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Antimicrobial Activity of Lemon Citrus Juice on Locally Pathogenic Bacteria

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Abstract:
Bacteria are among the most dangerous types of pathogens to humans because they are single-celled organisms that cannot be seen with the naked eye. They also attack various body systems and cause many bacterial infections and diseases of the digestive and respiratory systems, among others. Bacteria are regularly finding new behaviors to attack antibiotics, and these ways are spreading. For centuries, plants have been a chief in medicinal handlings for a variety of ailments due to their ability to synthesize hundreds of chemical compounds that play a role in defense against bacteria, fungi, and insects. Lemon (Citrus aurantifolia) is an important plant that typically grows in tropical climates. In this work, the antibacterial activity of citrus juice was evaluated on two nominated pathogenic bacteria, Staphylococcus aureus and Escherichia coli. 100% concentrated and 50% diluted fresh lemon juice tested and was compared with two types of antibiotics Amikacin and Piperacillin. To assay the antibacterial properties, agar well diffusion was followed. The results showed that concentrated lemon juice has an antibacterial effect on both Staphylococcus aureus and Escherichia coli, with the highest inhibition zone reaching 19 mm in Staphylococcus aureus, while the 50% diluted juice had no effect on Escherichia coli. The antibiotics used in this work did not appear inhibition zone in any species of bacteria which were used in this study. This indicates that citrus juice has antibacterial activity against bacteria that have drug resistance to some types of antibiotics because it has actual chemical compounds to eradicate bacteria.

Introduction

Maintaining respectable wellbeing is a top importance in today's domain, and it can be realized with effective medicines. However, the excessive and improper use of antibiotics has led to a growing threat of resistance in harmful microbes(Aggarwal et al., 2024). In some countries, the main way to protect antibiotics through stewardship programs is by controlling how they are sold, prescribed, and used (Manga et al., 2021). At first, scientists believed that bacteria became resistant to many antibiotics only when they had several resistance genes. Each of these genes would make the bacteria resistant to just one drug. But more recently, it has become clear that drug efflux pumps can often make bacteria resistant to many drugs (Massari et al., 2003).

Revisions of bacteria from permafrost demonstration they converted impervious without humans causing it. Bacteria can become resistant to some antibiotics naturally is an evolutionary process (Alfatlawi et al., 2021). When a bacterium is naturally resistant to an antibiotic, it's because of its own structure or how it works, which stops the antibiotic from working. Some antibiotics are pumped out of cells after entering through porin, while others can't get through the outer layer of the cell, so they can't reach where they need to go.(Jian et al., 2021). Bacteria are currently believed to acquire antibiotic resistance via active removal of the antibiotic from the cell, enzymatic modifications of the antibiotic, modifications of cell components which are the target of the

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antibiotic, overexpression of an enzyme inactivated by the antibiotic, a change in the permeability of bacteria cell membranes, production of an alternative metabolic pathway, an increase in the concentration of a metabolite which is an antagonist of the antibiotic (Urban-Chmiel et al., 2022). Antibiotic resistance genes (ARGs) can be passed down from parent to offspring or spread between bacteria through horizontal gene transfer (HGT) using mobile genetic elements (MGEs). The ways that ARGs are spread horizontally include transformation, conjugation transfer, and transduction, as well as through membrane vesicles (MVs) and DNA packaged like viruses. Bacterial mobile genetic elements (MGEs), like conjugative plasmids and integrative and conjugative elements (ICEs), are known to be important ways that disease-causing traits and resistance to antibiotics are spread. (Mutuku et al., 2022). The rise of bacteria that can resist many drugs is a worldwide issue. This problem is making infected people sicker and more likely to die. It is also making it harder to treat many different patients, such as those in the ICU, those who just had or are about to have surgery, those who had organ transplants, or those getting cancer treatment (Aljamali et al., 2021; Karnwal et al., 2023). The way Earth changes and stays alive depends on how humans, animals, and the environment work together, we are all connected, sicknesses can start when humans, animals, and the environment interact closely, these interactions are happening more and more makes people more likely to get current pandemics and global health problems like the COVID-19 pandemic it also increases antimicrobial resistance and the growing number of diseases.climate change, poverty, violence, and migration make these global health issues even worse (Ahmad et al., 2023).

In additional there is insistence regarding the lack of new treatments for pathogens. As the success rate of the drug development pipeline remains low, there is an urgent need for novel antibiotics to circumvent the threat from AMR, and our ability to treat common infections, the growing resistance of microorganisms to antibiotics highlights the need to investigate alternative solutions for disease prevention and management. Various plant extracts have demonstrated effective antimicrobial properties in numerous in vitro studies and even a few in vivo experiments (Delić et al., 2018; Rafsan et al., 2023). Antimicrobial agents can cause unwanted side effects, as well as microbial resistance. Because of this, researchers are becoming more interested in ethnopharmacology. Bioactive compounds are natural, found everywhere in most plants, and easy for people to get. Plant extracts offer many chances to discover new drugs because they have a wide variety of chemicals. As a result, there are thousands of medicinal phytochemicals that are safe and work better. For this reason, along with increasing microbial resistance to synthetic drugs, ethnopharmacology is quickly becoming recognized worldwide (Gupta et al., 2021) . Of these plant extracts, citrus fruit extracts show an acceptable antibacterial activity against a broad range of Gram-negative and Gram-positive bacteria. Therefore, they are recommended use(Álvarez-Ordóñez et al., 2013). Lemon (Citrus limon) is an edible fruit from a small tree or spreading bush in the rue family (Rutaceae). Citric acid may make up 5% or more of the weight of lemon juice, which is also high in vitamin C and low in vitamin B, particularly thiamin, riboflavin, and niacin. Lemon juice is a crucial by-product (Irshad Ali Saudagar et al., 2022). Investigation of lemon's antimicrobial properties showed that lemon is very effective at fighting Staphylococcus aureus, Klebsiella, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. Another study showed that extracts from grapefruit seeds and pulp could kill bacteria and fungi. Researchers found that Citrus extract from lemon, lime, and grapefruit could kill bacteria like Pseudomonas aeruginosa, Staph. aureus, proteus, klebsiella, and E. coli. They also found that lime and lemon juice were better at killing bacteria and worked as well as commercial antibiotic discs (Tawfik et al., 2010). Furthermore, a study found that fresh lemon juice works best against S. Paratyphi B and S. sonnei. Eating fresh lemons and drinking lemon juice has been linked to healthy gums and protection from gum disease (Katuromunda et al., 2024).

Other dealings can be cooperative in refining infection dealings, but we still necessity to generate new antimicrobials.

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Materials and Methods

-Sample Source and Preparation

Fresh lemon fruits were obtained from the local market in Al-Najaf Al-Ashraf province. According modified method (Rostikawati, 2020) .The fruits were thoroughly washed with tap water in the laboratory, and their surfaces were sterilized using 70% ethyl alcohol, followed by rinsing with sterile distilled water. The lemons were then aseptically cut using a sterile knife, and the juice was extracted into a sterile container. Subsequently, the juice was filtered into another sterile container to remove pulp and seeds. A volume of 5 mL of the raw juice was mixed with 5 mL of sterile distilled water to obtain a 50% concentration. The experiment was initiated immediately thereafter

-Microbiological Study

The Results of our work was directed in the Microbiology Laboratory at the College of Education for Women, University of Kufa. Pure local bacterial isolates of Staphylococcus aureus and Escherichia coli were used, which were obtained from the Al-Ameen Laboratory for Research and Biotechnology in Al-Najaf Al-Ashraf. The identification of the isolates was confirmed using the vitek 2 impact system

The antibacterial activity of the lemon juice at both concentrations was evaluated using the agar well diffusion method, three wells were drilled using a 6 mm diameter cork drill and the first was filled with concentrated lemon juice, the second with diluted lemon juice, and the third with distilled water as a negative control. based on the modified Kirby-Bauer technique. In addition, commercial antibiotic discs were used, including Amikacin (30 mg/disc) and Piperacillin (30 mg/disc).

All plates were incubated at 37°C for 24 hours. After the incubation period, the diameters of the inhibition zones around the wells and antibiotic discs were measured in millimeters using a ruler. All tests were performed in triplicate to ensure accuracy.

Results and Discussion

Bacterial diagnosis using the Vitec 2 impact system confirmed the species of bacteria as shown in tables 1 and 2

Table (1): Vitec 2 impact system diagnosis of Staph. aureus

Selected Organism	99% Probability	Staphylococcus aureus																	
	Bionumber: 010402062763231												Confidence:		Excellent identification				
SRF Organism																			
Analysis Organisms and Tests to Separate:																			
Analysis Messages:																			
Contraindicating Typical Biopattern(s)																			
Biochemical Details																			
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-		
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+		
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-		
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+		
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+		
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-		
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-		
64	OPTO	+																	

Table (2): Vitec 2 impact system diagnosis of E.

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Selected Organism	95% Probability	Escherichia coli		
	Bionumber: 0405611150524610		Confidence:	Very good identification
SRF Organism				
Analysis Organisms and Tests to Separate:				
Analysis Messages:				
Contraindicating Typical Biopattern(s)				
Escherichia coli	dSOR(90),PHOS(81),			

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

coli

Results of antibacterial activity of Citrus juice comparative with standard antibiotics discs were shown on Table (I). antibacterial activity was noticed in 100% (v/v) concentrations on Staph. aureus was 19 and 15 ml respectively but in the 50% concentration was on Staph. aureus only with 12 ml . and no effect on E.coli. on the other hand there is no effect for the Amikacin 30 mg/disc and Piperacillin 30 mg/disc on the two species of bacteria under study.

Table (1): Comparative the Diameter of inhibition zone antimicrobial activity of citrus juice concentrations and commercial antibiotic

Bacterial isolates	100% concentration	50% concentration	Amikacin 30mg/disc	Piperacillin 30mg/disc
Staph. aureus	19	12	-	-
E. coli	15	-	-	-

* Diameter of zone of inhibition in mm. *Data represented the callous of three reading

The most effect antibacterial activity was noticed of citrus juice with concentration 100% and 50% on Staph. aureus as shown in figure(1)



Figure 1: Antibacterial activity of citrus juice on Staph. aureus

This effect may be due to the composition of the cell membrane layers of Gram positive bacteria which consist of peptidoglycan, the polysaccharide wall can be penetrated easily, in

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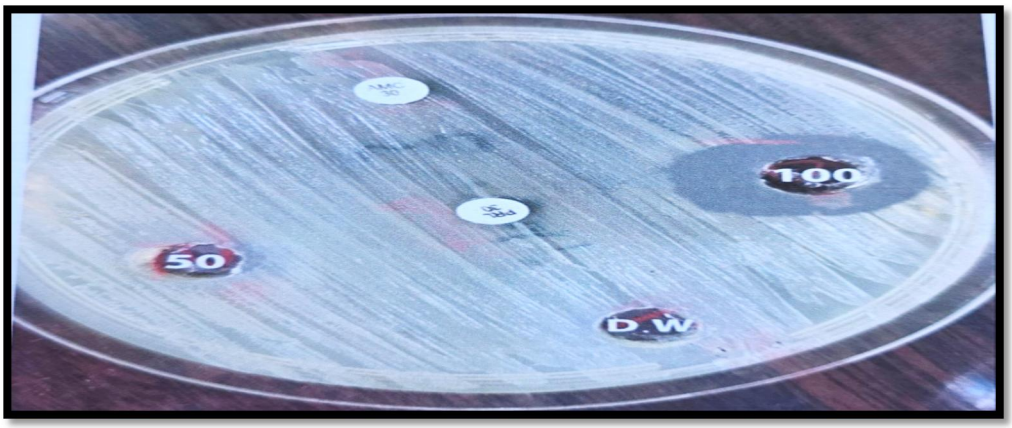
addition, the cell wall of Gram-positive bacteria contains teichoic acid, which has a polar polymeric nature, so polar compounds such as flavonoids can easily penetrate the polar peptidoglycan (Rostikawati, 2020). Especially since flavonoids are an important class of natural products in citrus fruits (Wang et al., 2022) furthermore to alkaloids, terpenoids, and tannins (Katuromunda et al., 2024). In E. coli, the effect of citrus juice was somewhat less than in Staph. aureus, where the concentrated juice 100% was the most effective, while the diluted juice was ineffective as shown in figure (2).

Figure 2: Antibacterial activity of citrus juice on E.coli

The reason for the weak effectiveness of lemon juice may also be due to the nature of the cell wall, as the cell wall in Gram-negative bacteria consists of an outer layer of phospholipids and some proteins, a quantity of polysaccharides, and a small amount of peptidoglycan located between the outer and inner layers of the membrane, the phospholipid layer is non-polar, so it is difficult for flavonoids to bind to it, and thus the effectiveness of these substances is less on Gram-negative bacteria. Additionally, there are enzymes in the periplasmic space that break down foreign molecules that try to enter cells (Ngo et al., 2023). Bacterial resistance to antibiotics is a major challenge in the treatment of diseases and thus negatively affects public health Therefore, researchers resort to finding an alternative method for treatment, such as using living organisms, including plants such as citrus fruits (Guedes et al., 2024). In this study, it was found that citrus juice has an inhibitory effect on Gram- positive negative and Gram-negative bacteria. The results of this study were similar to the results obtained by the researchers (Tawfik et al., 2010). The concentrated citrus juice is more effective than diluted juice, and this is consistent with what the researchers have concluded (P Chea & P Momo, 2021). Many parts of the lemon tree, including the fruit, have various therapeutic properties, including antiviral, antifungal, anti-inflammatory, antioxidant, anticancer, anti-ulcer, and anti-kidney stone properties. Secondary metabolites are potential sources of drugs due to their medicinal value, and these secondary metabolites are found in abundance in medicinal plants (Prakash et al., 2025). Lemon is an important source of flavonoid compounds with diverse biological effects (Walc et al., 2022). Citrus juice has a low pH due to its organic acid content, as it is rich in citric acid, which gives it



various biological activities, including its antibacterial properties (Tiencheu et al., 2021). Citric acid inhibits the growth of bacteria, and lemon contains a large percentage of tannin, which gives a high inhibition rate to the growth of bacteria by preventing the adhesion of microbial cells and enzymes and inhibits the transport of proteins within bacterial cell membranes. Tannin also targets the peptidoglycan layer in the formation of the cell membrane, which leads to the lack of integrity of the bacterial membrane due to the osmotic and physical pressure, which leads to the death of bacterial cells (Amelia et al., 2024) .

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