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Antibacterial Activity Test of Tembelekan Leaf Extract (*Lantana camara* linn) against *Edwardsiella tarda bacteria*

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Abstract: The purpose of this study was to analyze the optimal dose of Tembelekan leaf extract (*Lantana camara* Linn) which has antibacterial properties in inhibiting or killing *Edwardsiella tarda bacteria*. The method used in this research is Completely Randomized Design (CRD). The results of the research yield obtained from tembelakan leaf extract amounted to 8.9%. The positive phytochemical test results contained active compounds, namely flavonoids, alkaloids, tannins, triterpenoids, and saponins. GC MS analysis of tembelekan leaf extract showed that the most components were *4H-1-Benzopyran-4-one, 5,7-dihydroxy-6-methoxy-2-(4-methoxyphenyl)-,2,1,3-Benzothiadiazole, Benzisot hiadiazole , 3,4-Benzo-1,2,5-thia diazole*. This compound is a class of isoflavonoids compounds. The MIC (*Minimum Inhibitory Concentration*) test results obtained the lowest absorbance value at a concentration of 100 mg/L with a value of 0.602 close to the positive control. Results of discs with doses of 50, 100, 150, 200, and 250 mg/L. The disc results showed that the dose of 200 mg/L had the strongest inhibition zone with a value of 12.63 mm. Tembelekan leaf extract which contains dominant compounds such as flavonoids and alkaloids can be useful as an antibacterial which inhibits the growth of *E. tarda bacteria*

Keywords: Alkaloids; *Edwardsiella tarda;* Flavonoids; *Lantana camara* linn

Introduction

The natural product being developed at this time is the tembelekan plant with the Latin name Lantana camara L. This plant grows in the wild and has various secondary metabolites, especially in the leaves, such as terpenoids, including volatile compounds, flavonoids, phenolic compounds, saponins, alkaloids, Steroids, Tannins and Quinones (Kotala et al., 2019). Putri et al. (2018), stated that secondary metabolites in tembelakan leaves have potential as antibacterial compounds.

Flavonoid compounds in the leaves of the tembelekan plant can be extracted using 96% ethanol. Flavonol compounds themselves have strong antibacterial potential, because they can interfere with the permeability of bacterial cell walls, microsomes, and lysosomes (Marfuah et al., 2018).

According to research by Iwan et al. (2011) leaf extract of the tembelekan plant has an inhibition zone high against gram-positive and gram-negative bacteria namely *S.aeureus* and *E.coli bacteria.* Based on studies on the potential of *Lantana camara* Linn as an antibacterial, many references have been carried out, but its use as an alternative antibacterial against *E.tarda* bacteria not yet optimal, thus, it is necessary to carry out further research and study in detail the active compound content of *Lantana camara* linn which is useful as an antibacterial.

Bacteria *This E. Tarda* causes a disease called *Edwardsiellosis* (Kerie et al., 2019). In general, *Edwardsiella* is a disease that can attack fish farming systems. Therefore, it is necessary to prevent, treat and control the invasion of E. *tarda*. These problems can be overcome by optimizing the use of herbal or natural ingredients that are rarely used as alternative medicines to control E. *tarda.*

Method

Research Materials

The materials used in the extraction activities are tembelekan leaves *(L. camara Linn) and* solvents for maceration namely ethanol with pro-analysis (PA) quality. Culture bacteria and test Power resistor antibacterial, material Which usedis *E. Tarda* bacteria.

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Draft Study

The design in this study was Completely Randomized Design (CRD). The treatment used in this study was the same dose different, control positive, control negative and test as much 3 time. Treatment the is as following:

Treatment A	= Leaf extract *(L. camara Linn)* 50 ppm
Treatment B	= Leaf extract *(L. camara Linn)* 100 ppm
Treatment C	= Extract tabelapan leaf *(L. camara Linn)* 150 ppm
Treatment D	= Leaf extract *(L. camara Linn)* 200 ppm
Treatment E	= Leaf extract *(L. camara Linn)* 250 ppm
Treatment K-	= Treatment of bacterial infections and without giving extracts
Treatment K+	=Treatment infection by giving antibiotics

Research Procedure

Making Extract Tembelekan Leaves (L. camara Linn)

Making extract stembelakan leaves begin with Leaf samples were taken from fresh tembelekan plants (*L. camara Linn).* The leaf samples were then washed and dried in the sun. The dried leaves were weighed and then blended. Furthermore, tembelekan leaf powder was macerated with 96% ethanol as much as 2000 ml for 3 x 24 hours. Maserate is filtered with filter paper. Tembelekan leaf extract was evaporated using *a Rotary evaporator* with a temperature of 40 ^{o}C. (Lolodatu et al., 2019).

Test Identification Compound

Which method used in study This. Among them is test phytochemicals, test spectrophotometer *ultraviolet visible* (UV-Vis),test spectrophotometer *fouries transform-infrared* (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) test. Identification phytochemicals done use method Which done by Simaremare et al. (2014), with steps as following:

Test Alkaloids

As much as 4 grams of the refined sample was added with chloroform and pulverized again. Add 10 ml of ammonia and 10 ml of chloroform. The solution was filtered into a test tube, the filtrate was shaken regularly. Transfer the top layer into 3 test tubes. Each test tube is given 1-2 drops of Dragendorff reagent. The second tube is added 1-2 drops of Wagner's reagent and the third tube is added 1-2 drops of Mayer's reagent as well. A positive result is shown in Wagner's reagent, a brown precipitate will form and Dragendroff will form a red precipitate and in Mayer's reagent, a white precipitate will form in the test tube.

Flavonoid Test

The sample that has been heated with 96% ethanol is taken as much as 10 ml of the filtrate, added 0.5 grams of Mg powder, 2 ml of concentrated HCL, and 20 ml of amyl alcohol and then shaken until smooth, if a layer of amyl alcohol is formed which is red, yellow or orange in color 3 minutes then indicates the presence of Flavonoid content.

Saponin Test

A sample of 1 ml was taken and heated for 2-3 minutes with distilled water, 10 ml of the filtrate was taken into a test tube, shaken vigorously for 10 seconds and left for 10 minutes. Positive results for the presence of saponins with the formation of stable foam.

Test Tannins

10 ml of sample was taken into a test tube and added 2-3 drops of FeCl31%, positive results were shown in dark blue or greenish black.

Spectrophotometer UV-Vis (Ultraviolet-Visible)

The leaves of the stembelakan are diluted return with solvent 96% ethanol. Dilution This need done can determine the absorbance of the compound being characterized, UV light Which fired in spectrophotometer must pass sample so that the characterized sample should not be too dark. If the sample is too dark so ray UV Which emitted No can pass sample. Method spectrophotometer UV-Vis done with method measure spectra long wave absorbance from radiation super violet with use from extract of *L. camara linn* with spectrophotometer long wave 190 nm- 1100 nm. The result form spectrum uptake form chart comparison between absorbancewith long wave (Sahri et al., 2019).

Spektrofotometer FT-IR (Fouries Transform-Infrared)

Analysis use FT-IR This can identify clusters function in a compound represented as a wave number or wavelength (Kumayas et al., 2015). Compound analysis using FT- IR, namely by taking a sample of 0.5 mg of tembelekan leaves *(L. camara linn)* Then mixed with 180 mg KBr (Potassium Bromide) And homogenizeduntil it forms a pellet. The sample pellets were then measured using FTIR spectrum with a wavelength of 4000-400 cm $^{-1}$ and the results were analyzed (Triyasmono et al., 2020). Results from FTIR This form *peak* Which done identification for now characterization from sample.

Gas Chromatography Mass Spectrometry (GC-MS)

Gas Chromatography Mass Spectrometry (GC-MS) is a gas chromatography technique used in conjunction with mass spectrometry. Gas chromatography is used to search for volatile compounds under high vacuum and low pressure while heating. Mass spectrometry is used to determine molecular weight, molecular formula and produce charged molecules (Darmapatni et al., 2016).

The preparation of tembelekan leaf extract uses the maceration or soaking method. Then it was put into *a micro tube* containing 0.5 g of powder and 1.5 ml of ethanol solvent, then vortexed for 1 minute, then centrifuged for 3 minutes at 9000 rpm. The supernatant formed was continued for GC-MS testing. The time is set for 60 minutes with an injector temperature of 260°C, detector 250°C, and column 325°C. The carrier gas used is helium gas as the carrier at a constant flow rate of 1 ml/min. The identification process using the GC-MS tool to produce several bioactive compounds can be seen from the peaks of the chromatogram as identification of data from chromatography and mass spectrometry (MS) results seen from the mass spectrum with the molecular weight of each bioactive compound.

Antibacterial Activity Test

Media Which used for culture and rejuvenation of *E. tarda bacteria* is TSB. agar media5 ml and put into a test tube. Cultivation of pure culture of *E. tarda bacteria* was carried out using aseptic loops on TSB media. After planting, the media was then incubated at temperature 31^{o}C during 24 hours.

Test MIC (Minimum Inhibition concentration)

The first thing to do is to prepare a sterile test tube. The test tube was filled with 4.5 ml of sterile TSB media. Then 0.5 ml of tembelekan leaf extract (Lantana camara linn) was added to each test tube containing TSB at a predetermined dose.

Disc Test

Disc test uses paper discs which function as a place to collect antimicrobial substances. The filter paper containing the antimicrobial substance is placed on the agar plate which has been inoculated with the test microbe and then incubated at a certain time and temperature, according to the optimum conditions of the test microbe, namely at 31 ^{o}C for 18-24 hours. Radical zone, which is an area around the disk where absolutely no bacterial growth is found. Antibacterial potency was measured by measuring the diameter of the radical zone. Irradical zone is an area around the disk where bacterial growth is inhibited by antibacterials.

SEM test (Scanning Electron Microscope)

Making preparations *E. tarda* done with 2 treatments. TreatmentFirst *E. tarda* is treatment normal, treatment second bacteria *E. tarda* given extract stye leaves (*Lantana camara linn*) with dose. Then second preparations the Ready observed use SEM (*Scanning electrons Microscope*).

Results and Discussion

Yield of Tembelekan Leaf Extract

Table 1. Yield of Tembelekan Leaf Extract

Solvent	Total Solvent	Sample Weight	Extract Weight	yield
Ethanol	2000.0 ml	200.0 gr	17.8 gr	8.9%

The yield of tembelekan leaf extract which was macerated using 96% ethanol solvent above was obtained by comparing the weight of the extract with the weight of tembelekan leaf powder. The yield obtained from the tembelakan leaf extract was 8.9%. Extract yield calculations are carried out to determine the ratio of the amount of extract obtained from a material to the initial weight of the simplicia material (Fajar et al., 2020).

The yield of an extract can be affected by several factors, one of which is the type of solvent and its concentration (Siswanto et al., 2020). Ethanol solvent is the most effective and largest solvent for extracting compounds contained in natural ingredients such as phenolic compounds and flavonoids, and the process is faster. Ethanol is a universal solvent that dissolves compounds in polar, semipolar and nonpolar natural materials. 96% ethanol is the concentration of ethanol used to accelerate the evaporation of the extract. Ethanol solvent is also effective in binding active compounds such as flavonoids (Mulyani et al., 2021).

Phytochemical Screening

The results of the phytochemical test for tembelekan leaf extract are presented in Table 2.

Table 2. Results of Phytochemical Tests on Tembelakan Leaves

Compound Identification	Characteristics	Results
Flavonoids	Orange, Brick Red, Pink, Dark red	(+) Positive
Alkaloids		
meyer	White Precipitate	(+) Positive
Dragendrof	Orange deposit	(+) Positive
Bouchardat	Chocolate Precipitate	(+) Positive
Tannins / Phenol	Dark Brown, Dark Blue	(+) Positive
terpenoids		
Steroids	Bluish Green	(-) Negative
Triterpenoids	Orange, Orange Brown	(+) Positive
Saponins	Permanent Foam	(+) Positive

Phytochemical screening was carried out to determine the presence or absence of compounds such as alkaloids, flavonoids, tannins, steroids, and saponins contained in tembelekan leaf extract. The results of the identification of the color reaction can be seen that the results of the color reaction of the ethanol extract of tembelekan leaves contain positive active compounds namely flavonoids, alkaloids, tannins, triterpenoids, and saponins. These results are supported by research by Putri et al. (2018), the phytochemical results of tembelekan leaves with ethanol solvents yielded flavonoids, alkaloids, saponins, triterpenoids, and tannins.

Uv-Vis Spectrophotometry

The next test was to analyze the extract using a UV-Vis spectrophotometer. The results of the test found several peak points at the wavelength as presented in Figure 1. Wavelength peak point data generated from UV-Vis analysis is presented in Table 3.

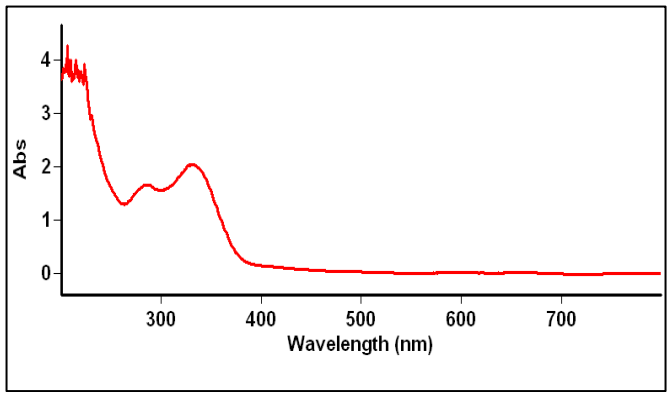

Figure 1. Uv-Vis Results of Tembelekan Leaf Extract

Table 3. Uv-Vis Wavelength Peak Data

Wavelength (nm)	absorbance	Compound	Literature
662.0	0.013	Flavonoids	Krisnawan et al 2022
330.0	2040	Carotene and its derivatives	Krisnawan et al 2022
287.0	1.656	Flavonoids	Kumar and Pandey, 2013
230.0	2.958	Flavonoids	Ritna & Anam. (2016)
223.0	3.935	Flavonoids	Ritna & Anam. (2016)
219.1	3.790	Flavonoids	Ritna & Anam. (2016)
217.0	3.805	Flavonoids	Ritna & Anam. (2016)
214.0	4.010	Flavonoids	Ritna & Anam. (2016)
211.1	3.718	Flavonoids	Ritna & Anam. (2016)
209.0	4.000	Alkaloids	Hammado dan illing 2013
206.0	4.278	Alkaloids	Hammado dan illing 2013
204.0	3.937	Alkaloids	Hammado dan illing 2013
202.0	3.847	Alkaloids	Hammado and illing 2013

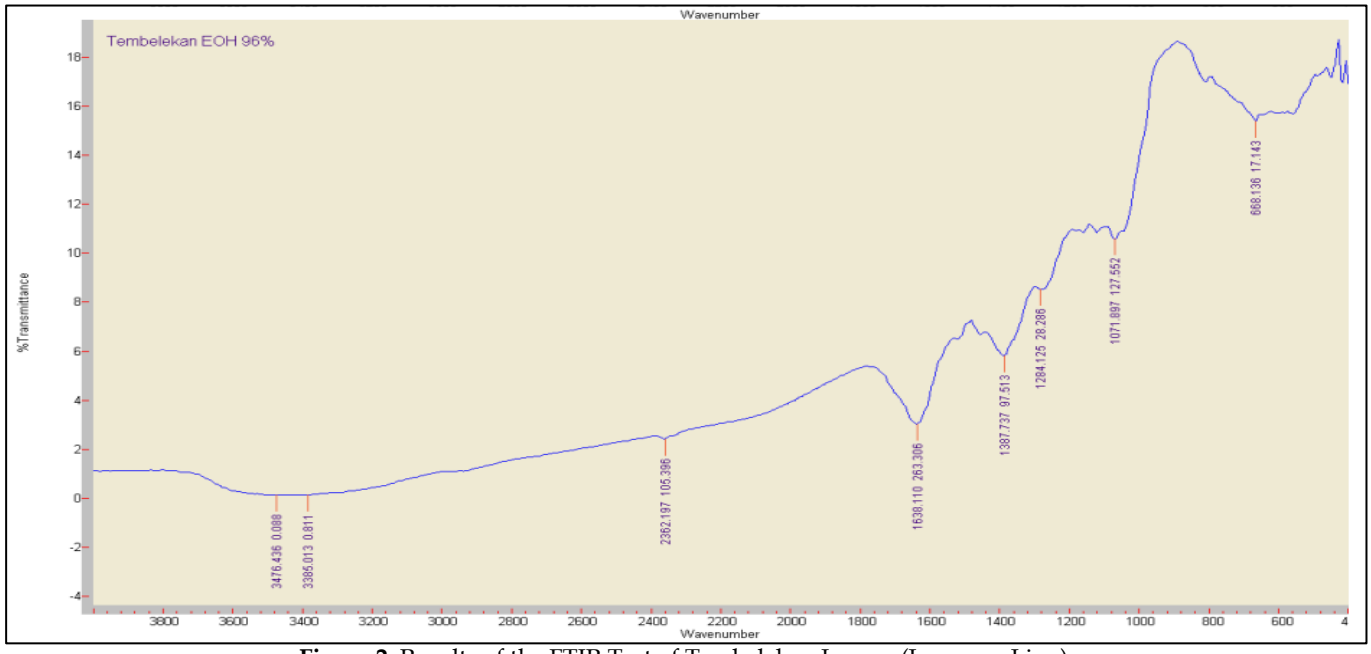

Figure 2. Results of the FTIR Test of Tembelekan Leaves (L. *camara* Linn)

The results of the Uv-vis spectrophotometric test data above can be concluded that the dominant compounds come from the flavonoid and alkaloid groups with large absorbance. Absorbance is the ratio of

the intensity of the absorbed light to the intensity of the incident light. This absorbance value will depend on the levels of substances contained therein. According to Neldawati et al. (2013), the more levels of a substance contained in a sample, the more molecules will absorb light at a certain wavelength so that the absorbance value is greater or in other words the absorbance value will be directly proportional to the concentration of the substance contained in a sample.

FTIR Spectrophotometry

FTIR can confirm at the functional group level of an extract. From UV-Vis and FTIR it can be seen that there are similarities in the results of the analysis of the active compound content contained in the tembelekan leaf extract. FTIR is used to see the absorption values obtained and the functional groups displayed in the graph. The results obtained are in the form of 8 absorption band frequency regions with different functional groups. The absorption band results of the FTIR spectrophotometer for tembelekan leaves are presented in Figure 2 as well as the absorption band data of the FTIR spectrophotometer analysis for tembelekan leaves as presented in Table 3.

Table 4. Results of Tembelekan Leaf FTIR Test analysis (L. *camara* Linn)

Absorption band frequency of tembelekan leaf extract (L. camara linn) (cm^{-1})	Frequency absorption band	Compound type	Group function (bonding)	Literature	Group
3476	3500-3000	Alcohol and Phenol	Help OH	Nandiyanto et al., 2019	Flavonoids
3385	3500-3000	Alcohol and Phenol	Help OH	Nandiyanto et al., 2019	Flavonoids
2362	2700-1725	Carbonyl	Aliphatic CH	Wahdaningsih., 2022	Flavonoids
1638	1650-1400	Alkene	C=C Aromatic	Nandiyanto et al., 2019	Flavonoids
1387	1470-1340	Alkane	CO Bend	Rasyida et al., 2014	Alkaloids
1284	1300-1000	Alcohol Ether	CO Bend	Rasyida et al., 2014	Alkaloids
1071	1300-1000	Alcohol Ether	CO Bend	Rasyida et al., 2014	Alkaloids
668	1000-550	Alkene	CH Aromatic	Rasyida et al., 2014	tannins

The results of FTIR spectrophotometer observations found the presence of hydroxyl groups (OH), C=O, C=C aromatic, aliphatic CH indicating the presence of flavonoid compounds. So it can be concluded that the dominant content of tembelekan leaf extract is the type of phenol and its derivative compounds.

Alkaloid and flavonoid compounds contained in herbal plants can damage the bacterial membrane by destroying the outer membrane of gram-negative bacteria. This is because the flavonoid compounds will react with the DNA in the cell nucleus and will cause damage to the DNA lipid structure so that the bacteria will lyse and the cell will die (Ernawati & Kumala, 2015). Alkaloid compounds as antibacterial work by destroying the peptidoglycan constituent components in bacterial cells, causing the cell wall layer to not form as a whole and causing bacterial cell death (Erlyn, 2016).

GC MS Analysis of Tembelekan Leaf Extract (L. camara linn)

Gas chromatography is able to read compounds with the lowest concentrations so that secondary metabolites in plants can be identified with the results in the form of chromatograms and mass spectra (Al-Rubaye et al., 2017). The results of the GC-MS analysis of the tembelekan leaf extract showed that there were 25 peaks and 150 possible components of the compound that were successfully extracted from the ethanol solvent (Table 5).

Table 5. compounds identified by GC-MS from tembelekan leaf extract (*L. camara* linn.)

Peaks	Real Time	Hits 1	Hits 2	Hits 3	Ret. Areas (%)
1.	2.490	*Propanal*	*2,3-dihydroxy-*	*alpha beta - Dihydroxypropionaldehyde*	1.96
2.	3.025	*Ethanimidic acid, ethyl ester*	*Ehtyl acetimidate*	*Ethyl ethanimi doate*	1.37
3.	3.928	*1,2,3-Propanetriol*	*Glycerol, Glyrol, Glycerin*	*Osmoglyn*	2.73
4.	4.679	*2-Octanone, Octan-2-one*	*Methyl hexyl ketone*	*Hexyl methyl ketone*	1.12
5.	5.144	*Pyrimidine-4,6-diol, 5-methyl-*	*5-Methyl-4*	*6-pyrimidinediol*	0.67
6.	6.069	*4H-Pyran-4-one*	*2,3-dihydro-3*	*5-dihydroxy-6-methyl-*	2.11
7.	7.014	*1,2-Benzenediol*	*Pyrocatechol*	*BRENZCATECHIN, Fourrine 6*	1.97

Peaks	Real Time	Hits 1	Hits 2	Hits 3	Ret. Areas (%)
8.	7.132	*4-vinylphenol*	*p-vinylphenol*	*1-Ethenyl-4-hydroxybenzene*	1.91
9.	7.243	*2-Furancarboxaldehyde, 5-(hydroxymethyl)-*	*2-Furaldehyde*	*5-(hydroxymethyl)-*	3.30
10.	8.375	*2-Methoxy-4-vinylphenol*	*Phenol, 4-ethenyl-2-methoxy-*	*4-ethenyl-2-methoxy- aiacol*	5.92
11.	8.841	*Phenol, 2,6-dimethoxy-*	*Pyrogallol 1,3-dimethyl ether*	*Syringol*	5.10
12.	10.196	*2,1,3-Benzothiadiazole*	*Benzisothiadiazole*	*3,4-Benzo-1,2,5-thiadiazole*	20.19
13.	10.794	*beta.-D-Glucopyranose, 1,6-anhydro-*	*Anhydro-d-mannosan*	*Levoglucosan*	3.41
14.	12.093	*4-(t-Butyl)-3*	*3-dimethyl-2(3H)-*	*thiophenone*	1.11
15.	12.281	*3-Chloro*	*-2-hydroxypropyl*	*2-methylisobutyrate*	1.47
16.	12.364	*(Z)-1,2-dimethylcyclohexanol*	*s-1,2-dimethylcyclohexanol*		1.03
17.	12.684	*Benzaldehyde*	*4-hydroxy-3,5-dimethoxy-*	*Gallaldehyde 3,5-dimethylether*	1.56
18.	13.566	*4-((1E)-3-Hydroxy-1-propenyl)*	*2-methoxyphenol*		8.10
19.	14.136	*Guaiacol glycerol ether*	*1,2-Propanediol*	*3-(2-methoxyphenoxy)- (CAS)*	1.37
20.	14.462	*11-NOR-8-HYDROXY-9-DRIMANONE*			0.97
21.	15.741	*Hexadecanoic acid (CAS)*	*Palmitic acid*	*Palmitinic acid, Prifrac*	3.64
22.	16.165	*trans - sinapyl alcohol*	*trans - 4 hydroxy - 3*	*5 dimethoxy - cinnamyl alcohol*	1.17
23.	21.793	*2,6-bis[3'-(Hydroxoxymethyl)*	*pyrazo l-1'-yl]pyrazine*		1.80
24.	24.948	*4H-1-Benzopyran-4-one*	*5,7-dihydroxy-6-methoxy-2-*	*(4-methoxyphenyl)- (CAS)*	24.23
25.	29.986	*Benzene*	*p-di-tert-pentyl-*	*1,4-i-tert-pentylbenzene*	1.79

Note: Positive control (K+) using 5 ppm Tetracycline, Negative Control (K-) without extract administration.

The highest compound components in tembelekan leaves in ethanol extract are located at *peak* 24 and *peak* 12. *Peak* 24 with a *retention area value of* 24.23% and peak 12 with a *retention area value* of 20.19%. With the three compounds present in *peaks* 24 and 12, namely *4H-1-Benzopyran-4-one, 5,7-dihydroxy-6-methoxy-2-(4-methoxyphenyl)-,2,1,3-Benzothiadiazole, Benzisot hiadiazole, 3,4-Benzo-1,2,5-thia diazole.*

According to the pubchem website that the compound *4H-1-Benzopyran-4-one, 5,7-dihydroxy-6-methoxy-2-(4-methoxyphenyl)-* has synonymous names *5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3 dihydrochromen-4-one* . This compound is a class of isoflavonoids compounds. According to Yulifianti et al. (2018), Isoflavones are secondary metabolites with antioxidant and anti-inflammatory abilities. This is supported by the research of Rahma et al. (2014), that Isoflavones One of the prominent physiological activities of isoflavones is antioxidant activity as a substance that can delay or prevent the occurrence of free radical anti-oxidation reactions.

MIC Test (Minimum Inhibitory Concentration)

The MIC test results were obtained from the absorbance value where the absorbance value indicated the ability of the extract to inhibit bacterial growth. The results of the MIC test using tembelekan leaf extract on E. tarda bacteria are presented in Table 6.

Based on Table 6 the MIC test using tembelekan leaf extract obtained the lowest absorbance value at a concentration of 100 mg/L with a value of 0.602; close to positive control. This shows that the tube concentration of 100 mg/L has inhibited the growth of *E. tarda bacteria* due to the presence of antibacterial compounds in tembelekan leaf extract.

Table 6. MIC Test Results

Concentration	Absorbance Value
Control (-)	0.986
1mg/L	0.845
10mg/L	0.778
100mg/L	0.602
500 mg/l	0.683
1000mg/L	0.622
Control (+)	0.036

Based on the table above, tembelekan leaf extract at a dose of 100 mg/L was chosen as the minimum dose reference for determining the disc test dose. the spectrophotometer was unable to distinguish between the turbidity of the extract color and the turbidity of the bacteria. According to Gress et al. (2019), the spectrophotometer is unable to distinguish color turbidity from bacterial turbidity so the OD value obtained is a combination of the two.

Disc Test

The purpose of the disc test is to see how much clear zone is formed around the disc paper. The clear zone indicates the ability of tembelekan leaf extract to inhibit the growth of E. tarda bacteria. The disc test was carried out for 24 hours of incubation and 48 hours of incubation with doses of 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, and 250 mg/L. The results of clear zone measurements are presented in Table 7.

Table 7. Clear zone measurement results

Concentration (mg/L)	Average Diameter of Clear Zone(mm)		Clear Zone Classification
	24 hours	48 hours	
K (-)	0.00±0.00	0.00±0.00	Weak
50	0.04±10.34 [a]	0.04±10.24 [a]	Currently
100	0.05±10.75 [b]	0.04±10.63 [ab]	Currently
150	0.28 ± 11.39 [c]	0.26 ± 11.30 [c]	Strong
200	0.68 ± 12.63 [b]	0.68±12.53 [b]	Strong
250	0.05±11.79 [b]	0.03 ± 11.68 [b]	Strong
K(+)	0.26±13.595 [d]	0.26 ± 13.695 [d]	Strong

Note: Classification of zones of inhibition, weak 0-5 mm, moderate 5-10 mm, strong 10-20 mm and very strong >20 (Indri & Januartha, 2020)

The results of measuring the diameter of the inhibition zone at all doses with 24 hours intervals indicate that the number of doses of the extract given can increase the diameter of the inhibition zone. Extract concentrations of 50 mg/L and 100 mg/L were included in the Medium and lower categories with inhibition zones of 10.34 and 10.75 mm while the strong inhibition zones were found at concentrations of 150 mg/L, 200 mg/L and 250 mg/L with an inhibition zone of 11.39; 12.63; and 11.79mm. concentration of 200 mg/L has the highest inhibition zone compared to other treatments. concentration of 200 mg/L was able to inhibit the growth of E. tarda bacteria. This was indicated by the formation of the highest inhibition zone around the disc paper.

The diameter of the inhibition zone with a time of 48 hours showed a decrease in the inhibition zone in all treatments. So in this case the material of tembelekan leaf extract can be categorized as an antibacterial which is bacteriostatic, which is only able to inhibit bacterial growth. Tembelekan leaf extract is not bactericidal, namely antibacterial which can kill bacteria. This is in accordance with the statement of Rabekka et al. (2016), that the content of antibacterial compounds in tembelekan leaves such as flavonoids and alkaloids can inhibit bacterial growth (bacteriostatic) in culture media.

SEM Test

The SEM test showed the effect of giving tembelekan leaf extract (*L. camara* linn) on changes in the structure of the bacterial cell which is presented in Figure 3. Figure A shows the morphology of E. tarda bacteria without any damage to the cell wall, while figure B shows that the administration of antibacterial metabolites of tembelekan leaf extract causes damage to E.tarda bacterial cells. This is due to the influence of antibacterial compounds that can damage the bacterial cell membrane. According to Nimah & Shofiatun (2012), that triterpenoid compounds can damage cell membranes.

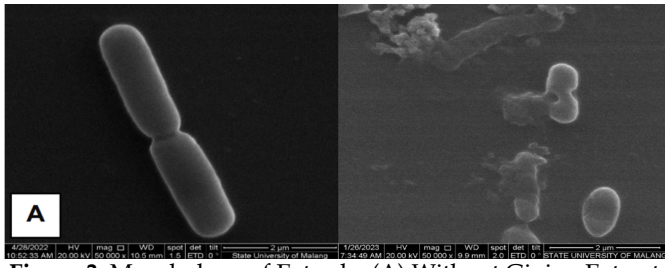

Figure 3. Morphology of E. tarda; (A) Without Giving Extract (B) Giving Red Galangal Extract (Research Documentation, 2023)

The surface of the cell is damaged where the cell looks hollow with different length and diameter. This situation indicates that the metabolites of tembelekan leaves are able to cause changes in membrane permeability, and result in cytoplasmic fluid seeping out so that spaces between cells are formed which will increase membrane porosity due to weakening of the cell wall. The lysis of the cell wall results in the release of large amounts of fluid and causes the cell to shrink and die.

This change is thought to be due to compounds in tembelekan leaf extract which can cause loss of the basic constituents of cells, resulting in swelling which results in cell death. According to Ariani & Riski. (2018), alkaloid compounds play a role in destroying the peptidoglycan component of bacterial cells which results in lysis of the cell wall layer.

Conclusion

Based on the results of the study of the Antibacterial Activity Test of Tembelekan Leaf Extract (*Lantana camara*

linn) against E. tarda bacteria, it can be concluded that tembelekan leaf extract at a dose of 200 mg/L is the best treatment for inhibiting the growth of E. *tarda bacteria.*

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Author Contributions
Research related to natural ingredients has begun to be investigated by scientists because they are considered to be an alternative for disease control in order to minimize the use of antibiotics. One of the natural ingredients currently being developed is the tembelekan plant with the Latin name Lantana camara L. This plant grows wild and has a variety of secondary metabolites. Putri et al. (2018), stated that secondary metabolites in tembelakan leaves have potential as antibacterial compounds. but its use as an antibacterial alternative to E.tarda and its application in fish treatment is not yet optimal, thus, it is necessary to carry out further research and study in detail the active compound content of Lantana camara L. which is useful as an antibacterial.

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Conflicts of Interest
The authors declare no conflict of interest.

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