

Accumulation of Potential Photo-Protective Compound Groups in Mangrove (*Sonneratia caseolaris* (L.) Engler.) Leaves

Haviah Hafidhotul Ilmiah, Tri Rini Nuringtyas, Laurentius Hartanto Nugroho*

ABSTRACT

Objective: To analyze the accumulation of potential photo-protective compound groups, include phenol, flavonoid, and tannin in the leaf tissues of *Sonneratia caseolaris* (L.) Engler.

Methods: The research used *Sonneratia caseolaris* (L.) Engler leaves as materials. The total of compound groups and photo-protective ability level of each tissue was measured using spectrophotometry, while detection of compound group accumulations in the leaf tissue were observed using histochemical assay. **Results:** Epidermis extract contained the highest content of total phenolic compounds (phenol, flavonoid, and tannin), followed by those of mesophyll and whole leaf. Moreover, Sun Protection Factor (SPF) value of epidermis was also higher than those of mesophyll and whole leaf, considering that there were no significant difference of SPF value between mesophyll and whole leaf. Result of histochemical assay of *S. caseolaris* fresh leaf sections showed that phenol, flavonoid, and tannin were highly accumulated in the epidermis and some parts of mesophyll. **Conclusion:** Potential photo-protective compound groups of *S. caseolaris* leaves were accumulated in epidermis and mesophyll. However, epidermis showed the highest level of potential photo-protective compound groups than those of other tissues. Photo-protective ability of epidermis also showed higher value than those of mesophyll.

Key words: *Sonneratia caseolaris* (L.) Engler, Secondary metabolite, Photo-protective, SPF, Leaf anatomy.

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INTRODUCTION

Sonneratia caseolaris (L.) Engler is one of mangrove species found abundantly in Java Island northern beach, Indonesia. The plant is known having several usages in traditional medicine. People who are living in coastal-area often use the stem for wound-curing. The flower can be used as smallpox medicine, whereas the leaf is used to recover fever and stop bleeding. Moreover, it has been successfully isolated two flavonoid compounds from the leaves of *S. caseolaris*. They are luteolin and luteolin 7-O- β -glucoside.¹ Luteolin has an ability to protect human skin from UV A, UV B, and UV C radiations.² Although potential photo-protective of *S. caseolaris* has not many studied yet, because of its luteolin content, it can be predicted that *S. caseolaris* has protection ability to UV-ray radiation.

Instead of luteolin, *S. caseolaris* leaves also contain other phenolic compounds such as simple phenol and phenolic acid.³ The compounds is proven for having photo-protective potency.⁴ Photo-protective ability of phenolic group is consisted of two mechanisms, those are: absorption of UV ray and inhibition of Reactive Oxygen Species (ROS) formation.^{5,6}

Naturally, *in vivo* photo-protective mechanism occurs in plant tissue and has function to protect the tissue from UV radiation. Based on several researches, it has

proved that the application of phenolic on human skin also gives photo-protective effect. Hence, phenolic compound has potency to be developed as a sunscreen matter. The reason using natural resource to be used as a sunscreen matter because the natural resources are not only absorb UV radiation, but also able to inhibit oxidative stress inside skin. Level of photo-protective ability of certain matter is usually defined by SPF (Sun Protection Factor) value.⁷

Compounds that have photo-protective potency such as phenol, flavonoid, and tannin could be found in leaf epidermal tissue.⁸ Moreover, flavonoid quercetin and kaemferol were accumulated in epidermis vacuoles in some other species. Another research reported that the concentration of phenolics in leaf mesophyll was depending on UV radiation intensity.⁹ Accumulation of phenolics is different from one species to another. It was affected by plant habitat.

Anatomical structure of *S. caseolaris* leaf is arranged by adaxial epidermis, mesophyll, and abaxial epidermis. The mesophyll is consisted of adaxial-palisade, sponge, vascular bundle, and abaxial palisade.¹⁰

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Photo-protective compounds are expected accumulated in epidermis. Therefore, further research should be carried out.

The separation of epidermis from mesophyll should be done in order to analyze secondary metabolites of each tissue. Carborundum Abration (CA) technique is one of procedure that can be used.^{11,12} Basic principle of the technique is abrasion of epidermis layer using carborundum (Silicon carbide/SiC) powder resulted the separation of epidermis and mesophyll. Therefore, both tissues could be extracted separately.

The extracts from each tissue to be used to analyze the location of photo-protective compounds accumulation. Accumulation pattern of *S. caseolaris* leaf potential photo-protective compound has not studied yet. Whereas, knowledge of the accumulation of bioactive compound is important to study the biosynthesis and crucial enzyme involved, resulted in the efficiency the usage of *S. caseolaris* as a sunscreen resources. Therefore, the first step to optimize the utilization of the compounds in *S. caseolaris* leaf, studies about the location of phenol, flavonoid, and tannin accumulation in the epidermis and mesophyll of *S. caseolaris* leaf and its SPF values were carried out in this research.

MATERIALS AND METHODS

Sample collection

The samples of fresh leaves of mangrove (*Sonneratia caseolaris* (L.) Engler.) were taken from Wonorejo Mangrove Eco-Park, Surabaya, Indonesia. The leaves were taken during April to July 2015. This research used the leaves from 1st nodes until 3rd nodes, which were not infected by pest and plant diseases.

Epidermis and mesophyll extractions

Epidermis was extracted using carborundum abrasion (CA) method with modification.¹¹ Three kinds of materials were used in leaf abrasion to give optimum tissue separation. Those were pure carborundum powder (Fischer Sci) grit 320, sandpaper (Sikens) grit 400, and whetstone powder. Each adaxial and abaxial epidermis of leaf lamina was abraded 7 times by moderate pressure rubbing. Observation under light microscope was done to ensure the precision of epidermis layer separation. The abraded leaf was then collected in the conical tube contained 15 mL of 90% methanol followed by vortexing for 1 min. Moreover, leaf was taken out from conical tube, whereas the solution was centrifuged with 6000 rpm in speed for 10 min. Supernatant as the result of centrifugation was re-used to extract next 3 until 4 leaves lamina. The volume of the solution was maintained constantly 15 mL. Then the solvent of collected epidermis extract was evaporated using steam bath with the temperature 60°C. The abraded leaves which were defined as mesophyll, were ground immediately to be a powder. The powder was then macerated within 90% methanol for 24 h. Ratio between powder and solvent was 1:5. Mesophyll extract provided from filtering the macerated powder was evaporated using the same method of epidermis extract. The whole leaf without any abrasion was also extracted using the same method as previous extraction for comparison.

Phenols measurement

Total phenol was measured using spectrophotometry method with Folin-Ciocalteu reagent. Extract solution was reacted with the reagent. Then, absorbance was measured at 745 nm of wavelength.⁷ Total flavonoid was measured by using Aluminium chloride method, where the extract was reacted with AlCl₃ reagent and the absorbance was measured in 415 nm of wavelength. Total tannin was also measured with spectrophotometry method using Folin-Denis reagent.¹³

SPF value measurement

The measurement of SPF value used spectrophotometry method was adopted from Petro method.¹⁴ Determination of SPF value was done by measuring the absorbance of each extract, from 290 nm until 320 nm of wavelength gradually, so that the minimal absorbance equals to 0.05. Then, the absorbance is entered to this following formula.

$$\text{Log SPF} = \frac{\text{AUC}}{\lambda_n - \lambda_1} \times 2$$

Note:

SPF = Sun Protection Factor

AUC = Total value of absorbance's in λ_n and λ_n-1 , divided by 2

λ_n = Wavelength provided absorbance equal to 0,05

λ_1 = Wavelength of 290 nm

Histochemical assay

The histochemical assay was done by using cross-section of fresh *S. caseolaris* leaf. Phenol assay used 10% Ferric trichloride (FeCl₃) as a reagent. The cross-section of the leaf was then soaked in FeCl₃ and Sodium carbonate (NaCO₃) within 15 min in room temperature. The accumulation of phenol compound in leaf tissues was detected by the color changes from dark-green to black color under light microscope.¹⁵ Moreover, flavonoid assay was done by using 5% NaOH. The leaf cross-section was soaked in 5% NaOH solution for several min. The cross-section of leaf was removed on to object glass and observed under light microscope. The flavonoid content was detected by the yellow color of the leaf tissue. The assay of tannin used 10% FeCl₃ as the reagent. The leaf-section was soaked in 10% FeCl₃ for several min, followed by transferring to object glass and observed under light microscope. The changes of leaf tissue to be green, blue, or black color showed the availability of tannin.¹⁵

RESULTS

Carborundum abrasion technique (CA) was successfully used to peel epidermis of *S. caseolaris* leaves. The basic principle of the technique was the abrasion of the two epidermis (adaxial and abaxial epidermis) layers uses carborundum/silicon carbide powder. Optimization of CA technique have been used in this research. In the primary study, pure carborundum powder (Fischer Sci) grit 320 was used. However, the technique was not successfully made the epidermis peeled from the mesophyll. Then, sandpaper (Sikens) grit 400 was used to peel the epidermis, but it was resulted on the bumpy abrasion of epidermis. Moreover, the usage of sandpaper takes more times for epidermis abrasion. After trials and errors, it was found that the *S. caseolaris* leaf epidermis can be peeled using whetstone powder with 7 times of rubbing by moderate pressure. Abaxial epidermis of *S. caseolaris* leaf was more difficult to be abraded than adaxial leaf. Therefore, the abaxial epidermis cells just to be opened to take out the cell content to avoid mesophyll damage. Light microscope observation of *S. caseolaris* leaf cross-section, before and after epidermis abrasion are shown in Figure 1A and 1B.

The extraction process of epidermis, mesophyll and whole leaf of *S. caseolaris* were resulted of 0.93 g, 6.13 g, and 8.01 g extract respectively (Table 1). The Table shows that the weight of epidermis extract was lighter compared to those of mesophyll and whole leaf.

Total phenol

The total phenols of *S. caseolaris* leaf can be seen in Table 2. The total phenols of epidermis was the highest compared to those of two others materials, followed by mesophyll and whole leaf.

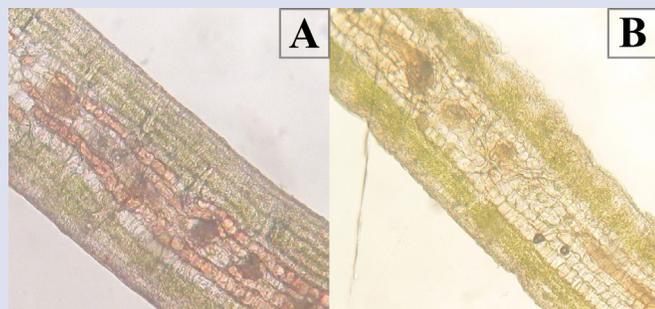


Figure 1: Cross-section of fresh *S. caseolaris* leaf with the magnification 10x10 (A= Before abrasion; B=After abrasion).

Table 1: Result of epidermis, mesophyll, and whole leaf of *S. caseolaris* leaf extraction.

No.	Extracts	Weight (g)	Colors
1.	Epidermis	0.93	Brown-blackish
2.	Mesophyll	6.13	Dark green-blackish
3.	Whole leaf	8.01	Dark green-blackish

Table 2: The total phenols of *S. caseolaris* epidermis, mesophyll, and whole leaf.

No.	Extracts	Phenol total (mg/g tissues) ± SD
1.	Epidermis	18.65 ± 0.14
2.	Mesophyll	17.71 ± 0.24
3.	Whole leaf	13.30 ± 0.24

Table 3: The total flavonoid of *S. caseolaris* epidermis, mesophyll, and whole leaf extracts.

No.	Extracts	Total flavonoid (mg/g tissues) ± SD
1.	Epidermis	79.95 ± 3.44
2.	Mesophyll	52.03 ± 0.57
3.	Whole leaf	47.66 ± 0.95

Table 4: Total tannin of *S. caseolaris* epidermis, mesophyll, and whole leaf extracts.

No.	Extracts	Total tannin (mg/g tissues) ± SD
1.	Epidermis	100.33 ± 4.42
2.	Mesophyll	64.46 ± 0.72
3.	Whole leaf	58.83 ± 1.22

Total flavonoid

The total flavonoid of *S. caseolaris* leaf is shown in Table 3. The total flavonoid of epidermis gives higher value than those of mesophyll, whereas the mesophyll total flavonoid was higher than those of whole leaf extract.

Total tannin

The total tannin of *S. caseolaris* extract is shown in Table 4. The total tannin of epidermis was higher than those of mesophyll, whereas mesophyll was higher than those whole leaf extract.

SPF value

The SPF value of *S. caseolaris* is shown in Table 5. SPF value of epidermis was higher than those of mesophyll, and SPF value of mesophyll was

Table 5: The SPF value of *S. caseolaris* epidermis, mesophyll, and whole leaf extracts.

No.	Solutions	SPF value ± SD	Photo-protective Ability
1.	Epidermis	1.538 ± 0.08	Minimal
2.	Mesophyll	1.516 ± 0.08	Minimal
3.	Whole leaf	1.517 ± 0.03	Minimal
4.	Sunscreen cream	1.601 ± 0.03	Minimal

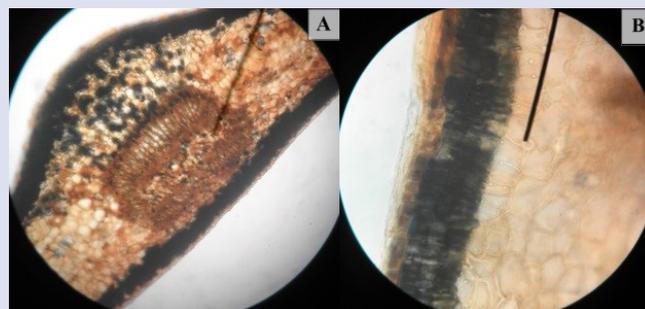


Figure 2: The phenol histochemical assay of *S. caseolaris* leaf cross-section. A=10x10 magnification; B=40x10 magnification.

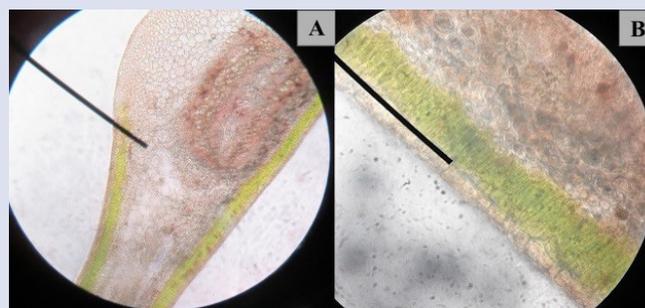


Figure 3: The flavonoid histochemical Assay of *S. caseolaris* leaf cross-section. A=10x10 magnification; B=40x10 magnification.

lower than those of whole leaf extract. Moreover, sunscreen cream used as a positive control showed the highest SPF value compared to those of extracts.

Histochemical assay

Result of histochemical assay of *S. caseolaris* leaf is shown in Figure 2A until 4B. Phenol assay is shown in Figure 2A and 2B. Phenol was reacted with $FeCl_3$ and $NaCO_3$ resulted in dark-green or black colors. Figure 2A represents that phenols were located in palisade cells and some places of sponge. The accumulation of phenol in epidermis is clearly showed in Figure 2B. Moreover, the flavonoid assay showed that the compounds were detected by yellow colors in epidermis and some cells of sponge (Figure 3A). Figure 3B shows that all epidermis cells were concentrated with flavonoid. Tannin assay (Figure 4A and 4B) shows that tannin react with $FeCl_3$ form dark blue and black colors. Figure. 4A shows that tannin were found in epidermis, palisade, and some idioblast cells in sponge tissue. The sponge cells that contain tannins are recognized as tannin cells. Figure 4B clearly shows the accumulation of tannins was in the palisade and epidermis.

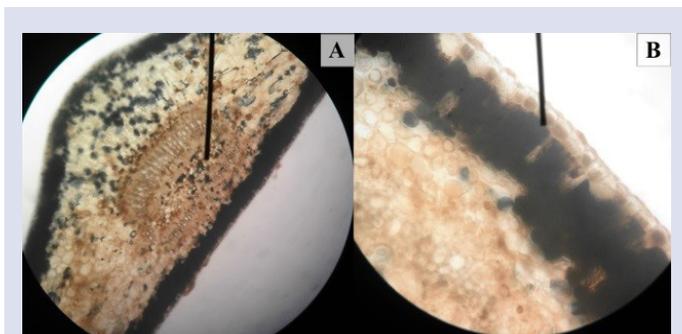


Figure 4: The tannin histochemical assay of *S. caseolaris* leaf cross-section. A=10x10 magnification; B=40x10 magnification.

DISCUSSION

Research on the localization of specific bioactive compounds or group of compounds nowadays to be an interesting topic for the simplification of the extraction material, for the research of biosynthetic route, or for the metabolic profiling. Instead of many high technology tools,^{16,17} simple separation method used nowadays was carborundum abrasion technique (CA). The separation of leaf tissue, for instance, could be done manually by applying carborundum as a material for the abrasion.¹¹ However, the success of peeling the epidermis from the mesophyll were affected by many factors such as the leaf anatomy, the thickness of cell wall, and the pressure level during the rubbing. Therefore, the current research failed to apply carborundum to separate epidermis and mesophyll. It may be due to the thickness of epidermis cell wall or the thickness cuticle layer of *S. caseolaris* leaf. The most success method was done by applying whetstone powder.

The research was continued by extracting the tissue with 90% methanol. The result showed that whole leaf produced the highest volume of extract followed by those of mesophyll and epidermis. It seems that the volume of extract had positive correlation with the volume of tissues considering that whole leaf had the highest volume of tissue followed by mesophyll and epidermis. In other words, the compound which could be extracted with 90% Methanol was distributed evenly in the leaf tissues.

However, the following results showed that phenolic compound, flavonoid and tannin were accumulated dominantly in the epidermis cells followed by mesophyll cells. It means the compounds which had main function for the protection were accumulated in the periphery tissues. Phenolic compounds in epidermis play an important role as early defense to protect plant tissue from high intensity of UV radiation. Moreover, when the UV-ray reach mesophyll, it will be recovered by the mesophyll phenolic through antioxidant mechanism. The phenolic compounds will inhibit the formation of reactive oxygen species (ROS). Therefore, the damage of DNA in plant tissue can be avoided.⁵

Moreover, the measurement of SPF showed that epidermis had the highest SPF value compared to those of mesophyll and whole leaf. From the result, it can be predicted that SPF value has positive correlation with the accumulation of phenolic compound, flavonoid and tannin. Other research showed that the accumulation pattern of photo-protective compounds i.e. phenol, flavonoid, and tannin have been proved shows high variety from one species to another.¹⁸

The result of histochemical assay (Figure 2A until 4B) shows that epidermis phenolic were distributed in all epidermis cells, while the phenolic was only contained in certain cells of mesophyll. Concomitant with this results, measurement of total phenolic compound in epidermis gives higher value than those of mesophyll.

Enzymes involved in the beginning of phenolic biosynthesis, include L-Phenylalanine Amonia-Lyase, 4-coumarate-KoA ligase, and S-adenosil-L-metionin-kafeat 3-o-metiltransferase could be found in epidermis and vascular bundle.¹⁹ Whereas rate limiting enzyme of flavonoid, include chalcone synthase, chalcone isomerase, flavonoid O-metiltransferase, and isovitexin arabinosiltransferase were found in mesophyll.¹⁹ Therefore, it can be predicted that there were certain mechanisms of intercellular transportation of phenolic compounds from mesophyll to epidermis.

CONCLUSION

Based on the result of this research, it can be concluded that potential photo-protective compounds of *S. caseolaris* leaf were accumulated in epidermis and mesophyll, where the epidermis showed higher content than those of mesophyll. Photo-protective ability represented by SPF value showed that SPF value of the epidermis also higher than those of mesophyll. Histochemical assay showed that photo-protective compounds distributed evenly in the epidermis cells while the compounds only distributed in specific cells of mesophyll.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS USED

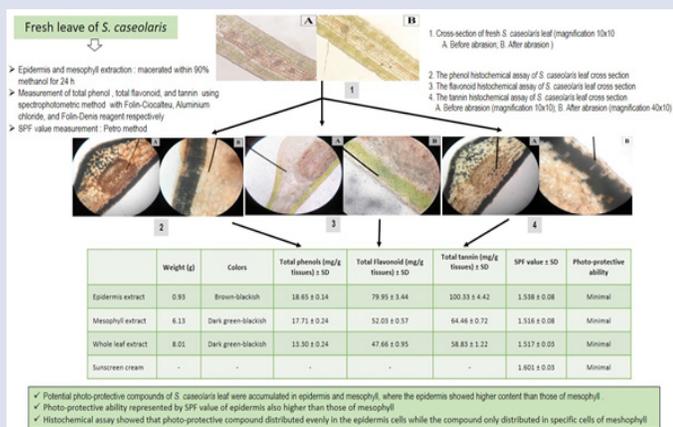
AlCl₃: Aluminium chloride; **AUC**: Area under the curve; **CA**: Carborundum abrasion; **DNA**: Deoxyribonucleic acid; **FeCl₃**: Ferric chloride; **NaCO₃**: Natrium carbonate; **NaOH**: Natrium hydroxide; **SiC**: Silicon carbide; **ROS**: Reactive oxygen species; **SPF**: Sun protection factor; **UV**: Ultra violet.

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GRAPHICAL ABSTRACT



SUMMARY

- Epidermis extract contained the highest content of total phenolic compounds (phenol, flavonoid, and tannin), followed by those of mesophyll and whole leaf.
- Sun Protection Factor (SPF) value of epidermis was also higher than those of mesophyll and whole leaf, considering that there were no significant difference of SPF value between mesophyll and whole leaf.
- Histochemical assay of *S. caseolaris* fresh leaf sections showed that phenol, flavonoid, and tannin were highly accumulated in the epidermis and some parts of mesophyll
- Potential photo-protective compounds of *S. caseolaris* leaf were accumulated in epidermis and mesophyll, where the epidermis showed higher content than those of mesophyll.
- Photo-protective ability represented by SPF value showed that SPF value of epidermis also higher than those of mesophyll
- Histochemical assay showed that photo-protective compound distributed evenly in the epidermis cells while the compound only distributed in specific cells of mesophyll.

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