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Research Article

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 ± 1.1 mm). 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 5×10^7 to 5×10^4 . But the activity on *Escherichia coli* just weaken concentration 5×10^7 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*.

ABSTRAK

Laurus nobilis has been known for a long time as a plant that is efficacious for preventing and treating several kinds of diseases. This study aims to determine the antimicrobial effect of *L. nobilis* leaves extracted using ethanol against *Staphylococcus aureus*, *Salmonellae typhi*, and *Escherichia coli*. The research method used agar diffusion to measure the zone of inhibition against the test bacteria. Gentamicin and DMSO were used as positive and negative controls. The results showed that *Laurus nobilis* extract had antibacterial activity against *Staphylococcus aureus* with an inhibitory zone (16.3 ± 1.5 mm), *Salmonellae typhi* with a zone (14.5 ± 0.5 mm), and against *Escherichia coli* (11.3 ± 1.1 mm).

Kata kunci: Antibakteri; *Laurus nobilis* etanol ekstrak; *Staphylococcus aureus*; *Salmonellae Typhi*; *Escherichia coli*.

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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.¹ 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 lauraceae 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 Aldhafer *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

NPs was greater against Gram-positive (*S. aureus*) bacteria than Gram-negative (*P. aeruginosa*) bacteria.¹⁹ Therefore, the main objective of this study was to evaluate the antimicrobial activity of *L.nobilis* leaves extract against *S. aureus*, *S. typhi* and *E. coli*.

MATERIAL AND METHODS

Preparation of the *L. nobilis* leaves ethanol extract.

Fresh *L. nobilis* leaves ethanol extract (Daun Salam) weighing 5 kilograms (kgs) were collected from a farm in Malang Indonesia in October 2019. Its were washed under running tap water, air dried and finely grinded with a blender. 500g of the finely grinded leaves were then soaked in 300mls of 70% ethanol in an airtight container for 24 hours. The mixture was filtered using filter paper 11µm and the solute was extracted with a rotary evaporator at 45°C were the final volum of extract 100ml then stored in -20 °C before used.^{20,21,22}

Antimicrobial assay by agar-well diffusion method

This study was an evaluation which was intended to assess the antimicrobial activity of *L. nobilis* leaves ethanol extract to *S. aureus*, *S. typhi* and *E. coli*. 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 activity was carried out using the agar well diffusion method according to Clinical Laboratory Standards Institute guidelines (CLSI).^{23,24} Three to five colonies of each bacterium were dissolved in 2 ml of physiological saline and the turbidity was adjusted to 0.5 Mac Farland's turbidity which is equivalent to 0.5×10^8 bacteria per ml of solution. a swab use to spread the bacteria on surface of MHA media and then applied 100µl of *L.nobilis* leaves ethanol extract at concentration 100% on labeled well and 100

µl of Dimethyl Sulfoxide put on another well as a negative control and Gentamicin (10µg) disc was used as a positive control. The plate were incubated at 37°C for 24 h and the antibacterial activity determined by an inhibition zone (IZ) that formed around the well. The IZ of *L.nobilis* leaves ethanol extract was measure using calipers and compared with IZ gentamicin.²⁵

RESULTS AND DISCUSSION

Through our experiments as shown in Fig. (1) and Table 1 the results of agar well diffusion assay showed the IZ of *L.nobilis* leaves ethanol extract to *S. aureus* (16.3 ± 1.5 mm), followed by *S. typhi* (14.5 ± 0.5 mm) and *E. coli* (11.3 ± 1.1 mm). This results were shown that the *S. aureus* were most sensitive against *L.nobilis* leaves ethanol extract. This finding was in tandem with the results published by Al-Ogaili (2020) which highlighted the great inhibition activity of *L.nobilis* leaves ethanol extracts to this Gram-positive bacterium.²⁹ As reported by Otsuka et al. (2008) the *L. nobilis* has antimicrobial activity against methicillin-resistant *S. aureus* (MRSA) through purified two compound flavonoids and kaempferol, that both compounds showed strong antimicrobial activity.³²

Table 1. Antibacterial activity of *L.nobilis* leaves ethanol extracts

Microorganism	Diameter of growth of inhibition zones (mm)	
	<i>L.nobilis</i> leaves ethanol extract	Gentamicin
<i>S. aureus</i>	16.3 ± 1.5	25.6 ± 0.5
<i>S. typhi</i>	14.5 ± 0.5	20.6 ± 1.1
<i>E. coli</i>	11.3 ± 1.1	19 ± 0.5

*Values, including diameter of the well (6 mm), are means of three replicates ± SD

Figure 1. Antibacterial activity of *L.nobilis* leaves ethanol extract against bacteria (A) *Salmonella typhi* (B) *E. coli* (C) *S. aureus*.

The active compound was seen against *S. aureus*, *S. typhi* and *E. coli*. One from this Flavonoid compound has antibacterial properties because it has the capability to produce transduction energy

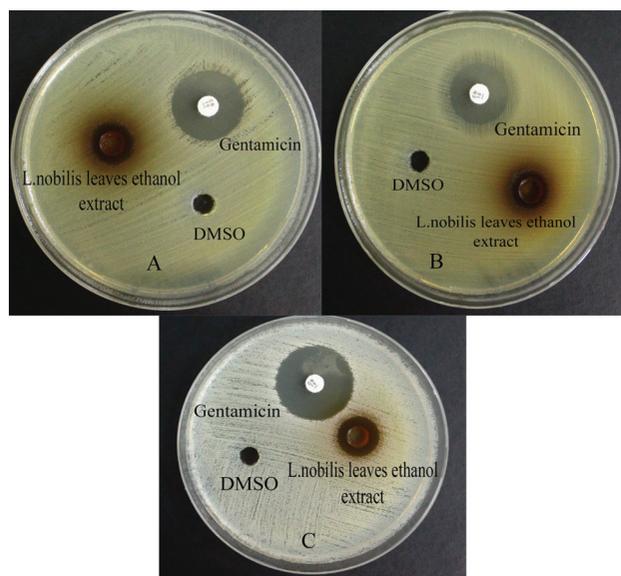


Figure 1. Antibacterial activity of *L.nobilis* leaves ethanol extract against bacteria (A) *Salmonella typhi* (B) *E. coli* (C) *S. aureus*.

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.¹⁴ Type of solvent used for extracting *L.nobilis* leaf has a major impact on their antibacterial activity. Extraction of *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 leaf 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 *S. aureus*.¹⁵ Also, El Malti and Amarouch (2009) found that the bay leaf extract has a significant antimicrobial activity against wide range of human pathogen.²⁸

Therefore, the result that we found confirmed that *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 *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.^{14,17} Also, the study of Siriken who demonstrated that the essential oil of *L. nobilis* had strong antibacterial activity against Gram-negative and Gram-positive food-borne pathogens.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.¹⁷ While study of Patil and Mule they found *S. typhi* sensitive to Cefixime, Ceftriaxone, and Azithromycin and based on average Minimal Inhibitory Concentration and MIC breakpoints.³⁰ Through the experiment conducted on Rats by Qnais *et al* (2012) which found *L. nobilis* aqueous extract has antidiarrheal agent.³¹

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

CONCLUSIONS

The result of this study demonstrated an antibacterial effects of *L. nobilis* leaves ethanol extract was proved a strong antibacterial activity against bacterial infections as they exhibited an antimicrobial effect against *S. aureus*, *S. typhi* and weak effect in *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.

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