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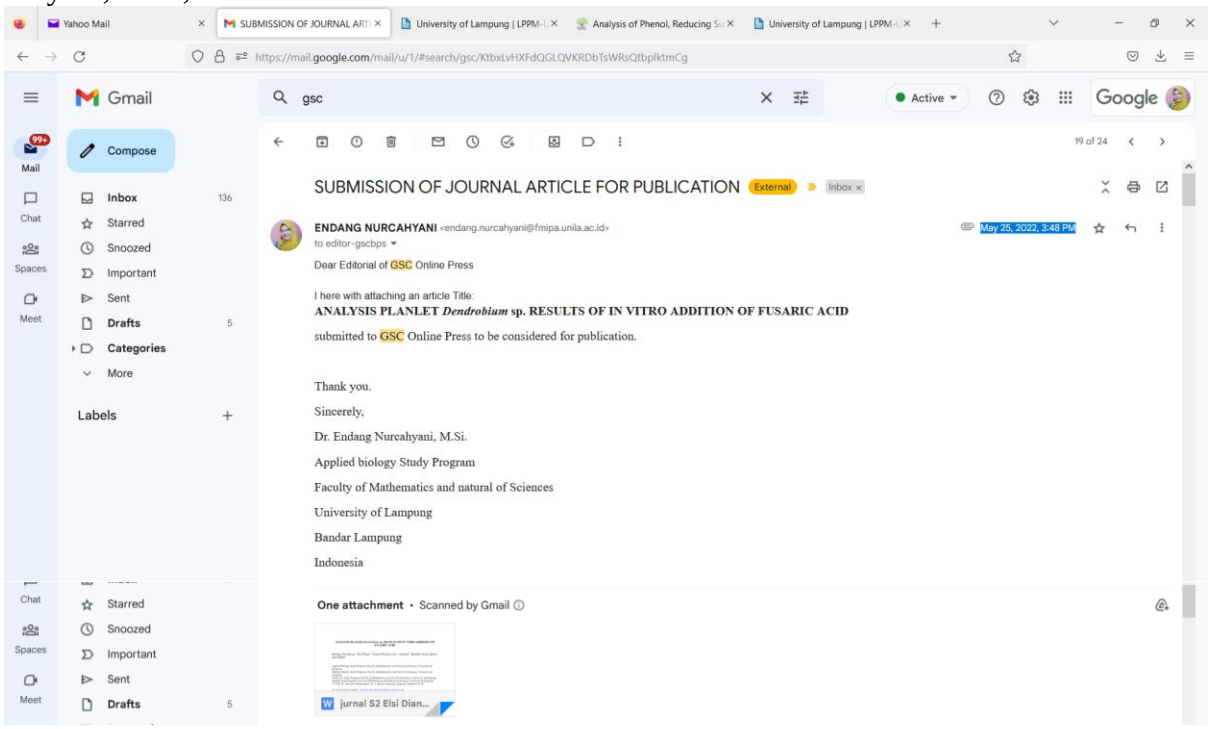

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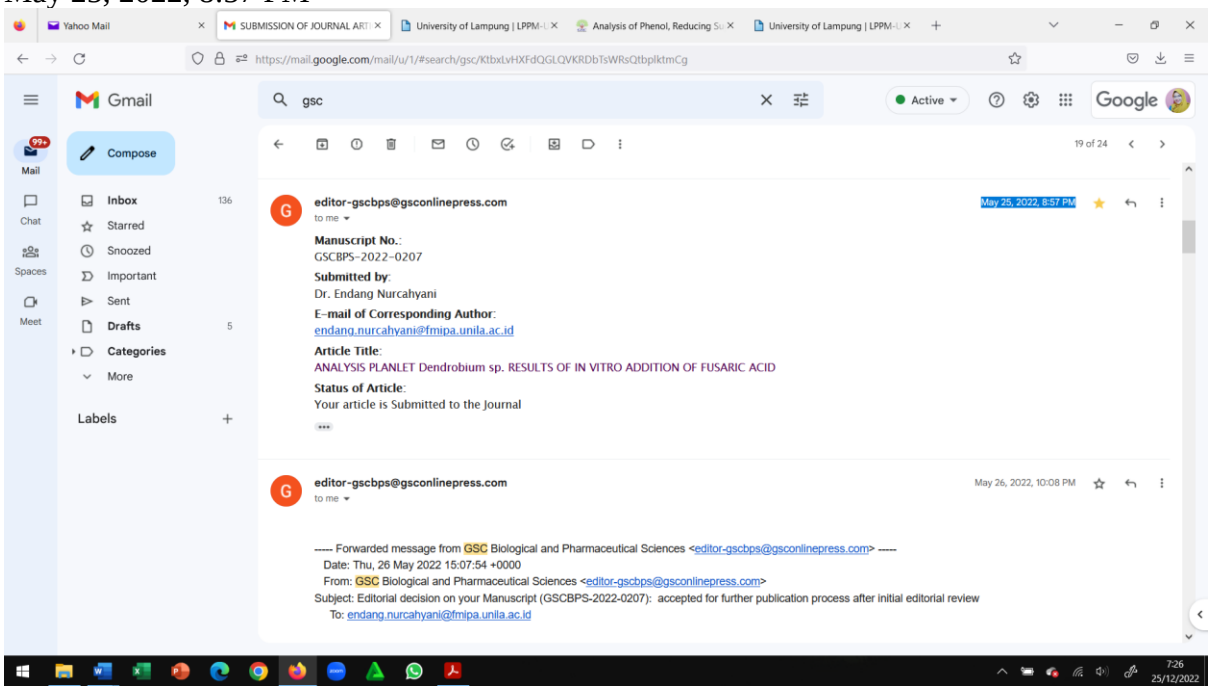

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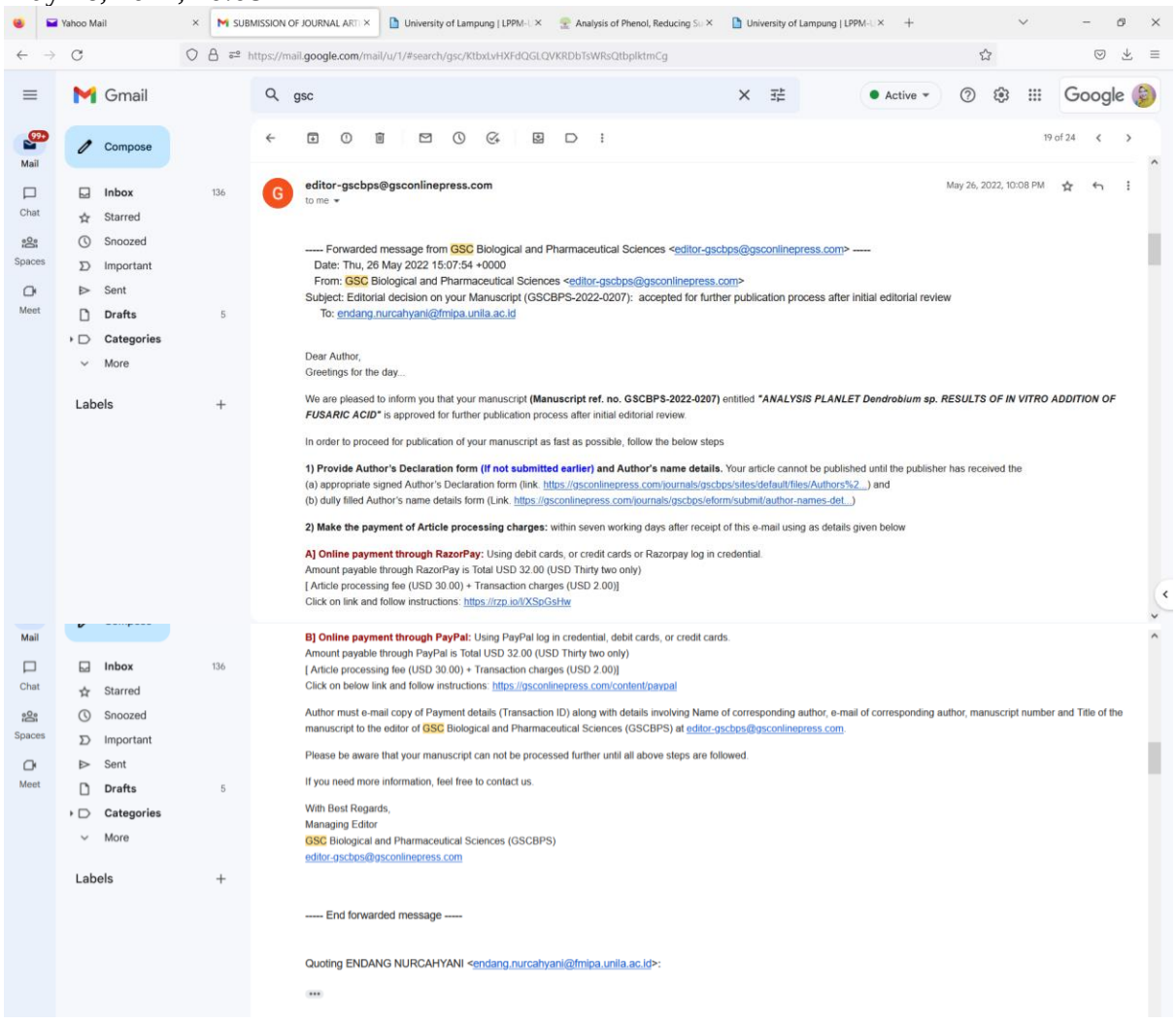

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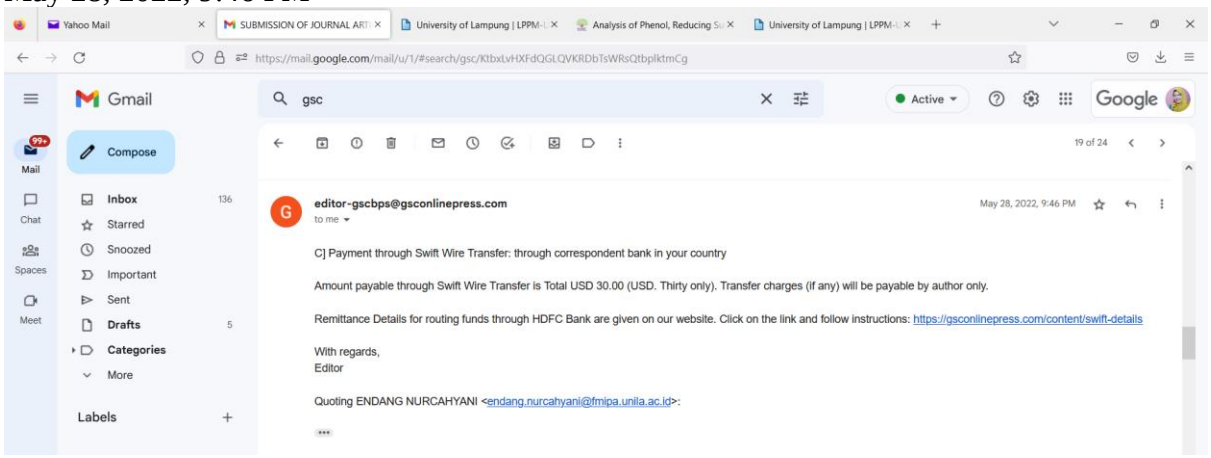

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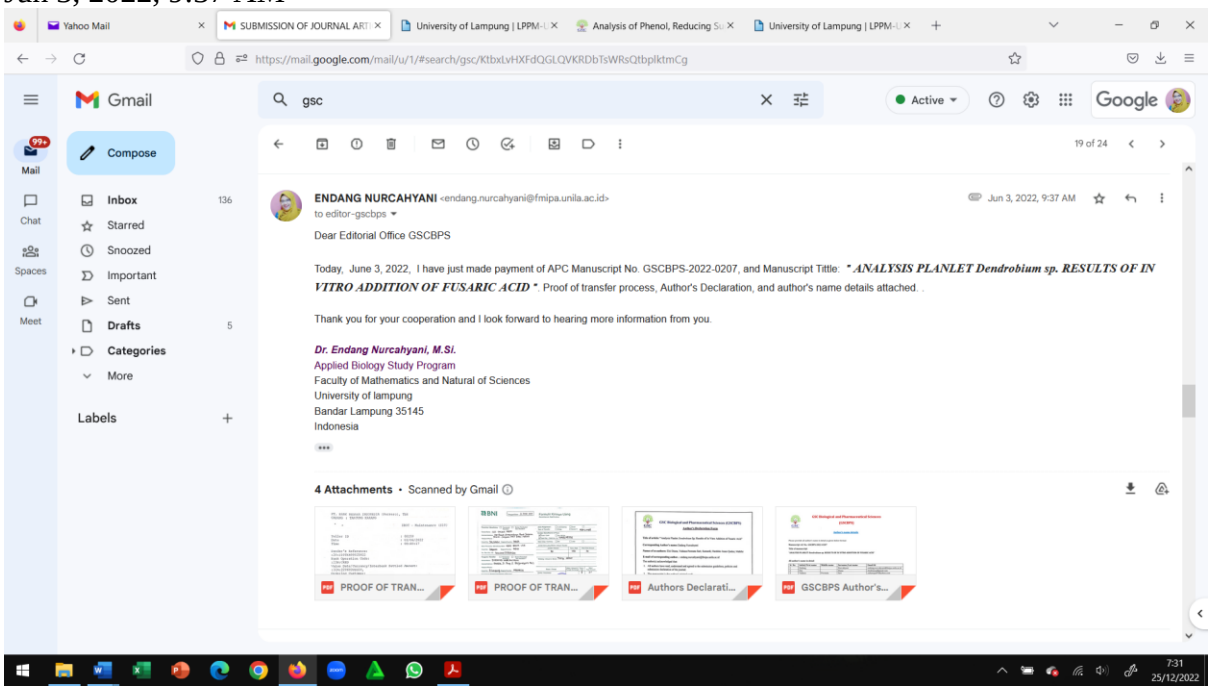

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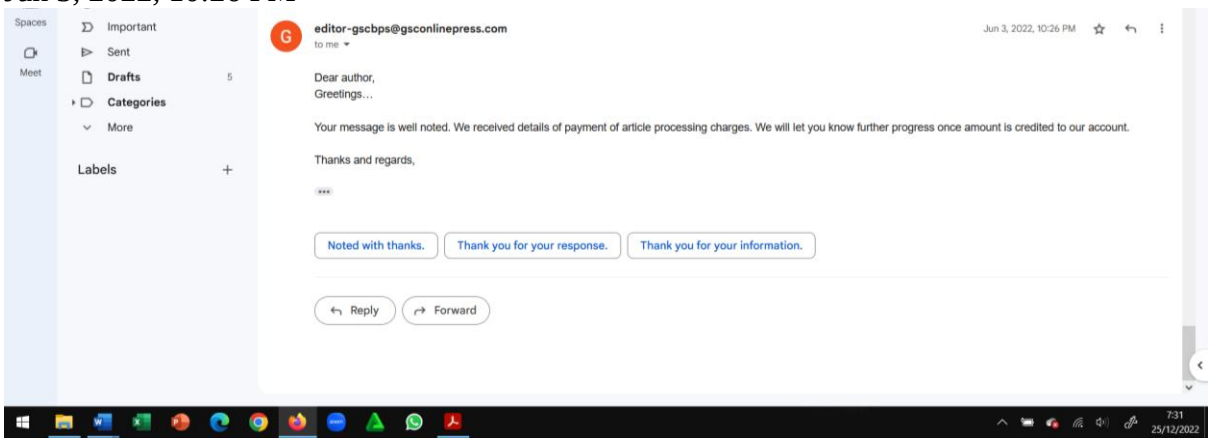

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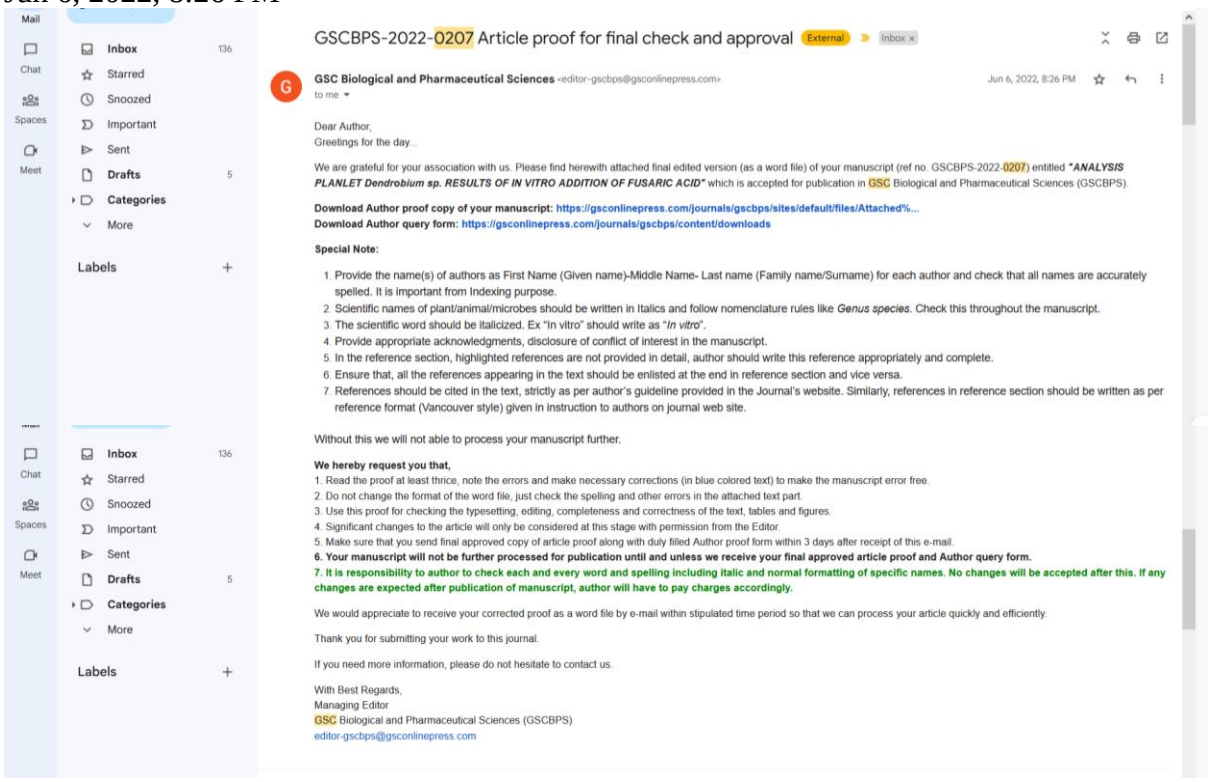

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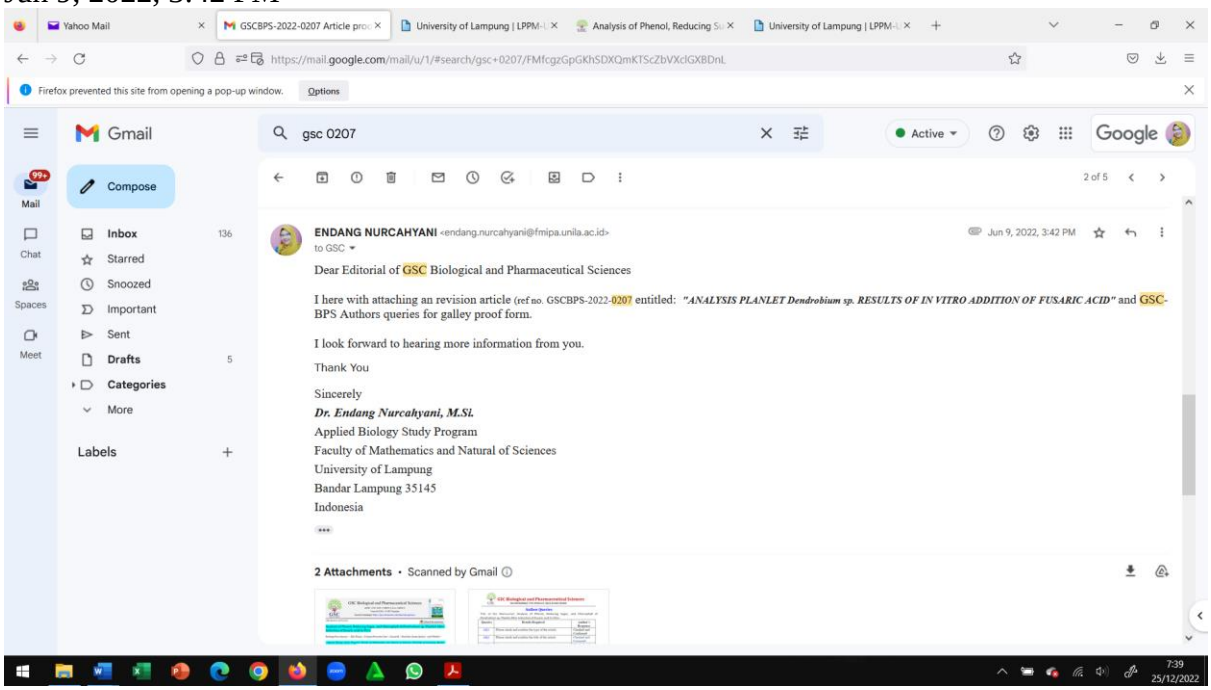

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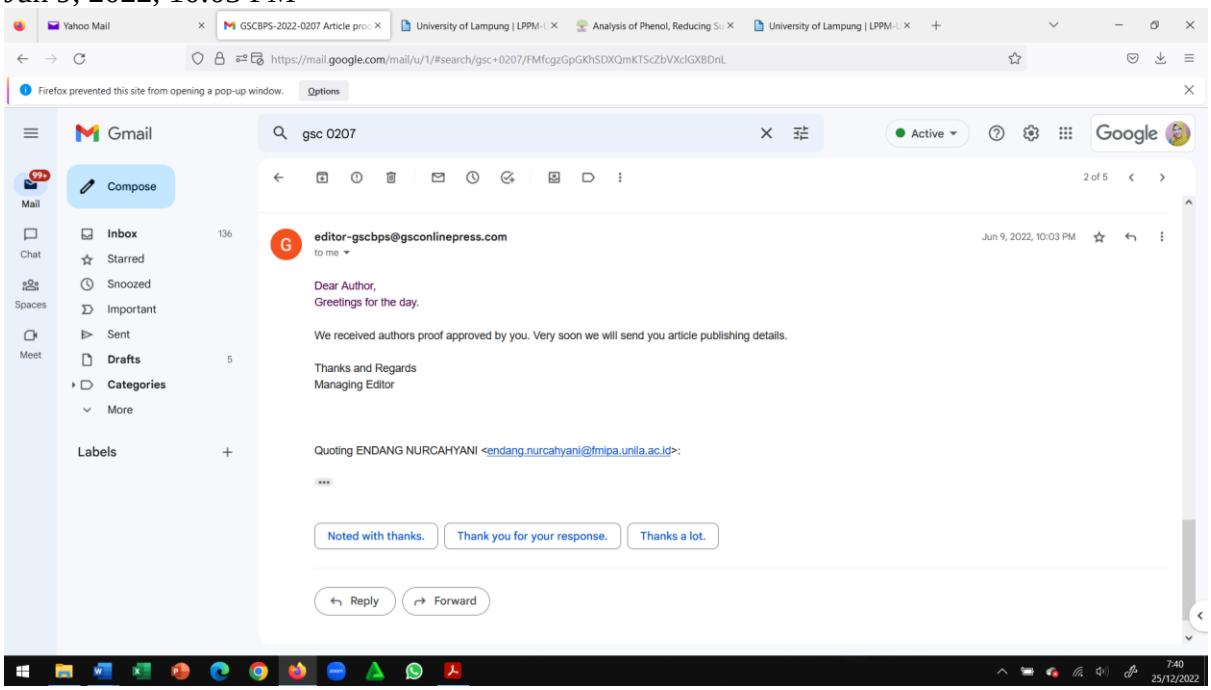

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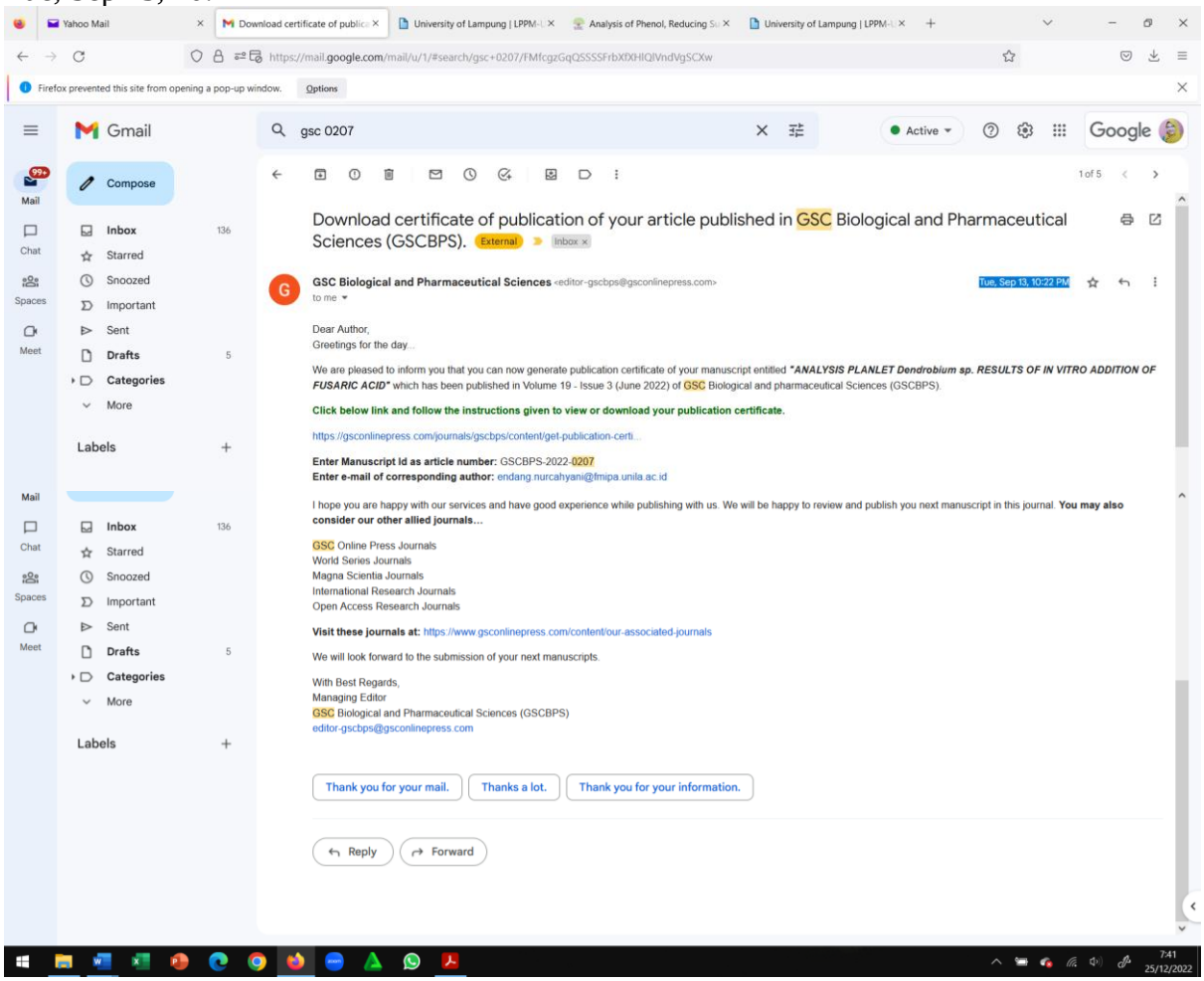

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Analysis of Phenol, Reducing Sugar, and Chlorophyll of *Dendrobium* sp. Plantlet After Induction of fusaric acid *In Vitro*

Endang Nurcahyani [1,*], Elsi Diana [2], Yuliana Permata Sari [2], Sumardi [2], Hardoko Insan Qudus [3] and Mahfut [4]

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Abstract

Orchids are ornamental plants with varied and beautiful flower colors and are much favored by the public. Orchid plants have a higher economic value when compared to other ornamental plants, both as cut flowers and potted flowers. In the process of growth, orchid plants are disturbed, namely Fusarium wilt disease caused by Genus *Fusarium* which can produce a toxin compound, namely fusaric acid which is widely used in *in vitro* selection of plants. Plant resistance can be formed through the induction of fusaric acid compounds. Induced resistance is an alternative way to obtain disease-resistant plants because plants are able to create natural resistance mechanisms. The purpose of this study was to determine the concentration of tolerant fusaric acid to select Dendrobium orchid plantlets with optimal growth and specific expression characters of Dendrobium orchid plantlets based on phenol content, reducing sugar content, and total chlorophyll content, chlorophyll a and chlorophyll b. The results of this study indicated that there was an increase in total phenol content, reducing sugar content, and total chlorophyll content, chlorophyll a and chlorophyll b along with the addition of fusaric acid concentration.

Keywords: Chlorophyll; *Dendrobium* sp.; Fusaric Acid; *In vitro*; Phenol; Reducing Sugar

1. Introduction

Orchid plants are included in the Orchidaceae family which based on the nature of their life are divided into 3 namely epiphytic orchids, semi-epiphytic orchids and land or terrestrial orchids. Epiphyte is a type of plant that lives by attaching to other plants that are not harmful to the host plant, its roots are attached and have aerial roots that are used to find food [1]. One type of orchid that is in great demand by the public is *Dendrobium* sp. because it has the characteristics of having varied flower shapes and flower colors, long-lasting flower freshness, and flexible flower stalks so that they are easy to assemble [2].

In the process of growth, *Dendrobium* sp. can be attacked by the disease. One of the compounds that can be used for plant resistance is fusaric acid produced by several species of fungi of the genus *Fusarium*. Fusaric acid (FA) will be more effective at low doses, namely at non-toxic FA concentrations so that plants will respond to resistance by

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producing phytoalexins to inhibit pathogen activity [3]. The plantlet *Dendrobium* sp. which has been given the addition of fusaric acid can be analyzed based on the content of phenol, reducing sugar and chlorophyll.

Phenol is a compound whose hydroxyl group (-OH) is directly connected to an aromatic ring group. Plants containing phenolic compounds are classified into simple phenols, lignins, phenolic acids, acetophenones, xanthones, flavonoids, coumarin bioflavonoids, stilbenes, hydroxycinnamic acid, tannins, tyrosine derivatives, and benzoquinone [4]. Sugars consist of reducing and non-reducing sugars which have important roles in central metabolic pathways and assist in the production of secondary metabolites that can enhance the medicinal properties of plants [5].

Chlorophyll is a green pigment that plays an important role in photosynthesis, chlorophyll can absorb sunlight and use its energy to synthesize carbohydrates [6].The purpose of this study was to determine the concentration of tolerant fusaric acid for the selection of Dendrobium plantlets with optimum growth and specific expression characters of Dendrobium plantlets based on total phenol content, reducing sugar content, and total chlorophyll content, chlorophyll a and chlorophyll b. The plantlet Dendrobium sp. which has been given the addition of fusaric acid with various concentrations: (A) 0 ppm, (B) 10 ppm, (C) 20 ppm, (D) 30 ppm, and (E) 40 ppm are presented in **Figure 1.**

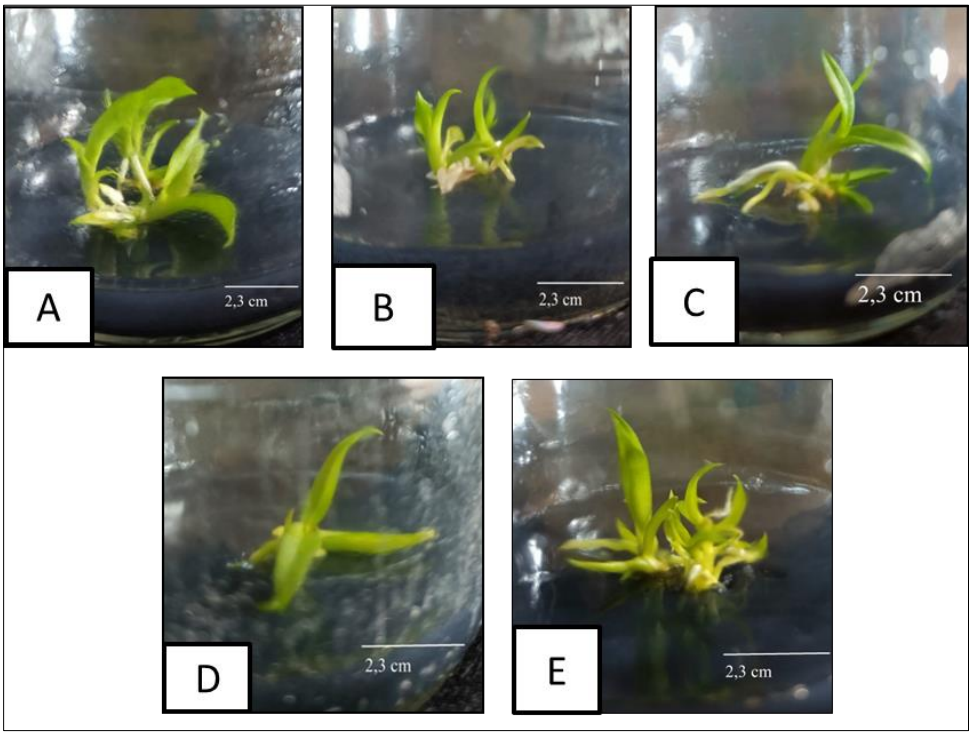

Figure 1 Control *Dendrobium* sp. plantlets (A) and *Dendrobium* sp. plantlets treated with various concentrations of fusaric acid: B.10 ppm; C.20 ppm; D. 30 ppm; and E. 40 ppm

2. Methods

This research was conducted from September 2021 to February 2022 at the *In Vitro* Culture Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, University of Lampung**.** The character of the *Dendrobium* orchid plantlets that have been given the addition of fusaric acid can be reviewed, including the total phenol content, reducing sugar content, and total chlorophyll content.

The Folin-Ciocalteu method can be used to analyze the content of total phenolic compounds [7] by making a fresh extract of Dendrobium orchid leaves in methanol, 0.2 mL of each extract solution is pipetted and then 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent are added. The solution was shaken until it became homogeneously clear yellowish then allowed to stand for 8 minutes, added 3 mL of 20% Na2CO3 solution and then shaken until homogeneous. The solution was allowed to stand for 30 minutes at room temperature until the solution turned blue. The absorption of the solution was measured with a Vis spectrophotometer at a wavelength of 749 nm. The phenol content obtained is mg gallic acid equivalent/gram sample. Calculation of the total phenol content using the formula:

$$\text{Total Phenol Content (mg/100g)} = \frac{\text{X. V. FP}}{\text{BS}}$$

Where, X = Concentration (ppm)
V = Volume of sample solution (extract) (mL)
FP = Sample solution dilution factor
BS = Sample weight (g)

Analysis of reducing sugar content in this study used Dendrobium plantlets that had been treated with fusaric acid and without treatment (control). The Nelson – Somogyi method can be used to analyze the reducing sugar content by making a sample solution in the form of 1 ml of Dendrobium orchid leaf extract taken from each concentration and then put into each test tube and then added 1 ml of Nelson's reagent in each test tube. The solution was heated on a boiling water bath for 20 minutes. Then the sample was cooled to room temperature. After cooling, added 1 ml of Arsenomolybdate reagent and 7 ml of distilled water, shaken until homogeneous. The solution mixture was put in a cuvette and the absorption of visible light was measured with a wavelength of 695 nm. The absorbance value obtained is reduced by the absorbance value of the blank so that the absorbance value of the sample is obtained. Then based on the standard solution regression equation, the absorbance value of the sample was converted to reducing sugar content (mg/ml).

Calculation of reducing sugar content [8] can use the formula:

$$\text{Reducing Sugar Content (\%)} = \frac{\text{X.FP}}{\text{BS}}\ x\ 100$$

Where, X = Concentration (ppm)
FP = Sample solution dilution factor
BS = Sample weight (g)

Analysis of chlorophyll content using plantlet leaves of *Dendrobium* sp. which has been induced by fusaric acid, using the Harborne method with a spectrophotometer [9]. The way it works is the plantlet leaves of *Dendrobium* sp. 0.1 g of uniform leaf was removed, then crushed with a mortar (pestle) then added 10 mL of 80% acetone. After that the solution was filtered with Whatmann paper No. 1, and put in a flakon and tightly closed. The sample solution and standard solution (80% acetone) were taken as much as 1 mL, then put in a cuvette. After that, the absorption readings were carried out with a UV spectrophotometer at wavelengths (λ) 646 nm and 663 nm. Chlorophyll content is calculated using the following formula:

Chlorophyll a = 12, 21 λ663 – 2, 81 λ646 mg/l
Chlorophyll b = 20, 13 λ646 – 5, 03 λ663 mg/l
Total Chlorophyll = 17, 3 λ646 + 7,18 λ663 mg/l

Where, λ646 = absorbance at a wavelength of 646 nm
λ663 = absorbance at a wavelength of 663 nm

3. Results and discussion

The effect of Fusaric Acid (FA) on *Dendrobium* sp. can be determined by the total phenol content. The content that can be observed in the leaves of the plantlet *Dendrobium* sp. who have been treated with FA and control are presented in Table 1.

Table 1 Results of Total Phenol Content of Planlet *Dendrobium* sp. that has been induced by FA

Treatment (ppm)	Total Phenol Content *Dendrobium* sp. (mg/100g)
0 (control)	10.66 ± 0.44[a]
10	10.00 ± 0.40[a]
20	10.18 ± 0.31[a]
30	13.19 ± 2.07[a]
40	15.25 ± 3.16[a]

Note: Numbers followed by the same letter, not significantly different at 95% confidence level

Based on Table 1, the total phenol content at a concentration of 0 ppm (control) was 10.66 mg/100g. The results of the total phenol content of *Dendrobium* sp. treated with fusaric acid (FA) showed that the higher the concentration of FA, the higher the total phenol content. The total phenol content at 10 ppm was 10.00 mg/100g, increased to 10.18 mg/100g at a concentration of 20 ppm, followed by 13.19 mg/100g at a concentration of 30 ppm, and 15.25 mg/100g at a concentration of 40 ppm. The results of this study are in line with research [10] on vanilla plantlets, the higher the concentration of FA, the higher the total phenol content.

Phenol compounds are one indicator of disease resistance. The results of the study [11] showed that the higher the phenol content, the more resistant the plant to pathogen attack. One of the results of plant metabolism that can function as a plant chemical resistance system that can prevent the growth and development of pathogens is phenol compounds [10].

Effect of Fusaric Acid (FA) on *Dendrobium* sp. can also be determined by the content of reducing sugars. The content that can be observed in the leaves of the plantlet *Dendrobium* sp. that have been treated with FA and control are presented in Table 2.

Table 2 Results of Reducing Sugar Content of Plantlet *Dendrobium* sp. that has been induced by FA

Treatment (ppm)	Reducing Sugar Content of Planlet *Dendrobium* sp. (%)
0 (control)	11.76 ± 2.20[a]
10	7.71 ± 2.49[a]
20	11.90 ± 1.34[a]
30	15.21 ± 3.16[a]
40	15.26 ± 1.81[a]

Note: Numbers followed by the same letter, not significantly different at 95% confidence level

Based on Table 2, the results of reducing sugar content at a concentration of 0 ppm (control) were 11.76%. The results of the reducing sugar content of *Dendrobium* sp. treated with fusaric acid (FA) showed that the higher the concentration of FA, the more reducing sugar content increased. The reducing sugar content at 10 ppm was 7.71%, increasing to 11.90% at a concentration of 20 ppm, followed by 15.21% at a concentration of 30 ppm, and 15.26% at a concentration of 40 ppm.

Table 3 The Chlorophyll Content of Plantlet *Dendrobium* sp. induced FA

Treatment (ppm)	The Chlorophyll Content of Planlet *Dendrobium* sp. (mg/g tissue)		
	Chlorophyll a content	Chlorophyll b content	Chlorophyll total content
0 (control)	0.39 ± 0.015^{a}	3.24 ± 0.257^{a}	3.63 ± 0.268^{a}
10	0.37 ± 0.020^{a}	3.96 ± 0.316^{a}	4.49 ± 0.342^{a}
20	0.45 ± 0.057^{a}	4.47 ± 0.170^{a}	4.84 ± 0.184^{a}
30	0.53 ± 0.066^{a}	5.18 ± 0.623^{b}	5.63 ± 0.588^{a}
40	1.11 ± 0.522^{a}	5.30 ± 0.668^{c}	6.41 ± 0.979^{b}

Note: Numbers followed by the same letter, not significantly different at 95% confidence level

Based on research [12] suggests that the Somogyi-Nelson method has higher sensitivity and accuracy than other methods, it is recommended to test the reducing sugar content using the Somogyi-Nelson method. In the Somogyi-Nelson method, there is a reaction between specific alkaline Cu (Cu^{2+}) reagent with reducing sugar to Cu^{+} which forms a brick red precipitate, then when arsenomolybdate solution is added, the reaction will turn blue-green.

The effect of Fusaric Acid (FA) on *Dendrobium* sp. can be identified by the chlorophyll content. Chlorophyll content can be observed in the leaves of the plantlet *Dendrobium* sp. who had been treated with FA and control. The method used to analyze the chlorophyll content is the Harboune method.

The results of the chlorophyll content of *Dendrobium* sp. induced FA is presented in Table 3.

Based on Table 3, it shows that the higher the concentration of FA, the higher the chlorophyll content in the *Dendrobium* sp. The highest chlorophyll content at 40 ppm FA concentration was at chlorophyll a 1.11 mg/g, chlorophyll b 5.30 mg/g and total chlorophyll 6.41 mg/g. The results of this study are in line with the results of studies [13] and [14] which showed an increase in chlorophyll a, chlorophyll b and total chlorophyll in soil orchids (*Spathoglottis plicata*) and moon orchids (*Phalaenopsis amabilis*) along with an increase in FA.

Chlorophyll is an important part in plants, is located in chloroplasts in large numbers and can be easily extracted into lipid solvents such as acetone and ether [9]. Chlorophyll in plants has a role in the process of photosynthesis which can produce carbohydrates with the help of solar energy. Plants can have high chlorophyll content because environmental factors are in the right conditions [14].

4. Conclusion

Based on the results of the research that has been carried out, it can be concluded that the induced fusaric acid with 5 concentrations, namely 10 ppm, 20 ppm, 30 ppm and 40 ppm can affect the *Dendrobium* sp plantlets, which can increase the total phenol content, reducing sugar content and chlorophyll content of plantlets compared to control (0 ppm).

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflicts of interest

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