

Total Phenol Content of *Avicennia marina* Leaf and Its Relationship to the Environmental Quality

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Abstract. Environmental dynamic in the coastal area is suggested to affect the metabolite concentration in mangrove plants. This research aimed to study the concentration of total phenol in *A. marina* leaf and to analyze the effect of environmental parameters on total phenol content dynamics. Environmental parameters studied in this research was temperature, pH, DO, and salinity, as well as N, P, and C sediment content. Data analysis was carried out through multiple regression of natural logarithm transformed data. Laboratory analysis resulted the value of total phenol content in *A. marina* leaf ranging from 0.88 – 1.62% with the average concentration of $1.28 \pm 0.28\%$. Regression analysis resulted the significant effect of temperature, DO, and sediment content P and C expressed in the formula: $\ln(\text{TP}) = 31.229 - 7.224\ln(\text{T}) - 0.067\ln(\text{DO}) - 1.054\ln(\text{P}) - 1.241\ln(\text{C})$. The research implicated that the increasing value of those factors was approaching the suitable condition for *A. marina*. Thus, instead of increasing the phenol concentration, the parameters negatively effect the secondary metabolite. The result showed that increasing temperature, DO, and content of P and C reduced the stress in *A. marina* and reduce total phenol content. This suggests that low temperature, DO, P and C concentration provides more potential of phenolic products from *A. marina*.

Key words: Mangrove; Negative; Nutrient; Phenol; Regression

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INTRODUCTION

Secondary metabolites are complex chemical compositions synthesized by plants to support their growth development as well as to respond to various stresses (Guerriero et al., 2018). There are three major groups of secondary metabolites, namely alkaloids, phenolics, and terpenoids. Recent research showed that secondary metabolites play roles in the environmental processes, such as decomposition processes and microbial activities (Chomel et al., 2016).

Secondary metabolites provide chemical compounds which are used in various industries (Guerriero et al., 2018). Therefore, many researches are carried out to improve the productivity and effectivity of secondary metabolites extraction for the purposes of seeking alternative plants as the source of the compounds.

One of the secondary metabolites products of plants is phenol. Phenol is the largest and most variative secondary metabolite groups in plants with simple to complex chemical chain (Sharma et al., 2019). Phenol is much used in the pharmaceutical industry due to its antioxidant activity (Bibi et al., 2019). Within a plant, polyphenol has various roles, such as germination, cell division, and photosynthetic activities (Sharma et al., 2019).

Based on the basic chemical structure, phenolic compounds are classified into 17 classes, including simple phenols, benzoquinones, benzoic acid, acetophenones, phenylacetic acid, cinnamic acid, phenylpropene, coumarins, chromones, naphthoquinones, xanthenes, stilbenes, anthraquinones, flavonoids, lignans/neolignans, hydrolysable tannins, and lignins (Garcia-Salas et al., 2010). However, among the classes, 60% of phenolic compounds consist of flavonoid and 30% are phenolic acids (Chahar et al., 2011).

Biosynthesis of phenol mostly occur in the leaf. Different phenolic compounds profiles are found in different plant specieses (Li et al., 2018). Increased phenolic compound biosynthesis occurs when plants are exposed to environmental stress, such as pathogen attack or significant environmental change (Kocacaliskan et al., 2020). Thus, difference of growing location may affect the biosynthetic of phenol as well.

Phenol produced by plants is distributed to various plant organs, such as root and trunk. Specifically, root is vulnerable to environmental stress caused by the quality of soil as the growth medium. Microorganism attacks such as mycorrhizal colonization (Damodaran et al., 2010) or nematode infection (Dhakshinamoorthy et al., 2014) are among the stress drivers of plants. As a response, the accumulation of phenol in the root increases. However, there may be

more variables affecting phenol concentration in the root.

Mangrove plants are wetland species which grow mainly in the coastal and estuarine area. Unfortunately, nowadays mangrove are grown in the location where water circulation is limited, such as land and pond area as an integrated part of silvofishery (brackish water aquaculture combined with mangrove rehabilitation). Inhabiting stressful environment, mangrove ecosystem produces various usable chemical compounds. The usable resources are not only provided by mangrove, but also its associated bacteria, such as Actinomycetes which has antibacterial properties (Ryandini et al., 2018). However, planting mangrove in the location other than its habitat would cause significant modification in metabolism as well as its secondary metabolite production, such as phenol (Wang et al., 2019).

In the silvofishery system, mangrove species are planted in less suitable environment. Depending on the distance from the coastline, the environment quality of mangrove's planting location could be different significantly. Thus, mangrove plants are forced to adapt to its new environment. As the impact, mangrove should adjust its metabolism which later result in the modification of secondary metabolites production and accumulation.

Among the adaptations of mangrove is the rapid littering of mangrove leaves. Decomposition of mangrove leaf rapidly cause the release of various chemical compounds, including polyphenol and inorganic nutrients (Ariyanto et al., 2018). This is one of the mangrove features expected in the silvofishery pond.

The accumulation of secondary metabolites in plant's organs is affected by various factors. The increased biosynthesis of phenol usually occurs when plants are disturbed by pathogen and environmental stress (Kocacaliskan et al., 2020). Phenol biosynthesis is also improved in the nutrient deficient environment (Allahdadi & Farzane, 2018). Thus, the stressful environment of mangrove habitat, moreover on the silvofishery pond is suggested to increase the biosynthesis of phenol in mangrove.

Based on the background explained above, this research aimed to study the total phenol content in *Avicennia marina* leaf and to analyze the effect of environmental parameters to the concentration of total phenol in *A. marina* leaf. Therefore, the research was expected to provide information on which and how environmental factors induce stress on *A. marina*.

METHODS

Study area

The research was carried out through field observation and sampling process. Sampling stations were located in pond and coastal area of Mangunharjo Village, Tugu District, Semarang City, Central Java. Different site selection was expected to provide more variation of collected data. The research was carried out in July 2019. Sampling site is shown in Figure 1.



Figure 1. Location of Mangunharjo mangrove area with coastal (Long: 110°19'32.37"E; Lat: 6°56'41.18") and pond area (Long: 110°18'40.83"E; Lat: 6°57'17.32")

Data Collection

Three factors studied in this research were the secondary metabolite, sediment quality, and water quality. The research was carried out mainly to obtain information on the phenolic content of *Avicennia marina* leaf. Sediment quality such as N, P, and C concentration and the water quality such as temperature, pH, Dissolved Oxygen (DO), and salinity were also studied as the additional information.

Field observation was carried out to study the water quality, while sampling was carried out to sediment in the surrounding area and leaf of *A. marina* trees. Water quality parameters measurement was the first thing done, followed by sediment sampling. Sediment under mangrove stand was taken using shovel and put into a plastic bag. The following activity was the sampling of mangrove leaf. Leaf samples were put in a plastic bag, and then stored in the cool case. The obtained samples were then analyzed in the laboratory. Sediment samples were analyzed for N, P, and C concentration, while mangrove leaf was analyzed for total phenol concentration.

Data Analysis

Data analysis was carried out through descriptive and statistical analysis. Statistical analysis was

carried out by multiple linear regression analysis to evaluate the effect of water and sediment quality to the phenol concentration of *A. marina* leaf. Pre-processing for multiple regression analysis was carried out by transforming the parameters' data using natural logarithm (ln). Data transformation was expected to provide improvements on the statistical analysis result. The analysis was carried out at 95% of confidence level.

RESULTS AND DISCUSSION

Total phenol content

The main variable studied in this research was the secondary metabolite content, specifically phenol in *A. marina* leaf. According to the result of laboratory analysis, the total phenol content of *A. marina* leaf of the plants grown in the pond and coastal area of Tugu district was ranging from 0.88 – 1.62% with the average concentration of $1.28 \pm 0.28\%$. The result showed that there was a high variation of phenol content between samples. This indicated that the environmental condition affected the concentration of total phenol. However, further analysis was required to prove the significance of the effect.

Mangrove plants inhabits coastal ecosystem with high environmental dynamic. Various factors such as tidal activity, freshwater supply, and climate are driving the changes of environmental quality in the coastal area (Moser et al., 2012). The environmental dynamic is not only driven by natural factors, but also anthropogenic factor (Passeri et al., 2015). Therefore, mangrove plants are vulnerable to environmental disturbance.

Mangrove, as well as other terrestrial plants, respond to the environmental stress through metabolic adaptation, indicated by the change of secondary metabolites production (Iwuala & Alam, 2017). However, since mangrove is tropical plant and had been well adapted to the coastal ecosystem, the impact might be reduced. Therefore, the effect of environmental parameters to the secondary metabolites production is important to understand.

Mangrove leaf is the organ which contains highest concentration of secondary metabolites (Mangrio et al., 2016). According to the results obtained, the average of total phenol content is higher than those from a study by (Cucikodana et al., 2019) which showed the concentration of total phenol in *A. marina* leaf between $3.85 \pm 0.37\%$ to $6.97 \pm 0.23\%$ depending on the maceration time and solvent used.

Sediment quality

Nutrient in the sediment is suggested as an important factor to support the secondary metabolites biosynthesis. Analysis on the sediment samples

showed that phosphorus had the lowest concentration compared to nitrogen and carbon, while carbon had the highest concentration. Detailed result of the laboratory analysis of nutrient content of sediment under mangrove stands is shown in Table 1.

Table 1. Nutrient concentration in the sediment under mangrove stands

Nutrient	Value
Nitrogen (%)	0.19 – 0.28 0.23 ± 0.03
Phosphorus (ppm)	90.23 – 112.08 100.68 ± 8.48
Carbon (%)	1.83 – 2.14 2.01 ± 0.13

Table 1 shows that the variation of nutrient concentration in the sediment under mangrove stands was notably high. N concentration had the highest relative variation, followed by the P concentration and the latest was C concentration. Nutrient concentration is specific feature of mangrove ecosystem. Differences of nutrient concentration could be found in different locations (Sofawi et al., 2017). Moreover, it also respond to the change of vegetation state. The ratio between C:N:P increases as the trophic level decrease and vice versa (Scharler et al., 2015).

Nutrient concentration under mangrove plant is fluctuative as the result of environmental dynamics. Various factors such as salinity, soil texture, and inundation pattern affect the nutrient storage under mangrove plants (Alongi, 2018; Sofawi et al., 2017). The fluctuation of nutrient dynamics is also supported by the active nutrient cycling process and nutrient transfer rate (Scharler et al., 2015). Mangrove plants actively uptake nutrient from the soil and return it in the form of mangrove litters which act as source of organic matter (Alongi, 2014). Mangrove structure also has significant effect on the nutrient concentration. According to Deborde et al. (2015) rapid nutrient uptake under *Avicennia* and *Rhizophora* stands causes low concentration of N and P.

Water quality

Water quality also plays an important role in driving or stimulating the biosynthesis of secondary metabolites. Environment quality frequently suggested as the cause of plant's stress. Water quality measurement was carried out for several important parameters i.e. temperature, pH, DO, and salinity. The measurement result is shown in Error! **Not a valid bookmark self-reference.** shows that temperature, DO, and salinity were highly variative in the research location. The difference of parameters values was suggested as the effect of observation

stations distributions which took place in the pond and coastal areas. Thus, the hydro-oceanographic and climatic factors emphasized its impact on the hidrological dynamic of the studied area.

Table 2.

Error! Not a valid bookmark self-reference. shows that temperature, DO, and salinity were highly variative in the research location. The difference of parameters values was suggested as the effect of observation stations distributions which took place in the pond and coastal areas. Thus, the hydro-oceanographic and climatic factors emphasized its impact on the hidrological dynamic of the studied area.

Table 2. Water quality under mangrove stands

Parameter	Value
Temperature (°C)	30.91 – 34.21 32.68 ± 1.31
pH	7.11 – 7.67 7.40 ± 0.23
DO (mg/l)	2.97 – 7.40 4.95 ± 1.73
Salinity (ppt)	24.20 – 33.55 30.27 ± 3.16

Water quality is important factor affecting the growth of *A. marina*. As a pioneer mangrove, *A. marina* inhabit coastal area where tidal activity is dominant so that mangrove plants would get inundated for certain period,. Therefore, water properties would change accordingly. The changes of water quality may affect mangrove metabolism which later affect its growth.

Mangrove ecosystem exists in the coastal area which is affected by terrestrial and ocean dynamic. Therefore, fluctuation of environment quality in mangrove ecosystem could occur daily or even hourly. High fluctuation of water salinity in mangrove ecosystem is the result of freshwater-saline water mixture (Kumara & Kumar, 2011). However, since the freshwater and sea water have different properties, the mixture, depending on the proportion could affect any other environmental parameters.

Measurement of water quality parameters in the research showed typical environmental dynamic in mangrove ecosystem. Similar trend was shown by Manju (2012) in Kerala Coast mangrove ecosystem where pH variation was typically low, ranging from 7.1 – 7.4, DO variation was between 2.86 – 6.41 mg/l, and salinity 29.31 – 35.97 ppt. Among the observed water quality parameters, temperature, DO, and salinity had significant range and may have significant impact on phenol concentration.

Effects of environmental quality on total phenol dynamics

Statistical analysis relating the total phenol concentration in the leaf area to the environmental parameters was carried out through multiple regression analysis. According to the analysis result, the concentration of total phenol in *A. marina* leaf was affected by four environment parameters, i.e. temperature, DO, phosphorus (P) content, and carbon (C) content. The following formula showed the effect of respective parameter to total phenol concentration in *A. marina* leaf:

$$\ln(TP) = 31.229 - 7.224\ln(T) - 0.067\ln(DO) - 1.054\ln(P) - 1.241\ln(C) \text{ (i)}$$

Notation:

TP: total phenol (%)

T: temperature (°C)

P: phosphorus concentration in sediment (mg/kg)

C: carbon concentration in sediment (%)

The analysis resulted F value of 18,438.02 with probability of 0.006 ($p < 0.01$). Determination coefficient obtained from the analysis was 100%. The significance of respective parameter's effect on the total phenol concentration was shown by its t value and probability, including -194.56 and 0.003 for temperature, -23.41 and 0.027 for DO, -67.21 and 0.009 for P content, and -73.15 and 0.009 for C. The result suggested that the total phenol concentration is affected simultaneously by the variables. Particularly for *A. marina* leaf, it was affected by temperature, DO, P, and C.

Referring to the result of regression analysis, *A. marina* plants in the study location preferred higher temperature, DO, P and C concentration.. It was shown by the effect of temperature, DO, phosphorus, and carbon concentration that were negative toward the concentration of phenol in *A. marina* leaf. Generally, total phenol concentration would increase when plants undergo environmental disturbance such as higher salinity and higher temperature (Boestfleisch & Papenbrock, 2017; Padmaja & Srinivasulu, 2016). This suggests that higher temperature, DO, P and C concentration are favorable to *A. marina*.

The result suggested that the temperature had negative effect to the total phenol content of *A. marina* leaf. Temperature stress, both chilling and heating generally induce the alteration of phenolic compounds biosynthesis (Sharma et al., 2019). However, the assumption was not completely correct. Mangrove species are mostly tropical plants which preferred habitat is warm area (Quisthoudt et al., 2012). Therefore, it prefers warm environment (Cavanaugh et al., 2019). Therefore, increasing temperature should decrease total phenol content due

to reduced stress. Temperature would not have significant effect on the phenol concentration in mangrove leaf as long as the fluctuation is still in the natural range (Wang et al., 2019). This explained why temperature had negative impact to total phenol content of *A. marina*.

Negative impact of DO to total phenol content of *A. marina* leaf showed its preference to oxygen rich environment. Mangrove has a specific features compared to terrestrial plants, which is its capability to grow in the inundated area. Mangrove sediment is generally anoxic (Liu et al., 2019). Due to the habitat condition, mangrove plants have adapted root structures such as the development of pneumatophores which act as the media of gas exchange between plants and its environment (Scholander et al., 1955). Thus, mangrove has certain level of dependency to the DO in the water.

Nutrient deficiency is among the factors which affect the increase of phenol biosynthesis in plants. Increased nutrient availability was proven to reduce the biosynthesis as well as the accumulation of phenol concentration in plants (Allahdadi & Farzane, 2018). As seen in the regression formula, P and C concentration had negative effects on the phenol concentration of *A. marina* leaf. This indicated that mangrove stress was reduced as the increasing P and C concentration in the sediment. Both P and C are macronutrient required by plants to grow. Thus, sufficient amount of nutrient would support the plants' growth optimally. However, reduced or insufficient nutrient concentration would lead to plants' stress. But, excessive nutrient concentration also has negative impact to plants which may cause toxicity.

The result of the research suggested that the concentration of P and C in the sediment under mangrove stands were under the sufficient level. Increasing concentration of P and C led to the reduction of phenol concentration instead of increasing it. This indicated that the stress was reduced. It was proved that the accumulation of phenol in wheat tissue was higher in the P deficient environment (Pontigo et al., 2018). Similar result was shown in lentil with additional pattern of phenol accumulation in the root (Sarker & Karmoker, 2011).

Increased phenol biosynthesis is generally driven by pathogen disturbance, environmental condition abnormalities, and wound driven stress (Kocacaliskan et al., 2020). However, the indication of an increase in phenol concentration was not found in the *A. marina* plant samples. This indicated that the environmental quality was appropriate to the growth of *A. marina*. As the impact, increased phenol biosynthesis was not occurred.

The result of this research implied that the environmental condition of Mangunharjo Village was supportive to *A. marina* growth. Four environmental parameters including temperature, DO, and P and C content had negative impact on the concentration of total phenol in mangrove leaf, indicating a low pressure to the metabolism of *A. marina*. However, the current result was limited to dry season, neglecting the environmental dynamic of rainy season. Therefore, to fully understand the effect of environmental dynamic in the coastal area, further research is needed to accomplish data coverage.

Total phenol content in *A. marina* leaf is affected negatively by several environment parameters, including temperature, DO, P and C concentration. This suggests that only when temperature, DO, P and C are at low or deficient condition will the phenol content in *A. marina* increase. Therefore, the lower temperature, DO, P and C concentration, the more it benefits to the potential of total phenol content. However, every parameter should have its limit to effect the dynamic of total phenol content, that was not met in this research. Therefore, further study is required to understand the limit of the parameters in affecting total phenol biosynthesis of *A. marina*.

CONCLUSION

Total phenolic content of *A. marina* leaf grown in Mangunharjo area was between 0.88 – 1.62% with the average concentration of $1.28 \pm 0.28\%$. The concentration of total phenol in *A. marina* leaf was significantly affected by water temperature, DO, and P and C content. Therefore, the environmental condition during the research was supportive to the growth of *A. marina*.

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