Development of extract library from indonesian biodiversity: exploration of antibacterial activity of mangrove *bruguiera cylindrica* leaf extracts

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Abstract. Antibacterial drugs derived from natural sources play significant roles in the prevention and treatment of bacterial infections since antibiotics have become less effective against many infectious diseases. Mangroves are very potential natural antibacterial sources among great numbers of wild medicinal plants. *Bruguiera cylindrica* is one of the many mangroves species which spread along Indonesian coastline. The aim of this study was to explore the antibacterial activity of *B. cylindrica* wet and dried leaf extracts. The wet extracts study was conducted with three different solvents system (water, ethanol, and n-Hexane) against *Escherichia coli* and *Staphylococcus aureus*. While, the dried extracts study was conducted with four different solvents system (water, ethanol, chloroform and n-Hexane) against three types of bacteria, *Escherichia coli*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The study showed that ethanol was the best solvent for extraction of phenolic and flavonoid. Antibacterial actitivity was measured by zone of inhibition which obtained from agar-disk diffusion method. The widest area of zone of inhibition was showed by wet extracts with ethanol against *S. aureus* and E. coli are 14.30 and 13.30 mm, respectively. While, the zone of inhibition dried extracts with ethanol against *S. aureus*, *S. epidermidis* and *E. coli* are 9.32, 6.59 and 6.20 mm, respectively. In conclusion, both type of extracts showed significant antibacterial activity against gram-positive bacteria as crude extracts.

Keywords: antibiotics, antimicrobial, infectious diseases, medicinal plants, natural products

1. Introduction

Antibiotics are the most important drugs for fighting bacterial infections [1,2]. However, antibiotics have become less effective against many infectious diseases not only because many of them produce toxic reactions to the patient but also due to the emergence of drugs-resistant bacteria [3,4,5,6,7]. The need for new, effective and affordable drugs to treat infectious diseases in the developing world is one of the major issues facing global health today.

Antibacterial drugs derived from natural source play significant roles in the prevention and treatment of human infection diseases [1,8,9]. Natural bioactive compounds would be best solution to overcome the effects of antibiotics resistance bacteria with no or less side effects [10,11]. In recent

decades, many studies have been carried out on different plant species to discover compounds of possible interest for medicinal application against bacterial infections [12,13,14]. Among these studies, several have focused on the biological and phytochemical properties of different species of mangrove [15,16,17].

There are about 3.6 million hectares of mangrove forests which grew along 95,000 km of Indonesian coastline and it represent approximately 23% of the total mangrove forests in the world [18,19]. *Bruguiera cylindrica* is one of the many mangroves species which spread along Indonesian coastline. However, the antibacterial potentials of this Indonesian mangroves are yet to be discovered.

Another factor that renewed the exploration of mangrove is the rapid rate of plant species extinction in the past 20 years and the most threatened plant species are found in the tropics [20]. Mangrove forests suffered a shrinkage of about 1-2% every year [21]. Before any medicinal use or other important features can be assessed, it is likely that some species will extinct before they are even discovered.

The aim of this study was to explore the antibacterial activity potency of *B. cylindrica* leaf extracts against *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*.

2. Methods

2.1. Plant Materials

The leaves of *B. cylindrica* harvested from Kawasan Ekowisata Mangrove, Pantai Indah Kapuk, North Jakarta, Indonesia with the permission from Department of Marine, Agriculture, and Food Security Special Capital Region of Jakarta. *B. cylindrica* was identified by Indonesian Institute of Sciences (LIPI) taxonomist at Cibinong Science Center. Before used, the leaves were firstly cleaned with tap water to remove dirt and then rinsed with distilled water. The leaves were cut into transverse of approximately 2 cm width and dried by using oven (Memmert, Germany) at 45 ºC for 12 hours. The dried leaves were ground into powder using blender (Cucina Philips, Indonesia) and screened by using mesh number 35 (CISA Cedaceria Industrial, Spain). The powder was then transferred into glass bottles with rubber stopper, wrapped in almunium foil and stored in the freezer at -20 ºC prior to extraction.

2.2. Extraction

The method was based on method described by Dhayanithi et al. [22] with slightly modification. The leaf powder transferred into an erlenmeyer flask and immersed in solvent (1:10) for 24 hours in a shaker set at 125 rpm. The erlenmeyer flask mouth was sealed with aluminium foil and Parafilm and the whole flask was covered with aluminium foil to protect the content from light. After 24 hours maceration, the extracts were filtrated through cotton fine meshed cloth. The filtrates were then centrifuged (Hettich Rotina 35R centrifuge, Germany) for 10 minutes at 10,000 rpm, 25 ºC. The supernatants were then collected by using graduated pipette and prepared for further phytochemical analysis.

2.3. Determination of total phenolic content

The determination of total phenolic content (TPC) of extracts obtained was adapted from Banerjee et al., 2008 [23] with slightly modification and calibrated against galic acid as the reference standard. A 0.3 ml sample was mixed with 1.5 ml of Folin-Ciocalteu reagent and 1.2 ml of sodium carbonate (7.5%), consecutively. The mixture of each step should be mixed well by using vortex (Genie 2 mixer, Scientific Industries, USA) and allowed to stand for one hour in a dark chamber. Absorption was measured by using UV-Vis spectrophotometer (Genesys 10 UV-Vis spectrophotometer, Thermo Electron Corporation, USA) at 765 nm. The standard curve galic acid was prepared by diluting the stock standard with the extraction solvents to yield 50 to 200 ppm TPC. The results were calculated according to the calibration curve for galic acid and crude extract of TPC derives from quadruplicate analyses and expressed as galic acid equivalents (GAE mg/ g) of dry material (DM).

2.4. Determination of flavonoid content

The determination of flavonoid content (FC) was adapted from Do et al. [24] with slightly modification and calibrated against quercetin as the reference standard. The Leaf powder was first diluted with its solvent to reach dilution factor of 25 for test the FC in the samples. 1.5 ml of methanol in test tube was prepared. A 0.5 ml sample was mixed with 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water, consecutively. The mixture of each step should be mixed well by using vortex. The mixtures were incubated in a dark chamber for 30 minutes and absorbance was measured by using a UV-Vis spectrophotometer at 415 nm. The standard curve quercetin was prepared by diluting the stock standard with the extraction solvents to yield 20 - 200 ppm of FC. The results were calculated according to the calibration curve for quercetin and crude extract of FC derives from quadruplicate analyses and expressed as quercetin equivalents (QE mg/ g) of dry material (DM).

2.5. Antibacterial activity

The study of antibacterial activity was conducted in two conditions, the wet extracts study which held at Swiss German University and conducted by using three different solvents (water, ethanol, and n-Hexane) against two types of bacteria, *E. coli* and *S. aureus*. While the dried extracts study which held at Biopharmaca Tropica Research Center, LPPM IPB and conducted by using four different solvents based on the level of polarity (water, ethanol, chloroform and n-Hexane), against three types of bacteria, *E. coli*, *S. epidermidis* and *S. aureus*.

The Agar disk diffusion assay has been widely used to assay plant extract for antibacterial activity [25]. *E. coli*, *S. epidermidis* and *S. aureus* were adjusted to certain concentration, 1 x 10^8 CFU/ml or 0.5 mcFarland's standard [26], were inoculated onto the entire surface of a Mueller-Hinton agar (MHA) plate with a sterile cotton-tipped swab to form an even lawn. In this method, 6 mm sterilized filter papers disks (Whatmann No. 1) were soaked into *B. cylindrica* extract and air dried for one hour at bio safety cabinet. Each type of extracts replicated by three. The paper disks which was impregnated with diluted extracts were placed on the surface of each MHA plate by using a sterile pair of forceps/ pincers. Then the plates were incubated at 37^{o}C for 24 hours and diameter of the zone of inhibition (ZOI) obtained was measured by a ruler or caliper. The bigger diameter of the ZOI, the more susceptible of the microorganism to the extracts.

2.6. Statistical analysis

Data result was analyzed by Analysis of Variance (ANOVA) single factor. The P-two tail or P-Value below 0.05 ($P < 0.05$) indicated that the data were significantly different, while if P-Value above 0.05 ($P > 0.05$), the data were insignificantly different. The data graphic was built using Microsoft Excel 2010.

3. Results and Discussion

Extraction of plant materials depends on various factors such as solvents, methods, and extraction time to separate different quality and quantity of bioactive components in the crude extracts [27]. Polarity of solvent plays key role in the outcome of bioactive compound extracted from *B. cylindrica*. The wet extracts with ethanol showed the highest total phenolic content compared with water and n-Hexane, consecutively (Figure 1). It was because ethanol has the ability to bind with hydrophilic compounds such as flavonoid and phenolic compound [28]. Ethanol is chosen when water-insoluble constituents need to be extracted. However, higher ethanol percentages do not necessarily mean higher extraction activity [29].

The wet extracts with ethanol also showed the highest flavonoid content compared with water and n-hexane, consecutively (Figure 2). Less polar flavonoids (e.g., isoflavones, flavanones, methylated flavones, and flavonols) are best extracted with chloroform, dichloromethane, diethyl ether, or ethyl acetate, while flavonoid glycosides and more polar aglycones are best extracted with alcohols or

alcohol–water mixtures [30,31]. The combination of water and organic solvent facilitate the extraction of chemicals that are soluble in water and/or organic solvent [24].

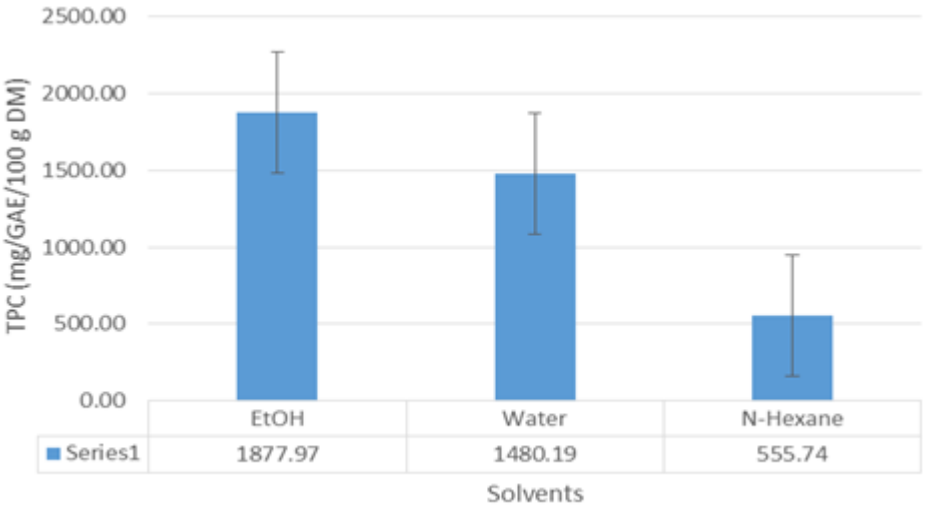

Figure 1. Total phenolic content of *B. cylindrica* leaf extracts

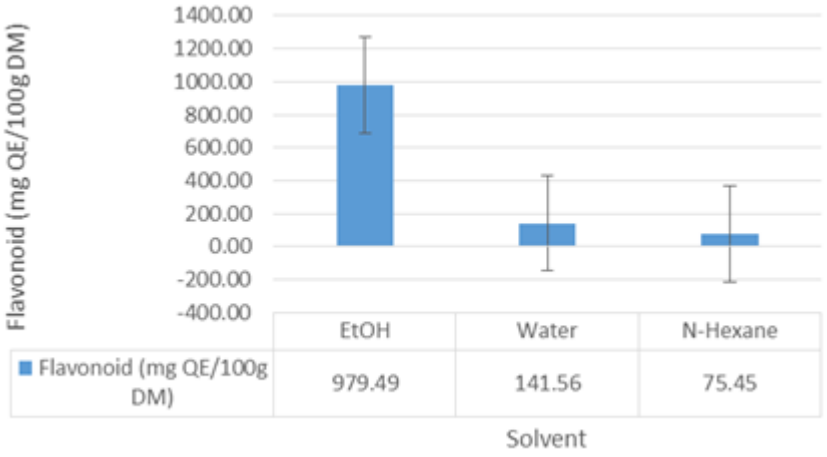

Figure 2. Flavonoid content of *B. cylindrica* leaf extracts

The mechanisms for phenolic toxicity to microorganisms include adsorption and disruption of microbial membranes, interaction with enzymes, and metal ion deprivation [32,33,34]. The higher FC was also expected to show the higher antibacterial activity since flavonoids are synthesized by plants in response to microbial infection [35,36,37,38]. Flavonoid involved in inhibition nucleic acid biosynthesis, inactivate microbial adhesins enzymes and cell envelope transport proteins [39,40]. Lipophilic flavonoids may also disrupt microbial membranes [13,36,41].

The ZOI obtained of the wet extracts with ethanol against *S. aureus* and *E. coli* were 14.30 and 13.30 mm, respectively (Figure 3). The dried extracts study also showed a consistent results with the wet extracts study. The ZOI of the dried extracts with ethanol against *S. aureus*, *E. coli*, and *S. epidermidis* were 9.32, 6.20 and 6.59 mm, as showed in Table 1. Taken altogether, the leaf extracts can be categorized as bacteriostatic due to the capability of bacterial growth inhibition. Quantitatively, the leaf extract can be classified as weak-to-moderate antibacterial since the ZOI obtained around 10

to 15 mm. While, the ZOI obtained by Ciprofloxacin, tetracycline and chloramphenicol were 41.2, 22.10 and 20.80 mm.

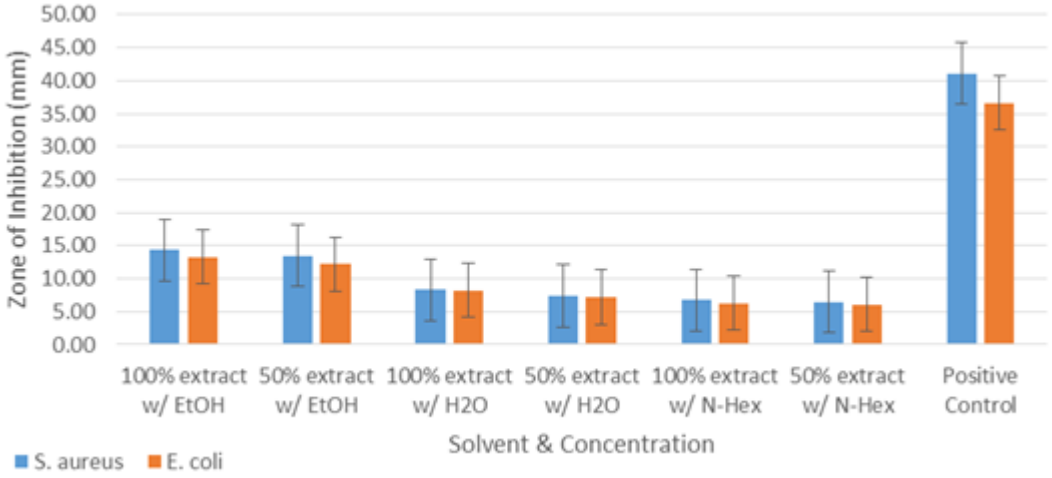

Figure 3. Zone of inhibition of wet extracts

Table 1. Antibacterial test of *B. cylindrica* leaf extracts againts *E. coli* and *S. aureus*

Conc	(+) mm		Extraction						(-) mm
(%)	Tetra	Chloram	b. Cyl ethx (mm)			b. Cyl chlx (mm)			DMSO
	22.10	20.80	SA	EC	SE	SA	EC	SE	
0.4			9.32	6.20	6.59	7.32	6.88	7.42	
0.2			8.32	6.00	6.32	7.00	6.52	6.26	
0.1			8.08	6.00	6.16	6.72	6.00	6.00	
0.05			6.40	6.00	6.20	6.56	6.02	6.52	
									6.00

Conc : concentration, tetra : tetracycline, chlor : chloramphenycol, b. cyl ethx : *B. cylindrica* ethanol extract, b. cyl chlx : *B. cylindrica* chloroform extract, r. muc ethx : *R. mucronata* ethanol extract, r. muc chlx : *R. mucronata* chloroform extract, DMSO = DMSO 20%, SA: *S. aureus*, EC: *E. coli*, and SE: *S. epidermidis*.

Since all the extracts consist of many different compounds, they presumably contain single compounds with higher activity than the value obtained as crude extract. In addition, isolation and characterization of the antibacterial compounds presented in these extracts seemed to be worthwhile [42]. The potential for developing antimicrobial agents from mangrove species appears rewarding, as it may lead to development of phytomedicine against pathogenic microbes [43].

Study with different sampling time, age of plant, plant part, solvent, method of extraction and time required for extraction would be necessary [44,45]. Because, many of the phytochemicals in plants can be detected at different concentrations because the amount and composition of secondary metabolites are not constant and their concentration depends on the tissue type and the age of the plant [46,47].

In general, both type of extracts showed significant antibacterial activity against gram-positive bacteria. This matter may be correlated with the cell envelopes composition. The cell envelopes of most bacteria fall into one of two major groups. Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharide. Gram-positive bacteria lack an outer membrane but are surrounded by layers of peptidoglycan many times thicker than is found in the Gram-negatives [48]. Therefore, gram-negative bacteria is more resistant than gram-positive due to the lipopolyproteins and peptidoglycan

composition in their cell envelope [49]. Gram-negative cell walls is strong to withstand three atm of turgor pressure, tough to endure extreme temperatures, pH and elastic to be capable of expanding several times their normal surface area [50].

Conclusions

The study of *B. cylindrica* leaf extract suggested that ethanol was the best solvent. The widest area of ZOI was showed by wet extracts with ethanol against *S. aureus* and *E. coli* were 14.30 and 13.30 mm, respectively. While, the ZOI dried extracts with ethanol against *S. aureus*, *S. epidermidis* and *E. coli* were 9.32, 6.59 and 6.20 mm, respectively. In conclusion, both type of extracts showed considerably high antibacterial activity against gram-positive bacteria as crude extracts.

Acknowledgments

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