Antimicrobial Activities of *Laurus nobilis* Leaves Ethanol Extract on *Staphylococcus aureus*, *Salmonellae typhi*, and *Escherichia coli*.

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ABSTRACT

*Laurus nobilis* is one of the most well-known, most frequently used plants is from Lauraceae family which contains up 2,500 species that grow in the subtropics and tropics of the Mediterranean region and Indonesia. This study was supposed to investigate the antimicrobial effect of *L. nobilis* leaves ethanol extract on *Staphylococcus aureus*, *Salmonellae typhi*, and *Escherichia coli*. This preliminary study examined the antimicrobial effect of *L. nobilis* leaves ethanol extract. The method used Agar-well diffusion for determination of the zone of inhibition and the minimum bactericidal concentration to investigate the activity of *L. nobilis* leaves ethanol extract at 100% concentration. The results revealed that extract of *L. nobilis* leaves had the antibacterial activity against *Staphylococcus aureus* with a zone of inhibition (16.3 ±1.5 mm), *Staphylococcus aureus* with (14.5±0.5 mm), and weak antimicrobial activity against *Escherichia coli* (11.3±1.1mm). Also, through the minimum bactericidal concentration experiment, the *L. nobilis* leaves ethanol extract had activity on *Staphylococcus aureus* and *Salmonellae typhi*, it’s killed the bacteria in all concentration start it from $5 \times 10^7$ to $5 \times 10^4$. But the activity on *Escherichia coli* just weaken concentration $5 \times 10^7$ and $10^6$. This research has concluded that the *L. nobilis* leaves ethanol extract exhibited a significant antimicrobial effect against *Staphylococcus aureus* and *Salmonellae typhi* then *Escherichia coli* that is considered a kind of multidrug-resistant bacteria.

Keywords: Antibacterial Activity; *Laurus nobilis* leaves ethanol extract; *Staphylococcus aureus*; *Salmonellae typhi*; *Escherichia coli*.
INTRODUCTION

In 2018, The WHO Global Antimicrobial Surveillance System reported that 500,000 people with suspected bacterial infections across the globe is attributed to the antibiotic resistance. *Staphylococcus aureus*, *Salmonellae typhi*, and *Escherichia coli* are the most common human pathogens that are consistently causing different sequelae of infection in both genders and all ages. These pathogens have also a significant number of morbidities and mortalities, particularly in developing countries. The bacteria develop a resistance to antimicrobials by different mechanisms whereby limiting uptake of the drug, enzymatic inactivation of the drug, modification of the drug target, and active efflux of the drug. Depending on the antimicrobial involved, the bacteria may use one or several of these resistance mechanisms.2

Pathogenic *E. coli* is resistant to various antibiotics and including the strain that extended-spectrum b-lactamase (ESBL).3 where *E. coli* considered the most pathogenic bacteria that causes of diarrhea in humans and animals.4 Increased resistant of the *S. typhi* to antimicrobial drugs was reported and may allow it to cross the intestinal mucosa to the bloodstream and infects deep organs such as the bones, joints, and meninges.5 Methicillin-resistant *S. aureus* (MRSA) is a major pathogen associated with serious community and hospital-acquired disease where these strains showed resistance to a wide range of antibiotics, thus limiting the treatment options to very few agents such as teicoplanin and vancomycin.6,7

Antibiotic resistance is an internationally recognized health problem. This problem, in recent years is greatly threatening because of emergence of Multi-Drug Resistant organisms (MDRO).8,9 New antibacterial agents from many sources including herbal products that are preferred over traditional medicines due to its wide biological activity, safety and lower cost. The herbal products contain groups of effective compounds that can be investigated for effectiveness as antimicrobials, antioxidants, antiseptic, and anti-inflammatory. Herbal products are increasingly used as a dietary supplement to fight against infection and lower the risk in population.10,11

*L. nobilis* is one of the most well-known and most frequently used plants and it is member lauracease family which contains up 2,500 species that grow in the subtropics and tropics of the Mediterranean region include Indonesia. Most species possess aromatic stems, roots, leaves, and fruits.12,13

As a medicinal plant, its leaves and fruits have been known since long time ago as a species that can be used for therapy against rheumatism, skin rashes, earaches, stomachache, astringent, carminative, diaphoretic, stimulant, emetic, emmenagogue and abortifacient.12 In addition, its Volatile oil is used by the cosmetic industry in creams, perfumes, and soaps. It has a lot of chemical properties that are useful in manufacturing of medicine, for instance, it represents a basic material in dentistry such as alkaloids, flavonols, phenolic, flavones (apigenin and luteolin), glycosylate flavonoids, cysterpine and soliterpinat to fight against or prevent common diseases.14,15

Several studies described and confirmed that extracting phytochemicals and active ingredients of herbal remedies give medicinal benefits more than the use of the herb itself.16 Many studies, for example Yilmaz *et al* (2017) and Aldhaher *et al* (2017) have found that the essential oil of *L. nobilis* leaves has strong antibacterial activity against Gram negative and Gram-positive bacteria.17,18 Ozcan *et al*. (2016) found that the green synthesis of zinc oxide nanoparticles using the aqueous leaf extract of *L. nobilis* (Ln-ZnO NPs) were has antibacterial activity of Ln-ZnO
This research was conducted in the Laboratory a BSL 3 Universitas Airlangga from November 2019 to December 2019. The used bacterial strains in this study are S. aureus (ATCC 25423), E. coli (ATCC 25922) and S. typhi (BSL 2 Lab. collection). Strains were overnight grown onto plates of Muller-Hinton agar (MHA).

Antimicrobial assay was carried out using the agar well diffusion method according to Clinical Laboratory Standards Institute guidelines (CLSI).

| Microorganism | L.nobilis leaves ethanol extract | Gentamicin |
|---------------|---------------------------------|------------|
| S. aureus     | 16.3 ± 1.5                      | 25.6 ± 0.5 |
| S. typhi      | 14.5 ± 0.5                      | 20.6 ± 1.1 |
| E. coli       | 11.3 ± 1.1                      | 19 ± 0.5   |

*Values, including diameter of the well (6 mm), are means of three replicates ± SD
that will affect the cytoplasm of the bacteria and slow down its motility, since it has an ability to interact directly with the Deoxyribonucleic acid (DNA) of the bacteria.\textsuperscript{14} Type of solvent used for extracting \textit{L. nobilis} leaf has a major impact on their antibacterial activity. Extraction of \textit{L. nobilis} leaf with ethanol resulted in a product with greater overall antibacterial activity. Study of Algabri that carried out on antibacterial activity of Libya bay leave extracted with methanol and n-hexane, it was observed that the n-hexane extract showed no antibacterial activity but the methanol extract had good inhibitory activity against \textit{S. aureus}.\textsuperscript{15} Also, El Malti and Amarouch (2009) found that the bay leave extract has a significant antimicrobial activity against wide range of human pathogen.\textsuperscript{28}

Therefore, the result that we found confirmed that \textit{L. nobilis} leaves ethanol extract has antimicrobial activity against microorganism, it’s that observed the antimicrobial activity during agar well diffusion and bactericidal activity experiment. These results concurred with the result of Aldhaher that found aqueous extract had good inhibitory activity against \textit{Streptococcus mutants} with MBCs range 30-60mg/ml. Also concurred with study of Yilmaz who found that antimicrobial activity of the essential oil against the tested panel of food-spoiling bacteria and one yeast strain.\textsuperscript{14,17} Also, the study of Sırıken who demonstrated that the essential oil of \textit{L. nobilis} had strong antibacterial activity against Gram-negative and Gram-positive food-borne pathogens.\textsuperscript{28} Study of Aljindan and Alkharsah, (2020) show, the resistance of Salmonella species to antimicrobial drugs increased from 24.6\% in 2011 to 37.8\% in 2018. The research study by in 2018 all Salmonella isolates were completely resistant to Cefalotin, Cefuroxime, and Cefoxitin, while they found some susceptibility to other Cephalosporins and Ciprofloxacin.\textsuperscript{17} While study of Patil and Mule they found \textit{S. typhi} sensitive to Cefixime, Ceftriaxone, and Azithromycin and based on average Minimal Inhibitory Concentration and MIC breakpoints.\textsuperscript{30} Through the experiment conducted on Rats by Qnais et al (2012) which found \textit{L. nobilis} aqueous extract has antidiarrheal agent.\textsuperscript{31}

Study of Nafis et al. exhibited notable potency regarding antimicrobial activity of (EOs) from \textit{L. nobilis} leaves had the highest activity against \textit{E. coli}, with MIC: 22.2 mg/mL and IZ 9.00 mm. while it had activity against \textit{S. aureus} with IZ 10.0 mm and moderate MIC: 5.55 mg/mL.\textsuperscript{32}

**CONCLUSIONS**

The result of this study demonstrated an antibacterial effects of \textit{L. nobilis} leaves ethanol extract was proved a strong antibacterial activity against bacterial infections as they exhibited an antimicrobial effect against \textit{S. aureus}, \textit{S. typhi} and weak effect in \textit{E. coli}, so that is considered a kind of drug development substance for multidrug resistant bacteria.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ. Acquired antibiotic resistance genes: an overview. Frontiers in microbiology. 2011 Sep 28;2:203.
2. Yılmaz EŞ, Aslantaş Ö. Antimicrobial resistance and underlying mechanisms in Staphylococcus aureus isolates. Asian Pacific journal of tropical medicine. 2017 Nov 1;10(11):1059-64.
3. Al-Talib H, Al-Khateeb A, Hassan H. Antimicrobial resistance of Staphylococcus aureus isolates in Malaysian Tertiary Hospital. International Journal of Medical. 2015 Apr 1;22(1):1-3.
4. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmai R, Kiflay R, Tesfu T. Methicillin-resistant Staphylococcus aureus (MRSA): prevalence and antimicrobial sensitivity pattern among patients—a multicenter study in Asmara, Eritrea. Canadian Journal of Infectious Diseases and Medical Microbiology. 2019 Jan 1;2019.
5. Alharbi NS, Khaled JM, Kadaikunnan S, Aloabaidi AS, Sharafaddin AH, Alyahya SA, Almanaan TN, Alsughayier MA, Shehu MR. Prevalence of Escherichia coli strains resistance to antibiotics in wound infections and raw milk. Saudi journal of biological sciences. 2019 Nov 1;26(7):1557-62.
6. Torkan S, Bahadoranian MA, Khamsehpour F, Anyanwu MU. Detection of virulence and antimicrobial resistance genes in Escherichia coli isolates from diarrhoeic dogs in Iran. Archivos de medicina veterinaria. 2016;48(2):181-90.
7. Aljindan NY, Alkharsah KR. Pattern of increased antimicrobial resistance of Salmonella isolates in the Eastern Province of KSA. Journal of Taibah University Medical Sciences. 2020 Feb 1;15(1):48-53.
8. Bryce A, Hay AD, Lane IF, Thornton HV, Wootton VM. Prevalence of multidrug-resistant Escherichia coli isolated from drinking water sources. International Journal of microbiology. 2018 Aug 19;2018.
9. Tambekar DH, Dahikar SB, Lahare MD. Antibacterial potentials of some herbal preparations available in India. Res J Med Med Sci. 2009;4:224-7.
10. Hidayat YW, Widodo AD, Dachan YP. The antimicrobial effect of Oxivarea against methicillin resistance Staphylococcus aureus and Pseudomonas aeruginosa. Internet Journal of Microbiology. 2019;16 (1):200-10.
11. Kaurinovic B, Popovic M, Vlaisavljevic S. In vitro and in vivo effects of Laurus nobilis L. leaf extracts. Molecules. 2010 May;15(5):3378-90.
12. Harismah K. Pemanfaatan Daun Salam (Eugenia Polyantha) Sebagai Obat Herbal Dan Rempah Penyedap Makanan. Warta Lpm. 2017 Feb 21;19(2):110-8.
13. Al-Ogaili N, Bilal R, Younis H, Khadim T. The examination of the water concentrates of Laurus nobilis leaves antibacterial activity utilizing various strategies for extraction (in vitro). International Journal
of Research in Pharmaceutical Sciences. 2020 Jan 6;11(1):66-9.

27. Otsuka N, Liu MH, Shiota S, Ogawa W, Kuroda T, Hatano T, Tsuchiya T. Anti-meticillin resistant Staphylococcus aureus (MRSA) compounds isolated from Laurus nobilis. Biological and Pharmaceutical Bulletin. 2008 Sep 1;31(9):1794-7.

28. El Malti J, Amarouch H. Antibacterial effect, histological impact and oxidative stress studies from Laurus nobilis extract. Journal of food quality. 2009 Apr;32(2):190-208.

29. Sirken B, Yavuz C, Güler A. Antibacterial Activity of Laurus nobilis: A review of literature. Medical Science and Discovery. 2018 Nov 30; 5(11):374-9.

30. Patil N, Mule P. Sensitivity Pattern of Salmonella typhi And Paratyphi A Isolates to Chloramphenicol and Other Anti-Typhoid Drugs: An in Vitro Study. Infection and Drug Resistance. 2019;12:3217.

31. Qnais EY, Abdulla FA, Kaddumi EG, Abdalla SS. Antidiarrheal activity of Laurus nobilis L. leaf extract in rats. Journal of medicinal food. 2012 Jan 1;15(1):51-7.

32. Nafis A, Kasrati A, Jamali CA, Custódio L, Vitalini S, Iriti M, Hassani L. A Comparative Study of the in Vitro Antimicrobial and Synergistic Effect of Essential Oils from Laurus nobilis L. and Prunus armeniaca L. from Morocco with Antimicrobial Drugs: New Approach for Health Promoting Products. Antibiotics. 2020 Apr;9(4):140.