Citrus Hystrix D.C Fluid Inhibits the Growth of *Escherichia Coli*, *Pseudomonas aerogenosa*, and *Bacillus subtilis*

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Abstract

Background: Citrus hystrix D.C is a plant traditionally used for herbs and medicinal plants in Indonesia. Citrus hystrix D.C fluid is proven to inhibit the growth of *Staphylococcus aureus*. The purpose of this study was to determine the ability of the fluid of Citrus hystrix D.C concentrations of 25%, 50%, 75%, and 100% in inhibiting the growth of *Escherichia Coli*, *Pseudomonas aerogenosa*, *Bacillus subtilis*.

Material and Method: This research is experimental with Posttest Only Control Group Design. The material used was local Citrus hystrix D.C, fresh and clean green, obtained from the Astambul area, Banjar Regency, South Kalimantan Indonesia.

Results: The results showed that there was a zone of inhibition of Citrus hystrix D.C fluid at a concentration of 25%, 50%, 75%, and 100% on the growth of *Escherichia Coli*, amounting to 7mm, 10mm, 13mm, 15mm. *Pseudomonas aerogenosa*, amounting to 6mm, 9mm, 13mm, 14mm. *Bacillus subtilis*, amounting to 6mm, 8mm, 11mm, 14mm.

Conclusion: It concluded that the fluid of Citrus hystrix D.C affected the growth of *Escherichia Coli*, *Pseudomonas aerogenosa*, *Bacillus subtilis* with the most significant inhibition zones respectively 15mm, 14mm, and 14mm.

***Keywords**: Citrus hystrix D.C; Antibacterial; Escherichia Coli; Pseudomonas aerogenosa; Bacillus subtilis*

Introduction

Unhealthy lifestyles and poor sanitation are factors that support bacterial infections. The bacterial infection is still a health problem in Indonesia. Bacterial diseases such as tuberculosis[1,2,3] and *Staphylococcus aureus*[4,5]. There are also problems with bacterial environmental contaminants such as *Salmonella*[6], E. coli[7], *Staphylococcus aureus*[8,9] and *Bacillus*[10,11].

Gram-positive *Bacillus subtilis* can cause bacteremia, septicemia, and endocarditis[12]. E. coli bacteria are Gram-negative bacteria that cause infections of the urinary tract and digestive disorders such as diarrhea[13] whereas P. aeruginosa is a Gram-negative bacterium that causes urinary tract infections, meningitis, diarrhea, necrosis of enterocolitis and pneumonia[14].

We are handling bacterial infections, in general, using synthetic bacteria. Synthetic antibacterial has side effects that can cause allergic reactions for users who are not suitable to use the antibacterial. So the manufacture of natural antibacterial originating from plants began to be investigated[15].

Indonesia is a country rich in herbal plants as medicinal plants such as Anredera cordifolia[16], Cananga odorata (Lamk). Hook[17], lime[18], Syzygium polyanthum[19], Carica papaya Linn[20], Jatropha curcas[21], Kaempferia galanga L.[22]. Cinnamomum burmannii[23], Eleutherine palmifollia (L) Merr[24], Hibiscus sabdariffa L.[25] Ocimum basilicum Linn[26], Citrus hystrix D.C.[27]. Citrus hystrix D.C. is an Indonesian endemic plant originating from the family Rutaceae, genus Citrus. The community has commonly used plants belonging to the genus Citrus for the treatment of various diseases[28].

Citrus hystrix D.C fruit fluid has a pH of 1.62, which shows very acidic, compared with lime (Citrus aurantifolia swingle) Research that has carried out on the fluid owned by the plant genus Citrus proves its activity

as an antioxidant and antibacterial (male). Citrus hystrix D.C fluid contains flavonoids which play a significant role in inhibiting bacterial growth[29].

The results of Kusumawardhani (2019) Citrus hystrix D.C fluid can inhibit the growth of *Staphylococcus aureus* at concentrations of 25%, 50%, 75%, and 100%, respectively 13.75mm, 17.25mm, 19.75mm, and 21.75mm. The inhibitory water of Citrus hystrix D.C against *Staphylococcus aureus* has known, but the inhibition has not been identified yet on *Escherichia Coli*, *Pseudomonas aerogenosa*, *Bacillus subtilis*. The purpose of this study was to determine the antibacterial ability of Citrus hystrix D.C fluid against *Escherichia Coli*, *Pseudomonas aerogenosa*, *Bacillus subtilis*.

Subjects and Method

The research carried out was an experimental study by examining the inhibitory power of Citrus hystrix D.C fluid with concentrations of 25%, 50%, 75%, and 100% when compared with the control group.

The material used in this study was Citrus hystrix D.C fluid with the criteria of local fruit, fresh and clean green, obtained from Astambul, Banjar Regency, South Kalimantan Indonesia.

The independent variable used is Citrus hystrix D.C. The Bound Variable used is the diameter of the bacterial inhibitory zone on Muller Hinton (MH). Determination test of Citrus hystrix D.C conducted at the Laboratory of Basic Mathematics, and Natural Sciences University of Lambung Mangkurat Banjarbaru Antibacterial activity test carried out using the diffusion method in a well with repeated work three times. This study uses sterile aqua dest as a thinner in various concentrations of Citrus hystrix D.C. The results obtained were read by measuring the inhibitory zone of Citrus hystrix D.C fluid against bacteria using a ruler.

Results

Testing the antibacterial activity of Citrus hystrix D.C fluid on bacteria showed variations in the inhibition zone. Data of Citrus hystrix D.C fluid inhibition zone data for bacteria presented in Table 1-3.

Table 1 The Zone of Inhibition of *Citrus hystrix* D.C fluid against *Escherichia Coli* at Various Concentrations.

Various Concentrations (%)	Zone of inhibition (mm)			Average (mm)
	1	2	3	
25	7	7	7	7
50	10	10	9	10
75	13	12	13	13
100	15	14	15	15

Table 2 The Zone of Inhibition of *Citrus hystrix* D.C fluid against *Pseudomonas aerogenosa* at Various Concentrations.

Various Concentrations (%)	Zone of inhibition (mm)			Average (mm)
	1	2	3	
25	6	6	5	6
50	10	9	8	9
75	13	13	10	13
100	15	14	14	14

Table 3 The Zone of Inhibition of *Citrus hystrix* D.C fluid against *Bacillus subtilis* at Various Concentrations.

Various Concentrations (%)	Zone of inhibition (mm)			Average (mm)
	1	2	3	
25	6	5	6	6
50	8	7	8	8
75	12	11	11	11
100	14	15	14	14

Discussion

Based on the data obtained in tables 1-3, it known that Citrus hystrix D.C. fluid can inhibit the growth of *Escherichia Coli*, *Pseudomonas aerogenosa*, *Bacillus subtilis*. The higher the concentration of Citrus hystrix D.C. fluid, the greater the diameter of the inhibition zone formed. This result shows that by increasing the level of Citrus hystrix D.C. fluid, the higher the content of active ingredients contained in Citrus hystrix D.C. fluid, which functions as an antibacterial, so the higher its ability to inhibit bacteria[30].

Citrus hystrix D.C. fruit fluid has a pH of 1.62, which shows very acidic so that when oxidized, the structure will change, and its function will decrease and even disappear[31]. In general, pathogenic bacteria cannot grow very slowly at pH below 4.6[32].

The Citrus hystrix D.C. fluid contains flavonoids which play a significant role in inhibiting bacterial growth[29]. Flavonoids are metabolites that often found in plants. One of the parts of flavonoids is antimicrobial and antiviral, so plants containing flavonoids widely used for traditional medicine[33]. Flavonoids are antimicrobial compounds because of their ability to form complexes with dissolved extracellular proteins and microbial cell walls. Flavonoids are lipophilic that are damaging to microbial membranes[34].

Chowdhury et al. (2009) stated that the methanol extract of Citrus hystrix fruit and some of its fractions had moderate to healthy antibacterial activity against some Gram-positive and Gram-negative bacteria[35]. The ethyl acetate extract and essential oil of Citrus hystrix rind have more effect on S. aureus than E. coli[36]. Citrus hystrix D.C. fluid used in this study also proved that the inhibition zone of E. Coli at a concentration of 100% was only 15mm, compared to the inhibition zone of *Staphylococcus aureus* in Kusumawardhani's study (2019) resulting in a higher inhibition zone at the same concentration of 21.75mm.

The limitation of this study is that no phytochemical tests carried out on the research material. The unknown chemical content of Citrus hystrix D.C., such as flavonoids, saponins, tannins, alkaloids, and others, is hidden.

Conclusion

There is a zone of inhibition of Citrus hystrix D.C. fluid at concentrations of 25%, 50%, 75%, and 100% on the growth of *Escherichia Coli*, amounting to 7mm, 10mm, 13mm, 15mm. *Pseudomonas aerogenosa*, amounting to 6mm, 9mm, 13mm, 14mm. *Bacillus subtilis*, amounting to 6mm, 8mm, 11mm, 14mm.

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Ethical Clearance: mTaken From Health Research Ethics Committee Politeknik Kesehatan Banjarmasin Indonesia

References

1. Thuraidah A, RA Widya Astuti, D Rakhmina. Anemia and duration of consumption of anti-tuberculosis drugs. Medical Laboratory Technology Journal. 2017; 3(2): 42-46 https://doi.org/10.31964/mltj.v3i2.157
2. Lutpiatina L, Wahidah, Nurhilaliah, D Rakhmina, Rifqoh. Sputum Quality Of The Anytime And Outset For Examination Acid-Resistance Bacilli, Medical Laboratory Technology Journal. 2018; 4 (1): 26-29 https://doi.org/10.31964/mltj.v4i1.183
3. Rifa'i, A., Muhlisin, A., Lutpiatina L. (2019). Erythrocyte Morphology of Tuberculosis Patients. Tropical Health and Medical Research, 1(1): 10-18. https://doi.org/10.35916/thmr.v1i1.3
4. Lutpiatina L, NN Agistni Eriana. *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* From The Diabetic Ulcer. Medical Laboratory Technology Journal. 2018; 4(1): 26-29 https://doi.org/10.31964/mltj.v4i1.182
5. Dwiyanti RD, A Muhlisin, A Muntaha. MRSA and VRSA in Paramedics at Ratu Zalecha Hospital Martapura. Medical Laboratory Technology Journal. 2015; 1(1): 27-33 https://doi.org/10.31964/mltj.v1i1.5
6. Yunus R, Ruth Mongan, Rosnani. Gram Negative Bacterial Contamination on Siomay Snacks in Kendari City. Medical Laboratory Technology Journal. 2017; 3(1): 11-16 https://doi.org/10.31964/mltj.v3i1.111
7. Roebiakto E, G Setiadi, YJ Supriyadi. Overview of Bacteriological Quality and Risk Level of Dug Well Water Pollution in Sungai Ulin Village, Banjarbaru City. Medical Laboratory Technology Journal. 2017; 3(1): 32-36 https://doi.org/10.31964/mltj.v3i1.150
8. Dwiyanti RD, L Lutpiatina. Bacteriological Quality of Pentol Tomato Sauce in Banjarbaru. Medical Laboratory Technology Journal. 2016; 2(1): 1-5 https://doi.org/10.31964/mltj.v2i1.31
9. Mustika Sari, P., Lutpiatina, L., Muhlisin. A. *Staphylococcus aureus* in Traditional Coconut milk Drinks. Tropical Health and Medical Research. 2019; 1(1): 33-38. https://doi.org/10.35916/thmr.v1i1.1
10. Lutpiatina L, Uricase Products from Bacillus sp. Laboratory Contaminants, Medical Laboratory Tecnology Journal. 2015; 1(2): 96-101 https://doi.org/10.31964/mltj.v1i2.22
11. Fahani, A., Dwiyanti, RD., Muhlisin. A. Contamination of Bacillus cereus in Elementary School Snack Food. Tropical Health and Medical Research 2019; 1(2): 56-61. https://doi.org/10.35916/thmr.v1i2.10
12. Samiullah & Bano, A. In Vitro Inhibition Potential of Four Chenopod Halophytes Against Microbial Growth, Pak. J. Bot. 2011; 43: 123-127.
13. Rahmaningsih, S., Wilis, S., & Mulyana, A. Bakteri Patogen dari Perairan Pantai dan Kawasan Tambak di Kecamatan Jenu Kabupaten Tuban, Ekologia. 2012; 12 (1): 1-5.
14. Foca, M. M. D., Jakob, K. R. N. B. S. N., Whitier, S., Latta, P. D., Factor, S. M.D., M. P. H., Rubenstein, D. M. D., et al. Endemic Pseudomonas aeruginosa Infection in A Neonatal Intensive Care Unit, The New England Journal of Medicine. 2000; 343 (10).
15. Astutiningrum, Theresia. Antibacterial Activity Test of Kenikir (Cosmos caudatus kunth) Leaf Extract Against Staphylococcus aureus Growth In-Vitro. Skripsi. Universitas Sanata Dharma, Yogyakarta; 2016
16. Dwiyanti RD, Nurlailah, I.K. Widiningsih. Water Effectiveness of Binahong Leaf Decoction (Anredera cordifolia) Against the Growth of Salmonella typhi. Medical Laboratory Technology Journal. 2015; 1(1): 1-6 https://doi.org/10.31964/mltj.v1i1.7
17. Nuryati A, Siti nuryani, AD wibowo. Effect of Simplicia of Kenanga Flowers (Cananga odorata (Lamk). Hook.) As Electric Mat Against Knock-Down of Aedes Aegypti Mosquitoes. Medical Laboratory Technology Journal. 2015; 1(1): 34-37 https://doi.org/10.31964/mltj.v1i1.6
18. Dwiyanti RD, L. Lutpiatina, Lime Soap as Bacteria Reducer. Medical Laboratory Technology Journal. 2018; 4(1): 6-20 https://doi.org/10.31964/mltj.v4i1.181
19. Dwiyanti RD, Rion Dediq, A Thuraidah. Effect of Simplicia of Kenanga Flowers (Cananga odorata (Lamk). Hook.) As Electric Mat Against Knock-Down of Aedes Aegypti Mosquitoes. Medical Laboratory Technology Journal. 2017; 3(1): 17-21 https://doi.org/10.31964/mltj.v3i1.151
20. Swastika D, Lenie Marlinae, Laily Khairiyati. Effect of Simplicia of Kenanga Flowers (Cananga odorata (Lamk). Hook.) As Electric Mat Against

Knock-Down of Aedes Aegypti Mosquitoes. Medical Laboratory Technology Journal. 2016; 2(2): 66-69 https://doi.org/10.31964/mltj.v2i2.98

21. *Ni Made Niagita Wiratni, I Nyoman Jirna, IGA. Sri Dhyanaputri. Potential Antifungal of Jatropha Stem Leaves Against the Growth of Candida albicans*. Medical Laboratory Technology Journal. 2017; 3(2): 63-67 https://doi.org/10.31964/mltj.v3i2.169
22. Annisa R, Erfan R, Lutpiatina L. Potential of Kencur Rimpang Extract (Kaempferia galanga L.) Inhibits the Growth of Candida albicans. Medical Laboratory Technology Journal. 2016; 2(2): 70-76 https://doi.org/10.31964/mltj.v2i2.94
23. Huda,N.,Dwiyanti,RD.,Thuraidah.A.Effectiveness of Cinnamon (Cinnamomum burmannii) Ethanol Extract Against *Staphylococcus aureus* Growth. Tropical Health and Medical Research. 2019; 1(2): 39-43. https://doi.org/10.35916/thmr.v1i2.6
24. Mahmudah, S., Muntaha, A., Muhlisin. A. Effectiveness of Dayak (Eleutherine palmifollia (L) Merr) Extracts Against *Escherichia Coli* In Vitro. Tropical Health and Medical Research. 2019; 1(2): 44-48. https://doi.org/10.35916/thmr.v1i2.8
25. Sutiany, A., Dwiyanti, RD., Oktiyani. N. Inhibition Strength of Rosella (Hibiscus sabdariffa L.) Boiled Water on Salmonella typhi in vitro. Tropical Health and Medical Research. 2019; 1(2): 62-67. https://doi.org/10.35916/thmr.v1i2.7
26. Pratama, HN., Lutpiatina, L., Dwiyanti, RD., Muhlisin. A. Growth of Malassezia furfur in Media with The Addition of Basil (Ocimum basilicum Linn) Powder. Tropical Health and Medical Research. 2020; 2(1): 26-33
27. Kusumawardhani, N., Thuraidah, A., Nurlailah. Citrus hystrix D.C Fluid Inhibits The Growth of *Staphylococcus aureus*. Tropical Health and Medical Research. 2020; 2(1); 34-38
28. Putra GMD, Satriawati DA, Astuti NKW. Standarisasi Dan Skrining Fitokimia Ekstrak Etanol 70% Daun Jeruk Limau. Jurnal Kimia. 2017; 12 (2): 188.
29. Azidi I, Kamilia M, Dahlena A. Chemical preliminary examination of the leaves of the skin and fruit of Citrus hystrix D.C, a local orange in South Kalimantan. Jurnal Sains dan Terapan Kimia. 2017; 11(2): 72-75.
30. Ajizah A. Salmonella typhimurium Sensitivity Against Psidium guajava L. Bioscientiae Leaf Extract. 2012; 1(1): 31-38.
31. Purnomo M. Isolation of Flavonoids from Beluntas (Pluchea indica L) Leaves which Have Antimicrobial Activity Against Causes of Sweat Odor. Universitas Airlangga; 2001
32. Arisman. Food poisoning. Nutrition Science Textbook. Jakarta, EGC; 2012
33. Robinson T. High Organic Plant Content. Bandung . ITB; 1998
34. Anggraini, ND., Roza M Rodesia, Fitmawati. The antibacterial activity of papaya leaf extract (carica papaya L) against *Escherichia Coli* and *Salmonela typhi*. Biologi FMIPA Universitas Riau; 2013
35. Chowdhury, A., Alam, M.A., Rahman, M.S., Hossain, M.A., dan Rashid, M.A. Antimicrobial, Antioxidant and Cytotoxic Activities of Citrus hystrix DC. Fruits, Dhaka Univ. J. Pharm. Sci. 2009; 8 (2): 177-180.
36. Chanthaphon, S., Chanthachum, S., dan Hongpattarakere, T. Antimicrobial activities of essential oils and crude extracts from tropical Citrus spp. against food-related microorganisms, Songklanakarin J. Sci. Technol. 2008; 30 (Suppl.1): 125-131.