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Research Article

The effect of inundation levels on secondary metabolites accumulation in *Avicennia marina* (Forsk.) roots under different salinity regimes

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Abstract: Salinity and inundation are factors that affect secondary metabolites. This research aims to study the range of typical secondary metabolite content in *Avicennia marina* growing at different salinity levels, analyze the level of inundation that causes peak stress, and examine the impact of inundation stress on *A. marina* under different salinity regimes. This study used a 2-factor factorial complete randomized design, namely salinity level (15, 20, 25, 30, and 35 ppt), and inundation level (10, 15, and 20 cm). The parameters measured were tannin content, total alkaloids, and total phenols in *A. marina* roots analyzed by spectrophotometry method. Data were analyzed by ANOVA and further tested with DMRT test. The concentration range of tannins, total alkaloids, and total phenols was 14.29–18.45%, 0.893–1.331 mgQE/g, and 62.7–8.75 mgGAE/g, respectively. Peak stress-induced by inundation in *A. marina* indicated by high secondary metabolite contents was differentiated based on the salinity regime. Peak secondary metabolite content was obtained from the combination of salinity and inundation of 25 ppt + 20 cm, 20 ppt + 15 cm, and 15 ppt + 10 cm for tannin, total alkaloid, and total phenol content with values of 18.26±0.17%; 1.301±0.021 mgQE/g; and 83.98±2.02 mgGAE/g. The research found that simultaneous effect of salinity and inundation impacted for all metabolites. Our result suggests that salinity has underlying effect on total alkaloid and total phenol concentration in *A. marina* roots, but not tannin. Inundation significantly affects tannin content, amplifying its effects on total alkaloid and total phenol content.

1. INTRODUCTION

Mangrove vegetation is an important component in the coastal area. One of the most important species among mangrove vegetation is *A. marina* acts as pioneer mangrove species (Naidoo & Naidoo, 2017) is frequently found in the newly developed mangrove ecosystem. It is also frequently used as preferred mangrove species for mangrove replanting in the degraded coastal area, such as in the flooded areas (van Bijsterveldt *et al*., 2022). As pioneer mangrove species, *A. marina* is able to sustain in the habitat with wide salinity variations and prolonged inundation (Etongo *et al*., 2022; Li, H., *et al*., 2020). Due to the dynamic environmental conditions, mangrove plants within mangrove ecosystem frequently undergo environmental stress (Limaye

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et al., 2014). The stress is typically induced by the hydrodynamic condition. This suggests that the development of mangrove ecosystem is different between geographic location.

Coastal area is the location where the ocean and land meets. Typically, it is characterized by saline environment. However, the salinity in the coastal area is different according to the geographic locations due to global sea water circulation (Liu & Wei, 2021). Additionally, local hydrological condition also have particular influence on the salinity of coastal waters, especially in the estuary areas (Pichler *et al*., 2017; Velmurugan *et al*., 2016). Local hydrology such as catchment area, land cover, as well as anthropogenic activities that lead to freshwater discharge is different among locations (Jiménez-Martínez *et al*., 2016; Li, B., *et al*., 2020). Therefore, due to the local hydrology alone, mangrove ecosystem in a location is considered as a specific unit.

Instead of the spatial variation, salinity in the marine areas also fluctuates temporally (Cloern *et al*., 2017; Shammi *et al*., 2017). Salinity would eventually go up and down gradually, forming a salinity fluctuation cycle (Liu & Wei, 2021). Temporal fluctuation of salinity is the result of seasonal cycle due to the change of freshwater supply and evaporation rate balance driven by the climate condition (Yu *et al*., 2020). It arises the question on how stress levels fluctuate in mangrove under different salinity regimes.

Instead of salinity, coastal areas are also characterized by the fluctuation of inundation level. The fluctuation of inundation is caused by tidal activities which cause the water surface to go high and low (Mawdsley *et al*., 2015). Due to tidal activities, the sea level continuously changes, while the changes can be seen hourly, daily and monthly (Anderson *et al*., 2021). The continuous fluctuation of sea level is the result of gravitational impact of the sun and the moon which changes due to the earth rotation (Haigh, 2017). Due to the everchanging environmental condition, mangrove plants in the coastal area are under continuous environmental pressures (Sofian *et al*., 2019). Due to salinity and inundation dynamics, mangrove habitat would never be in stable state, even for a day. However, the inundation fluctuation tends to have a greater role in inducing daily stress in mangrove since it fluctuates hourly, while daily salinity in the coastal area is typically uniform. Inundation is considered one of the most important aspects of mangrove survival, especially in the past decades. The vulnerability of mangrove toward inundation stress increases along with the occurring sea level rise (Di Nitto *et al*., 2014).

The everchanging environmental condition is suggested to have an impact on mangrove ecophysiological processes which is expressed in its secondary metabolites content (Yang *et al*., 2018). Mangrove plants typically produce various kind of secondary metabolites (Gajula *et al*., 2020). However, since the environmental condition of mangrove habitat significantly changes periodically, the secondary metabolites produced would change accordingly. High salt concentration causes various events that negatively impact. It has been reported that salt induced osmotic stress is responsible for the oxidative stress caused by reactive oxygen species (ROS) (Gengmao, *et al*., 2015). In response to such salinity stress, plant cells often produce a range of secondary metabolic like phenolic compounds. These phenolic compounds have been shown to be protecting biological systems against various oxidative stresses by playing a crucial role in the maintenance of redox homeostasis (Sadeghi, *et al*., 2024).

Understanding the impact of salinity and inundation on mangrove vegetation is important in order to properly manage mangrove ecosystems, especially for replantation. Under highly saline and frequent inundation, mangrove population is more vulnerable to possible collapse (Salmo & Juanico, 2015). Ambient salinity and inundation are important factors for individual mangrove development in the coastal area.

Referring to the previous explanation, the fluctuation of salinity level in the coastal area takes more time to show its significance. This research aimed to study the typical range of secondary metabolites content in *A. marina* growing in different salinity levels, to analyze the inundation level that causes *A. marina* to undergo peak stress, and to analyze stressability of inundation to *A. marina* growing in different salinity regimes.

2. MATERIAL and METHODS

2.1. Research Design

The research was conducted using an experimental approach. The experiment design was Completely Randomized Design with Factorial includes the application of different salinity and inundation levels. *A. marina* seedlings were planted in 80 L containers filled with mangrove soil and added with predefined saline water. The design for salinity variation was 15 ppt, 20 ppt, 25 ppt, 30 ppt and 35 ppt, while the design for inundation level was 10 cm, 15 cm, and 20 cm. The experiment was carried out in a greenhouse under ambient indirect lighting. The plant grew without direct sunlight. It was not exposed fully under the sunlight.

2.2. Sample Preparation

The plant used for the experiment was *A. marina* seedling. Planting media for *A. marina* seedling was a mix of mangrove mud and compost (2:1). Mangrove mud was retrieved from mangrove ecosystem located in Mangunharjo Village, Tugu District, Semarang City. Plastic barrel was used as planting container. The obtained media was placed in containers to the height of approximately 30 cm. *A. marina* seedling was then planted in the prepared media with density of 2 plants/barrel. Then, the barrel was filled with water with designed salinity to the designed inundation levels. The experiment was carried out for 60 days. Maintenance was carried out by adding saline water according to the treatment groups to maintain inundation level twice a week.

2.3. Determination of Total Tannin, Alkaloid, and Phenolic Components

Observation on secondary metabolites content was performed for mangrove roots. *Avicennia marina* roots were taken whole fully for laboratory analysis. Analysis of secondary metabolites content was performed quantitatively for tannin, total alkaloid and total phenol content using Spectrophotometry Method. UV/Vis double beam spectrophotometer and standard quarts cuvetts were used for all the absorbance measurement. Spectrofotometry methods are most commonly used for the quantification of tannin, alkaloid, and phenolic content (Tabasum, *et al*., 2016). The analysis procedure for each secondary metabolite according to Sarvade, *et al* (2020) and Tabasum, *et al* (2016) were:

a. Tannin

The sample was weighed as much as 50 mg, then 10 mL of diethyl ether was added to isolate the more polar tannin components, then the remaining diethyl ether was evaporated. 1 mL of sample solution was added 0.1 mL of Folin-Ciocalteu reagent. Added 2 mL of 20% sodium carbonate as a base buffer. The solution mixture was vortexed for 5 minutes so that all components react optimally. The color formed was then measured using a spectrophotometer with a wavelength of 760 nm. The next step is the preparation of a calibration curve with tannic acid as a standard. Tannic acid was weighed, then 10 mL of Folin-Ciocalteu reagent and 20% sodium carbonate was added. The color formed was then measured using a spectrophotometer with a wavelength of 760 nm. Tannin concentration can be determined by comparing the results with the standard calibration curve.

b. Alkaloid

Total alkaloid content was quantified by spectrophotometric method. This method is based on the reaction between alkaloid and bromocresol green (BCG). The plant extract (1 mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. 1 ml of this solution was transferred to a separating funnel, and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extract was collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The next step is the preparation of a calibration curve with quinine as a standard. Quinine was weighed as much as 10 mg, then the same steps starting from dissolving it in 2 N HCl, the pH of the phosphate

buffer solution was adjusted to neutral with 0.1 N NaOH, then 5 ml of BCG solution was added along with 5 ml of phosphate buffer. The mixture was shaken, and the complex formed was extracted with chloroform with vigorous shaking, then diluted to the same volume as the chloroform. Then the absorbance of each solution was taken at 470 nm using a spectrophotometer. The total alkaloid was determined with the help of a standard curve prepared from the quinine standard curve.

c. Phenol

The estimation of total phenol content in *A. marina* was measured spectrophotometrically with Folin-Ciocalteu reagent, using gallic acid as the standard and expresses the result as gallic acid equivalent (GAE) per gram of sample. The sample was weighed ± 50 mg, then 0.5 mL of Folin-Ciocalteu reagent was added. The reaction between phenol in the sample and the reagent produces a blue compound due to the oxidation process of phenol by the reagent. The blue color was measured spectrophotometrically at 765 nm. The next step is the preparation of a calibration curve with gallic acid as a standard. Gallic acid was weighed 10 mg, then added 0.5 mL of Folin-Ciocalteu reagent and 10 min later 1.5 mL of 20% sodium carbonate. Gallic acid standard solution was made with various concentrations (100; 75; 50; 25; 10; 5; 2.5; 1; 0.5; 0.2 ppm). After 10 minutes, the absorbance of each solution was taken at 765 nm using a spectrophotometer. The total phenol was determined with the help of a standard curve prepared from the pure phenolic standard curve (gallic acid).

2.4. Statistical Analysis

Data analysis was performed descriptively and statistically. Descriptive data analysis was performed through graphical illustration to represent laboratory analysis result of secondary metabolites content. Statistical analysis was performed through Analysis of Variance (ANOVA) with Factorial design to determine the effect of salinity and inundation on secondary metabolites content. Duncan's Multiple Range Test (DMRT) to further test.

3. RESULTS

The content of secondary metabolites varied throughout the research. Referring to the obtained data, average tannin content was highest (16.87%) under salinity of 30 ppt, but the variation was greatest in 25 ppt (14.29% – 18.45%). The average total alkaloid content on the other hand, was found highest in 20 ppt (1.224 mgQe/g), but the variation was greatest in 35 ppt (0.893 - 1.327 mgQE/g). The average total phenol content was highest of 15 ppt (79.35 mgQE/g), while the variation was greatest at 35 ppt (65.83-84.57 mgQe/g). Detailed laboratory analysis result of secondary metabolites content studied in this research is presented in Table 1. The highest content of tannin, alkaloid, and phenol was found in different salinity level.

Table 1. Secondary metabolites content in *A. marina* roots grown under different salinity regimes.

Salinity (ppt)	Secondary Metabolite Content		
	Tannin (%)	Total Alkaloid (mgQE/g)	Total Phenol (mgGAE/g)
15	14.70-17.44	0.899-1.323	72.55-85.65
	16.28±0.89[a]	1.086±0.122[a]	79.35±4.36[c]
20	14.97-17.47	1.126-1.331	70.19-76.70
	16.09±0.79[a]	1.224±0.071[a]	73.25±2.00[a]
25	14.29-18.45	1.023-1.282	62.77-74.36
	16.67±1.53[a]	1.157±0.092[a]	68.67±3.68[b]
30	15.60-18.33	1.006-1.301	68.30-80.42
	16.87±0.81[a]	1.177±0.123[a]	75.67±3.66[b]
35	14.90-18.27	0.893-1.327	65.83-84.57
	16.76±0.99[a]	1.132±0.166[a]	76.11±5.95[bc]

Note: different letter within the same column indicates significant difference

The research found that *A. marina* response to inundation stress changes according to the salinity setting. Detailed result on the response of *A. marina* toward inundation stress under different salinity regimes are showed in Figure 1 to Figure 3.

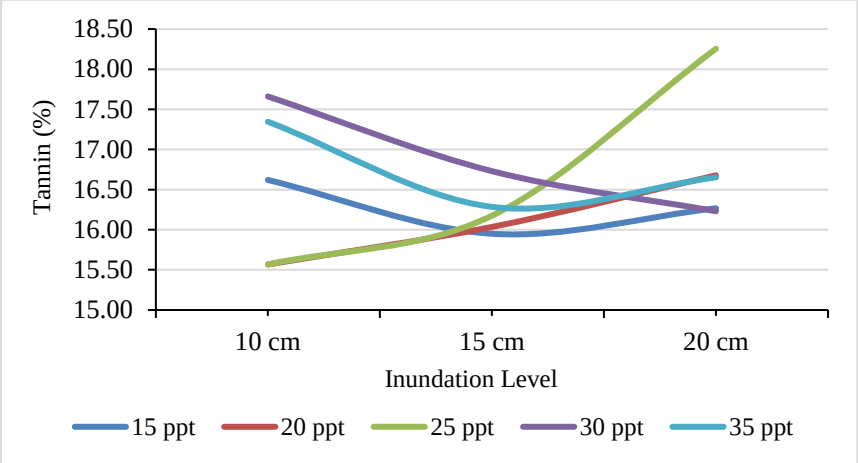

Figure 1. Tannin content of *A. marina* roots in response to the variation of inundation levels under different salinity levels.

Figure 1 shows the experiment result on tannin concentration in *A. marina* roots. Figure 1 shows, tannin concentration responded differently toward inundation stress under different salinity regime. Tannin concentrations were highest in the inundation level of 10 cm for *A. marina* grown in 15 ppt, 30 ppt and 35 ppt salinity levels. While 20 cm of inundation for those grown in the salinity of 20 ppt and 25 ppt. Refer to Figure 1, under salinity of 20 ppt and 25 ppt, there was the description of trends along with the increase of inundation levels to tannin content and a decreased effect trend of inundation level under salinity of 30 ppt. While under salinity of 15 ppt and 35 ppt, the trends of inundation effect have parabolic pattern. Refer to the finding of the result, peak tannin content in *A. marina* roots were 16.62 ± 0.46 %, 16.68 ± 0.73 %, 18.26 ± 0.17 %, 17.66 ± 0.52 %, and 17.35 ± 0.95% respectively for the salinity of 15 ppt, 20 ppt, 25 ppt, 30 ppt and 35 ppt. This suggests that under the salinity of 25 ppt, tannin content in *A. marina* roots was highest compared to other salinity levels.

Statistical analysis with Factorial ANOVA showed F value of 2.981 with probability of 0.003 which indicates that there was a simultaneous effect between salinity and inundation levels to tannin content in *A. marina* roots. However, there was no partial effect of salinity and inundation, showed by the F value of 1.783 and 2.274 with the probability of 0.149 and 0.115 respectively.

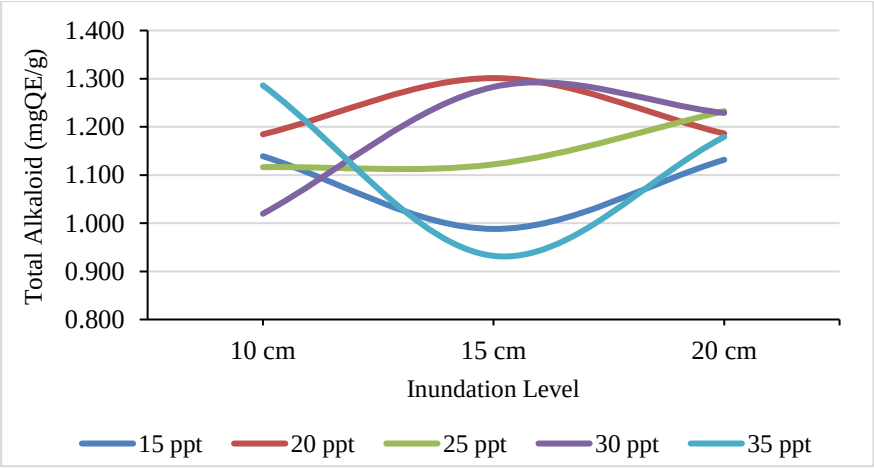

Figure 2. Total alkaloid content of *A. marina* roots in response of the variation of inundation levels under different salinity levels.

Different reaction of *A. marina* toward inundation stress under different salinity regimes was also shown by its total alkaloid content. The analysis result as presented in Figure 2 shows that the fluctuation of total alkaloid content toward inundation stress typically have parabolic pattern. Refer to the result, total alkaloid content was highest in 10 cm inundation for *A. marina* grown under 15 ppt and 35 ppt, 15 cm for those grown under 20 and 30 ppt, and 20 cm for those grown under 25 ppt. Refer to the analysis result, peak total alkaloid content in respective salinity levels is 1.139 ± 0.129 mgQE/g, 1.301 ± 0.021 mgQE/g, 1.233 ± 0.071 mgQE/g, 1.283 ± 0.016 mgQE/g and 1.286 ± 0.040 mgQE/g.

Statistical analysis with Factorial ANOVA showed that there was a significant simultaneous effect of salinity and inundation to total alkaloid concentration. The significance of simultaneous effect was showed by its F value and probability which were 9.915 and 0.000 respectively. The effect of salinity and inundation on total alkaloid was also observed in partial model. The effect of salinity was showed by its F value of 6.021 and probability of 0.001, while the effect on inundation was showed by its F value of 4.330 and probability of 0.019.

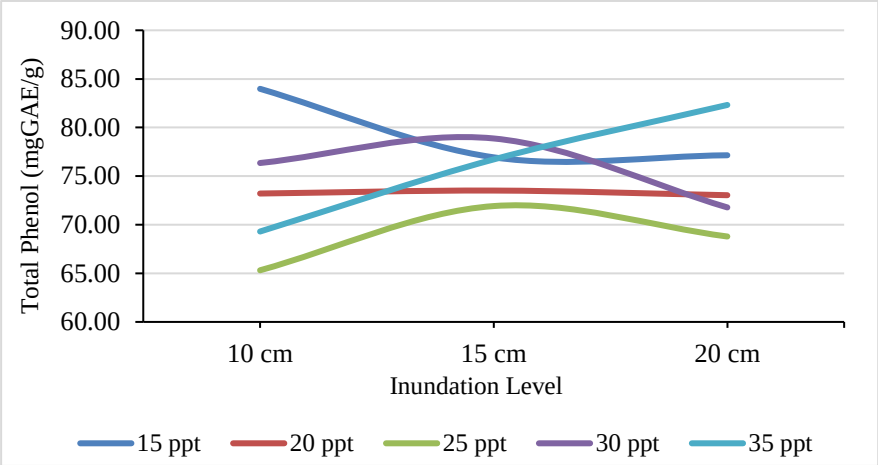

Figure 3. Total phenol content of *A. marina* roots in response of the variation of inundation levels under different salinity levels.

Based on statistical test with ANOVA was performed to emphasize the difference of peak secondary metabolites content induced by inundation stress at each salinity level. Figure 3 shows the response of *A. marina* toward inundation stress under different salinity regimes expressed by total phenol content. Various effect pattern between inundation level and total phenol content was obtained from the observation, including parabolic for 15 ppt, 25 ppt and 30 ppt and linear for 20 ppt and 35 ppt. Refer to the result, total phenol content in *A. marina* roots was highest in inundation level of 10 cm under 15 ppt salinity. While in 15 cm of inundation level, total phenol content was highest under salinity of 20 ppt, 25 ppt and 30 ppt. Lastly, under 35 ppt of salinity, total phenol content was highest in 20 cm of inundation. Refer to the analysis result, peak total phenol content in *A. marina* roots grown under salinity of 15 ppt, 20 ppt, 25 ppt, 30 ppt and 35 ppt are 83.98 ± 2.02 mgGAE/g, 73.52 ± 2.42 mgGAE/g, 71.92 ± 1.84 mgGAE/g, 78.87 ± 1.35 mgGAE/g and 82.32 ± 2.00 mgGAE/g.

Statistical analysis for total phenol concentration also indicated the significance of salinity and inundation effects, both partially and simultaneously. The significance of simultaneous effect was showed by its F value and probability of 16.395 and 0.000. Partially, statistical analysis for the salinity effect showed the value of 30.601 and 0.000 respectively, while the effect of inundation was showed by the value of 3.118 and 0.054 respectively for F and probability.

In order to further understand the distribution of peak secondary metabolites concentration in regards of the inundation levels, an extraction of values from each salinity group. Additionally, a statistical test with ANOVA was performed to emphasize the difference of peak secondary metabolites content induced by inundation stress at each salinity level. The comparison between inundation level and secondary metabolites content is presented in Figure 4 to Figure 6.

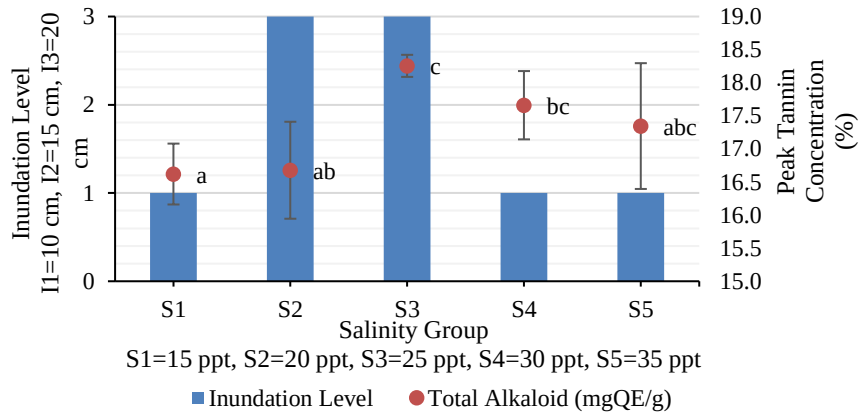

Figure 4. Peak tannin concentration in *A. marina* roots in accordance with inundation level at each salinity regime; S is salinity level.

Figure 4 shows the comparison between inundation levels and tannin content in *A. marina*. The result shows that tannin concentration was highest under salinity of 25 ppt and inundation level of 20 cm. Statistical analysis showed that peak tannin concentration in *A. marina* roots under salinity of 25 ppt was significantly higher than those below 25 ppt. Statistical analysis with ANOVA showed the F value of 4.864 and probability of 0.010.

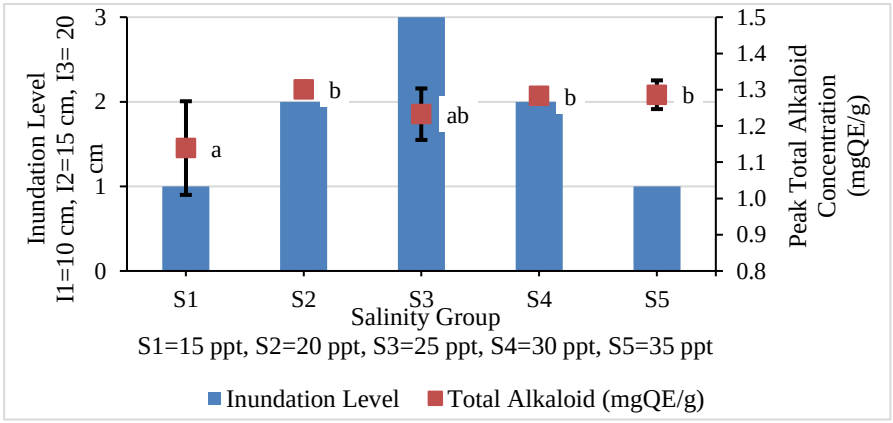

Figure 5. Peak total alkaloid concentration in *A. marina* roots in accordance with inundation level at each salinity regime.

Figure 5 shows the comparison between inundation levels and total alkaloid content in *A. marina* root. The result shows that total alkaloid content was highest under salinity of 20 ppt and inundation level of 15 cm which difference is insignificant to other treatments with higher salinity levels, but significant to lower salinity level. Statistical analysis with ANOVA showed the F value of 3.671 and probability of 0.028.

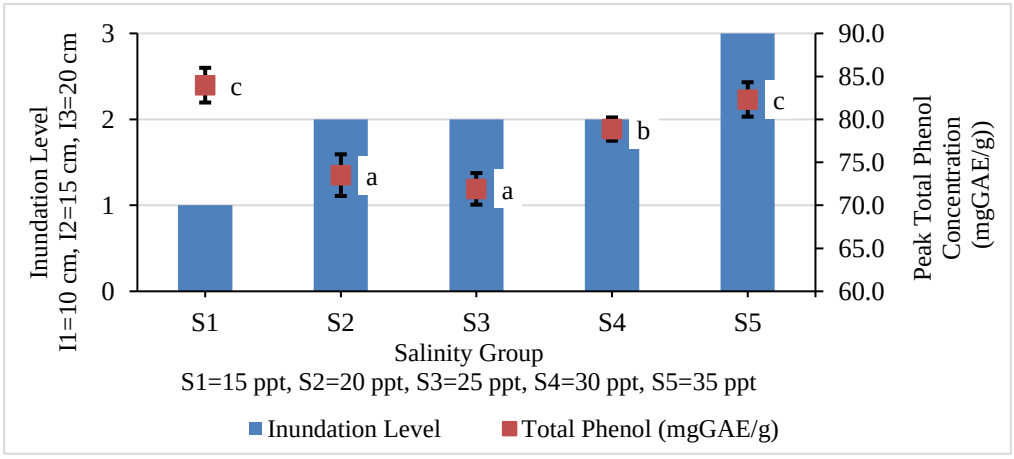

Figure 6. Peak total phenol concentration in *A. marina* roots in accordance with inundation level at each salinity regime.

Figure 6 shows the comparison between inundation levels and total phenol content in *A. marina* root. The result shows that total phenol content was highest under the salinity of 15 ppt and inundation level of 10 cm which content is indifferent from the treatment of 35 ppt salinity and 20 cm inundation, but different other salinity levels in between. Statistical analysis with ANOVA showed an F value of 29.288 and a probability of 0.000.

Further analysis was performed through ANOVA to differentiate the secondary metabolites concentration stored in *A. marina* roots under different salinity regimes and inundation levels. Univariate statistic test showed that there was significant combined effect of inundation and salinity to tannin, total alkaloid, and total phenol content in *A. marina* roots.

Univariate statistic test on tannin content showed F value of 2.981 and probability of 0.003, which indicates that the combination effect between inundation and salinity was significant. However, salinity that is considered as the predetermined condition, did not have an effect on the variation of tannin content. Therefore, inundation levels are considered as the factor that signifies the variation of tannin content. Referring to the analysis result, the highest tannin concentration was observed in the treatment of 25 ppt salinity and 20 cm inundation, while the lowest was obtained from 25 ppt salinity and 10 cm inundation. Detailed analysis result on tannin content is presented in Table 2.

Table 2. Homogenous subsets of tannin content in *A. marina* roots under different salinity and inundation levels.

Salinity	Inundation Levels		
	10 cm	15 cm	20 cm
15 ppt	16.62 ± 0.46^{abc}	15.95 ± 1.11^{ab}	16.27 ± 1.08^{abc}
20 ppt	15.57 ± 0.53^{a}	16.04 ± 0.79^{ab}	16.68 ± 0.73^{abc}
25 ppt	15.57 ± 1.16^{a}	16.18 ± 1.38^{ab}	18.26 ± 0.17^{d}
30 ppt	17.66 ± 0.52^{cd}	16.73 ± 0.38^{abc}	16.23 ± 0.76^{abc}
35 ppt	17.35 ± 0.95^{bcd}	16.29 ± 0.74^{abc}	16.65 ± 1.18^{abc}

Univariate statistic test on total alkaloid content showed F value of 9.195 and probability of 0.000, which indicates that the combination effect between inundation and salinity was significant. According to the analysis result, salinity has particular effect on the variation of total alkaloid content. Therefore, inundation levels is considered as the amplifying factors of the variation of total alkaloid content in *A. marina* roots. Referring to the analysis result, the highest total alkaloid content was obtained from the treatment of 20 ppt salinity and 15 cm inundation, while the lowest was obtained from 35 ppt salinity and 15 cm inundation. Detailed analysis result on total alkaloid content is presented in Table 3.

Table 3. Homogenous subsets of total alkaloid content in *A. marina* roots under different salinity and inundation levels.

Salinity	Inundation Levels		
	10 cm	15 cm	20 cm
15 ppt	1.139 ± 0.129^{c}	0.988 ± 0.061^{a}	1.132 ± 0.122^{c}
20 ppt	1.185 ± 0.038^{cde}	1.301 ± 0.021^{e}	1.186 ± 0.068^{cde}
25 ppt	1.116 ± 0.049^{bc}	1.122 ± 0.110^{bc}	1.233 ± 0.071^{cde}
30 ppt	1.020 ± 0.012^{ab}	1.283 ± 0.016^{de}	1.229 ± 0.060^{cde}
35 ppt	1.286 ± 0.040^{de}	0.932 ± 0.049^{a}	1.179 ± 0.095^{cd}

Univariate statistic test on total phenol content showed F value of 16.395 and probability of 0.000, which indicates that the combination effect between inundation and salinity was significant. According to the analysis result, salinity particularly has a significant effect on total

phenol content in *A. marina* roots. This suggests that the inundation levels act as the amplifier of total phenol content differentiation. Referring to the analysis result, the highest total phenol content was obtained from the treatment of 15 ppt salinity and 10 cm inundation, while the lowest was obtained from 25 ppt salinity and 10 cm inundation. Detailed analysis result on total phenol content is presented in Table 4.

Table 4. Homogenous subsets of total phenol content in *A. marina* roots under different salinity and inundation levels.

Salinity	Inundation Levels		
	10 cm	15 cm	20 cm
15 ppt	83.98 ± 2.02^{i}	76.93 ± 1.87^{efg}	77.15 ± 4.39^{fg}
20 ppt	73.21 ± 2.05^{cdef}	73.52 ± 2.42^{def}	73.04 ± 2.09^{cde}
25 ppt	65.32 ± 2.31^{a}	71.92 ± 1.84^{bcd}	68.79 ± 3.46^{ab}
30 ppt	76.35 ± 2.35^{efg}	78.87 ± 1.35^{gh}	71.78 ± 2.71^{bcd}
35 ppt	69.3 ± 2.89^{bc}	76.72 ± 1.89^{efg}	82.32 ± 2.00^{hi}

The result suggests that tannin content, total alkaloid content and total phenol content react differently toward salinity and inundation levels. Inundation levels could act as the signifying or amplifying factors of environmental stressors to *A. marina*.

4. DISCUSSION and CONCLUSION

Salinity and inundation are two major factors corresponding to stress in mangrove vegetation (Salmo & Juanico, 2015). In the coastal area, both variables are known to fluctuate in different cycles and the intersection creates unique ecological condition of mangrove ecosystem. According to Perri *et al*. (2017) the combination of salinity and inundation in mangrove ecosystem plays important role in determining mangrove's ability regarding water uptake, photosynthesis, stomatal conductance, gas exchange and nutrient availability. Referring to the finding of this research, salinity variation has partial impact on total alkaloid and total phenol content, but not on tannin content. Salinity is the main stress drivers to mangrove plants in the coastal area (Alhassan & Aljahdali, 2021). The salinity issue exists not only during the flooding period, but also prolonged to non-flooding period. Less-flooded mangrove ecosystem tend to have a saline sediment (Wunderlich & Pinheiro, 2013). Thus, even after land establishment, mangrove ecosystem will remain saline.

The research suggests that tannin content in *A. marina* root is not significantly affected by environment's salinity variation. Typically, tannin is an important metabolites that helps mangrove plants to cope with salinity stress (Zhu *et al*., 2023). Therefore, this finding suggests that the designed salinity variation (15 – 35 ppt) is still within the tolerance range of *A. marina*. However, tannin content in *A. marina* seedling during the experiment was signified by the inundation. Signified tannin content difference by inundation variation suggests that inundation amplifies the magnitude of stress factors related to tannin synthesis. Refer to Cui *et al*. (2022), tannin concentration in mangrove plants is related to biological stress such as bacteria. In addition, the microbial community in the mangrove ecosystem is more influenced by inundation than salinity (Chambers *et al*., 2016).

Total alkaloid content and total phenol content on the other side was also significantly partially affected by salinity variation. There was no particular trend of salinity's effect on total alkaloid content. However, a parabolic trend resulted in total phenol content. High total phenol content was obtained from the treatment with 15 ppt and 35 ppt of salinity, and parabolically decreased in between. According to Yang *et al*. (2018), alkaloid and phenol content in mangrove is related to temperature stress. However, Pant *et al*. (2021) found that the fluctuation of total alkaloid and total phenol contents are also related to other factors, such as light intensity, drought, salinity and soil fertility. Other than physical and chemical factors, total phenol content could

also be related to biological factors such as fungi abundance. According to Luo *et al*. (2018), under fungal disturbance, mangrove plants may excrete more phenol, causing higher availability of phenol in the sediment. In addition to the salinity factor, inundation also contributes to the amplification of total alkaloid and total phenol content in *A. marina* roots. Inundation may add other stressing factors to salinity variation. According to Li *et al*. (2020), inundation is important factor that lead to metabolic modification. Therefore, the ability of *A. marina* to sustain inundation comes with the cost of decreased photosynthesis rate.

Variation of tannin, total alkaloid and total phenol content in *A. marina* roots showed that mangroves undergo different pressures of combined salinity and inundation level. Accumulation of secondary metabolites is the mechanism of mangrove to improve its tolerance toward environmental pressures (Ravi *et al*., 2020). According to a research by Barnuevo and Asaeda (2018), the combination of salinity and inundation levels affect the development of mangrove plants both in short and long periods such as first leaf development and total height gain.

The finding of this research suggests that mangroves, especially *A. marina*, reacts differently toward the variation of salinity and inundation combination. This finding is important to determine which environmental condition is less stressful and which one is more stressful to *A. marina*. Therefore, a proper management strategy in nursery of mangrove breed could be applied. The finding is also important in case of the utilization plan of mangrove as source of secondary metabolites extract. According to Hurmat *et al*. (2020), environmental modification is frequently applied in the cultivation of medicinal plants. It is expected to provide metabolites products in a greater sum and/or better quality.

Secondary metabolites content in *A. marina* roots under varied environmental settings were between 14.29 to 18.45 % for tannin, 0.893 to 1.331 mgQE/g for total alkaloid and 62.77 to 85.65 mgGAE/g for total phenol. Peak stress induced by inundation in *A. marina* indicated by high secondary metabolites content was differentiated by salinity regimes, including 10 cm of inundation under 15 ppt, 30 ppt and 35 ppt salinity and 20 cm of inundation under 20 ppt and 25 ppt salinity for tannin content, 10 cm of inundation under 15 ppt and 35 ppt salinity, 15 cm under 20 ppt and 30 ppt salinity and 20 cm under 25 ppt salinity for total alkaloid content, as well as 10 cm under 15 ppt salinity, 15 cm under 20 ppt, 25 ppt and 30 ppt salinity, and 20 cm under 35 ppt salinity for total phenol content. Inundation plays an important role in inducing stress to *A. marina*, proved by the ability of inundation to signify and amplify the effect of salinity on the variation of secondary metabolites content.

In response in salinity and inundation stress, *Avicennia* increases the production of secondary metabolites like tannins, alkaloids, and phenolics, which act as antioxidants to detoxify ROS and protect cellular structures from oxidative damage. Secondary metabolites also helping to maintain cellular turgor and prevent dehydration. Stress hormones like abscisic acid (ABA) and ethylene are key regulators of secondary metabolite pathways. According to Reshi, *et al* (2023), salinity and inundation both trigger ABA accumulation, which stimulates the synthesis of secondary metabolites. Inundation increases ethylene production which can further activate stress response pathways, amplifying the effects of salinity on secondary metabolite production.

The conclusion of this research is the range of secondary metabolite content in *A. marina* has a different range for each metabolite. Tannin has the greatest variation in 25 ppt (14.29% – 18.45%), alkaloid in 35 ppt (0.893 - 1.327 mgQE/g), while phenol in 35 ppt (65.83-84.57 mgQe/g). The inundation level that causes *A. marina* to undergo peak stress was found based on peak total metabolite concentration. The highest tannin concentration was observed in the treatment of 25 ppt salinity and 20 cm inundation, alkaloid highest concentration was obtained from 35 ppt salinity and 15 cm inundation, while phenol highest concentration was found in 25 ppt salinity and 10 cm inundation. These findings highlight the amplifying role of inundation in the salinity-induced stress response of *A. marina*. The results have important implications for mangrove management strategies and the utilization of *A. marina* as a valuable source of

secondary metabolites. Further studies are needed to elucidate the biochemical mechanisms underlying these responses and to explore the performance of *A. marina* under more complex natural environmental conditions.

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The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Endah Dwi Hastuti: Organized research, Conception, Data collection and processing, Writing publication manuscript and report. **Erma Prihastanti**: Data collection, Laboratory sample testing, Analysis and interpretation.

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