**Tropical Health and Medical Research**
Vol.2, No.1, March 2020, pp.34-38
ISSN (Online) : 2684-740X
*Journal homepage:medlabtecnojournal.com*

# *Citrus hystrix* D.C Juice Inhibits The Growth of *Staphylococcus aureus*

***Novina Kusumawardhani, Anny Thuraidah, Nurlailah**

Medical Laboratory Technology Poltekkes Kemenkes Banjarmasin
Mistar Cokrokusumo Street 4a Banjarbaru, Indonesia.
*E-mail: novinakusuma@gmail.com

**Abstract**: *Citrus hystrix* D.C. is a citrus-type citrus tribal plant, one of the most favored and developed fruits by the people of South Kalimantan Indonesia. *Citrus hystrix* D.C. contains useful chemical compounds, one of which is flavonoids, which function as antibacterial and play a critical role in inhibiting bacterial growth. The purpose of this study was to determine the antibacterial activity of *Citrus hystrix* D.C. juice at 25%, 50%, 75%, and 100% concentration on the growth of *Staphylococcus aureus*. 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. The results showed that there was a zone of inhibition of *Citrus hystrix* D.C. juice on *Staphylococcus aureus* growth which began at 25%, 50%, 75% and 100% concentrations of 13.75mm, 17.25mm, 19.75mm, and 21.75mm, so it can conclude that the juice of *Citrus hystrix* D.C. influences the growth of *Staphylococcus aureus* which has the largest inhibitory zone at a concentration of 100%.
**Keywords:** *Citrus hystrix* D.C; antibacterial; *Staphylococcus aureus*

## INTRODUCTION

*Staphylococcus aureus* is one of the pathogenic bacteria that can cause swelling, redness, heat, and pain. This bacterium has a more muscular endurance[1]. Deaths caused by staphylococcal infections vary. Untreated bacteremia causes more than 80% of mortality. The death rate due to staphylococcal toxic shock syndrome ranges from 3 to 5%, while infection by coagulase-negative staphylococci causes only a few deaths[2].

*Staphylococcus aureus* growth inhibitors are antibacterial. The use of synthetic antibacterial 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[3].

One of the plants that have the potential to be developed as traditional medicine is *Citrus hystrix* D.C. *Citrus hystrix* D.C is an Indonesian endemic plant derived from the family Rutaceae, genus Citrus. The community very commonly uses plants belonging to the genus Citrus for the treatment of various diseases[4].

*Citrus hystrix* D.C fruit juice has a pH of 1.62, which shows very acidic, compared with lime (Citrus aurantifolia swingle) Research that has carried out on the juice owned

**Corresponding Author:** Novina Kusumawardhani
Medical Laboratory Technology Poltekkes Kemenkes Banjarmasin.
Mistar Cokrokusumo Street 4a Banjarbaru, Indonesia.
E-mail: novinakusuma@gmail.com

by the plant genus Citrus proves its activity as an antioxidant and antibacterial[4]. *Citrus hystrix* D.C juice contains flavonoids that play a critical role in inhibiting the growth of bacteria and active compounds from chemical contents such as essential oils and phenols, which are inhibiting the growth of bacteria such as *Staphylococcus aureus*. The bactericidal ability of phenols by denaturing proteins and damaging the cytoplasmic cell membrane. Instability in the cell wall and cytoplasmic membrane of the bacterium so that it causes the function of selective permeability, the role of active transport, and control of the composition of the bacterial cell protein become disrupted—impaired cytoplasmic integrity results in the escape of macromolecules and ions from the cell. Bacteria will lose their shape and undergo lysis depending on their concentration[5]. Rinaldy E. D et al. 2017 on the inhibitory test of *Citrus hystrix* (Citrus hystrix) against *Staphylococcus aureus*, showed inhibition zone activity which formed at 12.8mm, 11.8mm, 13.05mm, 13.0mm, 14.6mm[6].

Razak research results, 2013 on the effectiveness test of lime juice (*Citrus aurantifolia*) on the growth of *Staphylococcus aureus*, showed inhibitory zone activity at concentrations of 25%, 50%, 75%, 100% with an average inhibition zone formed of 5mm, 7.25mm, 13.25mm and 14.25mm[7]. Whereas in this study, researchers used *Citrus hystrix* D.C juice to the growth of *Staphylococcus aureus*. The purpose of this study was to determine the inhibitory antibacterial activity of *Citrus hystrix* D.C juice against *Staphylococcus aureus*.

## MATERIALS AND METHODS

The research conducted was experimental research that is by checking the inhibitory power of *Citrus hystrix* D.C juice with a concentration of 25%, 50%, 75%, and 100% than compared with the control group.

The material used in this study was *Citrus hystrix* D.C juice with the criteria of local fruit, fresh and clean green, obtained from Astambul, Banjar Regency, South Kalimantan Indonesia. The bacterium used in the study was *Staphylococcus aureus* ATCC 25923, collected from the Yogyakarta Indonesia Health and Calibration Laboratory.

The independent variable used is *Citrus hystrix* D.C. The Bound Variable used is the diameter of the inhibition zone *Staphylococcus aureus* 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 The antibacterial activity test carried out using the diffusion method in a well with repeated work four 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 juice against *Staphylococcus aureus* using a ruler. The standard interpretation of the antibiotic drug Ciprofloxacin against *Staphylococcus aureus* from (Clinical and Laboratory Standards Institute Antimicrobial Susceptibility Testing (M100), 2017):

Resistant: ≤ 15 mm
Intermediate: 16-20 mm
Sensitive: ≥ 21 mm

## RESULT AND DISCUSSION

Tests on the antibacterial activity of *Citrus hystrix* D.C juice against *Staphylococcus aureus* showed inhibition zone variations. *Citrus hystrix* D.C juice inhibition zone data against *Staphylococcus aureus*. Positive controls and negative controls presented in table 1.

Table 1 The Zone of Inhibition of *Citrus hystrix* D.C juice against *Staphylococcus aureus* at Various Concentrations.

| Various Concentrations (%) | Zone of inhibition (mm) P1 (mm) | P2 (mm) | P3 (mm) | P4 (mm) | Average (mm) | Category |
|---|---|---|---|---|---|---|
| 25% | 14 | 13 | 14 | 14 | 13,75 | Resistant |
| 50% | 18 | 17 | 17 | 17 | 17,25 | Intermediate |
| 75% | 20 | 20 | 19 | 20 | 19,75 | Intermediate |
| 100% | 22 | 22 | 21 | 22 | 21,75 | Sensitive |
| Positive Control (Ciprofloxaci) | 45 | 45 | 45 | 45 | 45 | Sensitive |
| Negative Control (sterile aquadest) | 0 | 0 | 0 | 0 | 0 | - |

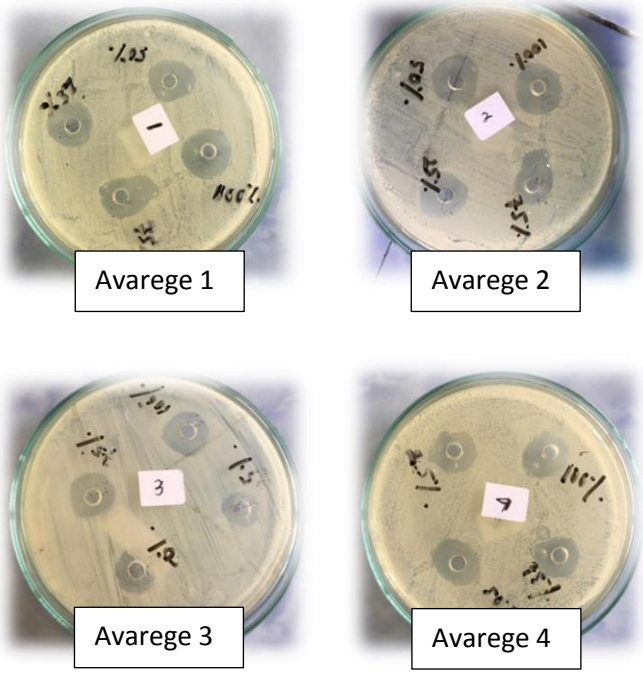


Figure 1. Inhibited Zone of *Citrus hystrix* D.C Juice Against *Staphylococcus aureus* with 4 Repetitions.

Based on the data obtained in table 1, it knows that *Citrus hystrix* D.C juice the ability to reduce the number of *Staphylococcus aureus* by inhibiting the growth of these bacteria. The higher the concentration of *Citrus hystrix* D.C juice, the greater the diameter of the inhibition zone formed. This shows that by increasing the level of *Citrus hystrix* D.C juice, the higher the content of active ingredients contained in *Citrus hystrix* D.C juice, which functions as an antibacterial, so the higher its ability to inhibit bacteria[8].

*Citrus hystrix* D.C fruit juice 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[9]. In general, pathogenic bacteria cannot grow or grow very slowly at pH below 4.6 [10].

This research uses *Citrus hystrix* D.C juice while for similar material, *Citrus hystrix*, a lot of research has done. The results research Ratna, Y., et.al. (2011) essential oils of *Citrus hystrix* leaf have antibacterial activity against *Staphylococcus aureus* with Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of 1 and 2%, respectively[11]. Chowdhury et al. (2009) reported that the methanol extract of *Citrus hystrix* fruit and some of its fractions had moderate to intense antibacterial activity against some Gram-positive and Gram-negative bacteria[12]. The ethyl acetate extract and essential oil of *Citrus hystrix* rind are more potent against *S. aureus* than *E. Coli*[13].

Research conducted by Nanasombat and Lohasupthawee (2005) shows that ethanol extracts and essential oils of leaves and *Citrus hystrix* fruit peels have antibacterial activity against some Salmonella and enterobacterial species[14]. The results of the research of Luangnarumitchai et al. (2007) indicated that fruit essential oil and *Citrus hystrix* leaf skin could inhibit the growth of 5 strains of Propionibacterium acnes[15]. The main components of Citrus hystrix essential oil are β-citronellal, monoterpenes (66.85% of total essential oils) followed by β-citronellol, linalool, and citronellol[16].

The limitation of this study is that the MIC and MBC values of *Citrus hystrix* D.C are not known because the method used is the diffusion well method. So this research cannot be compared directly with other studies that use MIC and MBC methods.

## CONCLUSION

There is a zone of inhibitory water of *Citrus hystrix* D.C on the growth of *Staphylococcus aureus*, which starts at concentrations of 25%, 50%, 75%, and 100% at 13.75mm, 17.25mm, 19.75mm, and 21.75mm.

## CONFLICT OF INTEREST

There were no conflicts of interest with related parties in this study.

## REFERENCES

1. Balakrishan KP, Narayanaswamy N, Subba PEH, Poornima. Antibacterial Activity of Certain Medicinsl Plants Againts Acne-including Bacteria. *International Journal Of Pharma and Bio Sciences*. 2011;3(2):476-480.
2. Herchline, T.E. *Staphylococcal Infections*, online. 2011. http://emedicine.medscape.com.
3. Astutiningrum, Theresia. *Antibacterial Activity Test of Kenikir (Cosmos caudatus*

*kunth) Leaf Extracts Against Staphylococcus aureus Growth In-Vitro*. Skripsi. Universitas Sanata Dharma: Yogyakarta; 2016
4. Putra GMD, Satriawati DA, Astuti NKW, 2017. Phytochemical Standardization and Screening of 70% Ethanol Extract of Lime Orange. *Jurnal Kimia.* 2017;12(2): 188.
5. 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. Preliminary Examination of Chemical Leaves, Skin and Fruit of *Citrus hystrix* D.C: Local Oranges in South Kalimantan. *Jurnal Sains dan Terapan Kimia.* 2017;11(2):72-75.
6. Rinaldy E.D, Herriyannis H, Vonny N.S Wo. Inhibition of the Test of Inhibition of Lime Juice (*Citrus aurantifolia*) on Staphylococcus aureus Growth. *Jurnal Ilmiah Farmasi*. 2017;6(1)
7. Razak A, Djamal A, Revilla G. Inhibition of the Test of Inhibition of Lime Juice (*Citrus aurantifolia*) on Staphylococcus aureus Growth. *Jurnal Kesehatan Andalas*. 2013;9(2).
8. Ajizah A. Salmonella typhimurium Sensitivity Against *Psidium guajava* L, *Bioscientiae*. 2012;1(1):31-38.
9. Purnomo M. *Isolation of Flavonoids from Beluntas (Pluchea indica L) Leaves which Have Antimicrobial Activity Against Causes of Sweat Odor*. Universitas Airlangga; Surabaya: 2001
10. Arisman. *Food poisoning. Nutrition Science Textbook*. Jakarta: EGC; 2012
11. Ratna Yuliani, Peni Indrayudha, dan Septi Sriandita Rahmi Antibacterial Activity Of Volatil Oil Of Small Aromatic Lemon Leaves (*Citrus hystrix*) against *Staphylococcus aureus and Escherichia coli. Pharmacon*. 2011;12(2):50-54
12. 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.
13. 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.
14. Nanasombat, S. dan Lohasupthawee, P., 2005, Antibacterial Activity of Crude Ethanolic Extracts and Essential Oils of Spices Against *Salmonellae* and Other *Enterobacteria*, *KMITL Sci. Tech. J*. 2005;5(3):527-538.
15. Luangnarumitchai, S., Lamlertthon, S., dan Tiyaboonchai, W., 2007, Antimicrobial Activity of Essential Oils Against Five Strains of *Propionibacterium acnes*. *Mahidol University Journal of Pharmaceutical Sciences*. 2007;34(1-4):60-64.
16. Loh, F.S., Awang, R.M., Omar, D., dan Rahmani, D., 2011, Insecticidal properties of *Citrus hystrix* DC leaves essential oil against *Spodoptera litura fabricius*. *Journal of Medicinal Plants Research*. 2011;5(16):3739-3744.