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Sensitivity Test of Bacterium (*Escherichia coli)* against Brotowali's Extract (*Tinospora crispa*)

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Abstract
In general, a bacterium such as *Escherichia coli* produces a kind of toxic protein which can disrupt intestinal wall. Livestock reacts to these toxins by pumping lots of water into the intestine in order to rinse or flush these toxins. As a result, the livestocks have diarrhea as a body response to remove the toxin in the digestive system. In the presence of these problems, breeders take a measure such as using antibiotics freely. Among breeders, antibiotics are often used freely without knowing the indication and the appropriate dose for the treatment of livestock diseases. It is caused due to the antibiotics easily obtained in animal drugstores which leading to bacterium resistance to these antibiotics. Another alternative done by the breeders is by using plants as medicinal herb such as brotowali (*Tinospora crispa*). The purpose of this research is to examine Escherichia coli bacterium sensitivity against brotowali extract. This research is descriptive with maceration technique as the method of extract production.The study was conducted by in vitro usingTinosporacrispaextract with a concentration of 0.5%, 2%, 8%, and 32%. A positive control using amoxicillin and negative control using DMSO10%. The result of the research shows that Tinosporacrispa extract with the concentration of 0.5% and 2.0% are not more sensitive than amoxicillin, whereas the concentration of 8.0% and 32% are more sensitive than amoxicillin which seen from formed inhibition zone. The content found in Tinosporacrispa such as alkaloids, flavonoids, and saponins are antibacterial which has a satisfying effect to kill *E.coli*.

Keywords : *Antibacterial* Tinosporacrispa *branches, Escherichia coli*

1. INTRODUCTION

Among breeders, antibiotics often used freely without understanding their effect and the appropriate dose for the livestock diseases treatment. One example where breeders' treatment could harm the consumer is to mix the milk from cow that is treated with antibiotic with a healthy cow's milk. As a result, the milk becomes infested because it contains antibiotic residues (Murdiati, 2004). Some antibiotics can work well on Gram-negative bacteria and some others are more effective on Gram-positive bacteria (Syamsuni, 2005). Uncontrolled use of antibiotics tends to increase the bacteria's resistance that initially sensitive (Noviana, 2004). *Tinospora crispa* contains effective chemical compounds to treat various diseases, namely abdominal pain, diarrhea, and fever. The roots contain alkaloids berberine and kolumbin. The leaves and stems contain alkaloids, saponins, and tannins. While the trunk contains flavonoids, alkaloids, and saponins (Kresnady, 2003). In-country communities often use herbs such as *Tinospora crispa* to treat animals illness, such as ulcers, fever, and even worm infestation (Apriani, 2013). Siregar (2010), states that *Tinospora crispa* extract has an antimicrobial effect against *Pseudomonas aeruginos*a, with a minimum inhibitory concentration

of 5% and 6% minimum levels of extermination. According to Zakaria *et al.*, (2006) *Tinospora crispa* have antibacterial activity against *Corynebacterium diphtheriae, Streptococcus pneumoniae, Shigella flexneri* and antifilarial, antipyretic and antihyperglycemic. Puspita 2010 stated one compound that purportedly acted as an antibacterial in brotowali is flavonoid. Brotowali extract (*Tinospora crispa*) was reported to hold the nitric oxide 9's release and synthesis, which plays a role in inflammatory processes (Pratt, 2015) Based on this, to determine the bacteria's sensitivity (*Escherichia coli)* to Tinosporacrispaextract, then the sensitivity test is applied to *Escherichia coli*.
Escherichia coli is a member of Enterobacteriaceae bacteria family which has Gram-negative, coccoid shape, irregular arrangement, nonspore, and most of them are mobile (flagellum Peritrik). The Colony's morphology is round, convex, and has flat edges (Simatupang, 2006). **Aims** ,this study aims to determine *Escherichia coli's* sensitivity to*Tinospora crispa extract*.

2. METHODS

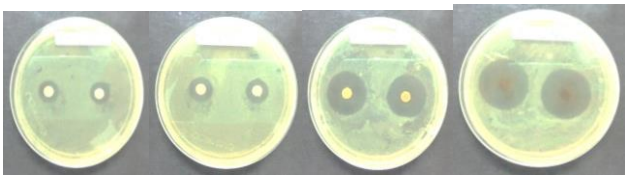
Tinospora crispa extraction by using ethanol 70%. Media used are *Eosin Methylene Blue Agar* (EMBA), Media Mullet Hinton Agar (MHA), Gram stain. Determination of *Escherichia coli*'s sensitivity to *Tinospora crispa* extract is by the standards of inhibition zone. Positive controls use Amoxicillin.

3. RESULT AND DISCUSSION

Tinospora crispa extraction process to obtain the desired active ingredient with 70% ethanol as ethanol is one of the solvents that can bind to the desired active substances such as flavonoids, alkaloids, and flavonoids content saponin. Flavanoid's concentration test with the addition of 0.1 mg of magnesium powder will form a layer of red. Saponins Testing is by foam-test. 2 grams of brotowali extract is added with distilled water and heated to visible foam. If the result is positive, for 30 minutes, the foam will be stable and remains. If Saponin is interacting with the bacterial cells, the bacteria will be damaged or lysis.

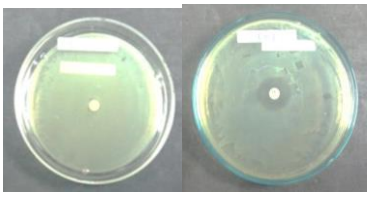
Flavonoids are a group of phenolic compounds that have a tendency to bind to proteins, thereby disrupting metabolic processes (Poeloengan *et al*., 2010). Alkaloids have an antibacterial ability, by interfering with peptidoglycan constituent component of the bacterial cell, so the cell wall layers are not fully formed and cause the cell to death (Santoso and Purwanto, 2012). *Escherichia coli* on media EMBA grow greenish with gloss metallic. Media EMBA has a certain specialty, that is it contains lactose and serves to sort out the microbes that ferment lactose (*Escherichia coli*) with bacteria that do not ferment lactose such as *Staphylococcus aureus.*
E.coli Sensitivity testing Against *Tinospora crispa* Stem.

Inhibition zone is formed by the interaction between the content of the *Tinospora crispa* extract with *E.coli*. In the inhibition zone, there is translucent zone formed around the disk.



A B C D

Figure 1. Zone of Inhibition With a concentration of 0.5% (A), 2% (B), 8% (C) and 32% (D)



A B

Figure 2. A.Negative control (ADMSO10%)and B positive control (Amoxicyllin)

Table 1. Results of Inhibition Zone Diameter from Tinosporacrispaextract test against *E. coli* (mm)

Concentration	Iteration				Median
	ZH1	ZH2	ZH3	ZH4	
0,5%	9	10	9	10	9,5
2,0%	10	11	11	11	10,75
8,0%	22	20	20	21	20,75
32%	25	30	22	23	25
Control (+)	19				
Control (-)	0				

ZH = Zone of Inhibition

Table 1 shows that a concentration of 0.5% with a diameter of 9-10 mm inhibition zone and 2.0% with inhibition zone diameter 10-11 mm, has a lower sensitivity compared to Amoxicillin 19 mm. Whereas the 8.0 % concentration of inhibition zone with a diameter of 20-22 mm and at a concentration of 32% inhibition zone with a diameter of 22-30 mm have a high level of sensitivity compared to Amoxicillin. The compound in *Tinospora crispa* has a great effect of killing the *E.coli*. Inhibition zone on the extract *Tinospora crispa* is larger than the inhibition zone formed on the positive control Amoxicillin.

Flavonoids are the largest of phenol and have a mechanism for obstructing the bacteria growth by doing proteins (enzymes) inactivation on in cell's membrane, resulting in protein structure becomes damaged. Saponins compound can perform obstruction mechanism by forming a complex compound with the cell membrane using hydrogen bonds, thus could disintegrate the cell's wall permeability and ultimately lead to cell death.

Alkaloids have the ability as an antibacterial, by interfering with peptidoglycan constituent component of the bacterial cell, so the cell wall layers will not be fully formed and cause cell death (Santoso and Purwanto, 2012).

4. CONCLUSION

Tinospora crispa extract had no effect on the bacteria *Escherichia coli*. At a concentration of 0.5% and 2.0% insensitive compared with Amoxicillin while at a concentration of 8.0% and 32% more sensitive. *Tinospora crispa* extract with a concentration of 8% can inhibit the growth of *E. coli.*

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