Jurnal Pembelajaran Dan Biologi Nukleus p-ISSN: 2442-9481
Vol 12 (3): 867- 887, September 2026 e-ISSN: 2685-7332

Antibacterial Activity of Ethanolic *Macaranga tanarius* Leaf Extract as Natural Lepat Binti Wrapper Against *Escherichia coli* Using Disk Diffusion

Putri Anisa(*)[1,3] , Henny Johan[1] , Aprina Defianti[1], Sipriyadi Sipriyadi[2], Arsela Eko Listiono[1]

[1]Department of Science Education, Faculty of Teacher Training and Education, Bengkulu University;
[2]Graduate School of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Bengkulu University;
[3]Undergraduate Student, Science Education Program, Faculty of Teacher Training and Education, Bengkulu University, Kandang Limun, Bengkulu 38112, Indonesia.

***Corresponding author:** nisaput010125@gmail.com

Submitted June 17 Th 2026, and Accepted August 15 Th 2026

Abstract

Background: *Traditional food products require wrapping materials that maintain quality and may reduce microbial contamination. Sapat leaves (Macaranga tanarius) are traditionally used as natural wrappers and contain antibacterial secondary metabolites. This study evaluated the antibacterial activity of 96% ethanolic extract of M. tanarius leaves against Escherichia coli and the organoleptic acceptability of fresh leaves as wrappers for lepat binti.* **Methodology:** *Extract concentrations of 5%, 25%, 50%, 75%, and 100% w/v (50–1000 mg/mL) were tested by disc diffusion using chloramphenicol and DMSO as positive and negative controls, with three replicates.* **Findings:** *Fresh M. tanarius leaves were compared with Musa paradisiaca L. leaves in a preliminary organoleptic test. Inhibition zones increased from 5.22±1.19 mm at 5% to 15.57±0.95 mm at 100%; the positive and negative controls produced 22.70±1.52 mm and 0.00±0.00 mm, respectively. Differences among concentrations were significant (Kruskal–Wallis, H= 13.06; p= 0.011), with a strong positive correlation between concentration and inhibition zone (Spearman, ρ = 0.961; p < 0.001). Phytochemical screening detected flavonoids, alkaloids, tannins, saponins, phenols, and steroids. Organoleptic acceptance for taste, color, aroma, and texture was 65.71%, 76.19%, 79.05%, and 67.62%, respectively, and remained acceptable for up to five days.* **Contributions:** *This study provides empirical evidence that M. tanarius leaves shows potential as a natural food wrapper with antibacterial activity against E. coli and acceptable organoleptic properties. Although further studies are needed to confirm whether extract activity reflects the effectiveness of fresh leaves during storage*

Keyword: *Antibacterial, Ethanol extract; M.tanarius leaves; E. coli, Natural wrapper*

https://doi.org/10.36987/jpbn.v12i3.9682

INTRODUCTION

Food safety is a critical component of public health, particularly for traditional food products that are often processed using simple methods and natural ingredients. Traditional foods are at risk of microbial contamination if their processing and storage are not properly managed. *Escherichia coli* was selected as the test organism because it serves as a relevant indicator of sanitation and fecal contamination and is commonly found as a food contaminant. Although normal *E. coli* can live as part of the human and animal gut flora, certain pathogenic strains can cause foodborne illnesses, such as diarrhea and hemolytic uremic syndrome (Bria et al., 2022).

Lepat binti is a traditional dish of the South Bengkulu community, served during condolence visits, tahlilan ceremonies, and communal prayers following a person's death. This dish is typically wrapped in natural materials, specifically banana leaves (Widiono et al., 2022). The use of leaves as wrappers not only serves to physically protect the food but also has the potential to provide natural antimicrobial effects that can help extend shelf life and ensure food safety (Thongphichai et al., 2023).

In addition to banana leaves, the people of South Bengkulu also use *M. tanarius* leaves to wrap foods such as rice and palm sugar. *M. tanarius* leaves are also commonly known as mara leaves, while the local community refers to them as sapat leaves. Besides serving as a wrapper, *M. tanarius* leaves are known to contain various secondary metabolites. A study by Musdalifah et al., (2017) reported the presence of secondary metabolites in *M. tanarius* leaves associated with antibacterial potential. This information supports further testing of *M. tanarius* leaf extracts and their application as food wrappers.

A similar study was previously conducted by Sari et al., (2015) on the antibacterial activity of *M. tanarius* leaves. However, information regarding the response of *E. coli* to varying concentrations of *M. tanarius* leaf ethanol extract using the disk diffusion method still requires further investigation. Therefore, this study was conducted to evaluate the antibacterial activity of *M. tanarius* leaf ethanol extract against *E. coli* at various concentration levels. The disk diffusion method was used to assess antibacterial activity based on the formation of inhibition zones around the disks, which were then measured to determine the extent of the extract's inhibitory effect on bacterial growth. This method was chosen because it is simple, practical, and commonly used in testing antibacterial activity (Intan et al., 2021).

Based on the above, *M. tanarius* has the potential to be utilized as a natural wrapper because it contains bioactive compounds with antibacterial activity. Although the antibacterial activity of *M. tanarius* extracts has been studied previously, information regarding the direct use of its fresh leaves as a food wrapper particularly in maintaining the sensory quality and acceptability of lepat binti during storage remains limited. In this study, an ethanol extract of *M. tanarius* leaves was specifically used to test antibacterial activity against *E. coli* at various concentrations, while fresh leaves were used directly as a wrapper for lepat binti without being applied as a coating. This study aims to analyze the relationship between extract concentration and the inhibition of *E. coli* growth, identify its secondary metabolite content, and evaluate changes in the organoleptic characteristics and acceptance level of lepat binti wrapped

in *M. tanarius* leaves during storage. The results of this study are expected to provide preliminary evidence regarding the potential of *M. tanarius* as an alternative natural wrapper for traditional foods.

METHODS

This study employed an experimental approach that included testing the antibacterial activity of *M. tanarius* leaf ethanol extract, phytochemical screening, and organoleptic evaluation of lepat binti. *M. tanarius* leaf extract was obtained through extraction using 96% ethanol; its antibacterial activity against *E. coli* was then tested using the disk diffusion method on Nutrient Agar (NA) medium. Chloramphenicol was used as a positive control, while DMSO served as a negative control. A 0.9% NaCl solution was used as an isotonic diluent in the preparation of bacterial suspensions, not as a growth medium. The secondary metabolites in the extract were identified through phytochemical screening for flavonoids, alkaloids, tannins, saponins, phenols, and steroids using appropriate reagents.

Antibacterial testing and the use of leaves as a wrapping were conducted as two separate stages. The ethanol extract of *M. tanarius* leaves was used specifically in testing antibacterial activity against *E. coli*, whereas fresh *M. tanarius* leaves were used directly as a wrapping for lepat binti without being applied as a coating. Organoleptic observations were conducted over seven days on lepat binti wrapped in *M. tanarius* leaves and *Musa paradisiaca* L. leaves by evaluating the parameters of taste, color, aroma, and texture using a hedonic scale.

Data Analysis

Data on the diameter of the inhibition zone for each treatment are presented as the mean ± standard deviation. Differences in antibacterial activity among extract concentration variations were analyzed using the *Kruskal–Wallis* test, while the relationship between extract concentration and inhibition zone diameter was analyzed using *Spearman's* correlation at a 5% significance level ($p < 0.05$). Phytochemical screening data were analyzed descriptively based on positive or negative responses to the reagents used. The results of the organoleptic test were analyzed descriptively based on the scores and the percentage of panelists' acceptance during the observation period.

Equipment and Materials

The equipment and materials used in the study included filter paper discs, inoculation needles, test tubes and test tube racks, droppers and micropipettes, measuring cylinders, beakers, Petri dishes, dark glass bottles, an analytical balance, an incubator, an oven, a rotary evaporator, a hot plate, a shaker, an autoclave, Erlenmeyer flasks, a blender, a vernier caliper, a mesh sieve, stirring rods, tweezers, scissors, a spirit lamp, aluminum foil, and other laboratory support equipment, while the materials used consisted of sapat leaves as the test material, distilled water, 96% ethanol, Nutrient Agar medium, chloramphenicol, DMSO, NaCl solution, and

E. coli ATCC 25922 bacteria obtained from the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Bengkulu University.

Sterilization of Equipment

The sterilization process in this study was carried out using moist heat and chemical sterilization techniques. Equipment resistant to high temperatures was sterilized in an autoclave at 121 °C and a pressure of 1 atm for 15 minutes to eliminate all microorganisms still present on the surface of the equipment.

Sample Collection and Preparation of Crude Extracts

The sample of *M. tanarius* leaves (3,000 gram) was collected from Suka Maju Village, Air Nipis Subdistrict, South Bengkulu Regency, Bengkulu Province. The leaves used were mature leaves that had reached physiological maturity; they were fresh, intact, undamaged, and free from pest infestation. Preliminary plant identification was based on leaf morphological characteristics, namely ovate-rounded leaf blades with a cordate base, wavy to serrated margins, a tapering apex, a dark green upper surface, and a light green lower surface, as described by Amirta et al., (2017).

Sample preparation began by cleaning the *M. tanarius* leaves, then cutting them into small pieces. The samples were then air-dried for approximately 3 days at room temperature (25 – 30 °C) and relative humidity (60 – 70%), protected from direct sunlight, until the material reached a dry state and a relatively constant mass. The dried leaves were then ground using a blender until they became a fine powder. The powder was sieved using a 60-mesh sieve to obtain a uniform particle size. Any raw material powder that did not pass through the sieve was ground again until a powder fine enough for use was obtained. The purpose of grinding the powder is to improve the efficiency of the extraction process, as smaller particle sizes result in a larger surface area. This allows for more optimal contact between the solvent and the material, enabling the active compounds to be extracted more effectively.

Sample Extraction

Extraction of *M. tanarius* leaves was performed using the maceration method, employing 200 g of fine powder soaked in 2 liters of 96% ethanol at a starting material to-solvent ratio of 1:10. The maceration process was carried out for three 24-hour periods at room temperature, protected from light, with routine stirring. The mixture was then filtered using *Whatman* filter paper No. 40, and the resulting filtrate was evaporated using a rotary evaporator at 50 °C and a speed of 60 rpm until a concentrated extract was obtained. 96% ethanol was chosen for its effective ability to dissolve phenolic compounds, flavonoids, tannins, and saponins, allowing for the maximum extraction of secondary metabolites. The use of ethanol helps ensure that all active compounds in the leaves dissolve properly and can be extracted. After the extraction process was complete, the weight of the resulting extract was calculated to determine the yield percentage relative to the weight of the sample used for extraction, using the formula cited by Nahor et al., (2020), as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of extract obtained}}{\text{Initial sample weight}} \times 100\% \quad \ldots\ldots (1)$$

Preparation of *M. tanarius* Leaf Extract Concentrations

Ethanol extracts of *M. tanarius* leaves were prepared in five concentrations: 50, 250, 500, 750, and 1000 mg/mL, which correspond to 5%, 25%, 50%, 75%, and 100% w/v, respectively. These concentrations were determined based on the mass of the extract relative to the final volume of the solution. Each extract was then dissolved in 10% DMSO (dimethyl sulfoxide) to a final volume of 10 mL and homogenized before being used in antibacterial activity testing against *E. coli*. The concentration variations were used to evaluate changes in the extracts' inhibitory effects on *E. coli* growth.

Preparation of Positive and Negative Control Solutions

The positive control solution was prepared using the antibiotic chloramphenicol at a concentration of 20 µg/mL to ensure that the testing procedure was proceeding correctly, as indicated by the formation of an inhibition zone. Chloramphenicol was chosen because it has a strong antibacterial effect against *E. coli* (Suryati et al., 2018). On the other hand, DMSO (dimethyl sulfoxide) was used as a negative control because it has no antibacterial activity.

Preparation of Nutrient Agar (NA) Medium

The Nutrient Agar (NA) culture medium was prepared by dissolving 0.4 g of Nutrient Broth (NB) and 0.75 g of agar in 50 mL of distilled water, then heating the mixture until all components were completely and uniformly dissolved. Next, the medium is transferred to an Erlenmeyer flask and sterilized in an autoclave at 121 °C for 15 minutes. Additionally, as a supplementary culture medium, a solution consisting of 0.2 g of NB in 25 mL of distilled water is prepared and sterilized using the same procedure (Maharani, 2022).

Revitalization of Pure Test Bacterial Cultures

Culture rejuvenation was performed by picking a single *E. coli* colony from the nutrient medium on a Petri dish using an inoculating loop, then inoculating it onto slanted agar medium in a test tube using a zig-zag streaking technique. The entire inoculation process was performed aseptically near a Bunsen burner flame to prevent contamination. Next, the test tubes were capped and incubated for 24 hours to allow the bacteria to grow optimally (Rather et al., 2017).

Preparation of the Test Solution Suspension

An *E. coli* suspension was prepared by picking a single bacterial colony with a sterile loop and inoculating it into a test tube containing 10 mL of 0.9% NaCl solution as an isotonic diluent. The suspension was then gently homogenized until a uniform turbidity was achieved. The turbidity of the suspension was adjusted to a 0.5 McFarland standard, equivalent to a bacterial density of approximately 1.5×10^8 CFU/mL. The suspension meeting this standard was then used as the inoculum in the antibacterial activity testing phase according to the research procedure (Aneja, 2018).

Bacterial Inhibition Zone Test

A study on the antibacterial effects of *M. tanarius* leaf extract against *E. coli* was conducted to measure the extract's effectiveness in inhibiting bacterial growth at various concentrations: 5%, 25%, 50%, 75%, and 100%. The method used was the disk diffusion method on Nutrient Agar, with each treatment repeated three times. To prepare the bacterial suspension, *E. coli* colonies were added to 10 mL of 0.9% NaCl solution, then stirred until evenly distributed before being applied to the medium. 6 mm paper discs were soaked in the extract solution for 3 minutes, then placed on the surface of the medium using sterilized tweezers. Chloramphenicol at a concentration of 20 µg/mL was used as a positive control because it exhibits antibacterial activity against both Gram-positive and Gram-negative bacteria, including *E. coli* (Suryati & Bahar, 2018).

Previous studies have indicated that a 0.1% chloramphenicol solution is capable of controlling the growth of both Gram-positive and Gram-negative bacteria (Utomo et al., 2018). Meanwhile, DMSO was used as a negative control because it functions as a solvent, is relatively neutral, and exhibits no antibacterial activity, thus not affecting bacterial growth bakteri (Rohama et al., 2023). All treatments were incubated aerobically at 37 °C for 24 hours. After the incubation process, the inhibition zones formed around the discs were measured using a vernier caliper in millimeters in both the vertical and horizontal directions. The diameter of the inhibition zone was then determined by accounting for the diameter of the disk. Differences in inhibition zones at extract concentrations of 5%, 25%, 50%, 75%, and 100% were analyzed using the *Kruskal–Wallis* test. The results of the analysis showed significant differences in the inhibition zones among the tested concentration groups ($H = 13.06$; $p = 0.011$). The measurement data from three replicates for each treatment were then analyzed descriptively to obtain the mean and standard deviation, while the diameter of the inhibition zone was calculated using the formula 2. The categories of inhibition zone diameters are found on table 1 refers to Susanto & Ruga (2012).

$$D = \frac{(Dv-Dc)+(Dh-Dc)}{2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Description,
D= Diameter of the Restriction Zone
Dv= Vertical Restriction Diameter
Dh= Horizontal Resistance Diameter
Dc= Paper Disc Diameter

Table 1. Categories of Antibacterial Inhibition Zone Diameter, refers by Susanto & Ruga (2012)

Diameter (mm)	Resistance Strength
≤ 5	Weak
6-10	Moderate
11-20	Strong
≥ 20	Very Strong

Phytochemical Screening Test

Phytochemical screening tests were performed qualitatively to identify flavonoids, alkaloids, tannins, saponins, phenols, and steroids. Positive results were determined based on color changes, the formation of precipitates, or the formation of foam/rings depending on the reagent used. Details of the qualitative indicators are presented in table 2.

Table 2. Details of Qualitative Tests for Secondary Metabolites

No	Secondary Metabolites	Primary Reagent	Positive Indicator
1	Flavonoids	Mg powder + acid alcohol + amyl alcohol	Color change to red/yellow
2	Alkaloids	Dragendorff; Wagner; Mayer	Red/orange; brown; or white precipitate depending on the reagent
3	Tannins	$FeCl_3$	Dark blue/black color change
4	Saponin	KOH-alcohol, shaking, 2 N HCl	Stable foam
5	Phenol	$FeCl_3$ 1%	Black/dark color
6	Steroids	Chloroform + anhydride/acetic acid + concentrated H_2SO_4	Blue-green ring

Organoleptic Test

The organoleptic test was conducted on lepat binti wrapped directly in fresh *M. tanarius* leaves and *Musa paradisiaca* L. leaves as a control. Ethanol extract of *M. tanarius* leaves was not used as a coating on the product. The evaluation involved three adult panelists who were in good health, accustomed to consuming lepat binti, had no allergies to the food ingredients being tested, were willing to participate in the entire observation process, and were able to provide consistent sensory evaluations.

In addition, an organoleptic test was conducted to determine the panelists' preferred option based on the parameters of color, texture, taste, and aroma. The test involved three panelists, and scores were assigned according to predetermined evaluation criteria. During the observation period, the samples were stored at room temperature (25–30 °C) with a relative humidity of approximately 60–70%. The evaluation scores are presented in table 3.

Table 3. Panelists' evaluation scores by Lawless & Heymann (1998)

Hedonic Scale	Number
Strongly Dislike	1
Dislike	2
Neutral	3
Like	4
Love	5

Before the testing began, the panelists received an explanation of the study's objectives and procedures and provided their consent to participate. Given that the testing involved only three panelists, the organoleptic results in this study are considered a preliminary sensory test and are not intended to represent consumer preferences on a broad scale. *Musa paradisiaca* L. leaves were used as a control to illustrate changes in the sensory acceptance of lepat binti wrapped in *M. tanarius* leaves during seven days of storage.

According to Syahroni (2023), the data obtained were then analyzed descriptively to examine changes in the panelists' acceptance levels of lepat binti over seven days under sample storage conditions of 25–30 °C and relative humidity of approximately 60–70%. The organoleptic test percentages are shown in table 4.

$$\text{Panelists' acceptance (\%)} = \frac{n}{N} X\ 100\ \% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (3)$$

Description,
% = Percentage score
n = Number of scores obtained
N = Maximum possible score

Table 4. Organoleptic test percentages by Anggraeni et al., (2025)

Percentage (%)	Category
81 - 100	Very Liked
61 - 80	Like
41 - 60	Somewhat Like
21 - 40	Don't Like
0 - 20	Strongly Dislike

RESULTS AND DISCUSSION

Phytochemical Testing of The Ethanol Extract of *M. tanarius* Leaves

Prior to conducting the phytochemical, antibacterial, and organoleptic analyses, *M. tanarius* leaves were prepared and extracted to obtain a concentrated extract to be used as the research material. The solvent used was 96% ethanol because it can extract various types of secondary metabolites and reduce the moisture content that can interfere with the extraction process. The preparation process included determining the wet weight, dry weight, weight of the extracted sample, weight of the resulting concentrated extract, and calculating the extraction yield. The results of the preparation and extraction stages are shown in table 5.

Table 5. Results of the preparation and extraction of *M. tanarius* leaves

No.	*M. tanarius* leaves	Results
1	Fresh weight	3000 grams
2	Dry weight	1620 grams
3	Pounded weight	600 grams
4	Weight of sample used	200 grams
5	Concentrated extract	33.12 grams
6	Extract yield	16.56 %

Table 5 shows that the extraction process of *M. tanarius* leaves involves a change in mass from the initial material to the concentrated extract. The wet weight of 3,000 grams decreased to 1,620 grams after the drying process, indicating a reduction in the moisture content of the material. The crushed sample was then used in the extraction process to obtain 33.12 grams of concentrated extract with a yield of 16.56%. The results of the phytochemical screening were used to determine the content of secondary metabolites present in the ethanol extract of *M. tanarius* leaves. In this study, six types of phytochemical tests were conducted to identify the groups of bioactive compounds contained in the extract, as shown in table 6.

Table 6. Results of Phytochemical Tests on The *M. tanarius* Leaf Extract

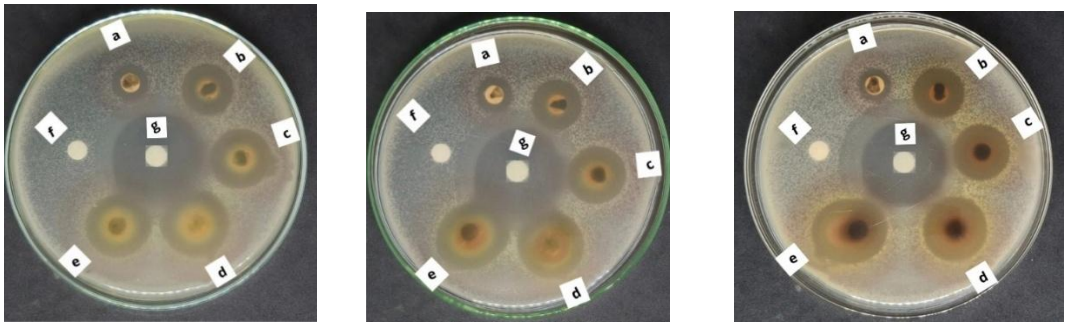
Phytochemical compound	Solvent	Reagent	Results	Remarks
Flavonoids	Distilled water	Mg metal + acid + alcohol + amyl alcohol (Red/yellow color change)	+ (Positive)	A yellow color change occurs
Alkaloids	Distilled water	a. Dragendorff (Precipitate + Red/orange)	+ (Positive)	a. There is a red precipitate
		b. Wagneir (Brown precipitate)	- (Negative)	b. No brown precipitate
		c. Mayer (White precipitat e)	- (Negative)	c. No white precipitate
Tannin	Distilled water	$FeCl_3$ (Turns dark blue or black)	+ (Positive)	A black color change occurs
Saponin	Distilled water	KOH-Alcohol + 2 N HCl	+ (Positive)	Present (foam present) layer of foam or froth
Phenol	Distilled water	1% $FeCl_3$ solution	+ (Positive)	A change to a deep black color (deep black discoloration)
Steroids	Chloroform	Acetic acid + sulfuric acid (Forms a blue-green ring)	+ (Positive)	Formation of a blue green ring

The phytochemical test results for the *M. tanarius* leaf extract shown in Table 6 indicate that six classes of compounds yielded positive results: flavonoids, alkaloids, tannins, saponins, phenols, and steroids. Alkaloids yielded a positive result only when

tested with *Dragendorff's* reagent. Conversely, when tested with *Mayer's* and *Wagner's* reagents, alkaloids yielded negative results.

Antibacterial Activity Testing of the ethanol extract of *M. tanarius* leaves

The results of the antibacterial activity test against *E. coli* growth using the disk diffusion method were indicated by the formation of inhibition zones or clear zones around the paper disks, signifying the presence of antibacterial activity, as shown in Figure 1. Figure 1 shows that the extract from *M. tanarius* leaves, which has an antibacterial effect against *E. coli*, forms an inhibition zone. This antibacterial activity test was conducted using the disk diffusion method. The experiment was conducted using various extract concentrations namely, 5%, 25%, 50%, 75%, and 100% as well as a positive control (K+) and a negative control (K-). Each treatment was repeated three times to improve data accuracy. Measurements of the inhibition zone diameters of the M. tanarius leaf extract against *E. coli* growth are shown in table 7.



Replication 1 Replication 2 Replication 3

Figure 1. Inhibition Zones Produced by *M. tanarius* Extract Against *E. coli*
Note: a = 5% concentration, b = 25% concentration, c = 50% concentration, d = 75% concentration, e = 100% concentration, f = Negative control (K-), g = Positive control (K+)

Table 7. Inhibitory Zone Result of *M. tanarius* Leaves Against *E. coli* (mm)

Concentration (%)	Replications			Mean±SD	Category
	1	2	3		
5% (50 mg/mL)	6.00	5.80	3.85	5.22±1.19	Weak Moderate
25% (250 mg/mL	10.80	10.60	10.00	10.47±0.42	Strong
50% (500 mg/mL)	13.25	13.25	11.95	12.82±0.75	Strong
75%(750 mg/mL)	15.75	15.05	14.05	14.95±0.85	Strong
100% (1000 mg/mL)	14.50	15.90	16.30	15.57±0.95	Strong
K+	23.60	23.55	20.95	22.70±1.52	Very Strong
K-	0.00	0.00	0.00	0.00±0.00	No Inhibitory

Note: K+ = positive control with chloramphenicol at 20 µg/mL; K− = negative control DMSO. The mean and SD were recalculated from the raw data of three replicates using the sample standard deviation.

Table 7 shows the inhibition zones measured using a vernier caliper in millimeters (mm). Measurements were taken three times in each direction, both vertically and horizontally. Subsequently, the average of all measurements was

calculated to obtain more precise, consistent, and objective data regarding the size of the inhibition zones. The inhibition zone increased as the extract concentration increased. A *Kruskal–Wallis* test on the five concentration groups revealed a significant difference (H = 13.06; p = 0.011). *Spearman's* correlation coefficient indicated a very strong positive relationship between extract concentration and the inhibition zone (ρ= 0.961; p < 0.001). These results support the existence of a concentration–response pattern, although the number of replicates for each concentration was only three, so interpretation should still be approached with caution.

Organoleptic Testing of Lepat Binti Wrapped (*M. tanarius* and *Musa paradisiaca*)

Organoleptic testing of lepat binti wrapped in *M. tanarius* and *Musa paradisiaca* L. leaves was conducted by three panelists over a seven day storage period. The results of the observations for each aspect including the taste, color, aroma, and texture are presented in graph of figure 2. Changes in the hedonic scores for the flavor aspect shown in Figure 2 indicate a decline in panelists' preference levels during storage for both types of wrappers. Lepat binti wrapped in *M. tanarius* leaves maintained a score of 5 until the second day, then decreased to 4 on the third and fourth days, 3 on the fifth day, 2 on the sixth day, and 1 on the seventh day.

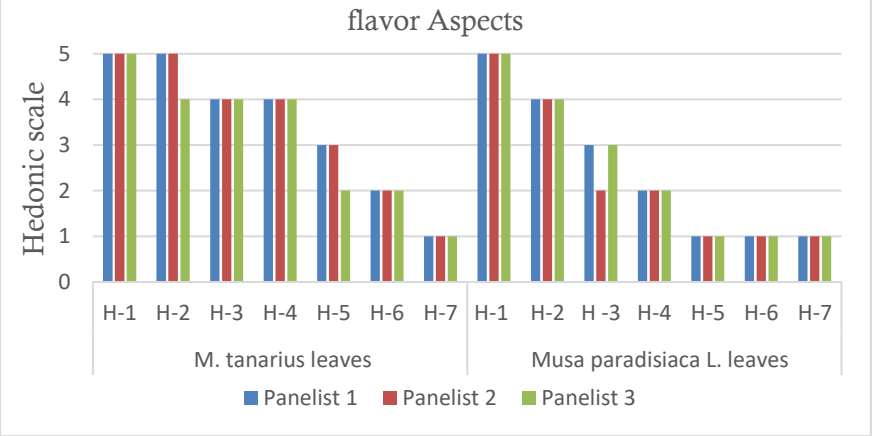


Figure 2. Comparison graph of the organoleptic test for the flavor of lepat binti wrapped in *M. tanarius* and *Musa paradisiaca* L. Leaves

In contrast, lepat binti wrapped in *Musa paradisiaca* L. leaves received a score of 5 on the first day, 4 on the second day, 3 on the third day, 2 on the fourth day, and 1 from the fifth through the seventh day. These results indicate that the use of *M. tanarius* leaves is able to preserve the flavor of lepat binti longer than *Musa paradisiaca* L. leaves. A comparison of the organoleptic test results for the color of lepat binti wrapped in *M. tanarius* and *Musa paradisiaca* L. leaves can be seen in figure 3.

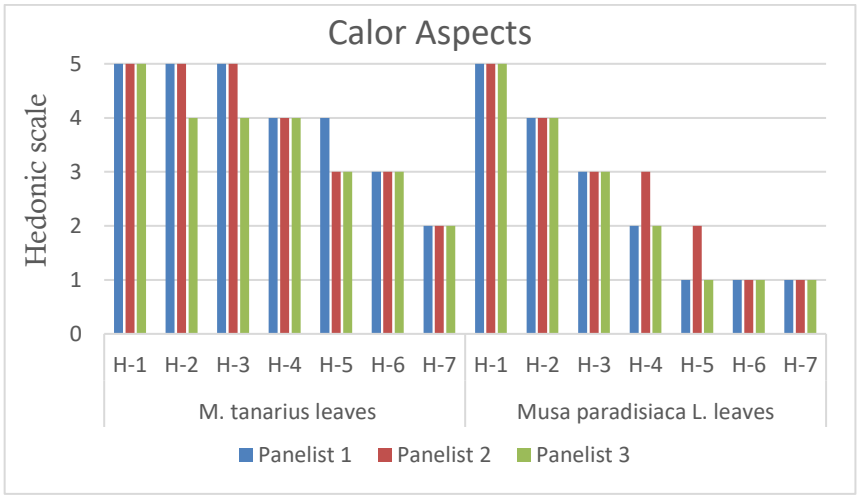


Figure 3. Graph comparing the organoleptic test for the color of lepat binti wrapped in *M. tanarius* and *Musa paradisiaca* L. leaves

Figure 3 shows that the hedonic score for the color aspect of lepat binti in both types of wrappings tended to decrease during storage. Lepat binti wrapped in *M. tanarius* leaves maintained a score of 5 through the second day. Subsequently, the score dropped to 4 on the third and fourth days, to 3 on the fifth and sixth days, and to 2 on the seventh day. In contrast, when wrapped in *Musa paradisiaca* L. leaves, the score of 5 lasted only through the first day, then dropped to 4 on the second day, to 3 on the third and fourth days, to 2 on the fifth day, and to 1 on the sixth and seventh days. These findings indicate that the use of *M. tanarius* leaves can help preserve the color of lepat binti longer than *Musa paradisiaca* L. leaves. A comparison of the organoleptic test results regarding the aroma of lepat binti wrapped in *M. tanarius* and *Musa paradisiaca* L. leaves is presented in the following graph.

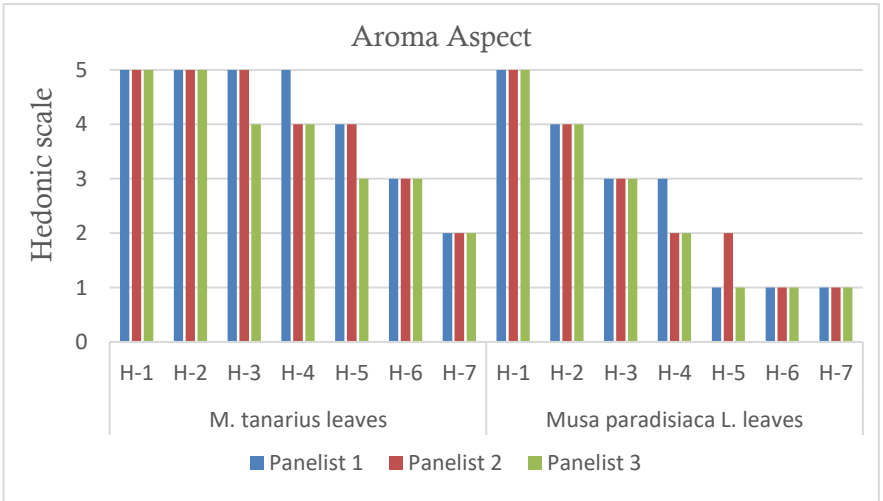


Figure 4. Graph comparing of the organoleptic test for the aroma of lepat binti wrapped in *M. tanarius* and *Musa paradisiaca* L. Leaves

As shown in Figure 4, there were changes in the hedonic scores for the aroma of lepat binti during storage in both types of wrappers. Lepat binti wrapped in

M. tanarius leaves maintained a score of 5 until the second day, then decreased to 4 on the third and fourth days, 3 on the fifth and sixth days, and 2 on the seventh day. Meanwhile, lepat binti wrapped in *Musa paradisiaca* L. leaves received a score of 5 on the first day, 4 on the second day, 3 on the third day, 2 on the fourth and fifth days, and 1 on the sixth and seventh days. These results indicate that the use of *M. tanarius* leaves is able to preserve the aroma of lepat binti longer than *Musa paradisiaca* L. leaves. The following graph presents a comparison of the texture of lepat binti wrapped in *M. tanarius* and *Musa paradisiaca* L. leaves.

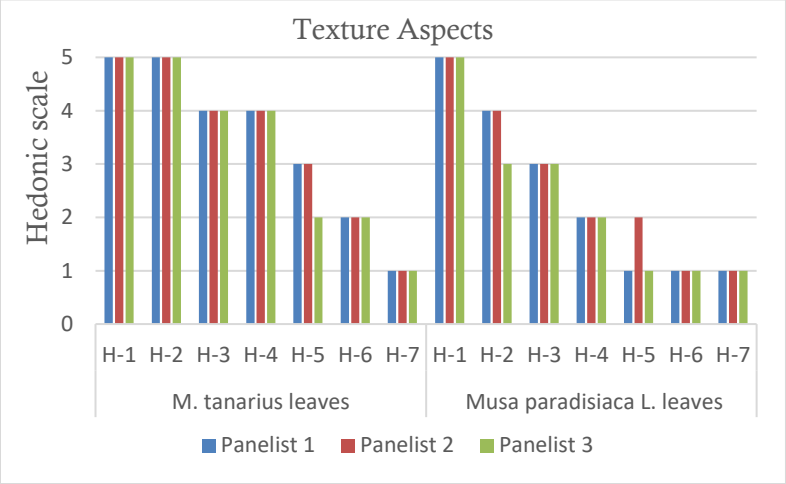


Figure 5. Graph comparing the results of the organoleptic test of the texture of lepat binti that wrapped in *M. tanarius* and *Musa paradisiaca* L. leaves.

Based on Figure 5, the hedonic scores for the texture of lepat binti in both types of wrappings tended to decrease during storage. Lepat binti wrapped in *M. tanarius* leaves maintained a score of 5 until the second day, then decreased to 4 on the third and fourth days, 3 on the fifth day, 2 on the sixth day, and 1 on the seventh day. In contrast, lepat binti wrapped in *Musa paradisiaca* L. leaves maintained a score of 5 only through the first day, then decreased to 4 on the second day, 3 on the third day, 2 on the fourth and fifth days, and 1 on the sixth and seventh days. These results indicate that the use of *M. tanarius* leaves is more effective at maintaining the texture of lepat binti during storage compared to *Musa paradisiaca* L. leaves. Table 8 presents the average results of the organoleptic test for lepat binti wrapped in *M. tanarius* leaves over seven days of storage.

Table 8. Organoleptic test results for lepat binti wrapped in *M. tanarius* leaves based on a hedonic scale over 7 days of observation

Parameter	Score Obtained (n)	Maximum Score (N)	Percentage (%)	Preference Category
Flavor	69	105	65.71	Like
Color	80	105	76.19	Like
Aroma	83	105	79.05	Like
Texture	71	105	67.62	Like

Based on the results presented in Table 8, the aroma parameter had the highest percentage at 79.05%, followed by color at 76.19%, texture at 67.62%, and taste at 65.71%. All of these parameters were classified in the "like" category. These findings indicate that *M. tanarius* leaves are effective at maintaining the quality of lepat binti during storage, with lepat wrapped in *M. tanarius* leaves remaining fresh for up to five days. Meanwhile, the average results of the organoleptic test for lepat binti wrapped in *Musa paradisiaca* L. leaves over a seven-day storage period are shown in table 9.

Table 9. Results of the organoleptic test of lepat binti wrapped in *Musa paradisiaca* L. leaves based on a hedonic scale over a 7 day observation period

Parameter	Score Obtained (n)	Maximum Score (N)	Percentage (%)	Preference Category
Flavor	50	105	47.62	Somewhat Like
Color	53	105	50.48	Somewhat Like
Aroma	53	105	50.48	Somewhat Like
Texture	51	105	48.57	Somewhat Like

The data in table 9 show that all parameters had lower acceptance percentages compared to *M. tanarius* leaves. This indicates that *Musa paradisiaca* L. leaves are relatively less effective at maintaining the quality of lepat binti. The highest acceptance percentage was for color and aroma at 50.48%, followed by texture at 48.57%, and taste at 47.62%, all falling into the "quite like" category. Furthermore, lepat binti wrapped in *Musa paradisiaca* L. leaves could only be preserved for up to two days.

Discussion

The results of the phytochemical analysis of *M. tanarius* leaf extract indicated the presence of flavonoids, alkaloids, tannins, saponins, phenols, and steroids. These findings confirm that *M. tanarius* leaf extract contains active compounds as secondary metabolites. For the flavonoid test, magnesium powder was added to the sample, followed by the addition of an acid-alcohol solution and an amyl alcohol solution. This treatment aims to break the bond between the flavonoid and its glycosidic group through a reduction process, so that the flavonoid is in its free form and can be detected. The appearance of a red or yellow color change on the surface of the amyl alcohol indicates a positive test result (Muthmainnah, 2017). In the flavonoid test, a yellow color change was observed, indicating that *M. tanarius* leaves contain flavonoids.

Alkaloid testing using *Mayer's, Wagner's*, and *Dragendorff's* reagents yielded a positive result only with *Dragendorff's* reagent, as indicated by the formation of a red orange precipitate. Meanwhile, *Mayer's* and *Wagner's* reagents yielded negative results because no precipitate formed. These findings indicate that the *M. tanarius* leaf extract contains alkaloids, even though not all reagents produced positive results. According to Rismawati et al., (2018), a positive reaction for alkaloids with *Dragendorff's* reagent is indicated by the formation of an orange to brick-red precipitate. These findings are consistent with the studies by Nurjannah et al., (2022), which showed positive results for alkaloids only with *Dragendorff's* reagent, while *Mayer's* and *Wagner's* reagents

yielded negative results. This suggests that *Dragendorff's* reagent is more sensitive in detecting alkaloids at low concentrations.

The saponin test in this study indicated the presence of saponins, as evidenced by the formation of stable foam in the test tube after the sample was mixed with a KOH–alcohol solution and then shaken with hot water for 10 seconds. The foam that formed remained stable and did not dissipate for 10 minutes even after the addition of 2 N HCl, indicating the presence of saponin compounds in the *M. tanarius* leaf extract. According to research by Putri et al., (2021), saponins can be identified by the formation of stable foam following agitation. Saponins are natural surfactants, enabling them to produce consistent foam in solution. These compounds are known for their potential as natural antibacterial and anti inflammatory agents.

Testing for tannin content in the *M. tanarius* leaf extract was conducted by adding a $FeCl_3$ solution as a reagent. Tannins belong to the group of phenolic compounds that are highly soluble in water and other polar solvents. The presence of phenolic groups in a sample can be identified using a $FeCl_3$ solution as a reagent, as phenolic compounds cause a characteristic color change. A positive reaction is generally indicated by the appearance of a dark blue or dark green color (Syeptiani, 2019). In this study, the addition of $FeCl_3$ to the *M. tanarius* leaf extract produced a dark blue color, indicating the presence of tannins in the extract.

The phenolic content test in this study yielded a positive result, indicated by the sample turning deep black after the addition of a 1% $FeCl_3$ solution. According to Ningsih et al., (2020), phenolic compounds react with $FeCl_3$ to produce a dark or blackish-green color. This color change occurs due to the interaction between the hydroxyl groups in the phenols and iron ions. This color change indicates the presence of phenolic compounds in the *M. tanarius* leaf extract sample.

The results of the steroid compound test indicated a positive reaction through a color change to bluish-green in the *M. tanarius* leaf extract solution. This color change occurred after chloroform, acetic anhydride, and concentrated sulfuric acid were added to the sample, causing a bluish-green ring to appear at the interface between the two solvents (Nugrahani et al., 2016). The formation of a bluish-green ring in the *M. tanarius* leaf extract solution indicates the presence of steroid compounds.

When compared to the study by Razoki et al., (2023), the results of the phytochemical screening of the 96% ethanol extract of Nephrolepis biserrata in the aqueous fraction revealed the presence of secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds, while no steroids, triterpenoids, or saponins were detected. In contrast, a study by Ningrum et al., (2021) indicated that Kabau fruit peel extract contains flavonoids, tannins, saponins, and steroids, while terpenoids and alkaloids were not detected. According to a study by Puspita et al., (2022), phytochemical analysis of *Premna serratifolia* leaf extract revealed the presence of alkaloids, flavonoids, and steroids that may function as anti inflammatory agents.

The results of the identification of secondary metabolites in this phytochemical study serve as the basis for investigating the antibacterial effects of *M. tanarius* leaf extract against *E. coli*. The objective of this study is to evaluate the extract's ability to inhibit bacterial growth.

The results of the antibacterial activity testing showed that the *M. tanarius* leaf extract formed an inhibition zone against *E. coli*, as observed using a Vernier caliper. Measurements of these inhibition zones revealed that DMSO, as a negative control, did not produce an inhibition zone; thus, it can be concluded that DMSO has no antibacterial effect against *E. coli*. Therefore, the inhibition zones formed at various extract concentrations were caused by bioactive compounds contained in the *M. tanarius* leaf extract. In contrast, chloramphenicol, as a positive control, produced an inhibition zone with a diameter of 22.70 ± 1.52 mm, indicating a strong ability to inhibit bacterial growth. The difference in results between the positive and negative controls demonstrates the inhibitory effect of the active compounds contained in the *M. tanarius* leaf extract.

The addition of *M. tanarius* leaf extract showed that the size of the inhibition zone increased in line with the increase in extract concentration. At a 5% concentration, the inhibition zone size was recorded at 5.22 ± 1.19 mm, which was categorized as weak inhibition. Meanwhile, at a 25% concentration, the inhibition zone enlarged to 10.47 ± 0.42 mm, falling into the moderate category. Furthermore, at concentrations of 50%, 75%, and 100%, the inhibition zone sizes were 12.82 ± 0.75 mm, 14.95 ± 0.85 mm, and 15.57 ± 0.95 mm, respectively, all of which fall into the strong category. These findings indicate that an increase in extract concentration is directly related to an increase in the inhibitory effect on bacterial growth. This is consistent with the results of a study by Yulisma (2018), which stated that an increase in extract concentration is accompanied by an enlargement of the inhibition zone, indicating higher antibacterial activity.

When compared to the study conducted by Saputra et al., (2025), the results of this study show similar findings, namely an increase in antibacterial activity as the extract concentration increases. In Saputra et al., (2025) study, the Meniran (*Phyllanthus niruri*) extract was able to inhibit the growth of *Propionibacterium acnes* at concentrations of 5%, 25%, 50%, 75%, and 100%, with inhibition zone diameters of 1.15 mm, 3.05 mm, 4.8 mm, 6.55 mm, and 9.5 mm, respectively.

Meanwhile, a study by Putri et al., (2021) showed an ethanol extract of *Ipomoea pescaprae* leaves could inhibit the growth of *Staphylococcus epidermidis* at concentrations of 5 mg/mL, 20 mg/mL, 35 mg/mL, and 50 mg/mL, with inhibition zone diameters of 5.92 ± 0.46 mm; 8.39 ± 0.56 mm; 10.22 ± 0.26 mm; and 11.40 ± 0.17 mm. These values were lower than those of the positive control using 0.25 mg/mL clindamycin (26.95 ± 0.33 mm), whereas the negative control using DMSO produced no inhibition zone. In addition to the bioactive compounds responsible for antibacterial activity, panelists' acceptance is also a key factor in the use of leaves as natural wrappers. Therefore, organoleptic tests were conducted to assess the level of preference and shelf life of lepat binti wrapped in *M. tanarius* leaves and *Musa paradisiaca* L. leaves.

The results of the organoleptic test using the Lepat Binti hedonic scale with Lepat Binti wrapped in *M. tanarius* leaves for seven days of observation showed a high level of acceptance among panelists. The aroma parameter had the highest percentage at 79.05%, followed by color at 76.19%, texture at 67.62%, and taste at 65.71%, all falling into the "like" category. These results indicate that *M. tanarius* leaves are

effective at maintaining the quality of lepat binti during storage, as lepat wrapped in *M. tanarius* leaves can last up to five days.

Meanwhile, the results of the organoleptic test using a hedonic scale for lepat binti wrapped in *Musa paradisiaca* L. leaves over a seven-day observation period showed that all parameters had lower acceptance percentages compared to those wrapped in *M. tanarius* leaves. These findings indicate that the ability of *Musa paradisiaca* L. leaves to maintain the quality of lepat binti is relatively lower. The highest acceptance percentage was found for the color and aroma parameters at 50.48%, followed by texture at 48.57%, and taste at 47.62%. All of these parameters fell into the fairly liked category. Additionally, lepat binti wrapped in *Musa paradisiaca* L. leaves could only be preserved for up to two days.

Based on this analysis, lepat binti wrapped in *M. tanarius* leaves received a higher acceptance rate from the panelists compared to those wrapped in *Musa paradisiaca* L. leaves. Furthermore, the shelf life of lepat binti wrapped in *M. tanarius* leaves reached five days, whereas those wrapped in *Musa paradisiaca* L. leaves could only maintain product quality for up to two days. These findings indicate that *M. tanarius* leaves are more effective as a natural wrapper because they can maintain quality while extending shelf life. This advantage is supported by the antibacterial activity of *M. tanarius* leaf extract, which effectively inhibits bacterial growth, thereby helping to maintain the quality of lepat binti during storage.

When compared to the study by Darmawan (2025) that tempeh wrapped in plastic had the highest compactness level with an average score of 3.84 (preferred category), followed by a combination of plastic and banana leaves with a score of 3.64, and banana leaves alone with a score of 3.52. However, an analysis of variance indicated that variations in packaging type did not significantly affect mold levels. Overall, the evaluation showed that panelists' preference levels fell within the somewhat like to like range (scores 2.76–4.08), with tempeh wrapped in plastic and the combination of plastic and banana leaves. Meanwhile, according to the study by Windarti & Saidi (2021), mustard greens flour stored in various types of packaging experienced a decline in organoleptic quality. Among the aspects evaluated by the panelists during the storage process namely aroma, color, and spreadability the most significant decline was observed in the aroma parameter, particularly in polyethylene (PE) packaging, while the greatest declines in color and spreadability were found in polypropylene and polyethylene packaging at 30 °C. Nevertheless, the results of the study indicate that the optimal storage temperature is 40 °C, and the packaging that best preserves the quality of mustard greens flour is polypropylene packaging.

CONCLUSION

Overall, this study revealed a concentration response pattern in the antibacterial activity of *M. tanarius* ethanol extract against *E. coli*. The net inhibition zone increased from 5.22 ± 1.19 mm at a 5% concentration to 15.57 ± 0.95 mm at 100%, with statistically significant differences between concentrations (*Kruskal Wallis* H= 13.06; p= 0.011) and a strong positive correlation between concentration and inhibition zone (ρ= 0.961; p < 0.001). Phytochemical screening detected six classes of

secondary metabolites: flavonoids, alkaloids, tannins, saponins, phenols, and steroids. In the fresh leaf wrapper test, *M. tanarius* showed the sensory acceptance of 65.71% for taste, 76.19% for color, 79.05% for aroma, and 67.62% for texture, as well as sensory acceptability up to day 5 in this study; *Musa paradisiaca* L. wrappers showed acceptability ranging from 47.62% to 50.48%, with sensory acceptability lasting until around day 2. These findings provide preliminary evidence of the potential of *M. tanarius* as a natural wrapper, but do not constitute definitive proof regarding shelf life or preservation mechanisms.

ACKNOWLEDGMENTS

We would like to thank the Research Team at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Bengkulu University; the MBKM Team, Natural Sciences Education Program, Faculty of Teacher Training and Education, Bengkulu University; and all parties who provided support, facilities, assistance, and guidance throughout the research process. The contributions from these various parties were instrumental in ensuring the smooth progress of every stage of the research, allowing this project to be successfully completed.

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How To Cite This Article, with *APA style* :

Anisa, P., Johan, H., Defianti, A., Sipriyadi, S., & Listiono, A. E. (2026). Antibacterial Activity of Ethanolic Macaranga tanarius Leaf Extract as Natural Lepat Binti Wrapper Against Escherichia coli Using Disk Diffusion. *Jurnal Pembelajaran dan Biologi Nukleus*, *12*(3), 867-887. https://doi.org/10.36987/jpbn.v12i3.9682

Conflict of interest : The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions : All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was submited by [**Putri Anisa**]. All authors contributed on previous version and revisions process of the manuscript. All authors read and approved the final manuscript.