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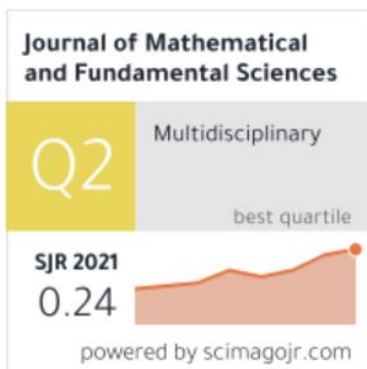
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249-260

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Antibacterial Activity of *Gyrinops versteegii* Fruit Extracts against *Staphylococcus aureus* and *Escherichia coli* and GC-MS Analysis

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Abstract. *Gyrinops versteegii* is widely used in the perfume, incense, and cosmetic industries as a raw material. Other potential uses of the plant are being explored, including in food and medicine. Therefore, this study carried out antibacterial assay and gas chromatography-mass spectroscopy of *G. versteegii* fruit extracts in three solvents with different polarities, i.e., *n*-hexane, dichloromethane, and methanol. The agar well diffusion method was then used to test the extracts against *Staphylococcus aureus* and *Escherichia coli*. The dichloromethane extract showed the most effective antibacterial activity against *S. aureus* at a concentration of 40%, with a zone of inhibition of 13.17 mm compared to *E. coli*, for which it was 7 mm. Furthermore, the bacterial species showed different responses to the extracts. Total and partial inhibition was shown by the *G. versteegii* fruit extracts against *S. aureus* and *E. coli*, respectively. Furthermore, GC-MS identified the following compounds in *G. versteegii* fruit extract: palmitic, oleic, and stearic acid, as well as bis-(2-ethylhexyl) phthalate, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, methyl octadec-9-enoate, squalene, and 2-monopalmitin derivatives. The results showed antibacterial potency of *G. versteegii* fruit extract against pathogenic bacteria. Additionally, the antibacterial activity of the extract was stronger against *S. aureus* compared to *E. coli*.

Keywords: agarwood; antibacterial; *Escherichia coli*; plant extract; *Staphylococcus aureus*.

1 Introduction

Antibiotics are commonly used to treat infectious diseases caused by microorganisms, but some types cause resistance [1,2,3]. Scientists have searched for new antibiotics for decades. Some of the efforts include modifying natural products (semisynthetic) [4] and exploring potential antimicrobe compounds of plant origin [5,6]. The tropical rainforest is an ecosystem that stores a lot of biodiversity, including potential plant sources of medicine to treat infectious diseases. One famous plant, *Gyrinops versteegii* (Gilg.) Domke, locally referred

to as agarwood, *gaharu*, or *ketimunan*, is endemic to Eastern Indonesia, especially the Lesser Sunda Islands [7]. In Lombok (West Nusa Tenggara), the flowering and fruiting season of wild *G. versteegii* is in November-December. However, cultivated types can flower and bear fruit twice in May, June-August, and November-December. Generally, the use of this plant is limited to resin, but the local people have begun to process *gaharu* leaves into drinks.

Several studies have been reported on the phytochemical analysis and antibacterial assay of leaf, stem, tree, and resin extracts on *G. versteegii*. Essential oil and leaf extract of agarwood showed wide biological activities, such as antibacterial [8-12], antifungal [13,14], antioxidant [15,16], anticancer [17], and cytotoxic [18]. However, no studies on antibacterial bioactivity and chemical compound analysis from the fruits have been reported.

Gas chromatography-mass spectrometry (GC-MS) is a tool commonly used to selectively detect the chemical composition of plants [19]. It has been used for identification of the chemical compounds of *Aquilaria malaccensis* and *G. versteegii* [20] and the functional groups and phytochemical constituents of *Amomum nilgiricum* leaf and rhizome [21]; analysis of the chemical compounds of the medicinal plant of *Senna hirsuta* leaf extracts [22]; and identification of volatile components of *Astragalus* [23].

This study analyzed the ability of *G. versteegii* fruit extract as an antibacterial agent against *Escherichia coli* and *Staphylococcus aureus* and to identify the chemical compounds of the fruit. The results of this study are useful as information for future investigations regarding the development of natural compounds from local plants to treat infectious diseases.

2 Methods

2.1 Sample Collection

Ripe fruits of *G. versteegii* were collected from the cultivation gardens in Punia Mataram (-8.594036 S, 116.104023 E), West Lombok, Indonesia, as shown in Figure 1. The sample was shade dried for 7 days to reduce water content and prevent photolysis. The dried fruits were then blended into powder form.

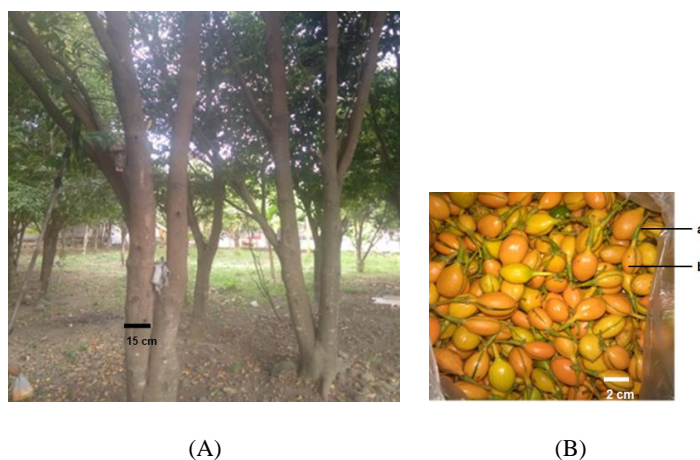


Figure 1 (A) *Gyrinops versteegii* in Punia Village, West Lombok with an average stem diameter of 15.5 cm. (B) Ripe fruits of *Gyrinops versteegii* collected from Punia Village (a: stalk and b: fruit).

2.2 Extraction and Dilution

The powder was macerated using *n*-hexane solvent for 24 h and filtered to separate the *n*-hexane filtrate and residue. Then, the filtrate was evaporated to obtain *n*-hexane extract. The same extraction procedure was applied for residue material using dichloromethane and methanol to afford dichloromethane and methanol extracts, respectively. Each extract was diluted to concentrations of 20%, 40%, 60%, and 80% using dimethyl sulfoxide (DMSO) [24].

2.3 Antibacterial Assay

Antibacterial assay of the fruit extracts was carried out using the agar well diffusion method against Gram-negative and Gram-positive bacteria, namely *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213, respectively. A total of 0.15 ml of bacterial suspension was spread on a nutrient agar plate (Himedia) and six wells with a diameter of 7 mm were made in each. Afterward, each well was filled with 100 μ l extract concentrations of 20%, 40%, 60%, and 80% (w/v), positive control (ciprofloxacin 0.03% (w/v)), and negative control (DMSO 99%). The plates were incubated at 37 °C and the zone of inhibition was observed and measured after 24 h. The zone of inhibition is the clear zone diameter around the well [5,24].

2.4 GC-MS Analysis

The *n*-hexane, dichloromethane, and methanol extracts were analyzed using GC-MS QP2010. The capillary column model was Rx-1 ms 100% dimethyl polysiloxane with a length, thickness, and diameter of 30 m, 0.25 μ m, and 0.25 mm, respectively. Afterward, the column oven and injection temperature were set at 40 °C and 260 °C, respectively. Sample injection was then carried out by splitting using helium gas, with a ratio of 51.0. The spectrum of the known components stored in the library of Wiley 7 software was used as a comparison for the spectrum detected in GC-MS QP2010.

2.5 Data Analysis

The experimental data of the plant extracts and concentrations were obtained by calculating the mean value of three replicates. The differences between the treatment groups were determined using F-test analysis of variance (IBM SPSS Statistics ver. 23) at a 5% level of significance ($p < 0.05$).

3 Results and Discussion

The fruit extracts of *G. versteegii* had a deep orange color compared to the RGB color chart, and the aroma was similar to tea extract, as shown in Figure 2. The residual mass obtained from 200 g dry fruit for the *n*-hexane, dichloromethane, and methanol extracts was 3.01%, 3.30%, and 7.93%, respectively.

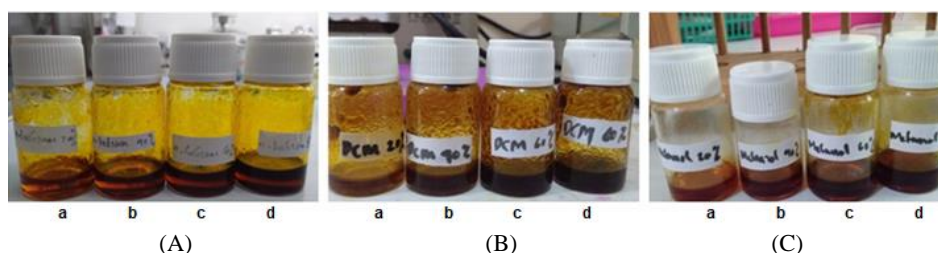


Figure 2 *Gyrinops versteegii* fruit extracts: (A) *n*-hexane extract, (B) dichloromethane extract, and (C) methanol extract at various concentrations: 20% (a), 40% (b), 60% (c), and 80% (d).

The *n*-hexane, dichloromethane, and methanol extracts, along with ciprofloxacin (positive control) and DMSO (negative control), were tested in vitro against *E. coli* and *S. aureus*. The different types of extract and concentration levels showed significant differences in the zone of inhibition, as shown in Table 1. The dichloromethane extract was the most effective at 40% compared to all concentrations of *n*-hexane, dichloromethane, and methanol. Furthermore, the dichloromethane extract *E. coli* inhibited the growth of *S. aureus* with a 13.17 mm and 7 mm zone of

inhibition, respectively. DMSO at 99% did not show inhibition against *S. aureus* and *E. coli*, while ciprofloxacin showed a 30 mm inhibition zone against *S. aureus* and *E. coli*, as shown in Figure 3.

Table 1 Mean and standard deviation of the zone of inhibitions of *n*-hexane, dichloromethane, and methanol of *Gyrinops versteegii* fruit extracts against *Staphylococcus aureus* and *Escherichia coli*.

Extracts (%) and control	Zone of inhibition (mm)	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
<i>n</i> -hexane (20%)	4 ± 0.0	4 ± 0.3
<i>n</i> -hexane (40%)	5 ± 0.0	5 ± 0.0
<i>n</i> -hexane (60%)	5 ± 0.0	5 ± 0.0
<i>n</i> -hexane (80%)	5 ± 0.0	5 ± 0.0
Dichloromethane (20%)	12 ± 0.0	8 ± 0.0
Dichloromethane (40%)	13.7 ± 0.29	7 ± 0.0
Dichloromethane (60%)	13 ± 0.0	6 ± 0.0
Dichloromethane (80%)	12.33 ± 0.1	3 ± 0.0
Methanol (20%)	9.83 ± 0.06	7 ± 0.0
Methanol (40%)	10 ± 0.0	8 ± 0.0
Methanol (60%)	10 ± 0.0	8 ± 0.0
Methanol (80%)	10 ± 0.0	8 ± 0.0
Ciprofloxacin (Control +)	30 ± 0.66	30 ± 0.5
DMSO (Control -)	0 ± 0.0	0 ± 0.0

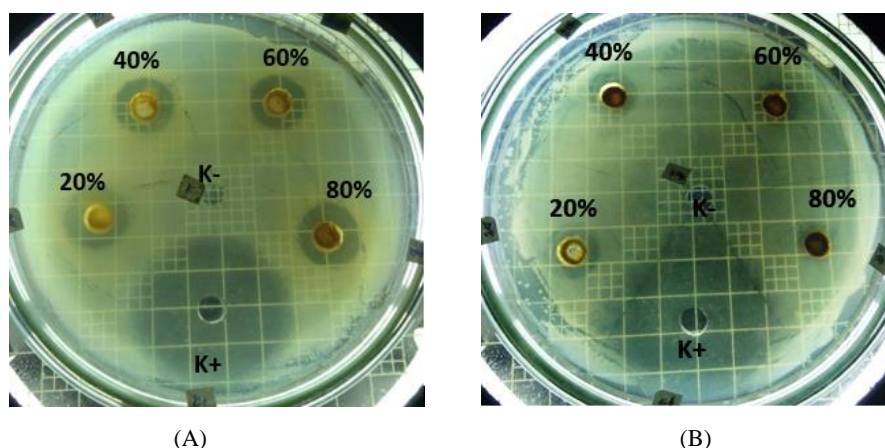


Figure 3 Zone of inhibition of dichloromethane extract of *Gyrinops versteegii* fruit for different concentrations against (A) *Staphylococcus aureus* and (B) *Escherichia coli*.

The chemical profile of *n*-hexane of *G. versteegii* fruit extract confirmed the presence of two components with different retention time values, as shown in Figure 4 and Table 2. The chemical profile of the dichloromethane extract was relatively different from that of the *n*-hexane extract, as shown in Figure 5. Furthermore, the GC-MS spectrum of the dichloromethane extract confirmed the

presence of palmitic, oleic, and stearic acid, as well as bis-(2-ethylhexyl) phthalates and squalene, as shown in Table 3. The chemical composition of the methanol extract (Figure 6) was also slightly different from that of the *n*-hexane and dichloromethane extracts. There were three fatty acids, namely oleic, palmitic, and stearic, as well as other unidentified compounds in the methanol extract, as shown in Table 4.

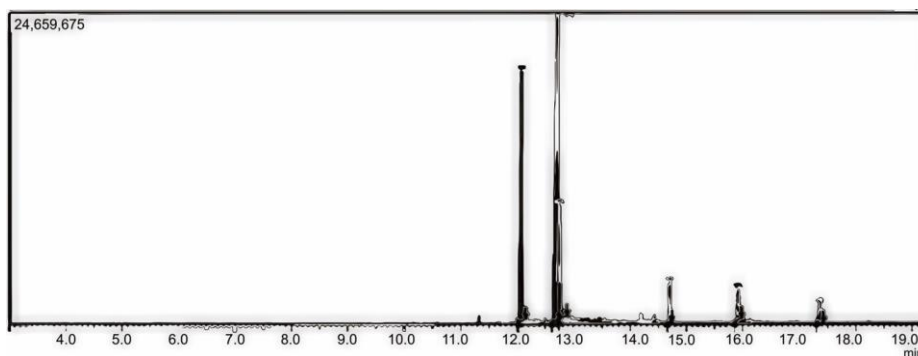


Figure 4 Typical GC-MS spectrum of *n*-hexane of *Gyrinops versteegii* fruit extract.

Table 2 Chemical components of *n*-hexane of *Gyrinops versteegii* fruit extract.

Peak	RT (min)	Peak Area (%)	SI (%)	MW	Molecular Formula	Name of Compounds
1	12.055	33.15	95	256	C ₁₆ H ₃₂ O ₂	Palmitic acid
2	12.680	66.85	96	282	C ₁₈ H ₃₄ O ₂	Oleic acid

RT = retention time, SI = similarity index, MW = molecular weight

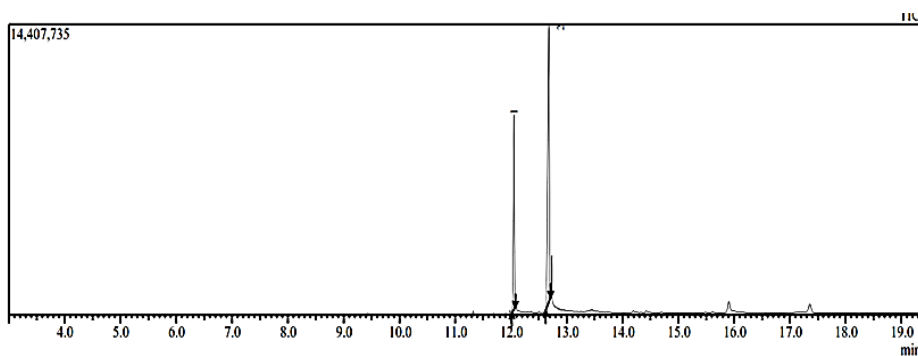
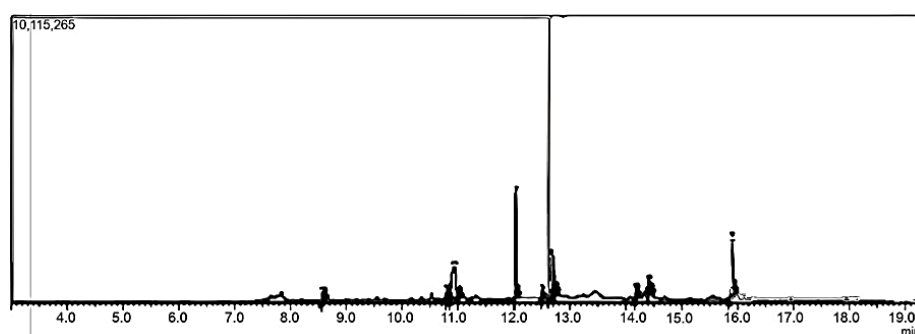


Figure 5 Typical GC-MS spectrum of dichloromethane of *Gyrinops versteegii* fruit extract.

Table 3 Chemical composition in dichloromethane of *Gyrinops versteegii* fruit extract.

Peak	RT (min)	Peak Area (%)	SI (%)	MW	Molecular Formula	Name of Compounds
1	12.080	27.12	94	256	C ₁₆ H ₃₂ O ₂	Palmitic acid
2	12.720	53.98	96	282	C ₁₈ H ₃₄ O ₂	Oleic acid
3	12.760	8.44	94	284	C ₁₈ H ₃₆ O ₂	Stearic acid
4	14.705	3.59	96	390	C ₂₄ H ₃₈ O ₄	Bis-(2-ethylhexyl) phthalates
5	15.920	4.02	88	356	C ₂₁ H ₄₀ O ₄	Not identified
6	17.365	2.85	94	410	C ₃₀ H ₅₀	Squalene derivative

RT = retention time, SI = similarity index, MW = molecular weight

**Figure 6** Typical GC-MS spectrum of methanol of *Gyrinops versteegii* fruit extract.**Table 4** Chemical composition in methanol of *Gyrinops versteegii* fruit extract.

Peak	RT (min)	Peak Area (%)	SI (%)	MW	Molecular Formula	Name of Compounds
1	8.580	1.22	96	144	C ₆ H ₈ O ₄	2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one
2	10.810	1.76	96	194	C ₇ H ₁₄ O ₆	Not identified
3	10.935	17.15	89	192	C ₇ H ₁₂ O ₆	Not identified
4	12.040	11.46	95	256	C ₁₆ H ₃₂ O ₂	Palmitic acid
5	12.500	1.12	96	296	C ₁₉ H ₃₆ O ₂	Methyl octadec-9-enoate
6	12.665	44.99	96	282	C ₁₈ H ₃₄ O ₂	Oleic acid
7	12.720	5.27	95	284	C ₁₈ H ₃₆ O ₂	Stearic acid
8	14.193	1.62	88	621	C ₃₉ H ₇₂ O ₅	Not identified
9	14.423	1.51	89	330	C ₁₉ H ₃₈ O ₄	2-monopalmitin derivative
10	15.912	13.89	88	356	C ₂₁ H ₄₀ O ₄	Not identified

RT = retention time, SI = similarity index, MW = molecular weight

All concentrations of the *n*-hexane, dichloromethane, and methanol extracts showed antibacterial activity. These extracts were more effective in inhibiting *S. aureus* than *E. coli*, as shown in Table 1. Furthermore, the *G. versteegii* fruit extracts showed a total zone of inhibition against *S. aureus*, while *E. coli* was partial. Total inhibition occurs when a clear zone forms around the well, while partial inhibition occurs when there is still growth of several bacterial colonies, as shown in Figure 3. Previous studies have shown that the essential oil of *Aquilaria sinensis* is better against *S. aureus* than *E. coli* [8,25]. Meanwhile, the differences in the cell wall and membrane structure in *S. aureus* and *E. coli* can cause different responses to antimicrobial agents. Gram-negative bacteria have more complex cell wall structures than Gram-positive ones [26]. A previous study on the effect of aqueous extract of *Aquilaria crassna* leaf on Gram-positive *S. epidermidis* showed inhibition of exopolysaccharides production, cell swelling, and distorting after 24 h of incubation [9]. The same can occur in *S. aureus* treated with fruit extracts of *G. versteegii*.

The GC-MS results of *G. versteegii* fruit extracts were dominated by fatty acid compounds such as palmitic, oleic, and stearic acid. Two other chemical compounds were detected in all extracts, namely palmitic and oleic acid. These compounds belong to the fatty acid class, with palmitic being a saturated fatty acid, while oleic is unsaturated. Palmitic and oleic acids are believed to influence the antibacterial activity of *G. versteegii* fruit extracts against *S. aureus* and *E. coli*. This suspicion was corroborated by the report of other studies that palmitic and oleic acid extracted from marine algae could inhibit the growth of Gram-positive bacteria, such as *S. aureus*, *Bacillus cereus*, *Bacillus subtilis*, as well as Gram-negative bacteria, such as *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Furthermore, the combination of palmitic and oleic acid, at a ratio of 1:1, showed better antibacterial activity against all tested bacteria compared to palmitic acid alone [27]. Fatty acid has a variety of bioactivities, such as antibacterial activities, and it is suspected to act by increasing plasma membrane permeability, disrupting the electron transport chain, and uncoupling oxidative phosphorylation. Its activity also inhibits membrane enzymatic and nutrient uptake [28].

The dichloromethane extracts showed a stronger effect on inhibiting the growth of the tested bacteria, especially against *S. aureus*, as shown in Table 1. The percentage of palmitic and oleic acids in the dichloromethane extract was lower compared to the *n*-hexane extract. However, the dichloromethane extract was the only source of squalene derivatives, at 2.85%. In the study of *Gyrinops walla*, squalene was found in the dichloromethane of the stem but not in the leaves [29]. Squalene is a linear triterpene widely found in fungi, algae, plants, animals, and microorganisms [30] and is widely used in disease treatment and therapy [31]. Squalene activities have been detected, such as antibacterial and antifungal

activities [32], and its derived nanoemulsions, such as squalene at cetylpyridinium chloride, are more effective against Gram-positive than Gram-negative bacteria and fungi. As studied in methicillin-resistant *S. aureus* (MRSA), the mechanism of action is to damage the cell membrane, leading to leakage of the DNA and cytoplasmic proteins [33]. The more varied content of antibacterial compounds is thought to cause the dichloromethane extracts to be more resilient against the tested bacteria.

4 Conclusion

Fruit extract of *Gyrinops versteegii* showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The dichloromethane extract at 40% concentration was the most effective in inhibiting the growth of *Staphylococcus aureus*, with an inhibition zone of 13.17 mm. Furthermore, GC-MS analyses of *Gyrinops versteegii* fruit extracts identified oleic, palmitic, and stearic acid, as well as bis-(2-ethylhexyl) phthalate, squalene derivative, 2-monopalmitin derivative, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, and methyl octadec-9-enoate.

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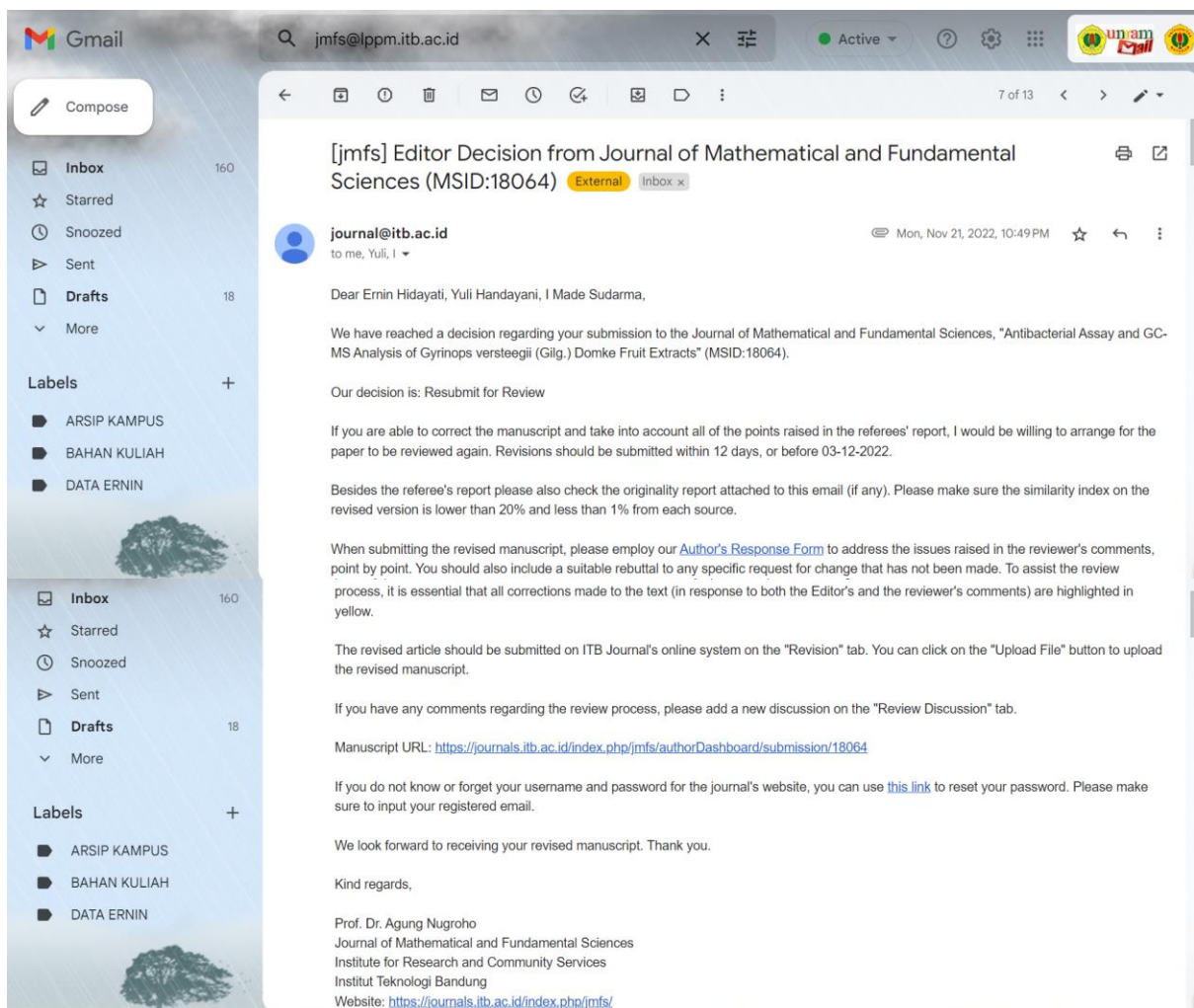
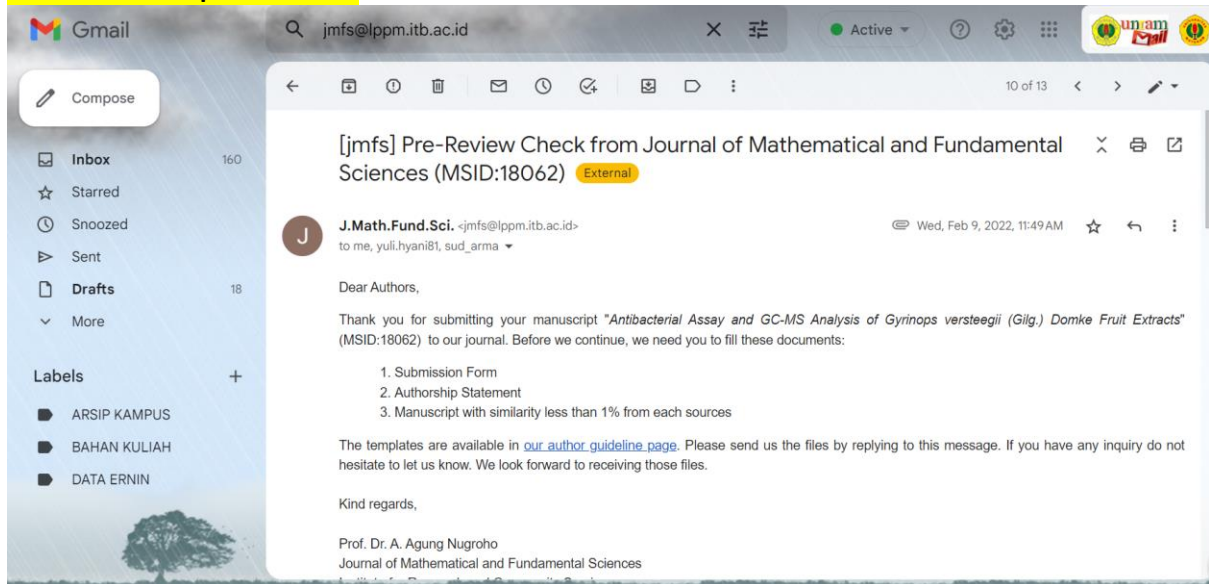
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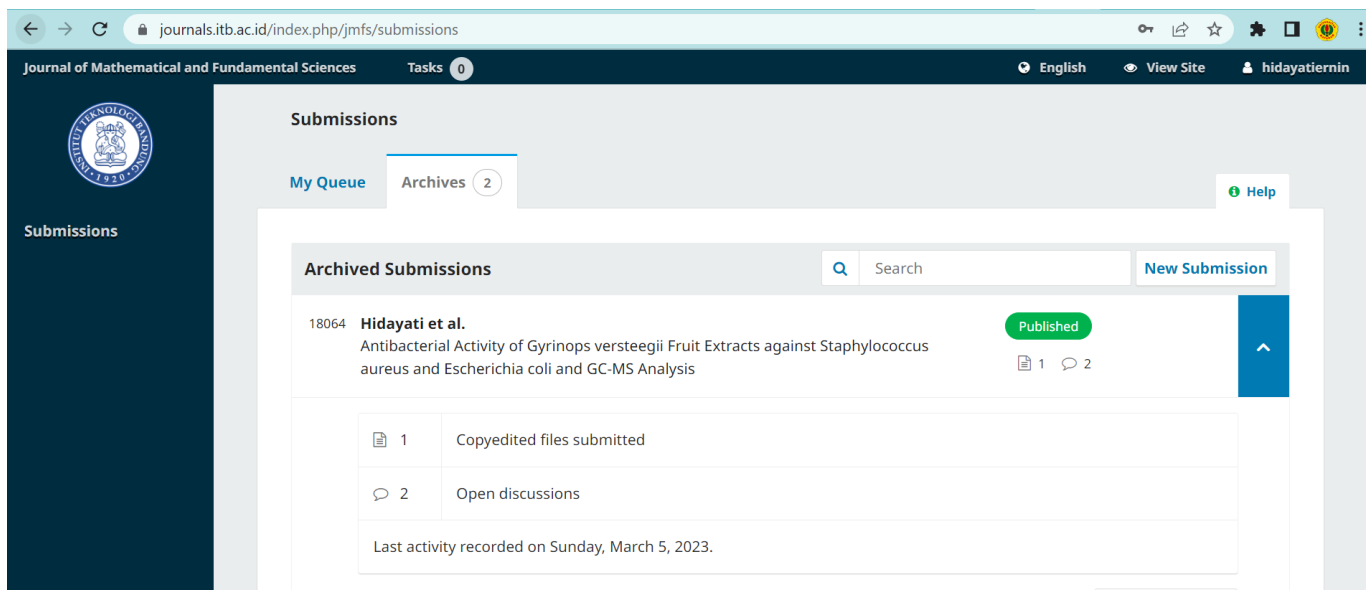
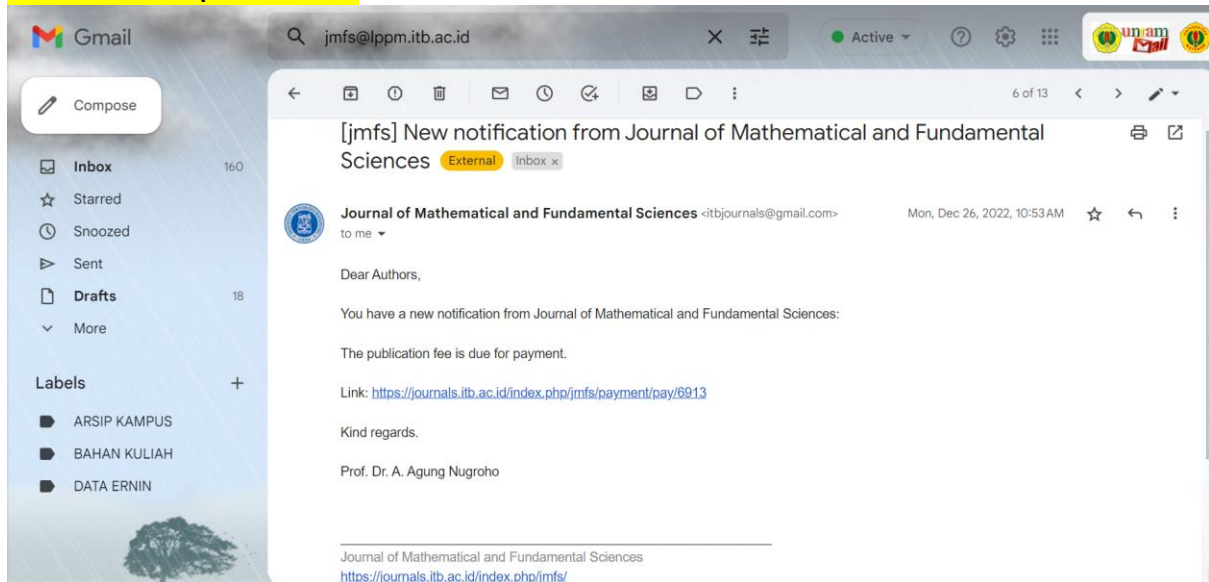
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