SCW are potential method to be used a s eco-friendly technology to obtain functional materials (including protein, sugar, flavonoid and saponin) from S. alba, R. mucronata and R. lamarckii. The R. lamarckii fruit extracts showed higher protein, flavonoid, saponin and also better antioxidant and antibacterial activities. Therefore, mangroves especially R. lamarckii could be used in the traditional medicines, and develop in foods as well as pharmaceutical industries.

5. References

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