Antibacterial Action of Citrus Fruits Against Selected Pathogenic Bacteria

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ABSTRACT

Objectives: This study's primary goal was to assess citrus fruits' antibacterial effectiveness against particular microorganisms.

Methods: The juice and peel of the seven chosen citrus fruits were extracted using a conventional qualitative method in an ethanol solvent. The resulting extracts were then diluted in an aliquot of 1 mL of 10% v/v DMSO. The antimicrobial test was conducted using the Agar well diffusion method. This study was conducted from February and April, 2022.

Results: The peel and juice extracts shown varying degrees of inhibitory zones on particular bacteria. *Citrus aurantium* juice extract was the most efficient citrus fruit extract against Staphylococcus aureus, with a 27mm zone of inhibition, out of the ones that were chosen. On the other hand, a 19mm zone of inhibition was demonstrated by the *Citrus reticulata* peel extract, which was more efficient against *Escherichia coli.*

Conclusion: Based on the findings, we conclude that citrus fruits may be helpful in preventing bacterial growth because of their strong antibacterial properties.

Keywords: Citrus fruits, Peel and juice extract, Ethanol solvent, Agar well diffusion, Zone of inhibition

INTRODUCTION

Citrus is a member of the Rutaceae family (Scazzocchio et al. 2001). Citrus fruits have a high acid content and are rich in nutrients, including vitamins, minerals, antioxidants, and other elements. The body benefits greatly from these nutritional values (Karapinar, 1985 and Nisha et al. 2013). Citrus fruits include flavonoids, which are phenolic and antioxidant chemicals. According to Nisha et al. (2013), they possess antibacterial, antiviral, antithrombic, antifungal, and anticancer properties. A high proportion of peel is represented by the fruits' total weights (Mandalari et al. 2006). Citrus peels are rich in polymethoxylated and flavonones, and their juices are primarily flavones and flavanones as well (Anita et al. 2016; Karapinar, 1985). Drinking citrus juice is popular because of its unique flavor and high vitamin content. As a flavoring agent, it is also utilized (Bourgou, 2012). Important citrus fruit varieties include Citrus sinensis (sweet orange), Citrus limon (lemon), Citrus aurantifolia (sour orange), Citrus maxima (pomelo), Citrus reticulata (mandarian), Citrus limetta (mausami), and others. Ascorbic acid, another name for vitamin C, is found in abundance in citrus fruits. Other bioactive substances found in it include alkaloids, vitamin B, limnoids, caretonoids, and essential oils. Fruits are 80–90% sugar and acid; the sap contains a lot of citric acid (Adode, 2002). The pulp inside the fruit is made up of various organic acids, fibers, ascorbic acids, soluble sugars, and potassium salt. It is this pulp that gives the fruit its distinctive citrine flavor (Roger, 2002). Juice from citrus fruits is useful for both maintaining good health and preventing sickness. According to Rekha et al. (2012) and Boudries et al. (2012), they have favorable health effects.

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METHODS

Study setting and fruits collection

The study was carried out from February to April, 2022 in the laboratory of the Department of Microbiology, D.A.V College. Fresh specimens of seven distinct citrus fruits—Citrus sinensis, Citrus limon, Citrus maxima, Citrus reticulata, Citrus limetta, Citrus japonica, and Citrus aurantium—were collected from Jawalakhel's local market.

Sample processing

After being collected, the fruits were properly cleaned in distilled water. Following washing, the fruit pulp was divided from the peel and mixed in a blender to produce juice. After being dried at 55°C, the peel was crushed to create a fine powder. Pure ethanol was used to extract the samples, or the peel and juices (10 g of powdered peel mixed with 100 ml of ethanol for peel extracts and 10 ml juice mixed with 90 ml of ethanol for juice extracts). The mixture was continuously stirred for 72 hours at 30°C.

Next, Whatmann no. 1 filter sheets were used to filter the extract. After that, the filtrate was centrifuged for 15 minutes at 4000 rpm. Once a sticky mass was formed, the solution was evaporated at 50°C. This mass was then weighed and dissolved in an aliquot of 1 milliliter of 10% v/v dimethyl sulfoxide (DMSO). Until it was needed again, the bulk was kept at 4°C (Shakya, et al. 2019).

Bacterial isolates

Bacterial isolates were isolated from the different medical samples in Sumeru city hospital. Isolation and identification were done in the hospital laboratory. All together five isolates were identified: Staphylococcus aureus, Salmonella Typhi, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae.

Assessment of antimicrobial properties

Using agar well diffusion, the antibacterial properties of citrus fruits were examined against test pathogens such as Salmonella Typhi, E. Coli, Pseudomonas sps, Klebsiella pneumonia and Staphylococcus aureus. 1000 mg of extracts were dissolved in 1 ml of 10% DMSO to create the extract sample. For twenty-four hours, the plates were incubated at 37°C. Citrus fruits were tested for their antibacterial activity by measuring the diameter of the zone of inhibition (ZOI).

RESULTS

Percentage yield of extracts

Citrus aurantium produced the highest yield of citrus fruit peel extract (21%) and Citrus japonica produced the lowest yield (6.5%) when citrus fruits were extracted as juice using ethanol as the solvent. Citrus sinensis produced the highest yield of citrus fruit juice (15.5%) and Citrus limetta the lowest (4.5%) (Figure 1).

Antibacterial activity of *Citrus aurantium*

Citrus aurantium peel extract shown more efficacy against Klebsiella pneumonia with a zone of inhibition of 12 mm, while the juice extract demonstrated greater efficacy against Staphylococcus aureus with a maximum zone of inhibition of 27 mm (Table 1).

Table 1: Antibacterial activity of *Citrus aurantium* against selected bacteria

Organism	ZOI (mm)	
	Juice	Peel
Klebsiella pneumoniae	16	12
Staphylococcus aureus	27	0
Escherichia coli	21	0
Salmonella Typhi	20	0
Pseudomonas aeruginosa	22	0

Antibacterial activity of *Citrus maxima*

The Citrus maxima peel extract had greater efficacy against Klebsiella pneumonia with a zone of inhibition of 13 mm, whilst the juice extract was more efficient against Staphylococcus aureus with a maximum zone of inhibition of 16 mm (Table 2).

Table 2: Antibacterial activity of *Citrus maxima* against selected bacteria

Organism	ZOI (mm)	
	Juice	Peel
K. pneumoniae	0	13
S. aureus	16	0
E. coli	0	9
S. Typhi	0	0
P. aeruginosa	15	0

Antibacterial activity of *Citrus sinensis*

Citrus sinensis peel extract did not exhibit any zone of inhibition against *Salmonella* Typhi, however its juice extract shown a greater degree of effectiveness with a maximum zone of inhibition of 11mm (Table 3).

Table 3: Antibacterial activity of *Citrus sinensis* against selected bacteria

Organism	ZOI (mm)	
	Juice	**Peel**
K. pneumoniae	10	0
S. aureus	0	0
E. coli	0	0
S. Typhi	11	0
P. aeruginosa	0	0

Antibacterial activity of *Citrus reticulata*

C. reticulata peel extract showed greater efficacy against *E. coli* with a zone of inhibition of 19 mm, but its juice extract demonstrated effectiveness against *Staphylococcus aureus* with a maximum zone of inhibition of 11 mm (Table 4).

Table 4: Antibacterial activity of *Citrus reticulata* against selected bacteria

Organism	ZOI (mm)	
	Juice	**Peel**
K. pneumoniae	0	10
S. aureus	11	0
E. coli	0	19
S. Typhi	0	0
P. aeruginosa	0	0

Antibacterial activity of *Citrus limon*

Citrus limon peel extract showed efficacy against *E. coli* with a ZOI of 10 mm, whereas citrus limon juice extract demonstrated greater performance against *S. aureus* with a maximal zone of inhibition of 25 mm (Table 5).

Table 5: Antibacterial activity of *Citrus limon* against selected bacteria

Organism	ZOI (mm)	
	Juice	**Peel**
K. pneumoniae	16	9
S. aureus	25	0
E. coli	16	10
S. Typhi	17	0
P. aeruginosa	20	0

Antibacterial activity of *Citrus limetta*

The juice extract of *Citrus limetta* showed more effective against *Salmonella Typhi* with the zone of inhibition 12mm while the peel extract of *Citrus limetta* did not show any zone of inhibition (Table 6).

Table 6: Antibacterial activity of *Citrus limetta* against selected bacteria

Organism	ZOI (mm)	
	Juice	**Peel**
K. pneumoniae	11	0
S. aureus	0	0
E. coli	9	0
S. Typhi	12	0
P. aeruginosa	9	0

Antibacterial activity of *Citrus japonica*

The juice extract of *C. japonica* showed more effectiveness against *S. aureus* and *K. pneumoniae* with zone of inhibition 15mm while the peel extract of *C. japonica* did not show any zone of inhibition (Table 7).

Table 7: Antibacterial activity of *Citrus japonica* against selected bacteria

Organism	ZOI (mm)	
	Juice	**Peel**
K. pneumoniae	15	0
S. aureus	15	0
E. coli	13	0
S. Typhi	0	0
P. aeruginosa	9	0

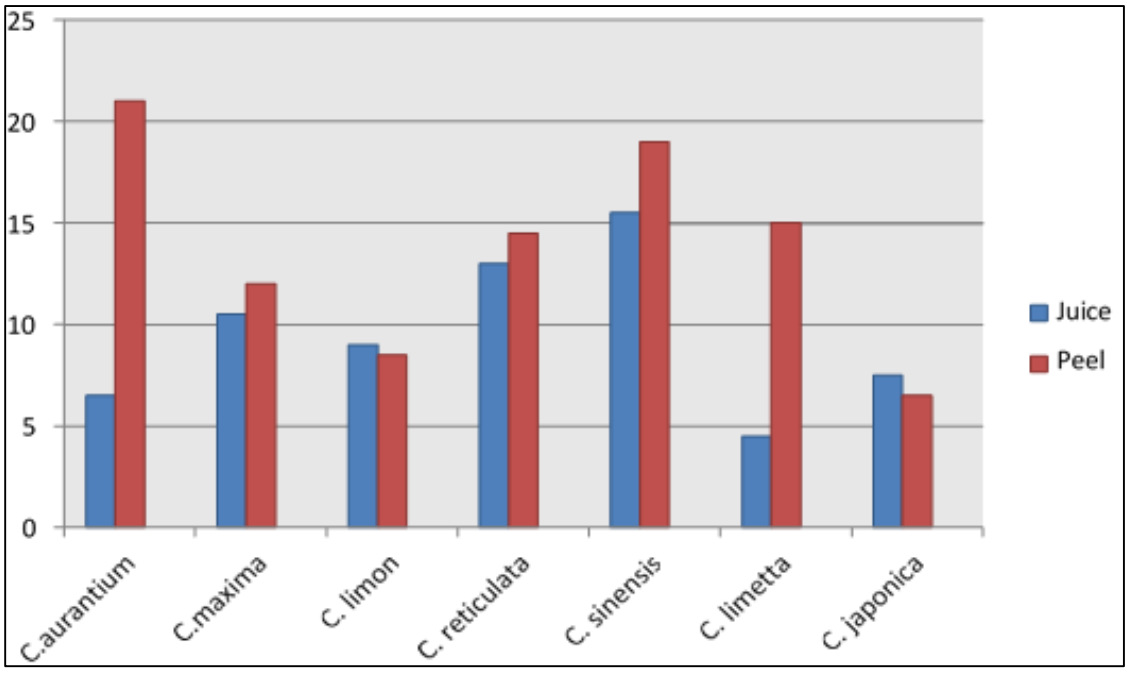

Figure 1: Percentage yield of Citrus fruits

DISCUSSION

In this study, we evaluated the antibacterial activity of citrus fruits such as *Citrus aurantium, Citrus limon, Citrus maxima, Citrus sinensis, Citrus limetta, Citrus reticulate* and *Citrus japonica* against selected bacteria. The juice extract of Citrus aurantium showed more effectiveness than its peel against *Staphylococcus aureus* with zone of inhibition 27mm while it showed lowest zone of inhibition with 16mm against *Klebsiella pneumoniae*. In the case of peel extract, the maximum zone of inhibition 12mm was showed by *Klebsiella pneumoniae* and it did not show any zone of inhibition against *Staphylococcus aureus, Pseudomonas aureus, Salmonella* Typhi and *Escherichia coli*. The Citrus aurantium showed highest antibacterial activity than other citrus fruits juices. Al-Ani et al, 2009 also reported that best antimicrobial activity was observed in juices of *Citrus aurantium*. They were active against the three types of bacteria used in their study. They include *Staphylococcus aureus, Pseudomonas aeroginosa* and *Proteus vulgaris* with 16- 20 mm inhibitory zones. This result was almost nearer to the value (16mm- 27mm) that we have obtained by our study.

The juice extract of *Citrus maxima* also showed more effectiveness against *Staphylococcus aureus* with the maximum zone of inhibition 16mm while it did not show any zone of inhibition against *Klebsiella pneumoniae*, *Salmonella* Typhi and *Escherichia coli*. Whereas the peel extract of *C. maxima* showed maximum zone of inhibition 13mm against *Klebsiella pneumoniae* but it did not show any zone of inhibition against *Pseudomonas aeruginosa*, *Salmonella* Typhi and *Staphylococcus aureus*. Similar study was done by Shakya et al, 2019. In her study, the peel extract of *C. maxima* did not show zone of inhibition against *Salmonella* Typhi, *E. coli* and *P. aeruginosa*. This study was slightly different from our study. This difference might be different in strain of *E. coli* and *S. aureus* chosen for study.

Here, the juice extract of *Citrus sinensis* were more effective against *Salmonella* Typhi with zone of inhibition 11mm while it did not show any zone of inhibition against *Staphylococcus aureus, E. coli* and *P. aeruginosa*. Likewise, their peel extract did not show any zone of inhibition against selected bacteria. Abalka et al, 2016 revealed that the peel extract of *C. sinensis* showed effectiveness against *Klebsiella pneumoniae, E. coli* and *P. aeruginosa* but no activity was shown by *Staphylococcus aureus* on the ethanol extract of *C. sinensis* peel at a different concentration of 50, 100, 150 and 200mg/l. This study did not similar to our finding. The reason for differences might be the collection of citrus fruits from different places. As the concentration

or chemical composition present in citrus fruits may be different in different places.

The juice extract of *Citrus limon* showed more effectiveness against *Staphylococcus aureus* with zone of inhibition 25mm while the least antibacterial activity against *Klebsiella pneumoniae* and *E. coli* with zone of inhibition 16mm. Whereas the peel extract of C. limon showed more effectiveness against *E. coli* with 10mm while the peel extract of C. limon did not show any zone of inhibition against *Pseudomonas aeroginosa, Staphylococcus aureus* and *Salmonella* Typhi. Shakya et al, 2019 revealed that the juice extract of *Citrus limon* was more effective than its respective peel extracts against both Gram positive and Gram negative bacteria. The extract of *Citrus limon* showed highest zone of inhibition against *Staphylococcus aureus* ATCC 25923 (17.66±0.577) which is similar to our study.

In the present study, the peel extract of *C. reticulata* found to be more effective against *E. coli* with maximum zone of inhibition 19mm, but the peel extract of C. reticulata did not show any zone against *P. aeruginosa*, *Staphylococcus aureus* and *Salmonella* Typhi. Likewise, their juice extract showed more effectiveness with maximum zone of inhibition 11 against *Staphylococcus aureus*, but it did not show any zone of inhibition against selected bacteria. Holem et al, 2020 reported that peel extract of mandarin has not significant effect to all bacteria. The area of inhibition of bacterial strain depends on the ability of the extract to diffuse uniformly through the agar.

The juice extract of *C. limetta* were more effective against Salmonella Typhi with zone of inhibition 12mm but S. aureus did not show any potential toward it. Whereas the peel extracts did not show any antibacterial activity. Mishra R. P. et al, 2012 revealed that the peel extract of *C. limetta* in 70% ethanol did not show any antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *E. coli*. This study was quite similar to our study.

In the present study, the juice extract of *C. japonica* showed higher antibacterial activity against *K. pneumoniae* and *S. aureus* with the maximum zone of inhibition 15mm while its juice extract did not show any antibacterial activity against *Salmonella* Typhi. Likewise, their peel extract did not show any antibacterial activity against selected bacteria. Similar study was done by Mahmound et al, 2019. In his study, the kumquat peel extracts were active against both Gram positive and Gram negative bacteria which did not match to our study. They also stated that the zone of inhibition was highly dependent on the type of extracts used in extraction process. The reason for difference in finding may be the different choice of solvent in extraction process. Pathan et al, 2012 observed that there is absence of the phytochemical such as flavonoids, steroids and tannin in ethanolic extract of peel whereas there is presence of the phytochemical in juice. The presence of phytochemical constituent helps in the effectiveness of juice extract of citrus fruits than that of peel.

In our study most of the Juice extract were found to be effective against *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*. This showed that these extract will be more effective against infection caused by these bacteria.

Conclusion

The intension of this project is to detect the antibacterial activity of juice extract of citrus fruits against selected pathogenic bacteria. This study concluded that the effectiveness of citrus fruits extract depends upon the particular types of citrus fruits juices. Different citrus fruits have their own antimicrobial property. The juice of *Citrus aurantium* and *Citrus limon* was found to be more effective in inhibiting the pathogenic bacteria. In upcoming world of antibiotic resistance, citrus fruits being as a natural product have proved to be effective against selected pathogenic bacteria.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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