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ANTIBACTERIAL ACTIVITY OF LEMON (*Citrus limon*) Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Citrus limon also referred as lemon is a fruit-bearing member of the *Rutaceae* family and a significant medicinal plant. Compounds derived from citrus limon from the extraction process are useful as antibiotic components to combat infections. As bacterial strains become more and more resistant to antibiotics nowadays, finding an alternative to antibiotics as a treatment for bacteria especially causing food poisoning and diarrhoea is crucial. The purpose of this study was to determine the antibacterial activity of *Citrus limon* peel extract against foodborne pathogens, mainly *S. aureus* and *E. coli*. The sem ethanol-based lemon extract was tested for antibacterial activity using the Kirby-Bauer disc diffusion method. The serial dilution was performed to estimate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) value of lemon peel extracts against *S. aureus* and *E. coli* which exhibited different values of MIC and MBC for *S. aureus* and *E. coli*. From the data, it clearly shows that ethanol extract of *Citrus limon* peel contains powerful inhibition towards pathogenic microorganisms especially *S. aureus* and *E. coli*. Increase the concentration of extracts is best recommended for upcoming studies of antimicrobial activity of fruit *Citrus limon*.

Keywords: medicinal plant, lemon peel extract, Citrus limon, antimicrobial activity, food poisoning.

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Introduction

Staphylococcus aureus and *Escherichia coli* are two types of bacteria which are dangerous and causes skin, bloodstream and foodborne diseases in humans and animals too. *S. aureus* strains are mainly resistant to multi drugs like penicillin (Khaled et al., 2021), ampicillin, quinolones, methicillin, and vancomycin. *E. coli*, a Gram-negative bacteria is known for its ability to infect the blood and meninges which leading to urinary tract infections (Mustafa et al., 2021) as well as other diseases. Antimicrobial resistance can be acquired through genetic mutations and Horizontal Gene Transfer (HGT), leading to fatal infections. The increasing threat of contagious microorganisms has raised concerns about an increase in antibiotic resistant pathogenic bacteria, particularly *S. aureus* and *E. coli*.

Citrus limon known as lemon is a part of the Rutaceae family, has been found to have antimicrobial, antioxidant, anticancer and anti-inflammatory properties. Lemon fruits consist of a variety of biologically important secondary metabolites, including flavonoids, limonoids, coumarins and furanocoumarins, sterols, volatile oils, organic acids, and alkaloids (Saeb et al., 2016). According to World Health Organization (WHO), medicinal plants, fruits, and their products can be used as a resource for obtaining various drugs. Natural extracts with antimicrobial properties are always efficient alternatives to chemical antibacterial products (Satyarup et al., 2022).

The National Antibiotic Resistance Surveillance Report in 2020 showed a decrease in resistance rates for *S. aureus* and *E. coli* strains, but high rates of resistance are still prevalent. The World Health Organization (WHO) notes that high rates of resistance are hospital-acquired or community-acquired,

with misuse of multidrug doses being a major contributor (Meer, 2019). A recent initiative to create new antibiotic types from fruits like lemon as a treatment for multidrug-resistant microorganisms was carefully chosen.

This study aims to determine and investigate the antibacterial properties of lemon peel extract against *S. aureus* and *E. coli*. The combination of lemon peel extract in ethanol solution is thought to enhance the antimicrobial property of antibiotic treatment against multidrug-resistant microorganism, particularly *S. aureus* and *E. coli.* Thus, it is expected that using lemon peel extracts for *S. aureus* and *E. coli* will aid in the reduction of resistance rates as well as the development of a new antibiotic using plant and natural fruit like lemon peel.

Methods

Preparation of Lemon peel extraction

Lemon fruits were collected from the local supermarket of Seremban. The lemon peels were oven dried at 55 °C, crushed into coarse powder by using a blender, and stored at room temperature for the next procedure. The yield peels powder was extracted using 80% ethanol by soaking method (10 gm powder + 80 ml ethanol) and the mixture were kept at 30 °C for 72 hours with constant stirring. Then, the extracts were filtered through Whatman no.1 filter papers. The filtrates evaporated using a rotary evaporator. Then, the extracts were weighed and stored at 5 °C (Sadat et al., 2021)

Preparation of bacterial suspension

A standardized $1x10^6$ cells/mL of *S. aureus* and *E. coli* suspension were used throughout the experiment. The standardized suspension was prepared by taking a loopful of freshly grown *S. aureus* and *E. coli* and dispensed in 5 mL of sterile nutrient broth. The turbidity of the cell suspension was adjusted to the equivalent of a MacFarland 0.5 standard by using spectrophotometer at 600 nm. 100 mL of the grown *S. aureus* and *E. coli* were inoculated onto MHA agar separately.

Determination of antimicrobial activity

The antimicrobial response toward lemon peel extract were evaluated by the disc diffusion method (Singh et al., 2020). The Kirby-Bauer susceptibility concept is applied for antibiotic testing. A blank disc with a diameter of 5 mm were dipped into the 10µl of each extract stock solution and placed on the surface of the MH agar of each Petri dish (Henderson et al., 2018). Gentamicin antibiotic disc was used as a positive control and blank disc was used as negative control. After 24 hours of incubation, the inhibition zones were determined using a measuring scale in millimetres (mm).

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) was measured by broth microdilution method on 96-well microtiter plate using Mueller Hinton broth. 100 µL of MH broth were dispensed into a microtiter plate label as Well 1 (W1) to Well 10 (W10). 100 µL of peel extracts concentration were added into W1. A serial two-fold dilution were carried out form W1 through W10. W11 was added with Gentamicin while W12 was added with peel extracts. Gentamicin as positive control and peel extract as negative control. The plate was gently shaken to mix the contents before being incubated at 37 °C overnight. The MIC value was the lowest concentration that prevented bacterial growth. 10µL from each test tube identified with MICs of the respective *S. aureus* and *E. coli* suspension were pipetted out and spread out onto MH agar surface and incubated at 37 °C for 18–24 hours. The concentration which exhibiting no growth observed were determine as the minimal bactericidal concentration (MBC).

Determination of percentage inhibition of diameter growth

The percentage inhibition of diameter growth (PIDG) was evaluated following antimicrobial susceptibility test (Latifah-Munirah et al., 2015). The percentage inhibitions of diameter growth (PIDG) values are determined according to the Equation 1 as described:

$$PIDGs(\%) = \frac{Diameter\ of\ sample\ (mm) - Diameter\ of\ control\ (mm)}{Diameter\ of\ control\ (mm)} \times 100 \qquad \text{Equation 1}$$

Result and Discussion

Determination of antibacterial activity of lemon peel

S. aureus and *E. coli* showed sensitive toward lemon peel extract at different level of concentrations used, with the presence of inhibition zones on Mueller-Hinton agar (MHA) plate after overnight incubation at temperature of 37 °C.

Figure 1 showed the diameter of inhibition zone (mm) of lemon peel extract against *S. aureus* and *E. coli*. In this study, lemon peel extract demonstrated better inhibition against *S. aureus* and *E. coli* at 100% concentration with mean of 18.22 mm and 16.22 mm, respectively. This result finding was similar with another study findings that reported the effectiveness of lemon peel extract toward *S. aureus* as its growth was weakly inhibited by ethanol extract of lemon peel (Harfouch et al. 2019). Another study by found lemon peel extract have moderate effectiveness toward *S. aureus* zone of inhibition 9.33 mm (Shakya et al.2019). A similar finding presented that zone of inhibition at 100% concentration is higher than lower concentration against Gram negative bacteria, *E. coli* with value 18.77 mm (Henderson et al. 2018). The resistance exhibits by both bacteria used are caused by the permeability of their cell walls or by the membrane accumulation mechanism which existed in both bacteria. Gram-negative bacteria have a high lipid content as a component of their cell wall, whereas Gram-positive bacteria have a permeable cell wall due to the presence of peptidoglycan as their outer cell layer. There is another explanation behind the effectiveness of lemon peel extract toward both bacteria and it's related to the presence of high concentrations of magnesium and zinc presence in lemon peel.

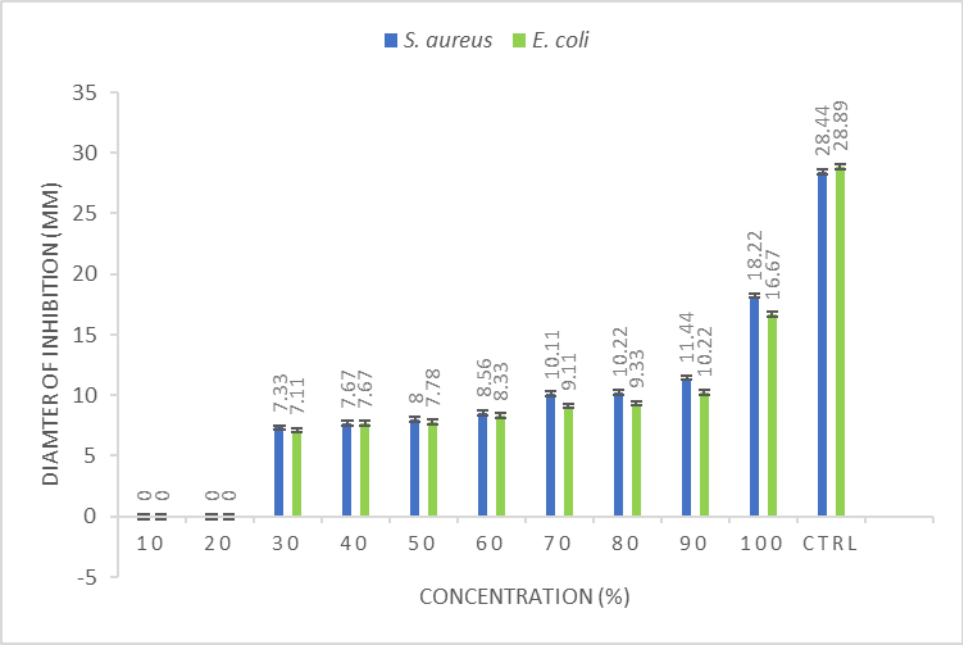

Figure 1. Diameter of inhibition zone (mm) of lemon peel extract against *S. aureus* and *E. coli*.

The MIC of lemon peel extract towards *S. aureus* and *E. coli* were determined as 75 mg/ml and 150 mg/ml, respectively while MBC value was determined as 50 mg/ml. MIC test was carried out for bacteria strain that showed sensitivity by forming zone of inhibition against lemon peel extract in lowest concentrations, while MBC test subjected to determine the lowest concentrations to kill bacteria tested. Based on the data of MIC and MBC obtained in Table 1, its explain that lemon peel extracts can acts as bactericidal against *S. aureus* and *E. coli*. The results of this study showed that lemon peel extracts exhibited antibacterial properties against *S. aureus* and *E. coli* with gradual increase in concentration

indicating the activity was concentration dependent.

Table 1. Determination of MIC and MBC value of lemon peel extracts via broth microdilution assay

Microorganisms	**Ethanolic extracts**		
	MIC (mg/ml)	**MBC (mg/ml)**	**Ratio**
Staphylococcus aureus	75	150	2
Escherichia coli	150	150	1

Figure 2 showed the PIDG graph of *S. aureus* and *E. coli* toward lemon peel extract. In this study, results obtained for the inhibition of zones were calculated into the formula for PIDG. These results can be seen in Figure 2 which shows that PIDG value of lemon peel extract concentrations were negative in reference to the PIDG value of positive control. Based on the PIDG value, *S. aureus* and *E. coli* has shown that lemon peel extract has little influence on growth inhibition compared to positive value used which is gentamicin.

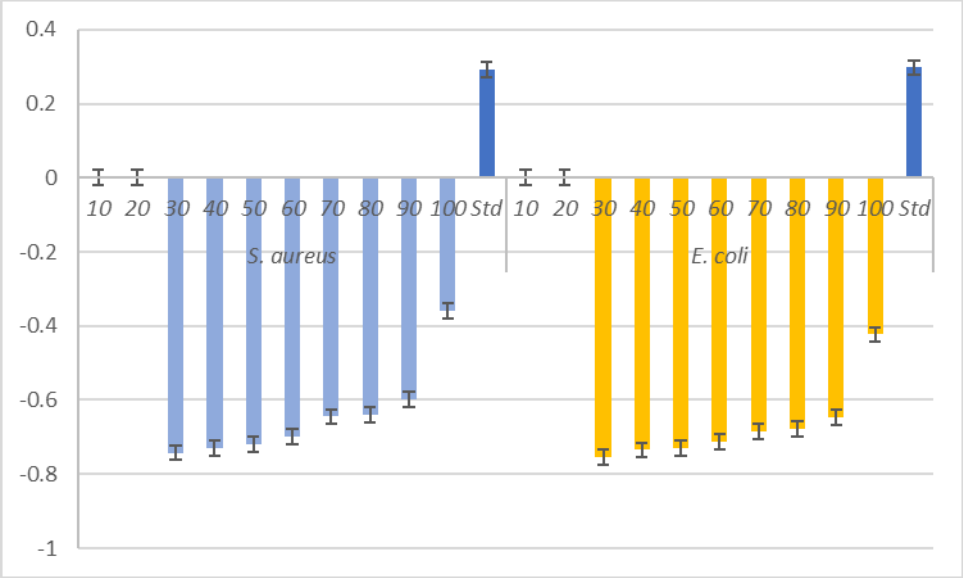

Figure 2. PIDG graph of *S. aureus* and *E. coli* toward lemon peel extract

Conclusion

This study concluded that ethanol peel extract of lemon possesses antibacterial properties on *S. aureus* and *E. coli*. Based on antimicrobial susceptibility test, MIC and MBC results, Gram negative bacteria which is *E. coli* were found to have low susceptibility against peel extract compared to Gram positive bacteria, *S. aureus* which shown intermediate susceptibility to peel extract. Besides, there was significance difference ($p<0.05$) detected on between *S. aureus* and *E. coli* toward lemon peel extract which can been seen at 100% concentration. Thus, it may conclude that lemon peel extract is more effective at Gram-positive bacteria, *S. aureus* than Gram-negative bacteria, *E. coli.* Lemon peel having the remarkable potential to function as a precursor of newer, more effective, and safer antimicrobial agents.

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Author Contribution

All authors contribute equally.

Conflict of Interest

Authors declare no conflict of interest.

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