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Usability of planted mangroves in the coastal area of Semarang, Indonesia, as the source of secondary metabolite extracts

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Abstract. *Hastuti ED, Izzati M, Darmanti S. 2023. Usability of planted mangroves in the coastal area of Semarang, Indonesia, as the source of secondary metabolite extracts. Biodiversitas 24: 2409-2415.* Mangrove plants are a potential source of secondary metabolites. So the secondary metabolites from mangrove plants must be assessed to promote their utilization. Considering this, the present study was carried out to analyze the alkaloid and phenol content in mangrove species in Tugu Sub-district, Semarang City, Central Java, Indonesia. Alkaloid and phenol content in five mangrove species (*Avicennia marina*, *Bruguiera gymnorrhiza*, *Rhizophora mucronata*, *Sonneratia alba*, and *Xylocarpus moluccensis*) were estimated using the spectrophotometry method. The study results revealed that the alkaloid content of *A. marina* was highest (0.23 ± 0.11%), while the highest concentration of phenol content was found in *X. moluccensis* (9.66 ± 1.36%). However, after including the other parameters, viz., abundance Index and accessibility Index, *A. marina*, was found to be a potential source of alkaloid extract, and *R. mucronata* was a potential source of phenol extract.

Keywords: Abundance, alkaloids, mangroves, metabolites, phenol

INTRODUCTION

Science and technology advancements have brought further improvement in the chemical industry (Hussain et al. 2012; Yaegashi et al. 2014). New chemical products are needed to support those industries, including those related to secondary metabolites. In this regard, there has been an increasing expectation of new alternative sources of secondary metabolites, leading to the exploration of new potential sources (Costa et al. 2014; Tiwari dan Rana 2015). It is increasingly acknowledged that mangrove plants are rich in natural products and new chemical compounds (Glasenapp et al. 2019; Bibi et al. 2019; Parthiban et al. 2022). Mangrove forest coverage accounts for approximately 137,600 km^2 worldwide and thrives in tropical and sub-tropical coastal areas (Hamilton dan Casey 2016). There are approximately 70 mangrove species that have been identified all over the world (Hogarth 2015). However, the species composition of mangrove species is different between locations. The geographical features, such as climate, soil structure, and tidal pattern, drive mangrove structural differences (Bryan-Brown et al. 2020). Several biologically active compounds with varying degrees of action, such as anticancer, antiulcer, antioxidant, antidiabetic, and antimicrobial properties, have been isolated from mangroves (Nurdiani et al. 2012; Gajula et al. 2020; Parthiban et al. 2022). The most valuable bioactive compounds obtained from mangrove plants include polyphenols, flavonoids, alkaloids, carotenoids, tannins, saponins, steroids, amino acids, carbohydrates, proteins, vitamins, terpenes, and glycosides (Bandaranayake 2002; Rouf et al. 2007; Wu et al. 2008; Bibi et al. 2019; Sachithanandam et al. 2019).

Despite the potential source of secondary metabolites products, utilization of secondary metabolites from mangroves is still limited. Moreover, mangrove plants exhibit wide variation in secondary metabolite concentration concerning location and species. For instance, Kumar et al. (2014) found phenol in the leaves of several mangrove species such as *A Avicennia marina*, *Rhizophora mucronata*, and *Ceriops tagal* were 0.72 ± 0.012 mg/mL, respectively 0.66 ± 0.009 mg/mL and 0.61 ± 0.012 mg/mL. The alkaloid content on the other side was 0.15 ± 0.019 mg/mL, 0.16 ± 0.017 mg/mL, and 0.10 ± 0.018 mg/mL. Ariyanto et al. (2018) found that mangrove tannin content varies according to the species. As much as 4.39 mg/g of tannin content in *R. mucronata* leaves, while in *A. marina*, it was only 0.85 mg/g and 7.09 mg/g in *Sonneratia alba*. Balakrishnan et al. (2016) found that tannin content in *R. mucronata* leaves was between 1.23 - 5.71 mg/g. Different concentration of secondary metabolites was also found between organs, such as Suh et al. (2014), who found that the concentration of total phenol in the leaf, stem, and root of *R. stylosa* as much as 9.32 ± 1.10 mg/g, 11.46 ± 0.56 mg/g, and 7.30 ± 0.76 mg/g respectively. These results showed that particular growing locations and species provide different potential secondary metabolite contents.

The effort to utilize mangroves as the source of secondary metabolites chemicals is faced by the geographical condition of the mangrove ecosystem and its ecological features (Pham et al. 2019). Mangrove plants typically grow in the coastal area affected by tidal activities. Established mangrove forests are often dense and aren't easy to access. Moreover, mangrove plants and the ecosystem have a protective role in the coastal area that cannot be neglected (Maza et al. 2021). Thus, the utilization of mangroves needs proper ecosystem management.

The coastal area is the main habitat of mangrove plants. Unfortunately, there has been a serious threat to the existence of the mangrove ecosystem due to the intensive exploitation of coastal and terrestrial areas (Kauffman and Donato 2012). Slowly, mangrove vegetation is losing its growing habitat, altering the risk of extinction. The pressure experienced by the mangrove ecosystem varies between locations due to various factors, such as geological features, hydro-oceanographic activity, and the intensity of anthropogenic activities.

Recently, there has been an increasing concern about the upcoming threats to the coastal area, which led to the altering awareness of the importance of the mangrove ecosystem (Dalimunthe and Putri 2017; Rahman and Asmawi 2016). As the impact, mangrove planting has become more distributed and no longer focused in the beach area (Bryan-Brown et al. 2020). Therefore, mangroves can be spotted in any potential location, such as pond areas, eroded areas, and estuaries.

Massive planting of mangrove vegetation has forced mangroves to inhabit unsuitable habitats. Therefore, only pioneer mangrove species could survive better due to the dynamic environment (Teutli-Hernández et al. 2019; van Bijsterveldt et al. 2022). However, mangrove plants must adapt properly to cope with the unsupportive environment. The adaptation could be identified by changing secondary metabolite productions (Rodrigues and Černáková 2020). Typically, secondary metabolite production increases when disturbances exist (Mahajan et al. 2020). That suggests mangrove has a chance of increasing secondary metabolite content. Therefore, along with the massive planting activity, the adaptation process of mangrove plants could be seen as a potential source of secondary metabolites production cycle.

Approximately 77.73% of mangrove coverage in Tugu Sub-district is severely damaged, while the remaining 22.27% is moderately damaged, leaving no coverage of mangroves in good condition (Ardiansyah and Buchori 2014). Therefore, the mangrove ecosystem in Tugu Sub-district is vulnerable to tidal flooding (Utami et al. 2021). Conversely, land subsidence also promotes the increased vulnerability of coastal areas (Husnayaen et al. 2018). Tugu Sub-district in Semarang City is where massive mangrove planting has been carried out during the last few decades. The planting activity made mangroves developed in Tugu Sub-district as an artificial ecosystem with *A. marina* and *R. mucronata* as dominant species (Tefarani et al. 2019). However, traces of original mangrove remnants can still be spotted in certain locations. This shows the greater option of mangrove utilization as the source of secondary metabolites products. However, the utilization of mangroves as a source of secondary metabolite products requires appropriate assessment. Therefore, this research aimed to identify the species of mangrove in the coastal area of Tugu Sub-district, Semarang City, to analyze the alkaloid and phenol content in mangrove leaves and assess its potential for utilization as secondary metabolite sources.

MATERIALS AND METHODS

Study area

The research was carried out in the coastal area of Tugu Sub-district, Semarang City, Central Java, Indonesia. The design of the research was qualitative with a case study method. The research object was mangroves in the coastal area of Tugu Sub-district.

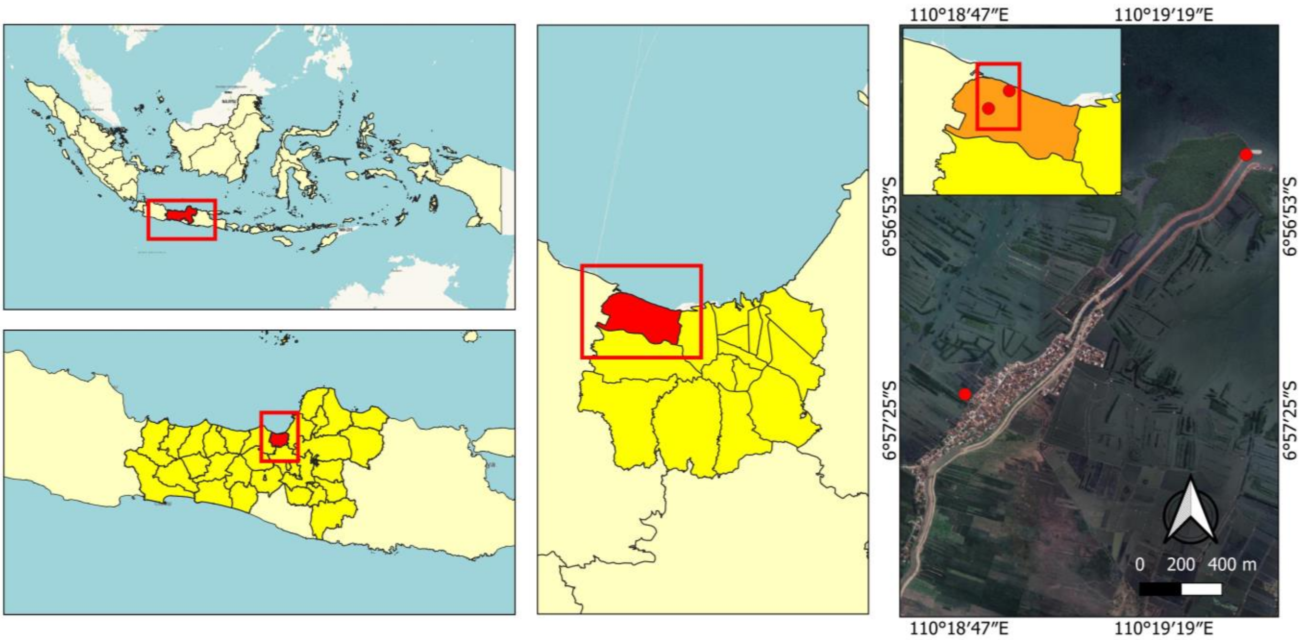

Figure 1. Map showing the sampling station in Tugu Sub-district, Semarang City, Central Java, Indonesia

Data collection of mangrove potential as the source of secondary metabolites was carried out through a field survey. An interview with the local community was conducted to identify the distribution of mangrove species in the coastal area of Tugu Sub-district along with its location. Based on the obtained information, a sampling of mangrove leaves was carried out. The sampling method was designed as purposive sampling. If mangrove species were found to have three or more plants, triplication of the sample was taken. However, samples were taken from existing plants when mangrove species were found in less than three plants. The samples were then preserved in cold storage. Later, the analysis of leaf samples was carried out in the laboratory. Analysis of secondary metabolite content was performed for total alkaloid and total phenol.

Phenol testing

The method used in phenol testing was spectrophotometry, while the Follin Ciocalteu was used in sample preparation. The laboratory uses the following procedure in phenol testing. First, as much as 50 mg of leaf sample was put in the test tube. Then, 0.5 mL of Folin ciocalteu phenol and 7.5 mL of aquadest were added. The mixture was shaken to dissolve the solution and cooled down for 10 minutes. Next, 1.5 mL of 20% Natrium carbonate was added, shaken, and cooled down for 10 minutes. Then, aquadest was added gradually until the solution reached a volume of 10 mL, 25 times the original volume, then diluted the solution. The diluted solution was then tested in the spectrophotometer at the wavelength of 760 nm for its absorption (LPPT-UGM Yogyakarta). The spectrophotometer used was Shimadzu UV - 1800.

The standard solution used 10 mg of gallic acid through a similar procedure. After that, the produced solution was diluted to the concentration of 0.2, 0.5, 1, 2.5, 5, 10, 25, 50, 75, and 100 ppm. Next, those solutions were tested in spectrophotometry for their absorption. The test results of the leaf sample solutions were then compared to the result of the standard solution. Thus, the actual phenol concentration was obtained.

Alkaloid testing

The method used in alkaloid testing was spectrophotometry. The following procedure was used in the alkaloid testing process. First, as much as 100 mg of leaf sample was taken, added with 5 mL of HCl_2N, and then shaken. The obtained solution was then washed thrice using 10 mL of chloroform in different funnels, and then the chloroform phase was wasted. The solution was then neutralized using 0.1 N of NaOH. The next step was adding 5 mL of BCG and 5 mL of Phosphate Buffer solution. The solution was then extracted using 5 mL of chloroform by stirring it using a magnetic stirrer at 500 rpm for 15 minutes. The extraction was repeated twice. The following step was collecting the chloroform phase and evaporating it using nitrogen gas while chloroform was slowly added until the solution volume reached 5 mL. The obtained solution was then tested in the spectrophotometer at 470 nm of wavelength.

The standard solution used 10 mg of quinine through a similar procedure. However, the dried chloroform phase was diluted until the solution volume reached 10 mL. The obtained solution was then diluted further to various concentrations, including 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 ppm. Then, an absorption test was performed using a spectrophotometer. The result of the samples test was then compared to the test result of the standard solution. Thus, the actual alkaloid concentration was obtained (LPPT-UGM Yogyakarta).

Assessment of mangrove usability

Assessment of mangrove usability as the source of secondary metabolites was carried out descriptively. The parameters used in the assessment included mangrove abundance, accessibility, and secondary metabolite contents. Mangrove abundance was categorized into five levels, and so was accessibility. Classification of mangrove abundance adopted the classification of plant abundance suggested by Braun-Blanquet, which divided plant community by cover percentage (McNellie et al. 2019). Therefore, this research classifies mangrove abundance into five categories and is weighted accordingly. Table 1 shows the abundance criteria applied in the research.

Accessibility represents the effort needed to reach certain goods or services. Accessibility is not limited to physical access but includes availability and safety aspects. Several factors, such as land use, transportation, and temporal components, are related to accessibility (Lucas et al. 2016). Unfortunately, no criteria have been related to the accessibility of the mangrove ecosystem. Therefore, the authors defined a classification regarding the accessibility of mangrove locations. Table 2 shows the category and criteria of mangrove accessibility.

Table 1. Criteria of mangrove abundance

Category	Range	Usability index
Very scarce	< 10 plants	1
Scarce	10 - 100 plants	2
Fairly abundant	100 - 500 plants	3
Abundant	500 - 1000 plants	4
Very abundant	> 1000 plants	5

Table 2. Criteria of mangrove accessibility

Category	Criteria	Usability index
Inaccessible	Plants exist in the middle of dense mangrove forest	1
Difficult to access	Plants exist in the inner side of inundated mangrove forest	2
Fairly difficult to access	Plants exist on the edge of inundated mangrove forest	3
Easy to access	Plants exist on the edge of mangrove forests with land access	4
Very easy to access	Plants exist in the pond area or near settlements	5

Classification of secondary metabolite content was carried out based on laboratory analysis results. Based on the obtained data, secondary metabolite contents were classified into five classes with constant intervals. Thus, the usability index between alkaloid and phenol content was different. Management strategy was arranged to improve the potential and sustainability of mangrove utilization as the source of secondary metabolites. The strategy was constructed based on the existing condition of mangrove potential.

RESULTS AND DISCUSSION

The results revealed the existence of five mangrove species in the coastal area of Tugu Sub-district, Semarang City *A. marina*, *R. mucronata*, *Bruguiera gymnorrhiza*, *S. alba, and Xylocarpus moluccensis.* However, there was a variation in the distribution and abundance of each mangrove species. Mangrove plants were found in the shore area and pond area and are typically planted vegetation. However, the mangrove population was concentrated in the shore area. Mangrove plants found in the pond area resulted from its integration into fish farms, namely silvofishery.

Three mangrove species, including *A. marina*, *R. mucronata*, and *B. gymnorrhiza*, were found in the shore and pond areas. In the shore area, *A. marina*, and *R. mucronata*, were found along the coastline. Therefore, both species were easily accessible. On the other side, *B. gymnorrhiza* was found in the inner side of a dense mangrove forest, making it difficult to access. In the pond area, *R. mucronata* occupied most of the pond dikes, and *B. gymnorrhiza, and A. marina*, were typically planted in the middle of the ponds. *S. alba*, and *X. moluccensis*, were found in the shore area and are considered natural existences. Among the five mangrove species found in the coastal area of Tugu Sub-district, only *A. marina*, and *R. mucronata*, had the most considerable abundance. In contrast, the other three were found to be very scarce.

Analysis of secondary metabolite content showed that each mangrove species had different concentrations of alkaloid and phenol content. For example, although the research found that the average alkaloid content was highest in *A. marina* and lowest in *B. gymnorrhiza*, analysis of average phenol content was highest in *Xylocarpus sp* and lowest in *B. gymnorrhiza*. Detailed results of secondary metabolite content in various mangrove species are presented in Table 3.

Analysis of the usability of mangrove species as a source of secondary metabolites was carried out through a deductive assessment of the pros and cons of respective species. Some parameters included in the consideration included the abundance, accessibility, and secondary metabolites content. Table 4 shows the assessment result of mangrove's usability as a source of secondary metabolites.

Based on the usability index analysis shown in Table 4, further analysis is carried out to describe the pros and cons of each species along with its usability potential. Finally, the result is presented in Table 5. Table 4 shows that *A. marina* and *R. mucronata* were the most potential mangrove species to be utilized as sources of secondary metabolites products. *A. marina* was the potential for an alkaloid source, while *R. mucronata* was the potential for a phenol source. The abundance and its growing location supported the potential. Unfortunately, the other mangrove species were considered not utilizable due to certain issues, such as the scarce abundance and sustainability. Therefore, the utilization of *A. marina* and *R. mucronata* must be considered.

Referring to the assessment result, the utilization of mangroves as the source of secondary metabolites should focus on the extraction of alkaloids from A. marina and phenol from *R. mucronata*. Both mangrove species are abundant, leaving a large space for future utilization. In addition, *X. moluccensis* had great potential as a phenol source. Unfortunately, currently, the species is threatened to be locally extinct. Therefore, it should be preserved to ensure its sustainability.

Table 3. Alkaloid and phenol content (%w/w) in Tugu Sub-district, Semarang City, Central Java, Indonesia mangrove leave samples

Species	Alkaloid	Phenol
A. marina	0.23 ± 0.11^{b}	2.18 ± 1.18^{a}
R. mucronata	0.14 ± 0.10^{ab}	3.90 ± 1.11^{b}
B. gymnorrhiza	0.09 ± 0.05^{a}	2.10 ± 0.25^{a}
S. alba	0.17 ± 0.09^{ab}	2.48 ± 0.25^{a}
X. moluccensis	0.11 ± 0.04^{ab}	9.66 ± 1.36^{c}

Note: different letters in the same column indicates significant difference

Table 4. Usability index analysis

Species	Abundance index	Accessibility index	Secondary metabolites content rank		Usability index	
			Alkaloid	Phenol	Alkaloid	Phenol
A. marina	5	4	5	1	100	20
R. mucronata	5	5	2	2	50	50
B. gymnorrhiza	2	1	1	1	2	2
S. alba	1	2	3	1	6	2
X. moluccensis	1	2	1	5	2	10

Table 5. Usability of mangrove plants as a source of secondary metabolites in Tugu Sub-district, Semarang, Central Java

Species	Prospect	Constrain	Usability
A. marina	Very abundant It has the highest concentration of alkaloid	Mostly grown in the shore area. It has a fair concentration of phenol	Utilizable with fair effort
R. mucronata	Very abundant Plants grow in the pond area It has a high phenol concentration	It has a fair alkaloid concentration	Utilizable with less effort
B. gymnorrhiza	-	Scarce Mostly grow in the middle of dense mangrove forest It has a low alkaloid concentration It has a fair phenol concentration	Not utilizable
S. alba	-	Very scarce Threatened natural existence It has a fair alkaloid concentration It has a fair phenol concentration	Not utilizable
X. moluccensis	It has the highest phenol concentration	Very scarce Threatened natural existence It has a low alkaloid concentration	Not utilizable

Discussion

The research found that *A. marina*, and *R. mucronata*, were potential secondary metabolites production sources in Tugu Sub-district, Semarang City, Central Java, Indonesia. Furthermore, *A. marina* is the potential for alkaloid production, while *R. mucronata* is the potential for phenol production. Therefore, the environmental condition could cause both species' high secondary metabolite contents. *A. marina* and *R. mucronata* are pioneer mangrove species that grow in the inter-tidal area (Syahirah et al. 2018). Compared to the other mangrove species, *A. marina* and *R. mucronata* have a better chance of survival while planted in a disrupted coastal area (Sari et al. 2019). Therefore, both species are frequently used in the planting activity for mangrove forest rehabilitation.

Typically, mangrove plants in Tugu Sub-district are the result of planting activity. Therefore, they were planted in the coastal area where environment quality dynamic exists. Unfortunately, the environment quality in the coastal area of Semarang City has been degraded, causing disturbances to mangrove plants. Therefore, secondary metabolite contents increase as a response to environmental disturbances (Bartwal et al. 2013; Yang et al. 2018). Different types of secondary metabolites produced in *A. marina* and *R. mucronata* indicates different kind of pressure attained by each species. Preferences and tolerance of each mangrove species determine environmental drivers that could cause disturbances to mangrove plants (Dasgupta et al. 2017).

Assessment of mangrove potential is expected to find mangrove species with high concentrations of secondary metabolites. Plants with high concentrations of secondary metabolites are expected to improve productivity and production efficiency (Salvi et al. 2019). When higher concentration raw material is used, the requirement for the raw materials could be reduced. Utilization of concentrated raw materials is beneficial for the sustainability of production (Seglah et al. 2019). The remaining source could be conserved for later utilization by utilizing a few resources. Conversely, utilizing a concentrated raw material is also more efficient. Since the required volume of raw materials could be reduced, the requirement of crop fields could be minimized. Less workforce is needed while processing time could be reduced, reducing production costs.

Typically, plants are exploited for their specific features or compounds. Refer to the finding of the research, *A. marina* was more suitable for alkaloid extraction (Dharmautama et al. 2017; Sormin et al. 2021), while *R. mucronata* was more suitable for phenol production (Arulkumar et al. 2020; Mangrio et al. 2016; Ramalingam and Rajaram 2018; Sungkar et al. 2019). Therefore, the utilization of each mangrove species should be designed for its respective potential. Plant abundance is an important factor supporting secondary metabolite production (Jayaraman dan Mohamed 2015). More population should provide more product volumes. On the other side, an abundant plants population should ensure the sustainability of the utilization (exploitation) activities. In order to provide continuous production, plants need to be cropped in cycles. A longer exploitation period could be attained when the resource availability is abundant, while the available resources could be used as the reference in designing the utilization plan. In addition, the accessibility of resources is a factor related to the exploitation plan. In order to be utilized, a resource should be accessible. While accessibility is absent, extra effort is needed to make it accessible. As the impact, more cost is needed as the capital or production cost.

The research finding suggested that using mangroves as the source of secondary metabolites, specifically for alkaloids and phenols, could be applied to *A. marina* and *R. mucronata*. Both species' abundance and secondary metabolites content were quite supportive. Actually, *X. moluccensis* had the potential to be used as the source of phenol product. Unfortunately, the plant's existence is threatened because the population is too low; because it is a remnant of original mangrove plants in the coastal area of Tugu Sub-district, preservation is needed, followed by population improvement. This should be viewed as a potential resource for future utilization. Arrangement of utilization design is needed to optimize the efficiency of mangrove exploitation as a source of secondary metabolites products.

In conclusion, five mangrove species are found in the coastal area of Tugu Sub-district, Semarang City, including *A. marina, R. mucronata, B. gymnorrhiza, S. alba, and X. moluccensis*. The concentration of secondary metabolites varied between plants, but the highest alkaloid concentration was found in *A. marina*, while phenol content was the highest in *X. moluccensis.* However, based on the abundance and accessibility, *A. marina* was a potential source of alkaloid extract, and *R. mucronata* was found to be a potential source of phenol extract. *B. gymnorrhiza* and *S. alba* could not be promoted because of their scarcity, located in the middle area and categorized as threatened in the study area.

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