OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Antimicrobial Evaluation of Different Types of *Aurantioideae* Extracts Against Some Pathogenic Bacteria

Orooba Mohammed Saeed Ibrahim[1*], Rawaa Saladdin Jumaa[2], Nibras Zeyad Yahya[1]

[1]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; [2]Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Abstract | The antimicrobial resistance phenomenon has recently emerged as the major public health problems, and efforts to create new medication to counter bacterial infections. Consequently, there is a gap in the market for novel adjuvants. The primary initial source of many medicines used to treat diseases today was plants. This study aims to ascertain the antibacterial activities of fruit *Citrus aurantium* and *Citrus limonum* juice on isolated *Staphylococcus aureus*, *Sterptococcus pneumoniae* and *Klebsiella pneumonia*. The antibacterial activity of fruit juice extract on bacterial strain was determined using macro-broth dilution, agar well diffusion methods and time kill curve. Results revealed that the used juice extracts were variously bacteriostatic and bactericidal against the examined bacteria depending on concentration. Phytochemicals analysis indicated the presence of alkaloids, phenol, saponins, flavonoids, tannins, and glycosides. The test organism was more sensitive to *C. limonum* compared to *C. aurantium*. The MICs ranged from 1.56 to 12.5mg/ml; MBCs, (3.125 - 25mg/ml) and MBC/MIC ratios (2.0-4.0). Furthermore, a time killing curve of each 4x MICs and 8x MICs concentrations of juice extract showed a distinguished bactericidal effect at 6th hr. Therefore, the use of citrus fruit juice as medicine to treat infections is supported scientifically by these findings.

Keywords | Fruit Citrus, Time killing, Macro-broth dilution, Bactericidal, MIC

Received | June 15, 2023; **Accepted** | July 20, 2023; **Published** | September 15, 2023
***Correspondence** | Orooba Mohammed Saeed Ibrahim, Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; **Email:** auroba.m@covm.uobaghdad.edu.iq
Citation | Ibrahim OMS, Jumaa RS, Yahya NZ (2023). Antimicrobial evaluation of different types of *Aurantioideae* extracts against some pathogenic bacteria Adv. Anim. Vet. Sci. 11(10): 1736-1743.
DOI | http://dx.doi.org/10.17582/journal.aavs/2023/11.10.1736.1743
ISSN (Online) | 2307-8316

INTRODUCTION

Plants have been of the utmost importance since antiquity for a variety of reasons. It is common knowledge that plants are a significant source of natural compounds that can be used to develop cutting-edge drugs that can protect humans against a range of harmful infections (Suntar et al., 2018; Ibrahim et al., 2020). Drinking fruit juice aids in the preservation of good health and the prevention of numerous diseases. Humans frequently consume citrus fruit juice because of its distinctive flavor and nutritional benefits. Juice's favorable health effects have been attributed in part to its abundance of vitamin C (ascorbic acid) (Ibrahim & Muhammad, 2016; Klimek-Szczykutowicz et al., 2020). Citrus fruits contain a wide range of bioactive substances, such as phenolics, flavonoids, vitamins, and essential oils. These bioactive substances have been used as anti-inflammatory, anticancer, anti-oxidative, and antibacterial effects (Ibrahim et al., 2015; Al-Ghareebawi et al., 2021). In addition, lemon juice has a high ascorbic acid and citric concentration, which gives its acidic behavior (Ekawati & Darmanto, 2019; Singh et al., 2020).

Plants can produce nitrogen compounds (amines, alkaloids), terpenoids (as well as carotenoids), vitamins, and endogenous metabolites (Henderson, et.al. 2018) in addition to aromatic molecules including quinones, flavonoids, phenolic acids, coumarins, stilbenes, lignans, and

tannins (Jumaa et al., 2021; Ecevit et al., 2022). These ingredients serve as exudates or resistance mechanisms for plants, shielding them from herbivores, bacteria, and other predators. According to Appelhans et al., (2021) the *Rutacea* (*Aurantioideae*) family of citrus varieties has about 140 genera and 1,300 species. *Citrus limon* (lemon), *Citrus sinensis* (sweet orange), *Citrus reticulate* (tangerine), *Citrus aurantium* (acrid orange), *Citrus grandis* (shaddock), *Citrus Paradise* (grape natural product), *Citrus medica* (citron), and *Citrus aurantifolia* are just a few of the notable products of the genus Citrus (Lime) (Chen et al., 2023).

Lemon (*Citrus limon*) juice includes a variety of bioactive substances, including terpenoids, flavonoids, carotenoids, limonoids, and tannins. The bioactive ingredients found in *Citrus limon* has an antibacterial effect (Berti, 2015). In addition to being an antibacterial, *Citrus aurantifolia* (lemon juice) serves as an antioxidant (Rao et al., 2018). Citric acid and Vitamin C are the two primary components of lemon (*Citrus limon*) juice (Berti, 2015). The pH of lemon juice (*Citrus limon*) increases due to the concentration of vitamin C and citric acid (Berti, 2015; Ogundele & Bolade, 2021). *Citrus aurantium*, a cross between *Citrus maxima* and *Citrus reticulata*, is referred to as the bitter (sour) orange (Benayad et al., 2021). This plant is a native of Southeast Asia and is one of the sour citrus fruits which have a sugar to acid ratio of less than 1(Maksoud et al., 2021).

Traditional antibiotic therapy has several undesirable side effects. For instance, it may result in skin irritation, hyperpigmentation, dryness, and redness. In severe cases, negligence may also result in bacteria that are resistant to antibiotics. Therefore, this study aims, to determine the efficiency of citrus juice extracts of two citrus fruit *Citrus limon* and *Citrus aurantium* using different concentration against some Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

Ethical Approval

This study doesn't include any animal experiments that conducted the authors.

Collection, Identification And Extraction Of Plants

Fruits of *Citrus aurantium* and *Citrus limonum* were fresh and collected from the local market in Baghdad City, Iraq during November 2022- February 2023 (25 for each fruit / month) and then three times washed using distilled water. The plant preparation was done in the Department of Physiology, Biochemistry and Pharmacology at the College of Veterinary Medicine**.** A plastic juice extractor was used to squeeze the juice out of ten fruits from each after they had been peeled. The extracted fruit juice underwent a double filtration process using a 0.45 µm membrane filter and No. 1 Whatman filter paper, respectively. Also, the filtrate was weighed into a Petri dish made of sterile materials, where it was evaporated to a paste and allowed to cool. The weight of the residue was then calculated. The fruit juice extract underwent 20 hours of UV sterilization (Kijpatanasilp et al., 2023). By plating an aliquot of the reconstituted extract on nutrient agar plates, the sterility of the sample was examined. Within two days of preparation, the extract was used for phytochemical analysis and pharmacodynamic effects and stored in sterile containers at 4°C.

Phytochemical Analysis

The phytochemical screening was done to characterize the chemical constituents present in extracts of *Citrus aurantium* and *Citrus limonum* by their color reactions with different reagents. Each extract was subjected for alkaloids, glycosides, tannins, diterpine, flavonoids, carbohydrates, protein, steroids, saponins using the standard procedure. This analysis revealed the presence or absence of the phytochemicals constitution in the extract. Chemical solutions and reagents are all provided by BDH, England (Harbone, 1973).

Bacterial Strains

The Department of Veterinary Microbiology at the University of Baghdad provided the bacterial strains, which included *Staphylococcus aureus* ATCC (12600), *Streptococcus pneumonia* ATTCC (49619), *and Klebsiella pneumonia* ATCC (13883) due to more common for respiratory and urinary diseases. By using conventional biological techniques, the authenticity and purity of the bacterial strains employed in this investigation were verified (Nimer et al., 2016).

Pharmacodynamics Analysis

Sensitivity Test: To test the antibacterial activity, an extract of fruit juice was created using a modified version method of the agar well diffusion (Okeke et al., 2015; Al-Jumaili and Ibrahim, 2021). To obtain turbidity of 0.5 McFarland units, bacterial strains were cultured at 37°C for 12 hours. 500 ml of sterile Mueller Hinton agar (Oxoid, England) was carefully mixed with a standard bacterial inoculum containing $1.5x10^8$ CFU/ml (Quinn et al., 2004). Each received 25 ml in the Mueller Hinton agar in sterilized Petri dishes. To allow the agar to solidify, it was allowed to set for 10 minutes. Then, 4 wells measuring 6 mm in diameter were poured into the agar in each of these plates. After that, the wells were completed with 0.1 ml of each concentration of fruit juice extract (25, 50, 100 mg/ml and distal water as control), which was allowed to diffuse for two hours at room temperature. To preserve the fruit extract in the wells at 37°C for 24 hours, later, the plates

were incubated in the upright position. For each concentration of extract, three replications were performed, and the activity was assessed by milli-metering the diameter of the inhibitory zone enclosing each well against the tested organism.

Minimum Inhibitory Concentration (MIC): The microdilution tests, according to Veiga et al. (2019), were carried out in sterile 96-well microplates with a U-shaped bottom. All the wells on the plates received 100 µL of Mueller Hinton broth. 100 µL of fruit extract solutions were poured into the first well of each column (The lines were passed 100 µL of wells from 1 to 10 to perform serial dilutions (0.125-256 µg/ml)), Then 10 µL of the corresponding standardized inoculum ($1.5*10^8$ cfu/ml of test stains) was added. Also, 200 µL of Mueller Hinton was added to the blank wells in the negative control (column 12), which was left without microorganisms. The plates were incubated at 35 °C for 22 hours. Following incubation, 20 µL of tetrazolium chloride (TTC) solution 0.125% (w/v) was added to each well, and the plates underwent another 2 hours of incubation. For each strain, this technique was repeated 3 times.

Determination Of Mic Index: The MIC index was computed using the macro-broth dilution MIC and MBC values and the well diffusion MIC values. The Fractional Inhibitory Concentration Index (FICi) as updated by Sanders et al. (1993) served as the basis for the MIC and MBC indices The ratio MBC/MIC, we appreciated antibacterial activity. If the ratio MBC/MIC ≤4, the effect was considered as bactericidal but if the ratio MBC/MIC > 4, the effect was defined as bacteriostatic (Mogana et al., 2020).

Bacterial Time Kill Curve: The time-kill curve assay of fruit extract against all bacterial strains was based on the National Committee for Clinical Laboratory Standards (NCCLS, 1999). Briefly, the suspension of bacteria equivalent to 0.5 Mcfarland (1.5×10^8 CFU/ml) was prepared from overnight bacterial culture.0.1 ml of the prepared bacterial suspension was diluted in 14.9 ml of Mueller-Hinton broth and incubated at 37°C for 1 h. to obtain 10^6 CFU/ml bacterial suspensions. Fruit extract had been dissolved in Mueller-Hinton broth to prepare 10 mg/ml stock solution, after that, fruit extract concentrations from 8x MIC to 0.5x MIC had been prepared. Bacterial colonies were calculated at 0,1, 2, 4, 6 and 24 h through the incubation time by making serial dilutions and spreading of 20 µl of each dilution on Mueller-Hinton agar plate (triplicate); colonies range 30-300 CFU/plate was accepted (Miles et al., 1938; Zykov et al., 2018).

Statistics Analysis

The data was analyzed and performed using the Microsoft Program (SPSS). The data points were obtained using Microsoft Excel spreadsheets, and the results were analyzed using the SPSS application to assess statistical differences using the Chi-square and the P Values P ≥ 0.05 (Leech et al., 2005).

RESULTS

Analysis of Phytochemical

The harvest of the fruit juice extracts was 0.38% and 0.42% (w/v) for *Citrus limonum* and *Citrus aurantium* respectively. The results of phytochemical detection were itemized in (Table 1). The fruit juice extracts screening exhibited the existence of the following phytochemicals: phenol, alkaloids, flavonoids, saponins, glycosides, tannins, and water-soluble vitamins. While anthracene glycosides and terpenoids were absent

Table 1: Phytochemical analysis of fruit extract

Phytochemical substances	*Citrus limonum*	*Citrus aurantium*
Alkaloids	++	+
Phenol	+	+
Saponins	+	+
Flavonoids	++	+
Tannins	+++	++
Cyanogenetic Glycosides	++	+
Anthracene glycosides	-	-
Carbohydrate	++	+
water-soluble vitamins	++	+
Terpenoids	-	-

+ = positive; ++ = good present; +++= strongly present; – = not detected

Antibacterial Activity of Fruit Juice Extracts

Different concentrations of *Citrus aurantium* and *Citrus limonum* extracts were used in an assay of agar well diffusion, producing different degrees of inhibition zones against *S. aureus, S. pneumoniae* and *K. pneumoniae*. The size of the inhibitory zones varied depending on the extracted content, and they proportionally grew larger as the concentration of fruit juice extracts increased (Table 2 and Figure 1). The results revealed that *S. aureus, S. pneumoniae* and *K. pneumonia* strains were more sensitive to *Citrus limonum* juice extract than *Citrus aurantium* in all the concentrations used in this study. There was a substantial increase ($P<0.05$) in the diameter of the inhibiting zone *S. aureus, S. pneumoniae*, and *K. pneumoniae* growth at all concentrations of *Citrus aurantium* extract when compared to the zone of inhibition of *Citrus limonum* extract.

Table 2: Fruit extract antibacterial activity at various doses against test organism

Fruit juice extract	Concentration mg/ml	Zones of inhibition (mm)		
		S. aureus	*S. pneumoniae*	*K. pneumoniae*
Citrus limonum	25	12.0	14.0	12.5
	50	20.6	21.5	18.8
	100	23.8	24.5	21.6
Citrus aurantium	25	10.2	11.4	10.4
	50	16.5	18.5	15.2
	100	19.6	20.0	18.8

Table 3: Fruit juice extract's Minimum Inhibitory Concentration (MIC) and Minimum Inhibitory Concentration (MIC) index as determined by the Agar well diffusion and Macro-broth dilution procedures

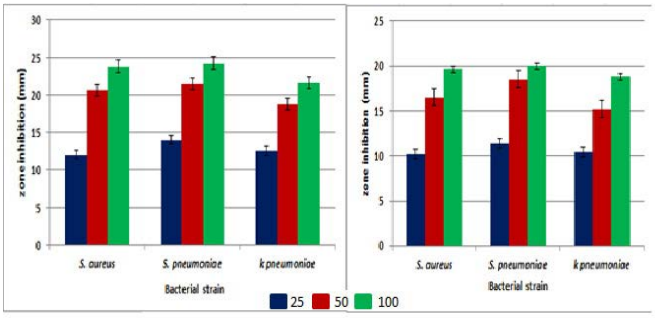
Test organism	MIC (Agar-well)		MIC (Macro-broth)		MIC index		Effect methods	
	F1 mg/ml	F2 mg/ml	F1 mg/ml	F2 mg/ml	F1	F2	F1	F2
S. aureus	12. 5	25.0	6.25	6.25	2	4	NS	NS
S. pneumoniae	6.25	12.5	1.56	6.25	4	2	NS	NS
K pneumoniae	12.5	25.0	3.125	12.5	4	2.0	NS	NS

Mean of triplicate experiments, MIC index "Agar well MIC\Macro-broth MIC"; S "significant (MIC index>4.0 or ≤0.125)"; NS "Non-significant (MIC index of >0.125 to 4.0)".

Table 4: Area under the time-kill curve of juice extract against ***bacterial Spp.*** (h*log CFU/ml).

Bacterial spp.	Extract	Control	0.5xMICs	1.0xMICs	2.0xMICs	4.0xMICs	8.0xMICs
S. aureus	*C.limonum*	142.5±0.23	118 ± 0.43	98.7±0.12	62.5±0.22	35.8±0.11	22.6±0.02
	C.aurantium	142.8±0.14	122.5 ±0.23	109.5±0.09	88.7±0.15	57.5±0.08	29.7±0.06
S. pneumoniae	*C.limonum*	142.2±0.23	120.5±0.23	100.1±0.23	78.5±0.23	42.5±0.23	30.5±0.23
	C.aurantium	142.3±0.14	128.3±0.14	112.8±0.14	82.8±0.14	62.8±0.14	44.8±0.14
K pneumoniae	*C.limonum*	142.2±0.21	135.4±0.23	110.3±0.23	85.3±0.23	52.5±0.23	28.5±0.23
	C.aurantium	142.3±0.12	132.3±0.14	115.2±0.14	91.9±0.14	59.8±0.14	34.8±0.14

Values represent mean ± S.E:
Means with significantly different (P≤0.05).



Citrus limonum *Citrus aurantium*

Figure 1: Proportional relationship between different concentration of fruit juice extract and the mean of inhibition zone (mm) against bacterial strain

Minimum Inhibitory Concentration (Mic) And Mic Index

According to the results of the microdilution method, the MIC results showed that the concentration of 1.56, and 6.25 mg/ml of *Citrus limonum* and *Citrus aurantium* respectively was the minimal concentration that inhibited the growth of *S. aureus*. The concentration of 6.25, and 12.5

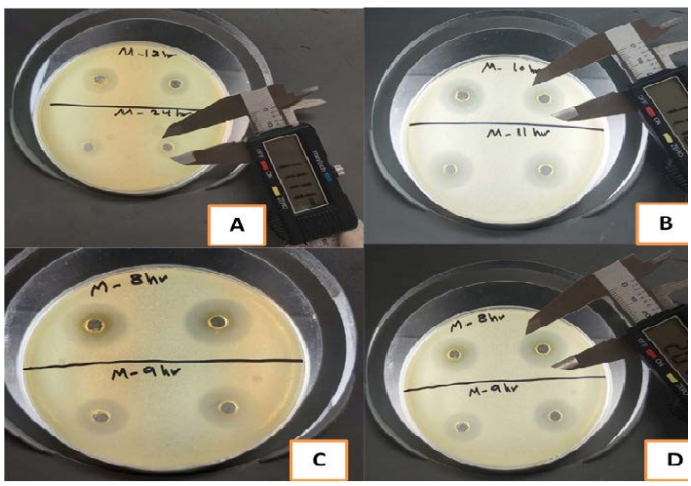


Figure 2: Sensitivity test of *S. aureus* (A), *S. pneumoniae* (B) and *K. pneumoniae* (C) to different concentrations of *Citrus limonum* and *Citrus aurantium* extracts

mg/ml of *Citrus limonum* and *Citrus aurantium* respectively was the minimal amount that inhibits the growth of *S. pneumoniae*. The concentration of 12.5, and 25.0 mg/ml of *Citrus limonum* and *Citrus aurantium* respectively was the minimal amount that inhibits the growth of *K. pneumoniae,* and that used in the test while other lower concentrations failed to inhibit the growth of the bacteria as shown in Table 3 and Figure 2. The fruit juice extract's macro-broth dilution MIC values were often lower than equivalent the values of agar well diffusion MIC 6.25-12.5mg/ml and 12.5-25.0 mg/ml for *Citrus limonum* and *Citrus aurantium* respectively. The MIC values produced by the two approaches differed, but not significantly.

Bacterial Time Kill Curve Kinetics

All concentrations from 8x (MICs) through 4x (MICs) achieved the bactericidal effect by reducing ≥3 log10 of the total number of CFU/ml of bacterial strains in comparison to control, 0.5x MIC and 1x MIC. While 2x MIC showed a drop in the growth curve at 1st six hours, then, there was continued inhibition of growth as reported at the 24th hour. The area under the time of killing curve of *Citrus limonum* and *Citrus aurantium* was calculated and compared to the control inoculum growth rate (Table 4). The results showed that all 4x (MICs) and 8x (MICs) achieved the highest significant bactericidal effect (P≥ 0.05) in comparison to other treatments. The 2x MIC concentration achieved a purely bacteriostatic effect (P≥ 0.05) in comparison to 1x MIC and 0.5x MIC concentrations and control groups. Both 1x MIC and 0.5x MIC failed to achieve a significant bacteriostatic or bactericidal effect (P≤ 0.05) in comparison to 2xMIC, 4xMIC and 8xMIC.

DISCUSSION

Citrus aurantium and *C. limonum* generally was an important medicinal plant and have several therapeutic potentials in folklore medicine (Falcinelli et al., 2020). In this study, the aqueous extracts of the fruits of *C. aurantium* and *C. limonum* revealed the presence of alkaloids, flavonoids, steroids, saponins, Cyanogenetic glycosides except anthracene glycosides and terpenoids which was absent. The results of this study corroborates the study of Dandekar et al. (2008) who reported the presence of tannins, flavonoids and saponins the absence of terpenoids, and phlobatannins in the aqueous and ethanol extracts of *C. auratium*. The study is also in agreement with the study of Babajide et al., (2023) who reported the presence of flavonoids, saponins and tannins in the aqueous extract of *C. auratium*. Furthermore, the presence of flavonoids in the study is in agreement with the report of He et al. (1997) who reported the presence of flavonoids and the types of flavonoids present. Maksoud et al. (2021) revealed presence of phytochemical in the current study when he found the presence of flavonoids, alkaloids, and tannins in *Citrus aurantium* and *Citrus limonum*. The research supports a study by Khudhair et al. (2017) that found tannins, flavonoids, and saponins in the watery extract of *C. auratium*.

The most suitable group of secondary metabolites in citrus products is flavonoids, which may naturally could improve human health by acting as anti-inflammatory, antimicrobial, anti-cholesterolemic, anti-diabetic, anti-carcinogenic, and anti-inflammatory (Chanet et al., 2010; Park et al., 2014). Therefore, *Citrus aurantium* and *Citrus limonum* extracts have effective antibacterial action against many bacterial strains, including *S. aureus, S. pneumonia*, and *K pneumoniae*, due to the presence of various phytochemical components. More and more reports of medicinal plants' antimicrobial qualities are coming in from all over the world (Hindi and Chabuck, 2013).

In the conducted research, each concentration of fruit juice extracts that are rich in vitamin C prevented bacterial growth (Okeke et al., 2015). In the present study, at the maximum concentration (100 mg/ml) of juice extracts, the biggest inhibitory zones against both Gram-negative and Gram- positive bacteria. Verghese et al. (2017a) reported similar results of higher ascorbic acid doses were reported to provide the biggest zones of inhibition in both Gram positive (*S. aureus*) and Gram negative (*E. coli*) in earlier research. According to Al-Talib et al. (2013) revealed the zone average of inhibition for *S. aureus* was greater than *E. coli*.

The MIC values of *Citrus aurantium* and *Citrus limonum* fruit juice extract as well as they could act as control positives for the antimicrobial activity in therapeutic plant extracts due to their wide-ranging antibacterial activity. The effectiveness of lemon juice extract against clinical and several strains of *S. aureus*, *S. pneumonia*, and *K. pneumoniae*, an organism infamous for its antibiotic resistance, is particularly intriguing (Van Eldere, 2003). As a result, compared to the Gram negative test organisms, the Gram positive test organism was more inhibited. The results of this study also showed that all bacterial strains were sensitive to orange extract. According to a review by Oikeh et al. (2020), *Citrus sinensis L.* is well known throughout the world due to its high Vitamin C content, which is linked to several important health benefits. Pepe et al. (2017) also revealed an antimicrobial effect in addition to these health benefits that found in plant extract.

One such alternative is vitamin C, which has been shown to have antimicrobial properties in numerous studies. It has a potent growth-inhibitory effect on the bacteria *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Campylobacter jejuni*, and *Enterococcus faecalis*, *Helicobacter pylori* (Vilchèze

et al., 2013). Initially, it was thought that vitamin C's ability to reduce pH was what caused it to have an antibacterial impact. This significant suppression may be due to the acidic pH of lemon extract, which can alter the charges of the amino acids that make up the peptidoglycan cell wall and disturb the active sites of enzymes. Additionally, vitamin C protects against free radicals and reactive oxygen species as a potent antioxidant (Mumtaz et al., 2023).

A low MBC/MIC ratio for the fruit juice extract suggests that the juice extract has a bacteriostatic concentration that is comparable to its bactericidal concentration. According to Sader et al. (2006) showed strong antibacterial capabilities are indicated by a low MBC/MIC ratio. The MIC values obtained using the agar diffusion approach were greater than those obtained using the macro-broth dilution method for the identical bacterial strains.

Although variations in the antimicrobial agent's diffusion rate in the agar have been put forth as an explanation for such discrepancies, this phenomenon still requires further research to reach acceptable standards for both techniques. Lemon fruit juice's antibacterial properties may be linked to its secondary metabolites, vitamins, organic acids, and their interactions with one another. Malic acid is absent from citric acid (Okeke et al., 2015).

Vitamin C is a crucial antioxidant that considerably lessens the harmful effects of reactive oxygen species (ROS). A startling finding was made about the pro-oxidant activity of vitamin C (Carr and Frei, 1999). According to Kallio et al. (2012), vitamin C functions as an antibacterial agent against a variety of bacterial infections, including *S. aureus*, *E. coli* (Verghese et al., 2017a), and *Klebsiella pneumonia* (Verghese et al., 2017b). It has been suggested that bacterial strain and concentration may both affect the antibacterial properties of vitamin C.

The most likely method by which vitamin C fights bacteria is based on oxidative stress brought on by the production of ROS. A fairly study by Ghosh et al. (2019) found that the Fenton reaction was triggered when vitamin C, an essential oil, and copper were combined. When combined with vitamin C, honey, especially honey, which was rich in polyphenols and transition metal ions (Fe and Cu), may cause bacterial cells to produce ROS within their own cells. Vitamin C in a honey solution quickly neutralizes the produced H_2O_2, which was a significant antibacterial component of honey, at sub-MIC levels. Due to variations in the metabolism or protective enzymes (ROS), the structure of bacterial cell wall, also the antibacterial activity of the vitamin C and honey combination was less efficient or ineffective when applied to specific Gram-positive bacteria in planktonic cultures (Majtan et al., 2020).

CONCLUSIONS AND RECOMMENDATIONS

Citrus fruit juice extracts are an efficient and secure antibacterial agent, according to this *in-vitro* investigation. Citrus fruit juice effectively suppressed the growth of bacterial strains include (Gram positive and Gram negative). Acidic pH was reported to have the highest zone of inhibition against all bacterial strains at concentration of 50 mg/ml for *Citrus limonum* and 100 mg/ ml for *Citrus aurantium* . Therefore, citrus fruit juice can be employed in the future as an assistant therapy option to treat infections in humans - brought on by bacteria that are resistant to multidrug after conducting safety and cytotoxicity evaluation. Lemon extracts have an important role as antimicrobial agents against microorganisms. They are natural, cheap, safe, and due to increase antibiotic resistance among bacteria.

ACKNOWLEDGMENTS

We would like to thank the Physiology, Biochemistry and Pharmacology and Microbiology departments for donating the bacterial strain at the College of Veterinary Medicine, University of Baghdad, Baghdad.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in this manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest that the authors of this manuscript claim should prevent this paper from being published.

NOVELTY STATEMENT

The novelty of the study is focus on antibacterial activity for Citrus limonum and Citrus aurantium extracts against bacterial resistance and efficient medicine for some diseases.

AUTHORS CONTRIBUTION

All the authors have contributed equally in terms of giving their technical Knowledge to frame the article.

REFERENCES

Al-Ghareebawi AM, Al-Okaily BN, Ibrahim OMS (2021). Characterization of Zinc Oxide Nanoparticles Synthesized

by Olea Europaea Leaves Extract (Part L). Iraqi J. Agricult. Sci., 52(3): 580-588. https://doi.org/10.36103/ijas.v52i3.1345

Al-Jumaili MAJ, Ibrahim OMS (2021). Pharmacodynamic Properties of Meropenem Against *Staphylococcus aureus*. Biochem. Cellul. Archiv., 21(1): 277–284.

Al-Talib H, Yean CY, Al-khateeb A, Ravichandran M (2013). Comparative evaluation of three different methods of genomic DNA extraction for *Staphylococcus aureus*. World Appl. Sci. J. 21: 424-427. https://doi.org/10.5829/idosi.wasj.2013.21.3.2850

Appelhans MS, Bayly MJ, Heslewood MM, Groppo M, Verboom GA, Forster PI, Duretto MF (2021). A new subfamily classification of the Citrus family (Rutaceae) based on six nuclear and plastid markers. Taxon, 70(5): 1035-1061.

Babajide AB, Adebolu TT, Oladunmoye MK, Oladejo BO (2023). Evaluation of antibacterial activity of Citrus aurantium L. leaf extracts on bacteria isolated from blood of Hepatitis B positive individuals in Ondo State, Nigeria. Microb. Infect. Dis., 4(1): 304-311.

Benayad O, Bouhrim M, Tiji S, Kharchoufa L, Addi M, Drouet S, Mimouni M (2021). Phytochemical profile, α-glucosidase, and α-amylase inhibition potential and toxicity evaluation of extracts from *Citrus aurantium* (L) peel, a valuable by-product from Northeastern Morocco. Biomolecules., 11(11): 1555.

Berti PL (2015). Antibacterial Lemon (Citrus limon (L.) Burm. (f)) on Porphyromonas gingivalis Domonant Periodontitis. Facult. Dentist., 4(1):6-9.

Carr A, Frei B (1999). Does vitamin C act as a pro-oxidant under physiological conditions? FASEB J., 13: 1007–1024. https://doi.org/10.1096/fasebj.13.9.1007

Chanet AD, Milenkovic C, Manach A (2010). Citrus flavanones: what is their role in cardiovascular protection. J. Agric. Food Chem., 60: 8809–8822. https://doi.org/10.1021/jf300669s

Chen X, Zhang J, Jiang D, Lu X, Xin X, Yin G (2023). Cryopreservation of Pollen Grains of Citrus and Other Aurantioideae. In Pollen Cryopreservation Protocols., 99-112.

Dandekar DV, Jayaprakasha GK, Patil BS (2008). Hydrotropic extraction of bioactive limonin from sour orange (Citrus aurantium L.) seeds. Food Chem J., 109: 515–520. https://doi.org/10.1016/j.foodchem.2007.12.071

Ecevit K, Barros AA, Silva JM, Reis RL (2022). Preventing Microbial Infections with Natural Phenolic Comp.. Fut. Pharmacol., 2(4): 460-498. https://doi.org/10.3390/futurepharmacol2040030

Ekawati ER, Darmanto W (2019). Lemon (Citrus limon) juice has antibacterial potential against diarrhea-causing pathogen. In IOP Conference Series: Earth and Environmental Science (Vol. 217, No. 1, p. 012023). IOP Publishing.

Falcinelli B, Famiani F, Paoletti A, D'egidio S, Stagnari F, Galieni A, Benincasa P. (2020). Phenolic compounds and antioxidant activity of sprouts from seeds of Citrus species. Agricult. J., 10: 33.

Ghosh T, Srivastava SK, Gaurav A, Kumar A, Kumar P, Yadav AS, Pathania R, Navani NK (2019). A combination of linalool, vitamin C, and copper synergistically triggers reactive oxygen species and DNA damage and inhibits Salmonella enterica subsp. enterica Serovar Typhi and Vibrio fluvialis. *Appl. Environ.* Microbiol J., 85: e02487-18. https://doi.org/10.1128/AEM.02487-18

Harbone JB (1973). Phytochemical Methods: Aguide to modern techniques of plant analysis. Chapman and Hall Ltd.,221-232.

He X, Lian L, Lin L, Bernart MW (1997). High-performance liquid chromatography– electrospray mass spectrometry in phytochemical analysis of sour orange (*Citrus aurantium* L.). J. Chroma. A., 791(1–2): 127.

Henderson AH, Fachrial E, Lister INE (2018). Antimicrobial activity of lemon (Citrus limon) peel extract against Escherichia coli. American Scientific Res. J. Engineer, Technol. Sci., 39(1): 268-273.

Hindi NKK, Chabuck ZAG (2013). Antimicrobial activity of different aqueous lemon extracts. J. Appl. Pharmaceut. Sci., 3(6), 074-078.

Ibrahim OMS, Abed AM, Yahea NZ (2020). Evaluation of Biological Activity of Greenly Synthesized Silver Nanoparticles Using Aloe Vera Gel Extract As Antibacterial Agent In-Vitro and In-Vivo. The Iraqi Journal of Agricultural Science, 51(6): 1706-1715. https://doi.org/10.36103/ijas.v51i6.1199

Ibrahim OMS, Muhammad MS (2016). Evaluation of Punica granatum Peels Extracts and its Phenolic, Alkaloid and Terpenoid Constituents Against Chemically Induced Diarrhoea in Rats. Adv. Anim. Vet. Sci., 4(3):161. https://doi.org/10.14737/journal.aavs/2016/4.3.161.168

Ibrahim OMS, Sarhan SR, Hameed AA (2015). In vivo and in vitro antibacterial activities of cranberry extract against E. coli O157: H7 in urinary tract infected rats. Adv. Anim. Vet. Sci., *3*(4): 233-244. https://doi.org/10.14737/journal.aavs/2015/3.4.233.244

Isela NNR, Sergio NC, Jose MSJ, Rene HD, Claudio CR (2013). Ascorbic acid on oral microbial growth and biofilm formation. Pharma Innovat., 2: 104-109.

Jumaa RS, Abdulmajeed DI, Karim AJ (2021). Evaluation of secondary metabolites of herbal plant extracts as an antiviral effect on infectious bursal disease virus isolates in embryonated chicken eggs. Vet. World., 14(11): 2971. https://doi.org/10.14202/vetworld.2021.2971-2978

Kallio J, Jaakkola M, Mäki M, Kilpeläinen P, Virtanen V (2012). Vitamin C inhibits *Staphylococcus aureus* growth and enhances the inhibitory e_ect of quercetin on growth of Escherichia coli in vitro. Planta Med., 78: 1824–1830.

Khettal B, Kadri N, Tighilet K, Adjebli A, Dahmoune F, Maiza-Benabdeslam F (2017). Phenolic compounds from Citrus leaves: Antioxidant activity and enzymatic browning inhibition. Complement. Integr. Med. J.., 14: 1–13. https://doi.org/10.1515/jcim-2016-0030

Khudhair AM, Abed AlA, Tareq RM, Osama KJ (2017). Phytochemical Analysis and Inhibitory Effect of *Citurs aurantium* L. (Bitter Orange) Leaves on some Bacterial Isolates *in vitro*. Dlyala J. Pure Sci., 13(1): 115 – 126

Kijpatanasilp I, Shiekh KA, Jafari S, Worobo RW, Assatarakul K (2023). Microbial Inhibition by UV Radiation Combined with Nisin and Shelf-Life Extension of Tangerine Juice during Refrigerated Storage. Foods, 12(14): 2725.

Klimek-Szczykutowicz M, Szopa A, Ekiert H(2020). Citrus limon (Lemon) phenomenon—a review of the chemistry, pharmacological properties, applications in the modern pharmaceutical, food, and cosmetics industries, and biotechnological studies. Plants., 9(1), 119.

Leech NL, Barrett KC, Morgan GA. (2005). SPSS for intermediate statistics: use and interpretation (2nd ed.). Mahwah, New Jersey: Lawrence Erlbaum Associates.

Majtan J, Sojka M, Palenikova H, Bucekova M, Majtan V (2020).

Vitamin C enhances the antibacterial activity of honey against planktonic and biofilm-embedded bacteria. Molecul. J., *25*(4): 992. https://doi.org/10.3390/molecules25040992

Maksoud S, Abdel-Massih RM, Rajha HN, Louka N, Chemat F, Barba F J, Debs E (2021). Citrus aurantium L. active constituents, biological effects and extraction methods. an updated review. Molecul. J., 26(19): 5832. https://doi.org/10.3390/molecules26195832

Miles AA, Misra SS, Irwin JO (1938). The estimation of the bactericidal power of the blood. Epidem. Infect. 38(6): 732-749. https://doi.org/10.1017/s002217240001158x

Mogana R, Adhikari A, Tzar MN, Ramliza R, Wiart C (2020). Antibacterial activities of the extracts, fractions and isolated compounds from Canarium patentinervium Miq. against bacterial clinical isolates. BMC Complement. Med. Therap., 20(1): 1-11.

Mumtaza S, Mumtaza S, Alia S, Tahira HM, Kazmib SAR, Mughalc TA, Younasd M (2023). Evaluation of antibacterial activity of vitamin C against human bacterial pathogens. Brazilian J. Biol., 83: 245.

NCCLS (1993). Methods for Dilution in Antimicobial Suceptibility Tests. Approved Standard M2-A5. National Committee for Clinical Laboratory Standards, illanova PA, 66-98.

Nimer NA, Al-Saada RJ, Abuelaish O (2016). Accuracy of the VITEK 2 system for a rapid and direct identification and susceptibility testing of gram- negative rods and gram-positive cocci in blood samples. Eastern Mediterranean Health J., 22(3): 193-200. https://doi.org/10.26719/2016.22.3.193

Ogundele OO, Bolade MK (2021). Biochemical characteristics and antioxidant properties of citrus juice from lemon (Citrus limon), lime (Citrus aurantifolia) and grapefruit (Citrus paradisi) as influenced by degree of ripening. Asian Food Sci. J., 20(3): 40-51.

Oikeh EI, Oviasogie FE, Omoregie ES (2020). Quantitative phytochemical analysis and antimicrobial activities of fresh and dry ethanol extracts of Citrus sinensis L. Osbeck (Sweet Orange) peels. Clin. Phytosci., 6: 46. https://doi.org/10.1186/s40816-020-00193-w

Okeke MI, Okoli AS, Eze EN, Ekwume GC, Okosa EU, Iroegbu CU (2015). Antibacterial activity of Citrus limonum fruit juice extract. Pak. J. Pharm. Sci., 28(5): 1567-1571.

Park K Il, Park HS, Kim MK, Hong GE, Nagappan A, Lee HJ, (2014). Flavonoids identified from Korean *Citrus aurantium* L. inhibit non-small cell lung cancer growth in vivo and in vitro. J. Funct. Foods., 7(1): 287–297.

Pepe G, Pagano F, Adesso S, Sommella E, Ostacolo C, Mantra M (2017). Bioavailable Citrus sinensis Extract: Polyphenolic composition and Biological activity. Molecules: A journal of synthetic chemistry and Natural product, 22(4): 623. https://doi.org/10.3390/molecules22040623

Quinn PJ, Carter ME, Markey B, Carter GR (2004). Clinical Veterinary Microbiology. Mosby. Edinburgh, Lomdon, New York, Oxord and Philadephia. USA. pp:21-63.

Rao N B, Kumari OS, Gajula RG, Anti-Microbial AO (2016). Phytochemical Analysis of Citrus Aurantium (Orange) Leaf Extract. IJRDO-J. Biolog. Sci., 2: 15-23.

Sader HS, Fritsche TR, Jones RN (2006). Daptomycin bactericidal activity and correlation between disk and broth microdilution method results in testing of Staphylococcus aureus strains with decreased susceptibility to vancomycin. Antimicrob. Agents Chemother., 50(7): 2330-2336. https://doi.org/10.1128/AAC.01491-05

Sanders CC, Sanders WEJR, Moland ES (1993). Decimal assay for additivity of drugs permits delineation of synergy and antagonism. Antimicrob. Agents Chemother., 37(2): 260-264.

Singh N, Jaiswal J, Tiwari P, Sharma B (2020). Phytochemicals from Juice as Potential Antibacterial Agents. Open Bioact. Comp. J., 8(1).

Suntar I, Khan H, Patel S, Celano R, Rastrelli L (2018). An overview on Citrus aurantium L.: Its functions as food ingredient and therapeutic agent. Oxidat. Med. Cellul. Longevit., 2018.

Van Eldere J (2003). Multicentre surveillance of Pseudomonas aeruginosa susceptibility patterns in nosocomial infections. J. Antimicrob. Chemother., 51(2): 347-352. https://doi.org/10.1093/jac/dkg102

Veiga A, Maria da Graça TT, Rossa LS, Mengarda M, Stofella NC, Oliveira LJ, Murakami FS (2019). Colorimetric microdilution assay: Validation of a standard method for determination of MIC, IC50%, and IC90% of antimicrobial compounds. J. Microbiolog. Methods., 162: 50-61. https://doi.org/10.1016/j.mimet.2019.05.003

Verghese RJ, Mathew SK, David A (2017b). Antimicrobial activity of vitamin C demonstrated on uropathogenic Escherichia coli and Klebsiella pneumoniae. J. Curr. Res. Sci. Med., 3: 88–93. https://doi.org/10.4103/jcrsm.jcrsm_35_17

Verghese RJ, Ramya SR, Kanungo R (2017). In vitro antibacterial activity of vitamin C and in combination with ciprofloxacin against uropathogenic Escherichia coli. J. Clin. Diagn. Res., 11: 1–5.

Verghese RJ, Ramya SR, Kanungo R (2017a). In vitro antibacterial activity of vitamin C and in combination with ciprofloxacin against uropathogenic Escherichia coli. J. Clin. Diagn. Res., 11:1–5. https://doi.org/10.7860/JCDR/2017/31251.10960

Vilchèze C, Hartman T, Weinrick B, Jacobs Junior WR (2013). Mycobacterium tuberculosis is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction. Nat. Commun., 4 (1): 1881. https://doi.org/10.1038/ncomms2898

Zykov IN, Samuelsen V, Jakobsen L, Småbrekke L, Andersson DI, Sundsfjord A, Frimodt-Møller N (2018). Pharmacokinetics and pharmacodynamics of fosfomycin and its activity against extended- spectrum-β-lactamase-, plasmidmediated Amp C-, and carbapenemase- producing Klebsiella pneumoniae and Escherichia coli in a murine urinary tract infection model. Antimicrob. Agents Chemother., 62(6). https://doi.org/10.1128/AAC.02560-17