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THE INHIBITION TEST OF FLAVONOID AND TANNIN IN NONI FRUIT (*Morinda citrifolia* Linn) COMPARED TO CEFTRIAXONE TOWARDS *Escherichia coli* IN VITRO

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ABSTRACT

Background: *Escherichia coli is one of the causatives of infection agents. Noni fruit that contains the antibacterial ingredients was predicted as an alternative medicine to reduce the resistance of antibiotics. Noni fruit extract can inhibit the growth of Escherichia coli. The absence of research on the active substances contained in this fruit which plays a role in inhibiting the growth of Escherichia coli encourages the researcher to conduct this research.*
Methodology: *The research was an experimental laboratory with post-test only control group design. The Escherichia coli grown on Mac conkey agar. Flavonoid and tannin in noni fruit (Morinda citrifolia Linn.) diluted with 3 concentrations, 50%, 70%, 100% and 2 control groups, positive control with ceftriaxone and negative control with Dimethylsulfoxide 10% (DMSO). The treatment group were incubated for 24 hours, then the inhibitory zone formed was measured in mm.*
Results: *The Kruskall-Wallis test showed significant difference in all concentration. The results obtained from this research showed that the average of inhibition towards Escherichia coli on positive control (22.83 mm) had the largest inhibition zone among flavonoid 100% (18.83mm), flavonoid 70% (14.67 mm), flavonoid 50% (6 mm), tannin 100% (17.33 mm), tannin 70% (10.83 mm), tannin 50% (7.50 mm), and negative control (2 mm).*
Conclusion: *Flavonoid and tannin in noni fruit (Morinda citrifolia Linn) were effective for inhibiting the growth of Escherichia coli. The 100% concentration is the most effective concentration that can be promoted as an alternative medicine.*

Keywords: *Noni fruit, Flavonoid, Tannin, Escherichia coli, Inhibition test*

INTRODUCTION

Infectious diseases is one of the main health problems that evolve over time and mostly caused by bacteria, fungi, virus, parasite, etc. *Escherichia coli* is one of bacteria type which causes infection in Indonesia. Gastrointestinal infection, urinary tract infection, pneumonia, meningitis is the diseases caused by *Escherichia coli* [1,2].

There has never been a specific gold-standard antibiotic for treatment against diseases caused by *Escherichia coli*. However, ceftriaxone serves as the most sensitive antibiotic due to numerous high resistance against *Escherichia coli* [3]. Ceftriaxone had mechanism to inhibiting synthesis of bacteria's cell wall and bacteria's enzyme performance. It is provided in a vial and is consumed by injection directly into muscle. Ceftriaxone is prone to the side of effects it caused including nausea, vomitting, stomachache, headache, glossitis, rash, itching, vaginitis, and weariness [4].

Aside from that, to prevent infectious disease, we can use modern medicines and traditional medicines. Modern medicines have many side effects. For example if the drugs not use as recommendation, then

the pathogen will developed a resistency. Traditional medicine is the national cultural heritage which were began to be used for medication. Traditional medicine is often used as an alternative therapy due to less side effects and relatively cheap if compared with modern medicine [5-7].

The traditional plant that popular in society and have many functions is noni fruit (*Morinda citrifolia* Linn)**.** One of that function is antibacterial agent. Noni fruit has antibacterial activity because the phytochemical components are flavonoid, tannin, fenol, terpenoid, steroid, anthraquinone, carbohydrate and glycoside which is proven as bacteriocide [8-10]. Flavonoid and tannin in noni fruit has the strongest anti-bacteria activity as flavonoid which can cause bacteria cell core not to form and tannin which can slow down bacteria enzyme performance [11,12]. Flavonoid and tannin effectively kills bacteria (bacteriocide) [11].

This study aims to compare the flavonoid and tannin of noni fruit (*Morinda citrifolia* Linn) with ceftriaxone to inhibit the growth of *Escherichia coli*.

MATERIALS AND METHODS

This is an in vitro experimental study with post test only control group design with *Escherichia coli* as samples. Sample were pure isolate of *Escherichia coli* grown at Microbiology Laboratory, Faculty of Medicine, Swadaya Gunung Jati University.

The subjects were divided into 8 groups: which were *Escherichia coli* given flavonoid extract in concentration of 50%, 70%, and 100%., and tannin extract in concentration of 50%, 70%, and 100%. A negative control group was given only DMSO 10% and the positive control group was given ceftriaxone. The data were then analysed using Kruskall-Wallis ~~methods (reference?)~~ [13].

Escherichia coli

Pure culture of *Escherichia coli* was taken by inoculation loop, transferred into a test tube and added 1 ml of 0.9% until the turbidity equal to Mc Farland's 0,5 (standard bacteria concentration). Using pour plate method, 1 ml of the dilution was taken, poured in Mac conkey agar as growth media and then homogenized. It was incubated at 37^0C [14].

Noni fruit (Morinda citrifolia **Linn***)*

Separation of active ingredients of Morinda fruit is conducted through some ways. Noni fruit is dried before it is then blended. After blending, ethanol solvent is added and if the maceration and evaporation stages are complete, the ethanol extract of noni fruit is obtained and then stored in the desiccator. After that, it is proceed to the screening stage repeatedly until the active ingredient is separated.

Total Flavonoid Test

Total Flavonoid Test Measurement : 50 mg of the sample was prepared. It was then inserted into a flask and 10 ml of 2N chloride acid was added into it. It was Refluxed for 30 minutes and was then cooled down. Extraction was done with 10 ml of diethyl ether, diethyl ether phase was taken afterwards. The extraction was repeated twice. And diethyl ether phase was vaporized with nitrogen current until it was dry. 0,3 ml of 5% natrium nitrite was then added. And after 5 minutes, 2 ml of 1 M natrium hidroxide was added. Then 10 of aquades was added into a volumetric flask. It was diluted 10 times and was then transferred it into a cuvet. It was then observed at a 510 nm wavelength.

Total Tannin Test

0,1 g of sample was extracted with 10 ml diethyl ether for 20 hours. Then the residue was filtered, boiled with 10 ml aquades for 2 hours, cooled down, and then filtered. The obtained extract was then added by aquades until the volume of the extract reached 10 ml. 1 ml of the extract was then added by 0,1 ml of reagen folin ciocalteu and was then vortexed. Then It was added by 2 ml of 20% Natrium Carbonate and was then vortexed once more. And then aquades was added until the volume reaches 5

ml. Absorbance is observed at 760 nm wavelength after it was incubated for 30 minutes at room temperature.

Determination of inhibition

Samples are incubated at the temperature of 37°C in 2 days and the antibacterial test used was well diffusion method on the Mac conkey agar. The inhibitory power is determined by measuring the diameter expressed in millimetres. The diameter of the inhibition zone is a clear zone without the growth of microorganisms formed around the well [15,16].

RESULTS

From a measurement of antibacterial activity in table 1 showed the flavonoid with 100% concentration has an average antibacterial diameter of 18.83 mm, flavonoid 70% with a diameter of 14.67 mm, flavonoid 50% with a diameter of 6.00 mm, tannin 100% with a diameter of 17.33 mm, tannin 70% with a diameter of 10.83 mm, tannin 50% with a diameter of 7.50 mm, K(-) with a diameter of 2.00 mm, while K(+) given ceftriaxone has an average of 22.83 mm. Flavonoid and tannin in noni fruit with the best antibacterial property is the concentration of flavonoid 100%, followed by flavonoid 70%, flavonoid 50%, tannin 100%, tannin 70%, and tannin 50%.

Table 1. Antibacterial effectiveness of flavonoid and tannin noni fruit on *Escherichia coli*

No	Groups	Diameter of antibacterial activity (mm)			Average (mm)	Antibacterial activity [15]	p value
		1	2	3			
1.	K (+)	3	3	2	22.83	Very strong	0,03
2.	K (-)	22	22	20	2.00	Weak	
3.	Flavonoid 50%	10	8	6	6.00	Weak	
4.	Flavonoid 70%	16	14	12	14.67	Strong	
5.	Flavonoid 100%	20	16	16	18.83	Strong	
6.	Tannin 50%	10	10	8	7.50	Weak	
7.	Tannin 70%	12	12	10	10.83	Moderate	
8.	Tannin 100%	18	16	14	17.33	Strong	

Normality test using Shapiro-Wilk showed p-value = 0.00 ($p>0.05$) on 5 groups, which means the population is not normally distributed. The data analysis was then continued using Kruskal Wallis. From Kruskal-Wallis test, it showed p value of 0.03 ($p<0.05$) which means that there was significant difference in antibacterial activity in all groups.

Table 2. Mann-Whitney post hoc test

	F100%	F70%	F50%	T100%	T70%	T50%	K(+)	K(-)
F100%								
F70%	0.105							
F50%	0.046*	0.050*						
T100%	0.487	0.261	0.050*					
T70%	0.043*	0.105	0.072	0.046*				
T50%	0.043*	0.046*	0.346	0.046*	0.099			
K(+)	0.068	0.046*	0.046*	0.046*	0.043*	0.043*		
K(-)	0.043*	0.046*	0.046	0.046*	0.043*	0.043*	0.043*	

In table 2. Mann-Whitney test result showed no significant difference between flavonoid 100% and flavonoid 70%, tannin 100% and K (+) with $p > 0.05$. This means that has similar strength in antibacterial activity to inhibit *Escherichia coli* growth.

DISCUSSION

The *Escherichia coli* bacteria are macroscopically yellowish white, convex round, smooth and shiny with flat edges [16-18]. Microscopically with Gram staining, it appears a bacterial colony in the form of pink *coccobacil*. *Escherichia coli* bacteria show pink results because they have a cell wall composition that is mostly composed of a layer of lipids that are easily damaged when washed with alcohol. No wonder, they cannot maintain crystalline violet dyes when colored and the safranin will turn pink when stained [19,20].

Based on data analysis using the Kruskall-Wallis alternative test, the results obtain $p < 0.05$, this shows the mean difference between treatment groups. The Mann-Whitney post hoc test obtains the value of $p < 0.05$ in the 20 pairs of treatment and control groups. It can be interpreted that there is a difference in the effectiveness of the group in inhibiting the growth of *Escherichia coli* bacteria. Then, 8 pairs of treatment and control groups show a p value > 0.05 which can be interpreted as having the same effectiveness in inhibiting the growth of *Escherichia coli* bacteria [13].

The antibacterial activity of flavonoid and tannin in noni fruit (*Morinda citrifolia* Linn) can be classified based on its inhibition zone diameter. Ponce et al, classifies antibacterial activity into 4 levels, those are weak (not sensitive), moderate (sensitive), strong (very sensitive) and very strong (extremely sensitive). Antibacterial activity is said to be weak if the inhibition zone diameter is < 8 mm, moderate is 8-14 mm, strong is 15-19 mm, and very strong if > 20 mm [15].

Additionally, the calculation of the zone of noni fruit flavonoids (*Morinda citrifolia* Linn) shows that the average diameter of the smallest inhibition zone is 6 mm with a concentration of 50%. Meanwhile, concentration of 70% shows a mean diameter of 14.67 mm and the largest diameter of inhibition zone is 18.83 mm with a concentration of 100 %. The results of this study indicate that the flavonoids of noni fruit (*Morinda citrifolia* Linn) have an antibacterial effect on the *Escherichia coli* bacteria. Flavonoids are antibacterial through 3 mechanisms, those are inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism. The mechanism of action of flavonoids in inhibiting nucleic acid synthesis is conducted through ring B on flavonoids which have an important role in the process of intercalation or hydrogen bonds by accumulating nucleic acid bases that inhibit DNA and RNA synthesis. Flavonoids inhibit the function of bacterial cell membranes through complex bonds with soluble extracellular proteins that can disrupt the integrity of bacterial cell membranes. The disruption in the permeability of the cell membrane will affect the electrochemical gradient of the proton that passes through the membrane. The electrochemical gradient of protons across membranes is very important for bacteria in synthesizing ATP, membrane transport and bacterial movement, so that the presence of flavonoids will cause disruption of proton motive forces which results in disruption of ATP synthesis, membrane transport and bacterial movement. Besides, the inhibition of bacterial energy metabolism by flavonoids is done by inhibiting the process of bacterial respiration so that the presence of inhibited energy will affect the activity of metabolite absorption and biosynthesis of bacterial macromolecules [12,21].

The calculation of the tannin inhibition zone noni fruit (*Morinda citrifolia* Linn) shows that the average diameter of the smallest inhibition zone is 7.5 mm with a concentration of 50%. Meanwhile, the concentration of 70% shows a mean diameter of 10.83 mm and the average diameter of the largest inhibition zone is 17.33 mm with 100% concentration. The results of this study indicate that noni fruit tannins (*Morinda citrifolia* Linn) have an antibacterial effect on the *Escherichia coli* bacteria. The content of tannins in noni fruit has antibacterial action associated with its ability to deactivate bacterial adhesives, inhibit the action of enzymes and inhibit protein transport in the cell sheath. The mechanism of action of tannin as an antibacterial agent includes destruction of bacterial cell membranes due to tannin toxicity and the formation of metal ion complex bonds from tannins which play a role in tannin toxicity. Bacteria that grow in aerobic conditions require iron for various functions, including reduction of ribonucleotide DNA precursors. The bond between tannins and iron will cause disruption of various bacterial functions. Samaranayake points out that *Escherichia coli* is an anaerobic facultative bacteria so

that they can still live in aerobic conditions and if living in aerobic conditions, the bacteria will be disrupted by the presence of tannin compounds as the study is conducted in aerobic conditions [12].

In this study, a concentration of 100% is the most effective concentration of both flavonoids and tannins of noni fruit (*Morinda citrifolia* Linn). This is in line with research conducted by Prabuseenivasan et al and Jantan et al that the extracts will work better at higher concentrations [22,23].

This study has 2 control groups, namely the negative control group and the positive control group. The negative control group uses DMSO 10% (*Dimethylsulfoxide*) solvents and a positive control group uses ceftriaxone. Negative control aims to see if there is a delay in the use of DMSO. Based on the results of this study, the negative control group has a mean inhibition zone with a diameter of 2 mm which can be interpreted as having a weak inhibition (not sensitive). Positive control aims to compare the effectiveness of negative controls and active substances of flavonoids and tannins of noni fruit (*Morinda citrifolia* Linn). The positive control group using ceftriaxone shows the average inhibition zone with a diameter of 22.83 mm which can be interpreted as having a very strong inhibitory power. Both of these indicate that the control group has antibacterial properties against *Escherichia coli* with both the weak and very strong inhibitory power. DMSO 10% of the research was not sensitive in killing *Eschericia coli* bacteria. DMSO can be bacteriostatic (inhibiting bacteria growth, but not killing it) if used at high concentration [24,25]. The inhibitory effect of ceftriaxone shows significant results. The mechanism of ceftriaxone as an antimicrobial is inhibiting cell wall synthesis where the cell wall functions to maintain the structure of bacteria. Damage to the cell wall or resistance to formation will cause cell lysis [26,27]. However, the continued use of ceftriaxone can have a negative effect in the form of resistance to the antibiotic. The occurrence of resistance is caused by the decomposition of antibiotic enzymes in microorganisms.

The results of this study indicate that the content of noni fruit (*Morinda citrifolia* Linn) can inhibit the growth of *Escherichia coli* bacteria and proved to be antimicrobial against the *Escherichia coli* bacteria. This proves that the hypotheses that have been compiled previously are correct. This research is expected to be an alternative for the treatment of diseases caused by *Escherichia coli*. We suggest the next research to observe the other active substance of noni fruit (*Morinda citrifolia* Linn) with the same of bacteria or other bacteria.

CONCLUSION

The group of flavonoids and tannins of noni fruit (*Morinda citrifolia* Linn) with a concentration of 100% has the greatest inhibitory power compared to other concentrations and flavonoids are more effective in inhibiting the growth of *Escherichia coli* bacteria compared to tannins and they have an equivalent effectiveness with ceftriaxone, making it an suitable natural remedy for bacterial infections.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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