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Antimicrobial Potential Combination Formulation of 1:2:3 Methanol Extract of Tambora Leaf (Ageratum conyzoides L), Sembalit Angin Leaf (Mussaenda frondosa L), and Turmeric Rhizome (Curcuma longa) Against Escherichia coli

Ratih Widyastuti[1], Noor Hujjatusnaini[1], Nurul Septiana[1], Astuti Muh. Amin[2]*

[1]*Departement of Biology Education, FTIK, IAIN Palangka Raya, Central Kalimantan, Indonesia.*
[2]*Departement of Biology Education, FTIK, IAIN Ternate, Ternate, North Maluku,* Indonesia.
**email: astutimuhamin@iain-ternate.ac.id*

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Abstract
This study aims to determine the potency of the combination formulation of Tambora leaves, Sembalit Angin leaves and turmeric rhizome using a 1: 2: 3 combination formulation and to determine its inhibitory power against the growth of Escherichiacoli bacteria in vitro which is measured based on the width of the inhibition zone or clear zone from the outermost side aper disc containing methanol extract, a combination formulation of Tambora leaves, Sembalit Angin leaves, and turmeric rhizome with Escherichiacoli bacteria colonies on the surface of the Na plate medium. Data collection and measurement of the growth of Escherichiacoli bacteria were carried out during the incubation period of 1x24 hours, 2x24 hours and 3x24 hours. The data obtained were then analyzed using the Anava one way analysis statistical test and continued with the 1% Duncan test. The results found that the combination formulation of 1: 2: 3 bioherbal methanol extract of Tambora leaves, Sembalit Angin leaves, and Turmeric rhizomes had a significant effect on the growth of Escherichiacoli at 1% significance, as evidenced by the sig value. 0.00 <0.01, so the 1: 2: 3 combination formulation can be recommended as the most effective combination extract formulation in inhibiting the growth of Escherichiacoli bacteria.

INTRODUCTION

Indonesia is a country that has various types of biological natural resources. One of province is Central Kalimantan, which is located at the equatorial latitude, making the potential for various forest products not only in the form of wood but also plants with lianas and ferns (Hujjatusnaini et al., 2021). Those plants have many benefits because they can contribute to humans as an ingredient of traditional medicine or infection. Infections are mainly caused by bacteria. Consequently, antibacterial is understood as a substance that can inhibit or even kill microbes that cause disease. Series of studies to determine the active substances contained in plants were carried out in order to find out what properties are contained in these plants (Handayani & Novaryatiin, 2016) (Friska et al., 2021).

These nutritious plants, such as Tambora, Sembalit Angin, and Turmeric, where each part of this plant has its own benefits. Tambora plant (*Ageratum conyzoides*) is a plant that lives in the wild, including herbaceous plants., This plant contains amino acids, organacids, pecticsubstances, coumarin essential oil, ageratochromene, friedelin, -sitosterol, flavonoids, saponins, stigmasterol, tannins,

sulfur, and potassium chloride, essential oils, alkaloids, and coumarins (Amin, 2019). The benefits of Tambora include healing new wounds, bleeding wounds, ulcers, and treating diseases caused by bacterial infections. The results of research by (Garg & Grewal, 2015) reported that Tambora leaf extract in petroleum ether and acetone had an effect on S.aureus, Bacillus subtilis, E.coli, and Pseudomonas aerogenase.

Another plant that is usually used for microbial infection is Sembalit Angin (Mussaenda frondosa). This plant which usually lives around the edges of small rivers, contains chemical compounds, such as alkaloids, saponins, glycosides, flavonoids and tannins. Sembalit Angin is a plant that is often used as a treatment for canker sores, diuretics, headaches, jaundice, and this plant can also be used to facilitate the birth process, and treat infections that accompany the process traditionally. Based on the results of research using Sembalit Angin leaf extract in white rats, it can heal wounds as antibacterial in Pseudomonas aeruginase, E.coli, S.aureus and S. Albus (Garvita, 2015).

Infections that accompany the traditional birth process due to bacteria can also be treated with turmeric rhizome. Turmeric is a natural ingredient that is often used in combination treatment because it contains essential oils, aryl-tumerone, artumerone, alpha and beta atlantone, kurlon kurkumol, zingiberen, bisabolen, seskuifellandren, aryl curcumin, humulen, kukuminoid, arbinose, fructose, starch, tannin, and resin (Sani & Wuryandari, 2019). Turmeric has benefits as a medicine for fever, diarrhea, liver, shortness of breath, inflammation of the nose, ulcers, eczema, and hypertension based on the results of Sandha's research using a combination of sambiloto and turmeric extracts, the effect on E.coli bacteria (Sandha et al., 2015). Many types of plants that exist can be combined into medicinal plants which are more efficacious, such as tambora, sembalit wind, and turmeric, where these plants grow wild and are often considered by the community as plants with minimal benefits. This is due to a lack of knowledge and little research on plants, one of which is tambora. Based on the results of previous research (Ardiansyah et al., 2021) that herbal medicine experts believe that the use of plant extracts by combining or mixing one plant with other types of plants, in certain compositions will have maximum healing potential than using only one type of plant. Mixing these plants has a better medicinal effect. The above facts are proven by research results that the antioxidant activity of the combination of jack fruit leaves and bandotan leaves is better than just one plant (Halimatussa'diah et al., 2014).

This formulation aims to prove whether the combination can increase the antimicrobial potential in it against E.coli, which is part of the findings in this study.

METHOD

This research was a laboratory experimental study where the measurement of research data was carried out after giving treatment. Antimicrobial potential analysis was carried out on the growth of E.coli bacteria by measuring the clear zone or inhibition zone formed between the sides of the paper disc containing the extract and E.coli bacteria on plate medium with observation times of 24 hours, 48 hours, and 72 hours. This research was conducted at the Microbiology Laboratory of IAIN Palangka Raya, Central Kalimantan.

The equipment used included an evaporator, autoclave, 1000 ml becker glass, 500 ml becker glass, 250 ml becker glass, 100 ml becker glass, 50 ml becker glass, test tube, erlenmeyer flask, petri dish, jam glass, inoculation needle, ironstirrer, glass stirrer, glass funnel, tweezers, magnetic stirer, micropipette, pipette, LAF, hot plate, incubator, digital balance, weighing scale, scissors, cutter, bunsen lamp, blender, basin, tray, gas stove, caliper, pan, tool stationery, cloth napkins, and a refrigerator.

The materials used included Tambora leaves, Sembalit Angin leaves, Turmeric rhizome, pure culture of E. coli, Nutrien Agar (NA) medium, beef extract, becto peptone, aquadest, chloramphenicol 0.1%, 70% alcohol, methanol, cotton, rubber bands, vaseline, filter paper, cover paper, gauze, label paper, blotting paper, lysol, laundry soap, cotton buds, and aluminum foil.

Combination formulation of plant methanol extract with a ratio of 1:2:3 in which 20% of Tambora leaves, 30% of Sembalit Angin leaves, and 50% of turmeric rhizome.

The concentration design in this study can be seen in the following Table 1.

Table 1. Combination formulation of plant methanol extract with a ratio of 1:2:3

Combination Formula 3:2:1	Description
Chloramfenicol(+) 0,1% Aquades (-)	2 ml *Chloramfenicol* + 198 ml Aquades
30%	6 gram combination extract+14 ml aquades
40%	8 gram combination extract +12 mlaquades
50%	10 gram combination extract+10 ml quades
60%	12 gram combination extract + 8 ml aquades
70%	14 gram combination extract + 6 ml aquades
80%	16 gram combination extract+ 4 ml aquades

Antimicrobial Potential Test of 1:2:3 Combination Formulation Against Escherichia coli

The first stage was culturing E.coli on liquid nutrient medium and incubated for 1x24 hours, then implanted on solid Na plate medium using a micropipette all over the surface of the plate medium in 10 petri dishes for 1x24 hours of adaptation period. Combination formulations of 1:2:3 extracts were prepared with concentrations of 30%, 40%, 50%, 60%, 70%, and 80%, after which soaking the paper discs for 1 minute at each concentration. Next, the paper disc containing the extract was put in the middle of the previous medium surface E.coli bacteria which had been implanted and then all research units are incubated. Observation of inhibition of growth of E.coli bacteria was carried out during the entire incubation time, by measuring the clear zone visible on the surface of the NA plate medium using a caliper. Data from observations of the effect of the 1:2:3 combination formulation extract on the growth of E.coli were analyzed by ANOVA and followed by Duncan's 1% test.

RESULTS AND DISCUSSION

E.coli growth measurement data were based on the results of measuring the diameter of the inhibition zone between the extract and the outer side of the clear zone, where the clear zone was a parameter of inhibition of E.coli growth in vitro, as shown in Figure 1.

The strength of the 1:2:3 extract combination formulation against E.coli is presented with the overall recapitulation data on the diameter of the growth inhibition zone (Table 2). The combination formulation of 1:2:3 extract had a significant effect on the growth inhibition zone of E.coli at a significance of 1%, during the entire incubation time in the study. The significance was confirmed by the comparison of the mean square that the 72 hour incubation was greater (Figure 2).

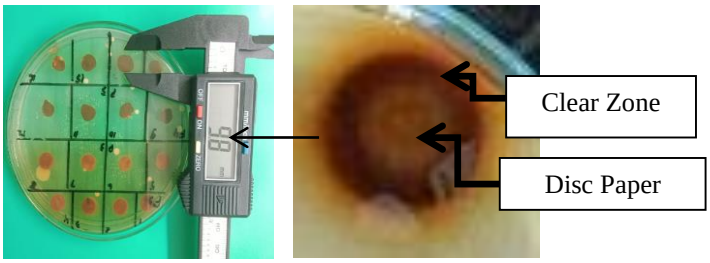

Figure 1. Measurement of the Growth Inhibitory Zone Escherichia coli

Table 2. Recapitulation of the Diameter of Escherichia coli Growth Inhibitory Zones Combination 1:2:3

Extract Combination Treatment 1:2:3	Average Inhibition Zone (mm)		
	24 hours	48 hours	72 hours
Chloramfenicol (+)	1,41 b	1,75 b	1,79 b
Aquades (-)	0 a	0 a	0 a
30% (6 gram combination extract+ 14 ml aquades)	2,81 b	3,12 b c	4,07 c
40% (8 gram combination extract+ 12 ml aquades)	1,78 b	3,79 c	4,24 c
50% (10 gram combination extract+ 10 ml aquades)	2,48 b	2,84 b c	4,15 c
60% (12gram combination extract+ 8 ml aquades)	2,35 b	2,84 b c	4,57 c
70% (14 gram combination extract+ 6 ml aquades)	2,17 b	3,28 b c	4,14 c
80% (16 gram combination extract+ 4 ml aquades)	2,41 b	4,03 c	4,56 c

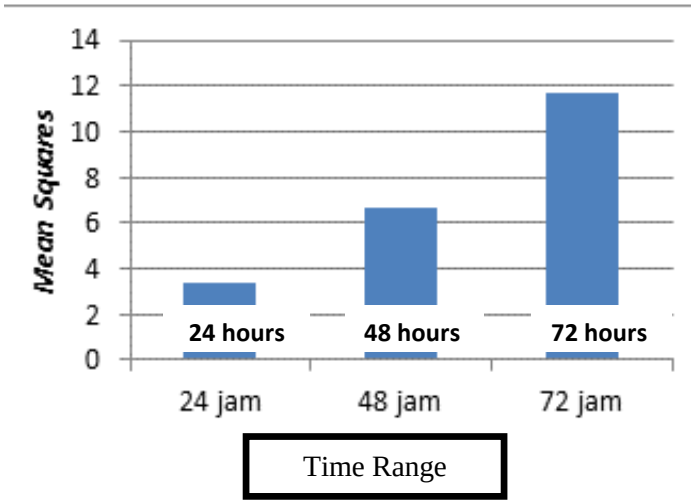

Figure 2. Mean Square Formula 1:2:3 Combination Escherichia coli

Based on the results of Duncan's 1% test at 24 hours incubation period, the treatment was not significantly different from the positive control (P1). Therefore, the concentration was interpreted to have the same ability as 0.1% Chloramphenicol. However, the concentration differed significantly when compared to the negative control of the study (P2). Because there is no significant difference between the lowest concentration of 30% (P3) compared to the higher concentration (80%), then the 30% concentration could state the effective and optimum concentration in inhibiting the growth of E. coli with an incubation period of 24 hours. The results of this observation were followed by the results of Duncan's test data 1% at an incubation period of 48 hours having almost the same interpretation, in terms of the effective concentration on the growth of E.coli, where the concentration of 30% was still the most effective.

The effectiveness of the minimum concentration at P3 was due to the fact that P3 was not statistically different from other concentration levels. P3, P5, P6, and P7 were not significantly different from P1, so they were interpreted to have the same ability as 0.1% Chloramphenicol. When Turmeric rhizome is used as the main composition, the optimum inhibition can only be carried out with a more concentrated concentration, which was 80%. The data on the significance of the effect of the

1:2:3 plant extract combination formulation on the growth of E.coli continued with observations with an incubation period of 72 hours showed that the positive control in the form of Chloramphenicol 0.1% (P1) experienced a very significant decrease in killing power, as evidenced by a different notation compared to the rest of the population. research treatment level. Although the higher concentration of P8 did not differ significantly, the test results at 72 hours incubation period showed empirical data that 30% concentration was the effective concentration and 60% was the optimum concentration in inhibiting the growth of E.coli bacteria.

Based on the recapitulation data that had been obtained, it showed that the results of observations on the combination treatment of bioherbal methanol extract combinations in 1:2:3 combinations were supported by the results of the analysis of variance that had a significance effect of 1% for all treatments. This was in accordance with the statement (Rodisa & Nasir, 2020) which states that combining several plants can increase the process of inhibiting the growth of a bacterium, because each plant contains compounds that function as antimicrobials and inhibit the growth of microorganisms.

This proves that the potency of the plant in the 1:2:3 combination formulation is quite good for use as an antibacterial. The content contained in the 1:2:3 combination formulation, namely 20% Tambora leaf extract, 30% Sembalit Angin leaf extract, and 50% Turmeric rhizome extract, where turmeric rhizome became the main natural ingredientbecause it had the potential to inhibit growth of E. coli compared to other plants. The results of observations on the 1:2:3 combination formulation were in line with the opinion of (Sandha et al., 2015) that the antimicrobial potential of turmeric is in the presence of a phenolic compound, namely curcumin. The mechanism of phenol as an antimicrobial at low doses is to damage the cytoplasmic membrane and cell nuclear membrane in bacteria, resulting in leakage of the cell nucleus, while at high doses these compounds coagulate against cellular proteins, resulting in decreased protein function. As a result, this functional deficiency inhibits the growth of E.coli bacteria. This was emphasized again by (Ulfah, 2020), who stated that in addition to the curcumin content, there are also other compounds such as essential oils, flavonoids, foliphenols and ascorbic acid that can function as antimicrobials and work by inhibiting the growth of these bacteria, one of which is E. coli.

CONCLUSION

The results of the analysis showed that the combination of bioherbal formulations with methanol extract with a ratio of 1:2:3 was proven to have an inhibitory power for the growth of E.coli bacteria. The 1:2:3 combination formulation is potential and can be recommended as an effective antimicrobial at a concentration level of 30%.

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