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# Antibacterial Activity Test Of Epazote Leaf Infusion (*Dysphania ambrosioides* L.) Against *Escherichia coli* Bacteria

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***ABSTRAK***

*Masalah kesehatan yang paling umum di Indonesia adalah infeksi bakteri. Dysphania ambrosioides L., juga dikenal sebagai daun epazote, adalah tumbuhan herbal yang memiliki potensi antimikroba. Penelitian ini dilakukan untuk melihat pada konsentrasi berapa terdapat aktivitas antibakteri Infusa daun epazote Dysphania ambrosioides L. terhadap bakteri Escherichia coli. Penelitian eksperimental laboratorium ini menggunakan desain penelitian racangan acak lengkap (RAL). Pengujian aktivitas antibakteri daun epazote dilakukan dengan menggunakan metode difusi kertas cakram dengan konsentrasi 50%, 75%, dan 100%. Daun epazote direbus dalam panci infus selama 15 menit, sampai suhu mencapai 90 derajat Celsius. kontrol positif ampicilin 10 µg/disc. Berdasarkan hasil pengujian metabolit sekunder Infusa daun epazote positif ada alkaloid, flavonoid, tanin, saponin, triterpenoid, dan fenolik. Hasil penelitian menunjukkan bahwa Infusa daun epazote menghambat bakteri Escherichia coli dengan diameter hambatan. Rata-rata pada konsentrasi 50%= 14,04 mm, 75%= 16,92 mm, 100%= 17,94 mm, yang mengartikan bahwa adanya aktivitas antibakteri dari Infusa daun epazote terhadap bakteri Escherichia coli.*

**Kata kunci:** *Dysphania ambrosioides* L.*, Antibakteri, Epazote, Infusa, E.Coli*

***ABSTRACT***

*The most common health problem in Indonesia is bacterial infection. Dysphania ambrosioides L., also known as epazote leaves, is an herbal plant that has antimicrobial potential. This research was conducted to see at what concentration there is antibacterial activity of epazote leaf infusion Dysphania ambrosioides L. against Escherichia coli bacteria. This laboratory experimental study used a complete randomized design (RAL) research. Antibacterial activity testing of epazote leaves was carried out using the disc paper diffusion method with concentrations of 50%, 75%, and 100%. Epazote leaves are boiled in an infusion pan for 15 minutes, until the temperature reaches 90 degrees Celsius. Ampicillin positive control 10 µg/disc. Based on the test results of secondary metabolites of positive epazote leaf infusion there are alkaloids, flavonoids, tannins, saponins, triterpenoids, and phenolics. The results of the study showed that infusion of epazote leaves inhibited Escherichia coli bacteria by diameter inhibition. Average at a concentration of 50% = 14.04 mm, 75% = 16.92 mm, 100% = 17.94 mm, which means that there is antibacterial activity of epazote leaf infusion against Escherichia coli bacteria.*

***Keywords***: *Dysphania ambrosioides* L., *Antibacterial, Epazote, Infusion, E.Coli*

## 1. INTRODUCTION

Bacterial infections are still common in developing countries. The unhealthy lifestyle of the people is a factor in the lack of awareness in maintaining health[1]. If the *Escherichia coli* bacteria gets outside the gut, it has the potential to cause infections of the urinary tract, bile ducts and other parts of the abdomen. It can also cause diarrhea and urinary tract infections[2].

Epazote leaves or often called sambote leaves are empirically used by the people of North Sulawesi, especially those in Taraitak

Langowan Village, to treat gout and reduce pain in the abdomen and head.

Previous research has stated that epazote leaves contain alkaloids, flavonoids, saponins, tannins, triterpenoids, phenolics, compounds, terpenes, sesquiterpene pigmols, xylosides, coumarins, and essential oils, which can provide antibacterial, antioxidant, and antifungal effects[3]. Metabolite compounds that have the potential to be antibacterial are saponins, tannins, alkaloids, flavonoids, triterpenoids and phenolics[4].

From the description above, researchers want to investigate how the antibacterial activity of epazote leaf infusion (*Dysphania ambrosioides L.*) against *Escherichia coli* bacteria can be tested by looking at the inhibition area formed. This study aims to see at what concentration there is antibacterial activity of epazote leaf infusion (*Dysphania ambrosioides L.*) against *Escherichia coli* bacteria.

## 2. RESEARCH METHODS

### Tools and Materials

Tools: Stationery, gloves, mask, lab coat, camera, container, knife, scale, beaker glass, measuring cup, bunsen, bunsen burner, stirring rod, spatula, flannelette, Erlenmayer, test tube, test tube rack, cotton swab, sterile gauze, incubator, autoclave, aluminum foil, blender, mortar and pestle, 9cm petri dish, 8mm paper disc, millimeter ruler, glass funnel, tweezers, cling wrap, pipette, tip and Infusion pot.

Materials: Epazote leaves (*Dysphania ambrosioides L.*), nutrient agar (NA), *Escherichia coli* bacteria Nutrient Broth (NB), 70% alcohol, distilled water, acetic acid, H2SO4, FeCl3 with 1% and 5% concentration, concentrated HCl, Magnesium, Dragendorff solution, Wagner solution, Mayer solution, McFarland 0.5 solution and ampicillin as positive control.

### Research Type and Design

This study is a type of laboratory experimental research that uses the Completely Randomized Design (CRD) method. by doing 3 repetitions with each of 5 treatments, namely 3 concentrations of epazote leaf infusion, 1 negative control and 1 positive control for one type of bacteria. This study used diffusion technique to test antibacterial. Kirby Bauer method, which involves the use of paper discs with agar diffusion media. The concentrations of Epazote (*Dysphania ambrosioides L.*) leaf infusion used were: 50%, 75%, 100% concentration, the positive control used was 10 µg/disc ampicilin antibiotic disc, while the negative control was sterile distilled water.

Description:

I : first test
II : Second test
III : Third test
A : Epazote Leaf Infusion 50%
B : Epazote Leaf Infusion 75%
C : Epazote Leaf Infusion 100%
D : Positive Control Ampicilin 10µg/disc
E : Negative Control Aquades

### Research Procedures

1. Sampling
   For this research, fresh green epazote leaf samples were taken from the Langowan area, precisely in Taraitak Village. Samples were cleaned, separated from the stems, dried, weighed as much as 20gr and then blended into smaller parts to facilitate the collection of active substances during infusion.
2. Preparation of Epazote Leaf Infusion
   Epazote leaves that have been weighed as much as 20gr, then extracted in Infusion using distilled water as a solvent as much as 30mL. From this Infusa process, a golden yellow liquid extract is obtained and then put into an erlenmeyer. The epazote leaf Infusa obtained is a 100% concentration. Then use the dilution formula to determine the concentration of 50%, 75% and 100% concentration.

Dilution of solutions in stoichiometric theory[5]:

Dilution formula: M1.V1 = M2.V2.....(1)

Description:

M1 =Stock concentration of epazote leaf Infusa
M2 =Final Concentration
V1 =Volume of epazote leaf Infusa used
V2 =Final volume

Extraction is a technique carried out in the extraction of secondary metabolite compounds that are targeted to be separated from the pulp or parts that are no longer needed, because they interfere with the presentation or interfere with the effectiveness of the efficacy of the active ingredient[6].

There are two types of extraction, namely, cold extraction such as maceration and percolation while hot extraction is carried out by

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reflux, soxhlet, steam distillation, decoction and infusa. The purpose of extraction is to extract or separate compounds from their mixtures or simplisia. Generally, when the surface of the simplicia powder comes into contact with the solvent, the extraction process is better[7].

**Table 1.** Dilution Volume of Epazote Leaf Infusion Concentration

| No | M1 | V1 | Vol Aquades | M2 | V2 |
|---|---|---|---|---|---|
| 1 | 100% | 5ml | 5ml | 50% | 10ml |
| 2 | 100% | 7,5ml | 2,5ml | 75% | 10ml |
| 3 | 100% | 10ml | - | 100% | 10ml |

**Preparation of Media for Test Bacteria**

1. Preparation of Test Bacteria Suspension
   Mix Nutrient Broth (NB) as much as 0.8gr with 100mL of distilled water in an erlenmeyer and then sterilized. Then pour 1ml of *Escherichia coli* bacteria into the NB solution, mix until homogeneous then pour into a test tube and incubate for 24 hours, take and shake until homogeneous. Then see the level of turbidity, which is comparing according to the McFarland 0.5 standard solution visually.
2. Preparation of Solid Media
   The first step is to mix 2.07 grams of nutrient agar (NA) with 90 ml of distilled water in an erlenmeyer. Mix then homogenized by stirring, then sterilized using an autoclave for 20 minutes at 121°C.
3. Preparation of Positive Control Solution
   Positive control solution is made by grinding and weighing 500mg ampicillin preparation so that 50mg ampicillin powder is obtained. The powder was then dissolved in 50ml of distilled water. As a negative control, pure distilled water was used.
4. Antibacterial Activity Testing
   Kirby-Bauer antibacterial test. The disc paper that has been photographed with the test solution with a concentration of 50%, 75%, 100%, positive control 10µg and distilled water, then dried. Place the disc paper on the test media in a petri dish that has been marked and incubated for one twenty-four hours in an inverted position at 37° Celsius. After twenty-four hours of incubation, observations were made to determine whether there was a zone of inhibition formed around the disc paper. Furthermore, a yardstick was used to measure the area of the zone of inhibition.

**Data Analysis**

The results of observation and measurement were processed and tabulated in the form of a test table of epazote leaf infusion against *Escherichia coli* bacteria. Furthermore, the data obtained was processed by variant analysis (ANOVA). If there is a significant difference, then continue with the Tukey HSD test to find out which treatment gives different effects[8].

## 3. RESULTS AND DISCUSSION

The results of phytochemical screening of epazote (*Dysphania ambrosioides L.*) leaf infusion are documented in Table 2. The examination of alkaloid, flavonoid, tannin, saponin, triterpenoid, and phenolic compound groups showed positive results. This indicates the presence of these compounds in epazote (*Dysphania ambrosioides L.*) leaf Infusa, which can function as antibacterial.

**Table 2.** Phytochemical Screening Results of Epazote Leaf Infusion (*Dysphania ambrosioides L.*)

| Compound Class | Reagents | Result | Color change |
|---|---|---|---|
| Alkaloid | "Dragendorff" | + | Orange deposits can be seen |
| | "Wagner" | + | Brown deposits are visible |
| | "Mayer" | + | Visible white deposits |
| Flavonoid | HCl Pekat dan Mg | + | Red color formed |
| Tanin | Etanol dan $FeCl_3$ | + | Formed a green color |
| Saponin | Aquades | + | Bubbles/ bubbles are formed |
| Steroid | Etil asetat dan $H_2SO_4$ pekat | - | No color change |
| Triterpenoid | Etil asetat dan $H_2SO_4$ pekat | + | Orange color formed |
| Fenolik | FeCl 5% dan $H_2SO_4$ pekat | + | Formed in brown orange color |

Ket : (+) There is a test compound
(-) Showed the tested compound did not contain epazote leaf infusion

Alkaloids work as antibacterial by how to interfere with the constituent components peptidoglycan on bacterial cells up to th The cell wall is not formed intact and causing cell death[9].

The results obtained in the phytochemical screening carried out using concentrated HCl and Mg reagents were formed in red. This shows that the epazote leaf infusion contains flavonoid compounds which are characterized by color change and positive results. According[10]. Positive results were shown in flavonoid testing with the occurrence of red color after the addition of Mg and HCl powders was shown by the sample. The metal fist of Mg and HCl functions to reduce the benzopyrone nuclei found in the flavonoid structure and form a red or orange flavilium salt.

The tannins obtained from the test results were positive, the tannin test was known from the color change that occurred after the addition of 1% FeCl solution, which was green color.

From the test results on the epazote leaf infusion sample, it was known to be positive for the presence of saponin compounds which were characterized by the formation of foam after shaking and did not disappear after 10 minutes. According to[11]. from the results of his research, the ability to form foam in hydrolyzed water into glucose and aglione compounds.

The test results of triterpenoid and steroid compounds produced are positive to contain triterpenoid compounds with orange color change after the addition of anhydride acetate and concentrated sulfuric acid.

Meanwhile, the testing of steroid compounds showed negative results. Negative results were shown because there was no color change. Based on previous research[12], the addition of acetic acid and sulfuric acid bound to terpenoid/steroid compounds can produce color changes to red, orange or purple.

Positive results in the testing of phenolic compounds occurred a brown-orange color change. According[13], the reaction of FeCl with the sample makes the formation of color in this test. The color change is due to the role of ions that undergo hybridization so that there is a color change from brownish-yellow to orange-brown which indicates the presence of phenolic compounds.

**Antibacterial Test Results**

The results show the diameter of the inhibition zone of each concentration of epazote leaf infusion shown in Figure 1, which has been tested on Petri dishes 1-3 has antibacterial activity that attacks *Escherichia coli* bacteria.

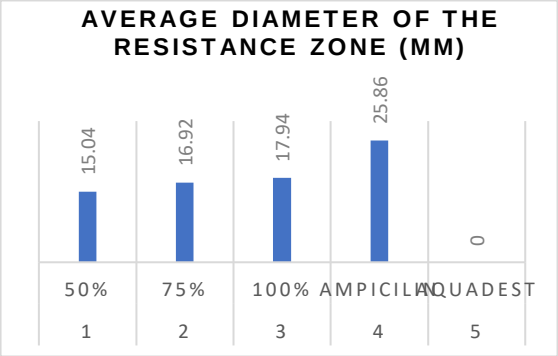


**Figure 1**. Inhibition Zone Diameter Chart (mm)

From Figure 1, it is shown that at a concentration of 50% with an average diameter of an inhibitory zone of 15.04 mm, a concentration of 75% with an average diameter of an inhibitory zone of 16.92 mm, a concentration of 100% with an average diameter of an inhibitory zone of 17.94 mm, a positive control of an ampicillin antibiotic with an average diameter of an inhibitory zone of 25.86, and an inhibitory control of aquadest has no inhibitory zone. Thus, each concentration of epazote leaf infusion has a different inhibitory zone.

With the presence of an inhibition zone against bacteria as seen from the average diameter of the inhibition zone of each treatment, it can be seen in the diagram of the epazote leaf infusion rod in figure 1.

In the bar diagram of Figure 1, it shows that the diameter of the infusion of epazote leaves against the growth of *Escherichia coli* bacteria. The inhibition zones formed at concentrations of 50%, 75%, and 100% are included in the medium category. Meanwhile, the control using aquadest is not in the form of an inhibition zone. Aquadest is a neutral compound that has no effect on bacterial growth and is free from contamination that can affect research[14].

From the results of the antibacterial test of epazote leaf infusion against *Escherichia coli* bacteria, it can be stated that the greater the concentration, the greater the inhibitory zone formed. The results also showed that the use of epazote leaf infusion at a concentration of 50% was sufficient to inhibit the growth of *Escherichia coli* bacteria, although seen from

different values or figures, but statistically did not have a significant difference.

Alkaloid compounds perform two functions: They stop the constituent parts of peptidoglycan in the bacterial cell wall and help the topoisomerase enzyme inhibit bacterial cell DNA[15].

Flavonoids act as antibacterials, producing complex chemicals dissolved in proteins that have the ability to damage the bacterial cell membrane, stop enzymes from working, and then remove the compound from the cell[16]. In addition, flavonoids have the potential to disrupt bacterial cell membrane function and bacterial energy metabolism. This occurs because the bacteria cannot utilize the oxygen and energy necessary to function[17].

Saponins disrupt the stability of the bacterial cell membrane. They damage the cell membrane and remove proteins, nucleic acids, and nucleotides from the bacterial cell, inhibiting bacterial survival[18].

Tannins inhibit the synthesis of bacterial cell walls, causing cell wall shrinkage and cell wall leakage, leading to bacterial cell lysis. Tannins attack the polypeptide wall of the cell wall, causing the formation of the cell wall to be incomplete, which inhibits the metabolic process of the bacterial cell, causing the cell to die[19].

The antimicrobial properties of triterpenoids allow them to damage the yeast cell membrane or impair lipid membrane synthesis. This results in membrane permeability, which allows parts of the cell to escape[20].

The data obtained from the infusion of epazote leaves against the growth inhibition of *Escherichia coli* bacteria then, the data were processed using SPSS software, and for statistical analysis, the parametric method used was one-way Analysis of Variance (ANOVA). Previously, normality and homogeneity tests must be performed on the data to be analyzed. If both conditions are met, further processing is done with the ANOVA test.

**Table 3.** Anova Test

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Between Groups** | 1065.905 | 4 | 266.476 | 45.667 | 0.000 |
| **Within Groups** | 58.352 | 10 | 5.835 | | |
| Total | 1124.257 | 14 | | | |

The results of the normality test of normally distributed data are seen from the significance of 0.069> 0.05, meaning that the data is accepted or the data meets the requirements. Based on the homogeneity test, it shows that the data entered is homogeneous. The data meets the requirements for normality and homogeneity tests because the significant value of 0.066 is greater than 0.05.

The results of the ANOVA test data in table 3, seen from the sig value = 0.000 <0.05. states that the data that has been tested has a significant difference in each treatment concentration of 50%, 75%, and 100%. Because there is a significant difference, the Tukey HSD test (truly significant difference) is used.

**Tabel 4.** Tukey HSD Test

| Treatment | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Negative Control | 3 | .0000 | | |
| Concentration 50% | 3 | | 15.0267 | |
| Concentration 75% | 3 | | 16.9200 | |
| Concentration 100% | 3 | | 17.9400 | |
| Positive Control | 3 | | | 25.8667 |
| Sig. | | 1.000 | .597 | 1.000 |

From the Tukey HSD analysis in Table 4, it can be seen that there is no significant difference between the effect of epazote leaf infusion on *Escherichia coli* bacteria. There are 3 treatment subsets, namely subset 1 by negative control treatment, subset 2 by 50%, 75% and 100% concentration treatment and subset 3 by positive control treatment. This means that concentrations with the same subset have almost identical inhibitory power and are not significantly different which is detected because they are included in the same group.

## 4. CONCLUSION

The results showed that the infusion of epazote leaves inhibited Escherichia coli bacteria. However, to make epazote leaves safer for public consumption, further research is needed.

Based on statistical tests of epazote leaf infusion at 50% concentration is the same as 75% concentration and 100% concentration, because it is in the same subset, in other words, the use of 50% concentration epazote leaf infusion is sufficient to inhibit the growth of

*Escherichia coli* bacteria, although the numbers and values are different, but there is no significant statistical difference.

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