The effect of mangrove types and leave maturity on the mangrove leaves (Sonneratia alba) and (Rhizophora mucronata) tea powder

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Abstract. Mangrove leaves are green plants that have high antioxidant potential and bioactive compounds such as flavonoids, alkaloids/tannins, phenols, catechins, saponins, triterpenoids, and many other compounds. Based on the many properties of the two types of mangroves, mangrove leaves have the potential to be used as raw materials for making alternative drinks such as tea. The research method used is an experimental design with a completely randomized design (CRD) with a factorial pattern with 2 factors (Rhizophora sp. leaves & extracts). Sonneratia sp.) and 2 test levels (old and young leaves). The results showed that mangrove leaf tea has the potential to be used as an alternative drink with high antioxidant activity (strong-very strong) with an IC50 value in the range of 49.76-50.12 with the highest antioxidant activity value obtained by leaves. Rhizophora mucronata (RM2) tea with old leaves as raw material.

Key Word: antioxidant; mangrove leaves; Rhizophora; Sonneratia; tea

1. Introduction

Mangrove leaves are green plants that have high antioxidant potential and bioactive compounds such as flavonoids, alkaloids/tannins, phenols, catechins, saponins, triterpenoids, and many other compounds. These phytochemical compounds are known to have many roles in health, beauty, and potential food. Mangrove leaves contain bioactive compounds such as antioxidants, anti-bacterial and anti-aging. Antioxidant compounds play a role in inhibiting free radicals that cause cell damage. Antioxidants are compounds or substances that can inhibit, delay, prevent or slow down oxidation reactions even in small concentrations [1]. Oxidation is a chemical reaction that can produce free radicals, thereby triggering a chain reaction. Meanwhile, antibacterial compounds play a role in inhibiting bacterial growth in several ways, such as damaging cell walls and lowering the pH and Aw of their living substrates. Tannins can inhibit bacterial growth. Tannins have the property of being able to shrink cell membranes, thereby interfering with the cell's permeability [2]. Several studies on mangrove leaves that have been carried out are mangrove leaves as potential food [3], the antioxidant effect of mangrove leaf tea (Rhizophora mucronata), Utilization of mangrove leaf extract (Rhizophora mucronata) with various solvents as active ingredients for preparations. pharmaceutical anti-cancer therapy [4]. Mangrove leaf extract was able to improve the growth performance of Vanname shrimp [5], Api api mangrove leaves (Avicennia sp.) as antibacterial [6].

The level of antioxidant activity in mangrove leaves is influenced by several factors, such as the place of growing environment, mangrove leaf species, and the age of mangrove leaves. In general, young mangrove leaves have a higher antioxidant content than old mangrove leaves. Sonneratia alba is a type of mangrove that is commonly found in the city of Dumai and is known to contain many bioactive substances contained therein. The results of previous studies that have been carried out on Sonneratia alba mangrove leaves, that the young leaves of Sonneratia alba mangrove extracted with methanol and ethanol using the
Soxhlet and maceration method contained bioactive components, namely phenols, flavonoids, steroids, saponins, triterpenoids, tannins and alkaloids and have potential as natural antioxidants [7]. While *Rhizopora* sp. is a mangrove leaf that acts as a phytopharma which contains active compounds in the form of flavonoids, alkaloids, tannins, saponins and terpenoids that function as antimicrobials because they can suppress the growth and development of *Vibrio* sp. bacteria. [8] flavonoids act as antimicrobial, antibacterial, antiviral and immunostimulants [9]. Based on the many benefits of the two types of mangroves, mangrove leaves have the potential to be used as raw materials for making alternative drinks such as tea. Green tea is the young leaf shoot of the tea plant (*Camellia sinensis* L.) which is processed without going through an enzymatic oxidation process which contains chemical compounds such as catechins, tannins, simple polyphenols and other phenolic compounds (flavanols). Based on the above background, it is expected that mangrove leaves can be used as a source of food or functional health drinks, so this study aims to determine how much influence the type and maturity of mangrove leaves have on antioxidant content and activity.

2. Material and Method
2.1. Materials
2.1.1. Sample preparation. The manufacture of mangrove leaf tea products was carried out using an experimental design method. This research was supported by using tools such as oven, hotplate, measuring flask, volume pipette, drying rack, tea bag, and blender. While the materials used are water, 1% solution of iron (III) chloride (FeCl$_3$), 96% alcohol, Aquades, 0.01 N iodine solution and 1% starch. The process of making mangrove leaf tea is carried out with three repetitions through the oven process at 110°C for 30 minutes and the second stage so that the mangrove leaves can be drier, then an oven process is carried out at 70°C for 1 hour with analysis of water content test, tannin test, flavonoid test, phenol test, vitamin C test, antioxidant activity and hedonic test.

2.1.2. Extract producing. Mangrove leaf powder (*Sonneratia* sp. and *Rhizopora* sp.) was weighed as much as 250 grams and put into a jar. Add 96% ethanol solvent, the amount of ingredients and solvent mixture is 1:7 (w/v), stirring constantly for 10 minutes then the glass jar is covered with aluminum foil and then wrapped with plastic wrap. After 2 x 24 hours, the jar was opened and stirred constantly with a stirring rod for 15 minutes. After stirring, filtered through a Buchner funnel. The filtrate is put into a different glass jar, then tightly covered with aluminum foil and wrapped with plastic wrap. The jar is stored in a place away from sunlight. The remaining filtered macerate is put into the initial jar, then 96% ethanol solvent is added, stirred constantly with a stirring rod for 10 minutes, then tightly covered with aluminum foil and then wrapped with plastic wrap. The jar is stored in a place away from sunlight. Repeat this maceration process for the next 24 hours so that the maceration stage is carried out for 3 x 24 hours After maceration 3 x 24 hours, all the filtrate was combined together, then filtered again. After filtering, the total volume of the filtrate is calculated. The filtrate is concentrated with a rotary evaporator at a temperature of 40°C to evaporate the solvent to obtain a thick filtrate. This is done until the filtrate that is evaporated remains about 100 ml for easy removal from the evaporator flask. The remaining filtrate is then concentrated again in a water bath with a temperature of 40°C until a thick and concentrated extract is obtained.

2.1.3. Testing. Making mangrove leaf tea can be done by preparing as much as 20 grams of each young mangrove leaf and old mangrove leaf cut into small pieces so that the heating process is easy in the oven. Then each of the young mangrove leaf tea and old mangrove leaf tea is heated in an oven at a predetermined temperature. To produce dry leaves that can be directly blended to form a powder [10].
2.1.4. Filtrate producing. Each sample was weighed as much as 2 grams, then added with 100 ml of water and boiled for 15 minutes cooled.

2.2. Method
2.2.1. Chemical characteristic analysis.
   a. Preparation of 50 ppm parent sample solution Ethanol extract of mangrove leaves (Sonneratia alba young and old & Rhizophora mucronata young and old) from maceration made a mother sample solution at a concentration of 50 ppm by weighing 5 mg of thick extract and then put into a 100-volume flask ml was then dissolved with 96% ethanol to the limit mark and shaken until homogeneous.
   b. Preparation of test sample solution from the 50 ppm master sample solution then diluted to make five test sample solutions each with a concentration of 2; 4; 6; 8 ppm.
   c. Preparation of 100 ppm DPPH parent standard solution. Concentration of standard solution of DPPH 100 ppm was made by weighing 10 mg of DPPH powder into a 100 ml volumetric flask and then dissolved with 96% ethanol to the mark and shaken until homogeneous.
   d. Preparation of 40 ppm DPPH working standard solution Concentration of 40 ppm DPPH solution was made by pipetting 40 ml of 100 ppm DPPH solution then put into a 100 ml volumetric flask and then dissolved with 96% ethanol to the limit mark and shaken until homogeneous.
   e. Determination of Maximum Wavelength 40 ppm DPPH solution was pipetted as much as 4 ml and then measured with a UV-Vis Spectrophotometer and observed for absorbance at a wavelength of 400-800 nm. From the spectrum the maximum wavelength is determined.
   f. Measurement of DPPH radical scavenging activity with UV-Vis spectrophotometer. Test solutions of different concentrations were then pipetted 2 ml each and put into six different test tubes and then added 2 ml of DPPH. As a comparison, in a different test tube, 2 ml of 40 ppm DPPH solution was pipetted and then 2 ml of 96% ethanol was added. All test tubes were shaken and allowed to stand for 30 minutes. After that, the absorbance was observed at a maximum wavelength of 516 nm with a UV-Vis spectrophotometer, all the results obtained were recorded.
   g. Determination of the IC$_{50}$ value and making a calibration curve From each concentration level tested and then calculating the percentage of attenuation then the results of the percentage of attenuation are plotted in a graph so that an equation $y = bx+a$ is obtained and the IC$_{50}$ value will be obtained by replacing $y = 50$ in the linear regression equation where x is the concentration (μg/ml) and y is the percentage of inhibition [11].

2.2.2. Hedonic test. The hedonic test is a more specific test that usually aims to determine the panelist's response to general organoleptic quality properties such as aroma, color, appearance, and so on [12] In accordance with SNI 01-2346-2006, the process of making mangrove leaf tea is brewed at a temperature of 85°C for 5 minutes which is carried out 2 times, namely in the oven drying process. The hedonic test was carried out using 30 panelists with the assessment scores given in the hedonic test from 1 to 9 (1 = Very much disliked, 2 = Very disliked, 3 = Disliked, 4 = Slightly disliked, 5 = Neutral, 6 = Somewhat like, 7 = Like, 8 = Very much like, 9 = Very much like). The hedonic test carried out on the treatment which includes taste, color, and aroma, obtained the average results of the panelist's assessment.

2.2.3. Phytochemical testing. Each phytochemical test treatment first weighed 5 mg gram of leaf powder, then dissolved it with three different solvents (methanol/ethyl acetate/n-hexane) as much as 5 ml in a glass beaker.
   a. Alkaloid compounds
      The sample solution is added as much as 1 ml of Dragendrof's reagent, observe the changes. If an orange to red brown color is formed, it indicates the presence of alkaloid compounds.
b. Flavonoid compounds
Test can be done by taking 5 ml of mangrove leaf filtrate, then put it in a test tube, add 0.05 Mg and 1 ml of concentrated HCL, after everything has been homogenized and shaken vigorously If the color changes, the results are declared positive for containing flavonoids [13].

c. Saponin compounds
A total of 2.0 mL of the sample solution was put into a test tube and then shaken for a few minutes, if it reacts positively, it will form a stable foam for 15 minutes.

d. Polyphenol (Tannin) Test.
A total of 1.0 mL of the sample was put into a test tube and then added with a few drops of 5% ferric chloride (FeCl₃) reagent if reacted positively, it would produce a brown precipitate.

e. Alkaloid Test A
Total of 1.0 mL of sample was put into a test tube and then added with 2-3 drops of Dragendorph's reagent, if it reacts positively, it will produce an orange precipitate.

f. Test for Steroids and Triterpenoids
In 1 mL of extract, 3.5 drops of chloroform were added, then 3-5 drops of acetic acid anhydride and 10 drops of concentrated sulfuric acid were added. Steroid positive test with a change in the color of the solution to blue or green. Triterpenoid positive test with a change in the color of the solution from brown to reddish brown.

2.3. Research method
This research was conducted with an experimental design. The data obtained from this study were presented in a quantitative descriptive manner using a 2x2 factorial randomized block design with 2 factors (*Rhizopora mucronata* leaves and *Sonneratia alba* leaves) and 2 test levels (old and young mangrove leaves) with 3 replications. Observational data were analyzed by means of variance test (ANOVA). If the treatment has a significant effect, it is continued by using Duncan's further test to find out the data that is significantly different. The data was processed using software SPSS Version 22 using ANOVA (Analysis of Varians). Significantly different data (P<0.05) was further tested using Duncan's test.

3. Result and discussion
Mangrove leaf powder samples used in this study were samples of mangrove leaves *Rhizopora sp.* (young and old) and *Sonneratia sp* (young and old). Figures 1 are samples of fresh leaves and after undergoing stages after being mashed.
Based on the data from Table 1, the results of phytochemical testing of mangrove leaf extracts Sonneratia sp and Rhizopora sp. with several different types of solvents, this aims to determine what types of antioxidant or antibacterial compounds are found in mangrove leaf extracts Sonneratia sp. and Rhizopora sp. that can be extracted with using different types of solvents, this is because the nature and polarity of each phytochemical compound is different.

Table 1 shows the phytochemical compounds found in the mangrove leaf species Sonneratia sp. are tannins, steroids, alkaloids, flavonoids, and triterpenoids. While the phytochemical compounds found in species Rhizopora spare tannins, saponins, alkaloids, and flavonoids. The results of another study, namely the research of [14], stated that the methanol extract of the leaves of Rhizophora mucronata contained phenolic compounds, saponins, terpenoids and flavonoids. The use of traditional medicine for the red Pedada plant (Sonneratia alba), is known to contain bioactive compounds such as flavonoids, steroids, phenol hydroquinone, tannins and two flavonoids namely luteolin and luteolin 7-0-β glucoside [15] [16].

3.1. Flavonoid content.
Based on figure 2 shows that the type of mangrove leaves and the age of mangrove leaves have a significant effect (P<0.05) on the value of flavonoid levels. The content flavonoid of mangrove tea derived from older leaves was higher than that of younger leaves. Explained that the leaves of this S. alba plant contain secondary metabolites such as flavonoids and tannins [18]. Flavonoids are the largest group of phenolic compounds that have effective properties to inhibit free radicals which have been used as a component of raw materials for medicines. The value flavonoid highest was obtained from mangrove leaf tea from leaf species Rhizopora derived from old leaves. The sample used in this study was the mangrove leaves of S. alba young and old, indicating that the yield of this study has the potential to have antioxidant activity because it uses samples of old leaves which are scientifically proven that the older a leaf, the higher the levels of phenolic and flavonoid compounds that function as antioxidants. The results of this study are the same as the results of research [19], that the ethanol extract of fresh young leaves has a higher total phenolic value, total flavonoid and antioxidant activity than the ethanol extract of dry young leaves, fresh old leaves and dry old leaves. The flavonoids in the leaves of dry young bird tenggek were 5.08±0.0015 mg/ml Eq. While, the old dried bird tenggek leaf was 4.68±0.001 mg/ml Eq, this result was also not too different from the results of the research that had been done.

3.2. Tanin content
Based on figure 3 shows that the type of mangrove leaves and the age of mangrove leaves have a significant effect (P<0.05) on the value of tannin levels. Tannins are phenolic compounds that can form complexes with proteins to form copolymers. The tannin content of mangrove tea from older leaves was higher than
that of younger leaves. The highest tannin value was obtained from mangrove leaf tea which was derived from leaf species *Rhizopora* derived from old leaves. The young leaves of the mangrove *Sonneratia alba* extracted with methanol and ethanol by the Soxhlet and maceration methods contained bioactive components, namely phenols, flavonoids, steroids, saponins, triterpenoids, tannins and alkaloids and had potential as natural antioxidants [7].

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### 3.3. Total phenolic content

Based on figure 4 shows that the type of mangrove leaves and the age of mangrove leaves have a significant effect (P<0.05) on the value of phenol content. Phenolic compounds work as antioxidants by breaking the chain reaction of radicals and donating hydrogen atoms to produce more stable free radicals. The phenol content of mangrove tea derived from older leaves is higher than that of younger leaves. The highest phenol value was obtained from mangrove leaf tea which was derived from leaf species *Rhizopora* derived from old leaves. Leaf age has a very significant effect on total levels phenol and flavonoid [20]. Young leaves have a high content of alkaloids and saponins and tend to decrease with leaf age, while the content of phenolic compounds and flavonoids in old leaves is higher than that of young leaves [20].

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leaves have a high content of alkaloids and saponins and tend to decrease with leaf age, while the content of phenolic compounds and flavonoids in old leaves is higher than that of young leaves[20].

3.4. Vitamin C level
Figure 5 shows that the type of mangrove leaf and the age of the mangrove leaf have a significant effect (P<0.05) on the value of vitamin C levels. The vitamin C content of mangrove tea from young leaves is lower than that of older leaves. The highest value of vitamin C was obtained from mangrove leaf tea from leaf species Rhizopora derived from old leaves. The highest vitamin C was obtained by samples of mangrove tea leaves Rhizophora mucronata with old leaves as raw material, which was 311.47 ppm. Vitamin C is an antioxidant compound that is very easily damaged by temperature, ripeness, environment, oxygen, etc. The vitamin C value of old leaves was higher than that of young leaves. Young leaves the primary metabolic activity is still high to support its growth, so the production of secondary metabolites is still relatively low. While in mature leaves the process Its growth begins to decrease so that more secondary metabolites are produced for defense from environmental stresses [21].

3.5. Moisture content
Based on figure 6 shows that the type of mangrove leaves and the age of mangrove leaves have a significant effect (P<0.05) on the value of water content. The water content of mangrove tea from young leaves is higher than that of older leaves. The highest water content value was obtained from mangrove leaf tea from leaf species Rhizopora derived from young leaves. The high and low water content in mangrove leaves is influenced by the level of maturity of the leaves where the older the leaves will decrease the amount of water content in them. The high water content in young plants because the young plants have more active cells when compared to old leaves [22].

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3.6. Antioxidant activity

Figure 7 is an inhibition calculation curve in excel. The curve shows that the average inhibition value shows a strong antioxidant activity value and can be further explained in table 2.

Based on the table above, it shows that the antioxidant activity of mangrove leaf tea is in the strong to very strong category because it is in the range of 49.76-50.12 ppm. The activity of mangrove leaves Rhizophora and Sonneratia young and old has different values because each has different phytochemical
content, level of aging/age and different growing conditions. The different antioxidant abilities are due to the different secondary metabolites contained in the mangrove *Rhizophora mucronata*, especially in the content of phenolic compounds, flavonoids and tannins which are responsible for supporting antioxidant compounds [23].

The level of antioxidant activity in the sample is influenced by the type of phytochemical content contained in the sample, the process of handling and processing the sample and the age of the sample. In this case, it shows that the sample of young mangrove leaves has higher antioxidant activity than old mangrove leaf tea. The best antioxidant activity was found in RM2 mangrove leaf tea (Tea from *Rhizophora mucronata* old) with 49.76 % inhibition (very strong) and 83.15% inhibition at 80% concentration. At IC₅₀ values of< 50 ppm, the antioxidant properties are very strong. IC₅₀ values of 50-100 ppm have strong antioxidant properties, IC₅₀ values of 100-150 are said to be moderate, and weak if the IC₅₀ value is 150-200 ppm [24] [25]. The IC₅₀ values are SA1 and SA2 almost the same as the results. The study that the old leaves of S. alba have a very strong antioxidant value with an IC₅₀ of 49.77 ppm (< 50 ppm) [26]. These results The older the leaf, the higher the antioxidant value because the higher the value of phenols and flavonoids which are bioactive compounds that act as antioxidants [20].

### Table 2. Quantitative antioxidant test result.

| Concentration of sample (%) | Means of absorbance (Mean±SD) | % Inhibition | IC₅₀ |
|-----------------------------|-------------------------------|--------------|------|
| SA2                         |                               |              |      |
| 20                          | 0,800±0,01                    | 20.4         |      |
| 40                          | 0,635±0,03                    | 42.7         | 49.87|
| 60                          | 0,552±0,02                    | 53.91        |      |
| 80                          | 0,333±0,04                    | 83.51        |      |
| SA1                         | Means of absorbance           | % Inhibition | IC₅₀ |
| 20                          | 0,745±0,03                    | 18.85        |      |
| 40                          | 0,538±0,03                    | 44.72        |      |
| 60                          | 0,467±0,01                    | 53.6         | 50.12|
| 80                          | 0,237±0,01                    | 82.35        |      |
| RM2                         | Means of absorbance           | % Inhibition | IC₅₀ |
| 20                          | 0,782±0,01                    | 17.380       |      |
| 40                          | 0,519±0,06                    | 48.320       |      |
| 60                          | 0,487±0,01                    | 52.090       | 49.76|
| 80                          | 0,223±0,01                    | 83.150       |      |
| RM1                         | Means of absorbance           | % Inhibition | IC₅₀ |
| 20                          | 0,745±0,03                    | 18.85        |      |
| 40                          | 0,538±0,03                    | 44.72        |      |
| 60                          | 0,467±0,01                    | 53.6         | 49.83|
| 80                          | 0,237±0,01                    | 82.35        |      |

### Table 3. Hedonic test of tea mangrove leaves.

| No. | Sample | Flavour | Color | Taste |
|-----|--------|---------|-------|-------|
| 1   | SA1    | 8,86±0,72 | 8,78±0,56 | 8,05±0,21 |
| 2   | SA2    | 7,78±0,15 | 8,05±0,21 | 7,20±0,45 |
| 3   | RM1    | 8,05±0,27 | 8,66±0,52 | 7,78±0,25 |
| 4   | RM2    | 7,54±0,18 | 7,89±0,28 | 7,02±0,55 |
3.7. Hedonic test
Based on the results of the hedonic test, it is shown that tea made from young mangrove leaves is more likely to be preferred than old mangrove leaves. Parameters of aroma and color tend to be accepted by panelists in all treatments. This is due to the aroma and color-forming components such as tannins and polyphenols as well as other antioxidant groups, whose composition is almost the same as the phytochemical compounds possessed by commercial tea so that in terms of color and aroma, they tend to be the same. Phytochemical compounds contained in tea leaves *Camellia sinensis* are phenols, flavonoids, tannins, alkaloids, inorganic acids, vitamins, and minerals.[27]

The taste parameters shown in Table 3 show that the most preferred flavor is *Sonneratia alba* mangrove tea leaves with young leaves as raw materials. The decrease in taste hedonic value in mangrove leaf tea derived from old leaf raw materials may be due to the high value of tannins, flavonoids and phenols contained in old leaves, causing the taste to tend to be more bitter and astringent than young mangrove tea leaves which contain tannins, phenols, and lower flavonoids [28]. Saponins and tannins are a class of plant active compounds that are phenolic, have astringent taste.

4. Conclusion
Based on the results of the study, it was shown that the type of leaf and the level of leaf maturity had an effect on phytochemical compounds and the level of panelists’ acceptance of mangrove leaf tea. Mangrove leaf tea could potentially be used as a functional beverage with a high vitamin C content and high antioxidant activity (in the range strong-very strong) with IC$_{50}$ values from 49.76 to 50.12 with the highest antioxidant activity values obtained from leaves *Rhizophora mucronata* (RM2) with old leaves as raw material.

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