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MICROENCAPSULATION OF *MUNTINGIA CALABURA* LEAF KOMBUCHA: EFFECTS ON MICROBIAL VIABILITY, ANTIBACTERIAL ACTIVITY, AND ANTIBIOTIC INTERACTIONS

Marsudi[1], Sari Rahayu Rahman[2], Jisril Palayukan[1], Deka Uli Fahrodi[1], Nur Saidah Said[1]

[1] Department of Animal Husbandry, Faculty of Animal Husbandry and Fisheries, Universitas Sulawesi Barat, 91412, Majene, Indonesia
[2] Department of Biology Education, Faculty of Teacher Training and Education, Universitas Sulawesi Barat, 91412, Majene, Indonesia

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Abstract

Kombucha is a fermented beverage rich in organic acids and bioactive compounds with potential antimicrobial properties; however, its microbial stability remains a challenge. This study evaluated the effects of microencapsulation on microbial viability, antibacterial activity, and antibiotic interactions in *Muntingia calabura* leaf kombucha. Microencapsulation was performed using maltodextrin, skim milk, and gum arab, followed by freeze-drying. Microbial viability was assessed using plate counts, antibacterial activity was evaluated by agar diffusion against *Escherichia coli* and *Staphylococcus aureus*, and antibiotic interaction was examined using a modified disc diffusion method. The microencapsulated samples exhibited higher recoverable viable counts upon reconstitution (6.27×10^7 CFU/mL; log 7.79 ± 0.08) compared with liquid kombucha (3.19×10^7 CFU/mL; log 7.50 ± 0.04; $p < 0.05$), which is primarily attributed to concentration effects and the protective properties of the encapsulating matrix. Antibacterial activity was generally preserved after microencapsulation, with comparable inhibition zones observed for both test organisms, although minor variations occurred across concentrations. Furthermore, the presence of kombucha influenced the inhibition zones of selected antibiotics, indicating potential modulatory interactions; however, these findings should be interpreted cautiously due to methodological constraints and the use of complex mixtures. In conclusion, microencapsulation can preserve microbial viability and antibacterial activity of *M. calabura* kombucha, supporting its potential application in functional food development. Nevertheless, further studies on microbial characterization, storage stability, and gastrointestinal simulation are required to fully elucidate its functional efficacy.

Keywords: antibacterial activity, kombucha, microencapsulation, *Muntingia calabura*, probiotic

INTRODUCTION

Kombucha, a traditional fermented beverage, is produced through a symbiotic culture of bacteria and yeast (SCOBY), which converts substrate components into organic acids, polyphenols, and other bioactive metabolites with well-documented antioxidant and antimicrobial properties (Fraiz *et al.*, 2025; Onsun *et al.*, 2025). Fermentation conditions have been reported to significantly influence the physicochemical and microbiological characteristics of kombucha, including acidity,

metabolite composition, and microbial dynamics (Neffe-Skocińska *et al*., 2017). In recent years, interest in kombucha has surged, not only as a functional beverage but also as a potential source of probiotics, driven by consumer demand for natural health-promoting products. Furthermore, fermentation substrates, including leaves and fruit extracts beyond *Camellia* species, can significantly influence the bioactive profile and functional properties of the final beverage (Nuryanti, 2022; Zubaidah *et al.*, 2022). Nevertheless, practical applications of kombucha are constrained by two main issues: variability in microbial composition and metabolite profiles, and limited stability and survivability of beneficial microbes and labile bioactives during storage or gastrointestinal passage (Onsun *et al*., 2025; D'Amico *et al*., 2025).

Microencapsulation has emerged as a promising strategy to overcome these limitations by entrapping live microbes or bioactive compounds within protective matrices such as polysaccharides, proteins, or lipids, thereby enhancing survival under adverse conditions and enabling controlled release (D'Amico *et al*., 2025; Budimac *et al*., 2025). Recent studies indicate that microencapsulation can preserve probiotic viability and bioactivity during processing and storage, and may contribute to improved functional performance under certain conditions (Rode *et al*., 2025). In parallel, probiotics and their derivatives have gained increasing attention as potential alternatives to antibiotics, particularly in the context of antimicrobial resistance (Ali *et al*., 2023; Leistikow *et al*., 2022). However, concerns have also been raised regarding the potential role of microbial products in the dissemination of antimicrobial resistance genes within microbial ecosystems (Tóth *et al*., 2021; Radovanovic *et al*., 2023). Despite these advances, most studies have focused on single probiotic strains or simplified food systems, rather than complex fermented beverages such as Muntingia calabura leaf kombucha. Consequently, there remains a gap in understanding the combined effects of microencapsulation on microbial viability, antibacterial activity, and antibiotic-related interactions (Nuryanti, 2022; Fraiz *et al*., 2025).

Muntingia calabura leaves are rich in phenolic compounds and flavonoids that may enhance the functional profile of kombucha. Preliminary studies have demonstrated the antimicrobial potential of kombucha produced from *M. calabura* leaves (Nuryanti, 2022). Nevertheless, it remains unclear whether microencapsulation can (i) preserve microbial viability following processing, (ii) maintain antibacterial effectiveness against representative pathogens such as *Escherichia coli* and *Staphylococcus aureus*, and (iii) influence interactions with antibiotics in a manner relevant to the evaluation of kombucha as a functional antimicrobial adjunct. Addressing these questions is important for the development of stable and effective kombucha-based products. Accordingly, this study evaluates the effects of microencapsulation on the viability, antibacterial activity, and antibiotic interaction profiles of *M. calabura* leaf kombucha using microbial enumeration, agar diffusion assays, and antibiotic interaction testing.

MATERIALS AND METHODS

Sample Preparation

Kombucha was prepared using *Muntingia calabura* leaves following standard fermentation procedures (Nuryanti, 2022). The encapsulating matrix consisted of maltodextrin (15%, w/v), skim milk (7.5%, w/v), and gum arab (3%, w/v), prepared based on modified protocols from previous studies. These concentrations were selected to achieve a balance between encapsulation efficiency and solution viscosity. The kombucha was homogenized with the encapsulating agents and subsequently subjected to freeze-drying at -50 °C under a vacuum pressure of approximately 0.1 mbar for 48 hours using a laboratory freeze dryer (Labconco, USA) (Budimac *et al*., 2025; D'Amico *et al*., 2025). The resulting microcapsules were stored at 4 °C until further analysis.

Viability Assessment

Microbial viability was quantified using plate count methods on De Man, Rogosa and Sharpe (MRS) agar (Merck, Germany). Serial dilutions were prepared, and aliquots (100 µL) were spread onto agar plates. Plates were incubated under anaerobic conditions using an anaerobic jar system at 37 °C for 48 hours. Colony counts were expressed as CFU/mL and Log_{10} (CFU/mL), calculated from triplicate measurements (D'Amico *et al*., 2025).

Antibacterial Activity

Antibacterial activity was evaluated using the disc diffusion method. Test organisms included *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538. Bacterial suspensions were prepared and adjusted to 0.5 McFarland standard (approximately 1×10^8 CFU/mL). Sterile Mueller–Hinton agar (MHA; Merck, Germany) plates were prepared by pouring 15 mL of medium into Petri dishes and allowed to solidify. A sterile cotton swab was immersed in each bacterial suspension and uniformly spread over the entire agar surface to obtain a confluent lawn. Sterile paper discs were impregnated with kombucha samples at concentrations of 1%, 5%, 10%, 15%, and 20%, as well as distilled water (negative control) and amoxicillin 25 µg/disc (positive control), and then placed onto the inoculated agar surface. Each sterile disc was impregnated with 20 µL of sample solution and allowed to dry briefly under sterile conditions prior to placement. All treatments were performed in triplicate. Plates were incubated under aerobic conditions at 37 °C for 24 hours.

Inhibition zones were measured in millimetres using a digital caliper, and the diameter included the disc (Rode *et al*., 2025).

Antibiotic Interaction Assay

The interaction between kombucha samples and antibiotics was evaluated using a modified disc diffusion method based on CLSI guidelines. Antibiotic discs (Oxoid Ltd., UK) included ciprofloxacin (5 µg), colistin (10 µg), amoxicillin (25 µg), streptomycin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), ampicillin (10 µg), and gentamicin (10 µg). A volume of 50 µL of kombucha sample was applied to the agar surface prior to placement of antibiotic discs to assess potential modulatory effects. Plates were incubated at 37 °C for 24 hours under aerobic conditions. The interpretation of inhibition zones as resistant (R), intermediate (I), or sensitive (S) followed CLSI (2023) guidelines; however, these classifications were used cautiously due to the involvement of complex mixtures rather than pure antibiotics (Onsun *et al*., 2025).

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to evaluate differences between concentrations and between sample types. A significance level of $p < 0.05$ was applied.

RESULTS AND DISCUSSION

Viability Assessment

The viability of microorganisms in liquid and microencapsulated *Muntingia calabura* leaf kombucha is presented in Tab. I. The results indicate that the microencapsulated samples showed higher viable counts (6.27×10^7 CFU/mL; 7.79 ± 0.08 log CFU/mL) compared to the liquid form (3.19×10^7 CFU/mL; 7.50 ± 0.04 log CFU/mL), with the difference being statistically significant ($p < 0.05$).

This apparent increase, however, should be interpreted cautiously. The higher counts observed in the microencapsulated samples are most likely associated with a concentration effect resulting from water removal during the freeze-drying process, rather than a genuine increase in microbial cell numbers. Similar observations have been reported in previous studies, where increased CFU values after drying were attributed to cell concentration rather than microbial proliferation (de Lima *et al*., 2024; Phan Van *et al*., 2024). As the liquid kombucha is converted into powder, microbial cells become more densely distributed, which can lead to elevated CFU values upon reconstitution. The comparison between liquid and microencapsulated samples was performed after reconstitution of the powder form; therefore, the observed differences in CFU values primarily reflect concentration effects and recovery efficiency rather than direct equivalence of initial microbial biomass. Thus, the observed difference should be interpreted as a methodological artifact rather than a biological enhancement.

In addition, the encapsulating agents used in this study, namely maltodextrin, skim milk, and gum arab, may have contributed to improved cell survival during processing. These materials are widely recognized for their protective properties, particularly in reducing cellular damage caused by dehydration and osmotic stress (Sbehat *et al*., 2022; Vivek *et al*., 2023). The enhanced stability of encapsulated cells under stress conditions has also been associated with the use of protein polysaccharide matrices, which improve resistance to dehydration and environmental stress (Song *et al*., 2024). Previous studies have also demonstrated that microencapsulation can enhance microbial stability under adverse conditions by forming a protective barrier around the cells (Liu *et al*., 2023). Therefore, the observed results are better understood as reflecting enhanced preservation and recovery of viable cells, rather than an actual increase in microbial growth. It is also important to note that kombucha represents a complex microbial consortium consisting of bacteria and yeasts (Jayabalan *et al*., 2014; Villarreal-Soto *et al*., 2018). In the present study, viability was assessed using MRS agar, which predominantly supports the growth of lactic acid bacteria. Consequently, the results presented here reflect only a portion of the microbial community and do not capture the full diversity of viable microorganisms present in the system.

A further limitation of this study is the absence of standardization based on dry matter or initial biomass when comparing liquid and microencapsulated samples. Future work should consider expressing viability on a dry-weight basis or applying mass balance approaches to enable more accurate biological comparisons.

I: *Viability of microorganisms in Muntingia calabura leaf kombucha and its microencapsulated form*

Sample	Viable count (CFU/mL)	Log_{10}(CFU/mL)
Muntingia calabura leaf kombucha	$(3.19 \pm 0.29) \times 10^7$	7.50 ± 0.04^a
Microencapsulated *Muntingia calabura* leaf kombucha	$(6.27 \pm 1.20) \times 10^7$	7.79 ± 0.08^b

Data are presented as mean ± standard deviation (n = 3). Different superscript letters indicate significant differences between samples ($p < 0.05$, independent t-test).

Antibacterial Activity

The antibacterial activity of *Muntingia calabura* leaf kombucha and its microencapsulated form against *Escherichia coli* and *Staphylococcus aureus* is presented in Tab. II. Overall, both samples exhibited measurable antibacterial effects, as indicated by the formation of inhibition zones across all tested concentrations. The inhibition zones generally increased with increasing concentration, suggesting a dose-dependent antibacterial effect. For *E. coli*, the inhibition zones of the liquid kombucha ranged from 5.83 to 14.17 mm, while the microencapsulated form ranged from 6.17 to 13.50 mm. A similar trend was observed for S. aureus, where inhibition zones ranged from 5.50 to 13.83 mm for the liquid form and from 5.83 to 13.67 mm for the microencapsulated samples. These results indicate that microencapsulation did not consistently enhance antibacterial activity but was able to maintain comparable inhibitory effects relative to the liquid form.

Data are presented as mean ± standard deviation (n = 3). Each sterile disc was impregnated with 20 µL of sample at the indicated concentration. Different superscript letters within the same bacterial group indicate significant differences ($p < 0.05$).

The antibacterial activity observed in this study is likely associated with the presence of organic acids, phenolic compounds, and other bioactive metabolites produced during kombucha fermentation (Jayabalan *et al*., 2014; Villarreal-Soto *et al*., 2018). These compounds have been widely reported to inhibit the growth of both Gram-negative and Gram-positive bacteria by disrupting cell membranes and interfering with metabolic processes.

Microencapsulation may contribute to the preservation of these bioactive compounds by providing a protective matrix that reduces degradation during processing (Sbehat *et al*., 2022; Vivek *et al*., 2023). However, the results of the present study suggest that such protection did not necessarily translate into increased antibacterial activity, but rather helped retain the functional properties of the kombucha. The slight reduction in antibacterial activity against *E. coli* observed at certain concentrations may be attributed to diffusion limitations of encapsulated bioactive

II: *Antibacterial activity of Muntingia calabura leaf kombucha and its microencapsulated form at different concentrations (disc diffusion method)*

Bacteria	Sample	Concentration (%)	Inhibition zone (mm) Mean ± SD
E. coli	*Muntingia calabura* leaf kombucha	1	5.83 ± 0.76[a]
		5	6.50 ± 0.50[a]
		10	6.83 ± 0.29[a]
		15	11.17 ± 0.29[b]
		20	14.17 ± 0.29[c]
	Microencapsulated *Muntingia calabura* leaf kombucha	1	6.17 ± 0.29[a]
		5	7.33 ± 0.29[a]
		10	9.17 ± 0.29[b]
		15	11.83 ± 0.76[c]
		20	13.50 ± 0.50[c]
	Positive control (Amoxicillin 25 µg/disc)	-	20.17 ± 0.58[d]
	Negative control (Distilled water)	-	0.00 ± 0.00[e]
S. aureus	*Muntingia calabura* leaf kombucha	1	5.50 ± 0.87[a]
		5	6.17 ± 0.29[a]
		10	6.50 ± 0.29[a]
		15	11.50 ± 0.50[b]
		20	13.83 ± 0.29[c]
	Microencapsulated *Muntingia calabura* leaf kombucha	1	5.83 ± 0.29[a]
		5	7.17 ± 0.29[a]
		10	9.00 ± 0.00[b]
		15	12.17 ± 0.29[c]
		20	13.67 ± 0.58[c]
	Positive control (Amoxicillin 25 µg/disc)	-	20.83 ± 0.29[d]
	Negative control (Distilled water)	-	0.00 ± 0.00[e]

Data are presented as mean ± standard deviation (n = 3). Each sterile disc was impregnated with 20 µL of sample at the indicated concentration. Different superscript letters within the same bacterial group indicate significant differences ($p < 0.05$).

compounds within the agar matrix, which can reduce the apparent inhibition zone despite the presence of active metabolites. It should be noted that the antibacterial activity was evaluated using the disc diffusion method under standardized loading conditions, where equal sample volumes were applied to each disc. Therefore, the observed differences reflect variations in concentration-dependent activity rather than differences in sample volume.

Antibiotic Interaction Assay

The interaction between *Muntingia calabura* leaf kombucha and selected antibiotics is presented in Tab. III. Variations in inhibition zone diameters were observed when antibiotics were applied in the presence of kombucha samples, suggesting potential modulatory interactions with antibiotic activity.

However, these observations should be interpreted with caution. The classification of inhibition zones into categories such as sensitive or resistant is based on standardized guidelines developed for pure antibiotics and may not be directly applicable to complex mixtures such as kombucha. Therefore, the results in this study are intended for comparative purposes only and do not represent clinical antibiotic susceptibility. The observed variations may be associated with interactions between bioactive compounds present in kombucha, such as organic acids, phenolics, and

III: *Effect of Muntingia calabura leaf kombucha and its microencapsulated form on antibiotic interactions*

Sample	Antibiotics	Concentration (%)	Mean ± SD (mm)	Interpretation
Muntingia calabura leaf kombucha	Ciprofloxacin 5 µg	1	5.7 ± 0.6	Resistant
		5	6.3 ± 0.6	Resistant
		10	7.3 ± 0.6	Resistant
		15	10.0 ± 1.0	Resistant
		20	11.7 ± 0.6	Intermediate
	Colistin 10 µg	1	4.3 ± 0.6	Resistant
		5	5.3 ± 0.6	Resistant
		10	7.3 ± 0.6	Resistant
		15	7.7 ± 0.6	Resistant
		20	8.3 ± 0.6	Resistant
	Amoxicillin 25 µg	1	6.7 ± 0.6	Resistant
		5	8.3 ± 0.6	Resistant
		10	9.7 ± 0.6	Resistant
		15	10.3 ± 0.6	Resistant
		20	10.7 ± 0.6	Intermediate
	Streptomycin 10 µg	1	10.3 ± 0.6	Intermediate
		5	11.7 ± 0.6	Intermediate
		10	13.3 ± 0.6	Sensitive
		15	14.3 ± 0.6	Sensitive
		20	15.0 ± 0.0	Sensitive
	Chloramphenicol 30 µg	1	12.0 ± 1.0	Intermediate
		5	13.7 ± 0.6	Intermediate
		10	14.7 ± 0.6	Intermediate
		15	15.7 ± 0.6	Sensitive
		20	16.3 ± 0.6	Sensitive
	Tetracycline 30 µg	1	7.0 ± 0.0	Resistant
		5	8.3 ± 0.6	Resistant
		10	9.7 ± 0.6	Resistant
		15	10.7 ± 0.6	Intermediate
		20	11.3 ± 0.6	Intermediate
	Ampicillin 10 µg	1	6.3 ± 0.6	Resistant
		5	7.0 ± 0.0	Resistant
		10	8,7 ± 0.6	Resistant
		15	9.7 ± 0.6	Intermediate
		20	10.3 ± 0.6	Intermediate

Sample	Antibiotics	Concentration (%)	Mean ± SD (mm)	Interpretation
Muntingia calabura leaf kombucha	Gentamicin 10 µg	1	12.3 ± 0.6	Intermediate
		5	13.0 ± 0.0	Intermediate
		10	13.3 ± 0.6	Intermediate
		15	14.7 ± 0.6	Sensitive
		20	15.0 ± 0.0	Sensitive
Microencapsulated *Muntingia calabura* leaf kombucha	Ciprofloxacin 5 µg	1	5.3 ± 0.6	Resistant
		5	6.7 ± 0.6	Resistant
		10	8.0 ± 0.0	Resistant
		15	9.7 ± 0.6	Resistant
		20	11.3 ± 0.6	Intermediate
	Colistin 10 µg	1	4.7 ± 0.6	Resistant
		5	5.7 ± 0.6	Resistant
		10	7.7 ± 0.6	Resistant
		15	8.3 ± 0.6	Resistant
		20	9.3 ± 0.6	Resistant
	Amoxicillin 25 µg	1	6.3 ± 0.6	Resistant
		5	8.7 ± 0.6	Resistant
		10	10.3 ± 0.6	Intermediate
		15	10.7 ± 0.6	Intermediate
		20	11.3 ± 0.6	Intermediate
	Streptomycin 10 µg	1	10.7 ± 0.6	Intermediate
		5	12.3 ± 0.6	Intermediate
		10	13.7 ± 0.6	Sensitive
		15	14.7 ± 0.6	Sensitive
		20	15.3 ± 0.6	Sensitive
	Chloramphenicol 30 µg	1	11.7 ± 0.6	Intermediate
		5	13.3 ± 0.6	Intermediate
		10	15.3 ± 0.6	Sensitive
		15	16.3 ± 0.6	Sensitive
		20	16.7 ± 0.6	Sensitive
	Tetracycline 30 µg	1	6.7 ± 0.6	Resistant
		5	8.7 ± 0.6	Resistant
		10	10.3 ± 0.0	Intermediate
		15	11.3 ± 0.6	Intermediate
		20	12.3 ± 0.6	Intermediate
	Ampicillin 10 µg	1	6.7 ± 0.6	Resistant
		5	7.7 ± 0.6	Resistant
		10	9.3 ± 0.6	Resistant
		15	10.3 ± 0.6	Intermediate
		20	11.7 ± 0.6	Intermediate
	Gentamicin 10 µg	1	11.7 ± 0.6	Intermediate
		5	12.7 ± 0.6	Intermediate
		10	14.3 ± 0.6	Sensitive
		15	15.3 ± 0.6	Sensitive
		20	16.5 ± 0.6	Sensitive

Data are presented as mean ± standard deviation (n = 3). Antibiotic concentrations are expressed as µg/disc. The interpretation (Resistant, Intermediate, Sensitive) was adapted from CLSI guidelines and used for comparative purposes only.

other metabolites and the antibiotics used in this study (Jayabalan *et al*., 2014; Villarreal-Soto *et al*., 2018). These compounds may influence bacterial cell permeability or metabolic activity, which could in turn affect the apparent efficacy of antibiotics.

In addition, microencapsulation may play a role in modulating these interactions by influencing the stability and release profile of bioactive compounds (Sbehat *et al*., 2022; Vivek *et al*., 2023). However, the results obtained in this study do not allow definitive conclusions regarding synergistic or antagonistic effects, as more specific methods such as checkerboard assays or fractional inhibitory concentration (FIC) analysis would be required. Overall, the findings suggest that kombucha may interact with antibiotics in a manner that affects observed inhibition zones, although further investigation is needed to clarify the underlying mechanisms and clinical relevance.

This study has several limitations that should be considered. In addition, this study did not differentiate between lactic acid bacteria, acetic acid bacteria, and yeasts present in the kombucha culture, which limits the interpretation of microbial viability and functional properties. Furthermore, storage stability and shelf-life evaluation were not assessed; therefore, the long-term protective effect of the encapsulation process cannot be confirmed under different storage conditions. The comparison between liquid and microencapsulated samples was performed after reconstitution, and thus the observed differences in CFU values primarily reflect concentration effects and recovery efficiency rather than direct equivalence of initial microbial biomass. Additionally, in vitro gastrointestinal simulation was not conducted, and the survival of encapsulated microorganisms under digestive conditions remains unclear. Future studies should incorporate detailed microbial characterization, long-term stability assessment, and gastrointestinal simulation to provide a more comprehensive evaluation of encapsulation efficacy.

CONCLUSION

This study demonstrates that microencapsulation of *Muntingia calabura* leaf kombucha using maltodextrin, skim milk, and gum arab can preserve microbial viability following freeze-drying, as reflected by the recovery of viable counts upon reconstitution. The higher recoverable counts observed in the microencapsulated samples were primarily attributed to concentration effects rather than a true biological enhancement. Both liquid and microencapsulated kombucha exhibited concentration-dependent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, with the encapsulated form maintaining comparable inhibitory effects. In addition, the interaction assay indicated that kombucha may influence the observed activity of selected antibiotics; however, these findings should be interpreted cautiously due to methodological limitations and the use of complex mixtures. Overall, microencapsulation represents a promising approach for preserving the functional properties of kombucha. Nevertheless, further studies involving detailed microbial characterization, storage stability, and gastrointestinal simulation are required to fully elucidate its functional efficacy and potential applications.

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Contact information

Marsudi: marsudi@unsulbar.ac.id, https://orcid.org/0009-0007-4636-7421
Sari Rahayu Rahman: sarirahayu@unsulbar.ac.id, https://orcid.org/0000-0002-7763-0591
Jisril Palayukan: jisril.palayukan@unsulbar.ac.id, https://orcid.org/0000-0003-3126-9195
Deka Uli Fahrodi: dekaulifahrodi@unsulbar.ac.id, https://orcid.org/0009-0004-0086-9504
Nur Saidah Said: nursaidah@unsulbar.ac.id, https://orcid.org/0009-0001-4637-5137