

POTENTIAL KOMBUCHA *ANNONA MURICATA* L. AND *CLITORIA TERNATEA* AS AN ANTIBACTERIAL OF *ESCHERICHIA COLI*

Kalyana Palupi, M. Rizal Fauzi, Dinda Dwi Anggita, Dini Sri Damayanti
Universitas Islam Malang

Email : 21701101019@unisma.ac.id

Abstract

Kombucha *Annona muricata* Linn and *Clitoria ternatea* contain organic acids, phenolic compounds, steroids/terpenoids, tannins, alkaloids and flavonoids which have the potential as antioxidants, anti-inflammatory and antimicrobial. The purpose of this study was to determine the potential kombucha *Annona muricata* Linn and *Clitoria ternatea* as an inhibitor of the growth of *Escherichia coli*.

Method: The preparation of *Annona muricata* Linn and *Clitoria ternatea* kombucha begins by boiling 1 L of water until it boils, then adding 100 grams of sugar and 2 grams of *A. muricata* Linn and *Clitoria ternatea* powder. Then cooled and put in a closed jar, then added 24 grams of SCOBY and covered with tissue paper. The fermentation process was carried out for 7 days. The Zone of Inhibition (ZOI) test used well method (three repetitions). The variations in boiling time are 5, 10 and 15 minutes. Inhibition zone measurement using OptiLab version 1.5 software application. Data analysis used *One Way ANOVA* test with a significance of $p < 0.05$.

Results: The ZOI diameter at kombucha *Annona muricata* Linn and *Clitoria ternatea* with 15 minutes of boiling time got the highest average inhibition zone of $14,52 \pm 1,7$ mm in *Escherichia coli* but not greater than gentamicin which was $22,43 \pm 0,3$ mm.

Conclusion: Kombucha *Annona muricata* Linn and *Clitoria ternatea* with variations in boiling time of 5, 10 and 15 minutes had strong antibacterial potential but lower than gentamicin.

Keywords: Kombucha, *Annona muricata* L, *Clitoria ternatea*, *Escherichia coli*.

*Corresponding Author:

Dr.dr. Dini Sri Damayanti, M.Kes

Jl. MT Haryono 193 Malang, Jawa Timur, Indonesia, 65145

Telp. +62341- 558959

e-mail: dinisridamayanti@unisma.ac.id

Kombucha is the result of fermenting a solution of high phenol leaves added to the symbiosis of acetic acid bacteria *Acetobacter xylinum* and the yeast *Saccharomyces* sp. Kombucha has a refreshing sour taste and is healthy for the body's metabolism. The microorganisms contained in it will change the sugar content into various types of acids, vitamins, and alcohol that are efficacious.¹ The content of organic acid compounds such as lactic acid, acetic acid, benzoic acid and propionic acid in kombucha has an antibacterial effect. The high acid content in kombucha makes pathogenic bacteria unable to survive in the intestinal tract. In addition, the content of raw materials in the basic ingredients of kombucha, especially flavonoids also has the ability to inhibit and kill pathogenic bacteria in the digestive tract.²

A high-fat diet for a long time will result in increased intestinal permeability and become susceptible to pathogenic microbes such as *Escherichia coli*, which will then trigger

metabolic endotoxemia. This is because a high-fat diet triggers an increase in free radicals, which disrupt the normal bacterial composition of the intestinal flora. The reduction in the number of normal bacterial flora compared to pathogenic bacteria causes a decrease in mucus secretion in the gastrointestinal epithelium, triggers a mild but chronic inflammatory process, weakens the intestinal barrier and results in systemic migration of intestinal bacteria.³

Soursop leaves and telang flowers contain phenolic compounds, steroids/terpenoids, tannins, alkaloids and flavonoids which have potential as antioxidants, anti-inflammatory and antimicrobial.⁴ The study was conducted in vitro on *Escherichia coli* bacterial culture and observed the inhibition zone that occurred due to the administration of kombucha soursop leaves and telang flowers with various variations of boiling time.

Methods

Research Design, Time and Place

Experimental research in vitro. Soursop leaf and telang flower kombucha was made at the Laboratory of the Faculty of Medicine, Islamic University of Malang (UNISMA) and ZOI (Zone of Inhibition) testing was carried out at the Biomedical Laboratory of the Islamic University of Malang (UMM). The study was conducted in August 2021.

Sampling

Soursop leaves are selected in the order of 3 to 5 from the base. Wash and chop then let stand for 12 hours, weigh the soursop leaves then dry at a temperature of $\pm 60^{\circ}\text{C}$ for 5-6 hours. Then blend until it becomes a powder.

Telang flowers are selected that are good then washed and drained. The telang flower is then dried using an oven at a temperature of $\pm 60^{\circ}\text{C}$ for 5-6 hours then blended. The two simplicia were mixed in a ratio of 1:1 and put into a tea bag.⁵

Soursop Leaf and Telang Flower Kombucha Making

Before the process of making kombucha all tools are sterilized first. Prepared soursop leaves and telang flowers that have been put in a tea bag with a weight of 2 grams each. 1000 mL of water is heated to boiling at 100°C . The bag containing simplicia soursop leaves and telang flowers is put into the water and wait for 5 minutes. Then added 100 g of sugar and stirred until dissolved and cooled in a glass jar. When the solution has reached room temperature ($25\text{-}30^{\circ}\text{C}$), 30 grams of liquid kombucha starter (SCOBY) is added. Cooling time should not exceed 4 hours. Jar covered with tissue paper and tied with rubber. Then the fermentation process is left for 7 days at room temperature and should not be exposed to direct sunlight. The same procedure was carried out for 10 minutes and 15 minutes of boiling.⁶

Zone of Inhibition (ZOI) Bacteria

The antibacterial activity testing method was carried out using the well-diffusion method with 3 replications. The test bacteria (*Escherichia coli*) were prepared in the form of a suspension in 0.85% NaCl solution. Preparation of the test bacterial suspension was carried out by means of test bacteria grown on a 24-hour sloping Nutrient Agar (NA) medium, 1-2 inoculation needles were taken and suspended in a sterile 0.85% NaCl solution and the density was equalized with the standard Mc. Farland 0.5.⁷

The next stage is the preparation of the test bacterial growth media, namely solid NA media is poured into a petri dish as the basic medium. A sterile backing with a diameter of 6 mm

was placed on top of the solidified base medium. The suspension of the test bacteria (*Escherichia coli*) was put as much as 1 mL into a semi-solid NA medium test tube which was still liquid and homogenized, then poured into a petri dish that had been placed in a buffer. After the semi-solid media has solidified, the backing is removed to form a pit.⁷ Soursop leaf kombucha was put into a well as much as 50 L. In this test, a positive control was used, namely gentamicin 10 mcg. The test medium was then stored in the refrigerator for 30 minutes to give the opportunity for kombucha as an antibacterial agent to diffuse into the media. After that, the test medium was incubated at 37°C for 24 hours. Observations were made by measuring the zone of inhibition using a caliper.⁷ The experiment was repeated 3 times.

Statistical Data Analysis

The measurement results of *Escherichia coli* inhibition were recorded in tabular form. Then the data were averaged for each group. Data analysis used one way ANOVA test with a significance of $p < 0.05$. Then the data continued with Tukey's Post Hoc test.

Results and Data Analysis

Escherichia coli . Bacterial Inhibitory Measurement Results

The results of the Inhibition Zone (ZOI) test for kombucha soursop leaves and telang flowers using the well method were compared with gentamicin against *Escherichia coli* bacteria measured using a caliper with an accuracy of 1 mm, shown in **Figure 1**.



Note : Figure 1. The results of measuring the inhibition of *Escherichia coli* bacteria

Description: A. Replication 1; B. Replication 2; C. Replication 3

The measurement of the inhibition zone was carried out on variations in the boiling time of kombucha soursop leaves and telang flowers, namely 5 minutes, 10 minutes and 15 minutes. The antibiotic gentamicin was used as a positive control. The inhibition zone was measured by measuring the diameter of the clear zone using a caliper. The measurement results can be seen in table 1.

After 3 repetitions, the highest yield was obtained in kombucha with a boiling time of 15 minutes with an average inhibition zone of 14.52 ± 1.7 mm. Meanwhile, the lowest yield was obtained at 10 minutes of boiling time with an average inhibition zone of 12.21 ± 0.23 mm. The average result of the 10 mcg gentamicin inhibition zone for comparison was 22.43 ± 0.3 mm, which indicates that soursop leaf kombucha and telang flower have a smaller inhibition zone than 10 mcg gentamicin. Based on statistical tests, with the Kolmogorov-Smirnov test, the data obtained were normal and homogeneous. Then Post Hoc Tukey was conducted to find a significant difference between soursop leaf kombucha and telang flower with gentamicin. While between boiling time there is no significant difference.

Table 1. *Escherichia coli* . Inhibitory Measurement Results

Replication	Soursop Leaf Kombucha and Telang Flower (mm)			Gentamisin (mm)
	5 minutes	10 minutes	15 minutes	
1	13,04629	11,92583	16,39775	22,08088
2	12,32394	12,34424	13,07911	22,34437
3	11,91132	12,33553	14,06976	22,83797
$\bar{X} \pm SD$	12,43 $\pm$ 0,57	12,21 $\pm$ 0,23	14,52 $\pm$ 1,70	22,43 $\pm$ 0,38*

Note: table 2 shows the results of measuring the inhibitory power (mm) of *Escherichia coli* by soursop leaf and telang flower kombucha with variations in boiling time of 5, 10, and 15 minutes compared to 10 mcg of gentamicin. Statistical analysis used one way ANOVA test with a significance level of $p \leq 0.05$. Different signs indicate a significant difference.

Discussion

Inhibitory Power of Soursop Leaf and Telang Flower Kombucha Using Well Method Against *Escherichia Coli*

The results of measuring the inhibition of *Escherichia coli* by soursop leaf and telang flower kombucha with boiling time of 5 minutes, 10 minutes, and 15 minutes showed the results of 12.20 - 14.51 mm. Based on the criteria proposed by Susanto, 2012 these results indicate that soursop leaf kombucha and telang flower with 5, 10 and 15 minutes of boiling time have strong inhibition against *Escherichia coli* because they have a clear zone diameter of more than 10 mm.⁸

The ZOI result of gentamicin 10 mcg as a positive control showed the number 22.42 ± 0.4 mm, which means that gentamicin has a strong inhibitory effect on *Escherichia coli*. The mechanism of action of gentamicin as an antibiotic is by binding to 16S rRNA on the 30S ribosomal subunit in the cytoplasm and disrupting mRNA translation so that the protein formed does not function. These results are consistent with the theory that gentamicin is effective against gram-negative bacteria such as *Escherichia coli*.⁹

The strong inhibitory ability was obtained from flavonoid compounds derived from soursop leaves and telang flowers. During fermentation, bacteria and yeast will degrade flavonoids by releasing the C chain and produce phenolic acids, namely phloronic acid and phenylacetic acid, as well as the release of flavonoids from acid-sensitive microorganism cells.¹⁰ Will inhibit the growth of bacteria. Active compound components contained in both herbs such as flavonoids, acetogenins, alkaloids, tannins and phenols will inhibit bacterial growth by damaging cell membranes, cell walls, and protein denaturation.^{11,12} Telang can be an alternative in preventing *Escherichia coli* bacterial infection.

The test results show that the longer the boiling time of kombucha, the wider the inhibition zone formed. This is because the longer the boiling time, the longer the solvent contact with soursop leaf powder and telang flower which causes the solubility of phenol compounds to be greater. If boiling is done more than 30 minutes, then the flavonoids will experience evaporation.¹³

Conclusions and Suggestions

Based on the results of statistical analysis and literature study, it can be concluded that:

1. Soursop leaf kombucha and telang flower with variations in boiling time of 5,10 and 15 minutes have antibacterial potential that is not significantly different

2. Soursop leaf kombucha and telang flower were significantly different from gentamicin in inhibiting the growth of *Eschericia coli* bacteria.

Suggestion

There are several suggestions to improve this research in the future, namely conducting in vivo research to test the effectiveness of soursop leaf and telang flower kombucha as antibacterial in experimental animals infected with *Eschericia coli*.

Thank-you note

The researcher would like to thank DIKTI, supervisory lecturer, Islamic University of Malang who have helped this research

References

- Naland, H. 2004. Kombucha Teh Ajaib: Pencegah dan Penyembuh Aneka Penyakit Tangerang: PT Agromedia Pustaka
- Gomes, A. C., Bueno, A. A., De Souza, R. G. M. H., & Mota, J. F. (2014). Gut microbiota, probiotics and diabetes. *Nutrition Journal*, 13(1).
- Gurung, M., Li, Z., You, H., Rodrigues, R., Jump, D. B., Morgun, A., & Shulzhenko, N. (2020). Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine*, 51, 1–9.
- Kardinan. 2004. Pestisida Nabati Ramuan dan Aplikasi. Penebar Swadaya. Jakarta.
- Folorunso, A. et al. 2019. Biosynthesis, characterization and antimicrobial activity of gold nanoparticles from leaf extracts of *Annona muricata*, *Journal of Nanostructure in Chemistry*, 9(2), pp. 111–117.
- Apriani, I. (2017) ‘Pengaruh Proses Fermentasi Kombucha Daun Sirsak (*Annona muricata* L.) Terhadap Kadar Vitamin C’, *Biota*, 3(2), p. 90.
- Y. Warbung, N.S. Vonny, N. Wowor, P. Jimmy. Daya Hambat Ekstrak Spons Laut *Callyspongia* sp terhadap Pertumbuhan Bakteri *Staphylococcus aureus*. Repository, Fakultas Kedokteran Gigi, Universitas Sam Ratulangi, pp. 1-3, 2013.
- Susanto, D., Sudrajat dan R. Ruga. 2012.
- Studi Kandungan Bahan Aktif Tumbuhan Meranti Merah (*Shorea leprosula* Miq) Sebagai Sumber Senyawa Antibakteri. *Mulawarmnan Scientifie*. 11 (2): 181-190.
- Chaves BJ, Tadi P. Gentamicin. [Updated 2021 Jul 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-.
- Jayabalan R, Marimuthu S, Swaminathan K. Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chem*. 2007;102(1):392-398.
- Lijon MB, Meghla NS, Jahedi E, Rahman MA, Hossain I. Phytochemistry and Pharmacological Activities of *Clitoria ternatea*. *Int J Nat Soc Sci*. 2017;4(1):1-10. www.ijnss.org
- Anggana, M.R. dan Happy, N.M. 2016. Phytochemicals and Antibacterial Activities of Soursop Leaf (*Annona muricata*) against *Edwardsiella tarda* (In Vitro). *Journal of Life Science and Biomedicine*. 6(1): 06-09.
- Ibrahim,A.M., Yunita dan H.S, Feronika. 2012. Isolasi dan Identifikasi Senyawa Flavonoid dalam Daun Beluntas (*Pluchea indica* L.). Laporan Penelitian. FMIPA UNSRAT. Manado.