# Evaluation of the antimicrobial effect of citrus pulp, peel, and juice extracts against *Streptococcus mutans* and *Lactobacillus acidophilus*

*Dharmashree Satyarup*[1], *Sailaja Panda*[1*], *Ramesh Nagarajappa*[2], *Shakti Rath*[3] & *Upasana Mohapatra*[1]

[1]Department of Public Health Dentistry, Institute of Dental Sciences, Siksha' O' Anusandhan (Deemed to be University) Bhubaneswar, Odisha 751003, India. [2]Dhruva Dental Care, Kasavanahalli, Bangalore-5600355, Karnataka, India. [3]Department of Microbiology & Research, Central Research Laboratory, Institute of Dental Sciences, Siksha' O' Anusandhan (Deemed to be University) Bhubaneswar, Odisha 751003, India

*Correspondence: sailajapanda10@gmail.com

Received: (21/11/2022), Accepted: (07/12/2022). DOI: https://doi.org/10.59049/2790-0231.1246

## ABSTRACT

Modern commercially available medicines are used extensively because of their guaranteed quick action, leading to an increase in the resistance of micro-organisms against these antimicrobials. Hence, there is a need to develop innovative formulations prepared from natural extracts which can replace these chemical antibacterials. The present study aimed to evaluate the antimicrobial efficacy of pulp, peel, and juice extracts of three different citrus fruits *(Citrus sinensis, Citrus limon, and Citrus limetta)* compared with Chlorhexidine (CHX) as positive control against oral pathogens like *Streptococcus mutans* and *Lactobacillus acidophilus.* The methanol extracts of three citrus fruits were prepared to estimate the Mean Zone of Inhibition in mm (ZOI), Minimum Bactericidal Concentration (MBC), and Minimum Inhibitory Concentration (MIC) to evaluate and compare their antimicrobial efficacy against these two oral pathogens. A comparison between the mean ZOI of these fruit extracts in comparison to CHX was made with the aid of an independent t-test. The present study revealed that *Citrus sinensis* (peel) showed a significantly higher mean diameter of ZOI against *L. acidophilus* (14.63±0.57) than CHX. *Citrus limetta* (peel) and CHX showed the highest antibacterial effect against *S. mutans* (MIC: 25 mg/mL, MBC: 50 mg/mL), but about *L. acidophilus*, all the extracts including CHX showed similar antibacterial activity (MIC: 25 mg/mL, MBC: 50 mg/mL) except for *Citrus limon*. All the fruit extracts were effective against both pathogens, while *Citrus sinensis* (peel) showed a significantly higher mean diameter of ZOI against *L. acidophilus.*

**Keywords:** Antibacterial, Dental caries, Chlorhexidine, Citrus fruits.

## INTRODUCTION

From the Vedic period till recent times, several natural products like plants, fruits, and their products have been used for medicinal purposes (1). According to the 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 (2). Further, with the evolution of multidrug-resistant strains, plant extracts are considered the best alternative to antibiotics, which are slowly losing their edge (3).

Citrus fruits are widely available in the market and possess various medicinal properties, especially oranges found mainly in tropical and subtropical regions (4,5). These fruits are rich in several vitamins like A, B complex, C, and E and flavonoids, phenolic compounds, carotenoids, and dietary fibers, which are helpful for the proper functioning of our immune system, digestive and cardiovascular systems (6). They act as antibacterial, anti-inflammatory, antiviral, and anti-cancerous agents (7,8). Each part of citrus fruits contains several bioactive substances, which are obtained from the fruit's peel, pulp, and juice and have been used by the pharmaceutical industry (6).

*C. Sinensis*, commonly known as sweet Orange, contains coumarins, peptides, flavonoids, carbamates, steroids, other chemical compounds, and various nutritional elements (9,10). Then the other common citrus fruits like *C.limetta* (Mosambi) and *C. Limon* (lemon) are also proven to have quite a good amount of antibacterial components like pectin, flavonoids, essential oil, polymethoxylated flavones, etc. (11,12). Several studies had been conducted earlier to assess and evaluate the antimicrobial effects of citrus fruit extracts. They have concluded that citrus fruits possess significant antibacterial actions against numerous microbes such as *S. aureus* and *epidermidis, P. aeruginosa, E. coli,* and *K. pneumonia* (7,8). Another study has concluded that *C. limetta* has antibacterial efficacy against *P. aeruginosa, E. coli,* and *S. aureus* (13).

Oral health is a fundamental component of general health and well-being. The presence of oral diseases like pain, infection, dental caries, mouth ulcer, periodontitis, and oral carcinoma can significantly influence general health. Out of all the dental problems, the most commonly occurring infectious and painful oral disease is dental caries affecting globally. *S. mutans* is responsible for initiating dental caries, while lactobacilli help in the progression of the carious lesion (14). So, in the current study, the test bacteria were *S. mutans and L. acidophilus*. Fluoride application and antimicrobial mouthwash use are effective preventive measures for dental caries. However, regular use of these antibacterial chemical compounds leads to various side effects. E.g., continuous use of CHX mouthwash can cause staining of teeth and increase the resistance of micro-organisms. Hence, there is a need to develop an effective formulation from natural or plant extracts with lesser adverse effects and costs that work as effective alternatives to commercial products. No previous studies have evaluated and compared the antimicrobial efficacy of citrus fruits' components (pulp, peel, and juice) against these dental caries-causing organisms.

Hence, the present study was conducted to evaluate and compare the antimicrobial efficacy of citrus pulp, peel, and juice extracts with that of CHX against *S. mutans and L. acidophilus* and to estimate the ZOI, MBC, and MIC of these extracts so that it can be added to the different formulation for the treatment of dental caries.

## METHODS

The instruments which were used for conducting the current study were an autoclave (Relitech, India), petri dish (Tarson, India), micropipette (Tarson, India), incubator (Universal, India), hot air woven (Universal, India), centrifuge (Remi, India), filter papers (Whitman no.1, Tarson, India), Laminar Air Flow (Yorco horizontal cabinet, India) and 96-welled (12×8) microtiter plate (Tarson, India).

The chemicals employed in the current investigation are methanol (Merck, India), DMSO (Dimethyl sulfoxide), triphenyl tetrazolium chloride (TTC), Mayer's reagent (Sigma, India), and Dragendorff reagent (Sigma, India). This study uses cultural mediums such as Mueller Hinton Broth and Nutrient Agar (HI media).

### *Collection of bacteria*

The test bacteria used in this trial were collected from the Central Research Laboratory of the institution. Two micro-organisms, i.e., *S. mutans* and *L. acidophilus,* were examined against pulp, peel, and juice extracts of *C. Sinensis, C. Limon,* and *C. limetta* for measuring ZOI, MBC, and MIC.

### *Collection and identification of fruit samples*

Three different citrus fruits, i.e., *C.* Sinensis (sweet Orange), *C. Limon* (lemon), *and C. limetta* (mosambi), were collected from the local market in Bhubaneswar city, Odisha. After being washed with distilled water, the peels of these fruits were removed (15). A juice extractor extracted the juice, and the pulps were collected. The pulp and the peel were dried in shadow at room temperature for 7 days. A sterilized mixture grinder is used to convert this dried pulp and peel into fine powder. 5 g of powdered peel and pulp sample and 50 ml of juice were dissolved in an aliquot of 100 ml of methanol and incubated at 4°C for a week with stirring. For each fruit sample, the same steps were repeated. Using sterilized Whatman No.1 filter paper, these extracts were filtered and stored in the sterilized container and refrigerated at 4 $^{0}$c (16,17).

### *Bacterial Strains*

*S. mutans* (MTCC 497) and *L. acidophilus* (MTCC-10307) were obtained from Microbial type culture collection and gene bank, IMTECH, Chandigarh, India. Two bacteria recognized as pathogens of dental caries were the micro-organisms chosen for testing in the current investigation. These strains were procured from *S. mutans*, a gram-positive, facultatively anaerobic coccus typically found in human oral cavities and plays a substantial role in tooth decay. It helps in caries initiation (18). *L. acidophilus* is a rod-shaped, gram-positive anaerobic microbe that advances a carious lesion that already exists (14).

***Agar-well diffusion method for antibacterial assays of fruit extracts***

With the help of the agar-well diffusion method, the antibacterial action of methanol extracts of pulp, peels, and juice extracts of collected fruits was estimated (18,19). Methanol was selected as the solvent for extraction based on a literature survey which suggested it was the best solvent and had the maximum extraction yield value (20,21). Both the identified bacterial strains monitored the extracts' antibacterial effects. Agar plates having 6mm thickness of agar were punched fully, bacterial colonies were made to appear on a petri dish, and after 30 mins, 6-8 wells were created. 50 µl molten Nutrient agar was used to line each well. 30 mg/mL solvent-extracts of the fruit were diluted by adding 10% (Dimethyl sulfoxide) DMSO solution to produce 100 µl aliquots to fill each agar well. The agar plates were incubated at 37°C for 18-24 hours. To evaluate the antibacterial activity, the mean ZOI was measured. Two agar plates were used for the experiment of every methanol extract of fruits, and the procedure was repeated three times. An aliquot of 100 µl of 2% CHX is considered the positive control, while 10% DMSO solution was the reference control. The 10% DMSO solution showed no activity.

***Estimation of MIC (Minimum Inhibitory Concentration)***

MICs are the lowest antimicrobial concentration that will inhibit a micro-organism's visible growth after overnight incubation (22). MIC of active pulp, peel, and juice extract prepared with methanol was estimated by diluting each fruit extract with 10% DMSO solution to attain concentrations of 0, 1.562, 3.125, 6.25, 12.5, 25, 50, 75, 100, 125, 150 and 200 mg fruit-extract/ml. A 96-well (12×8) microtiter plate was used to determine MICs. To each well on this plate, 80 µl of every dilution of fruit extract was added, which was then inoculated with 20 µl bacterial inoculum (109 CFU/ml) and mixed with 5 µl-aliquot of 0.5% Triphenyl tetrazolium chloride (TTC) and 100 µl MH broth (HI Media). Following this, each micro-titer plate was incubated for 18 hours at 37°C. The appearance of pink with the addition of TTC into a well was recorded as an indicator of bacterial growth. The MIC value was recorded where no color change was observed, which meant no bacterial growth.

***Estimation of MBC (Minimum Bactericidal Concentration)***

At each level of dilution, bacteria were subcultured from the well of the microtiter plate, and the level at which no bacterial growth was recorded was considered the MBC value for the extract (15). This process was repeated twice, and the readings recorded for the $2^{nd}$ time were considered.

***Qualitative phytochemical analysis***

The presence of different phytochemical agents was estimated by conducting a chemical test on every fruit extract. For testing the presence of reducing sugars aliquot of 2 ml of the pulp, peel, and juice extract in a test tube was added with equal volumes of Fehling's solutions I and II (5ml) and for 2 minutes heated in a water bath. A precipitate of brick-red color between the aqueous extract and Fehling solutions I and II indicated the presence of reducing sugars (13). For saponins, a mass of 0.5 g of the pulp, peel, and juice extract was dissolved in distilled water to get an aliquot of 10 ml which was agitated for 30 seconds and allowed to rest for 45 minutes—frothing when warmed confirmed saponins being present. For flavonoids, a ferric chloride solution of 10% was mixed with 0.5 ml of the dissolved pulp, peel, and juice extract. The appearance of blue-green color confirmed that flavonoids were present. Similarly, for testing the presence of tannins, the water of 5ml was added to 0.5 g of the pulp, peel, and juice extract. Then 10% ferric chloride solution was added to this mixture. A blue-black, green, or blue-green precipitate confirmed tannins' presence (13). To know the presence of steroids/terpenes, a mass of 500 mg of the concentrated mass of pulp, peel, and juice extract was added to 2 ml of acetic anhydride and got dissolved. Then 12 N sulfuric acid ($H_2SO_4$) was added to this mixture. After this, cooling of the mixture was done at 0 to 4°C. A change in the color of the solution from violet to blue-green color confirmed the presence of a steroidal nucleus. In the case of alkaloids, a mass of 0.5 g of pulp, peel, and squeezed juice was stirred with an aliquot of 5 ml 1% HCl in a steam bath. Then the filtration of this mixture was done. Mayer's reagent and Dragendorff reagent was added to this 1ml of the filtrate. The turbidity in the tubes indicated that alkaloids were present in the extract (23). An aliquot of 2 ml of glacial acetic acid (1.048 g/mL) and a drop of 1% $FeCl_3$ solution was added to the 5 ml pulp, peel, and juice extract to test the presence of glycosides, and mixing was done thoroughly. 1ml of sulfuric acid like 12N $H_2SO_4$ was mixed with this mixture. The formation of a brown ring at the interface confirmed that glycosides were present. All these tests were repeated for confirmation (23).

### *Statistical analysis*

Data was entered through a personal computer, and statistical analysis was performed using SPSS version 20 (IBM, Chicago, USA). The zone of inhibition was expressed in mean and standard deviation (SD). A comparison between the mean ZOI of three citrus fruit extracts with CHX was made with an independent t-test. The confidence interval is 95%, and a p-value ≤0.05 was considered statistically significant.

## RESULTS

The mean diameter of ZOI of methanol extracts of three citrus fruits, i.e., *C. Sinensis*, *C. Limon and C. limetta*, and CHX, are mentioned (table 1). Based on the results of the current study among all the fruit extracts, *C. limetta* (juice) showed the highest mean ZOI (11.80±0.49) against *S. mutans* followed by *C. Sinensis* (peel) (11.53±0.86) and lowest mean ZOI was shown by *C. Limon* (peel) (6.98±0.13). However, the positive control CHX exhibited the highest mean ZOI (11.98±0.09) against *S. mutans* compared to all other fruit extracts (table 1). DMSO (negative control) could not inhibit the growth of *S. mutans*.

In the case *of L. acidophilus*, *C. Sinensis* (peel) showed the highest mean ZOI (14.63±0.57) even in comparison to CHX (13.95±0.08) followed by *C. limetta* (juice) (14.03±0.10) and the lowest mean ZOI was shown by *C. Limon* (peel) (6.98±0.13). Again, DMSO showed no activity against *L. acidophilus* (table 1).

**Table (1):** Mean diameter of inhibition zone in mm (SD) against *S. mutans* and *L. acidophilus*.

| Group | Material | *S. mutans* (Mean±SD) | *L. acidophilus* (Mean±SD) |
|---|---|---|---|
| Pulp | *C. sinensis* | 8.59±0.81mm* | 12.05±0.13 mm* |
| | *C. limon* | 8.03±0.10 mm* | 9.80±0.41mm* |
| | *C. limetta* | 10.93±0.15 mm* | 11.98±0.17 mm* |
| Peel | *C. sinensis* | 11.53±0.86 mm | 14.63±0.57 mm# |
| | *C. limon* | 6.98±0.13 mm* | 11.05±0.20 mm* |
| | *C. limetta* | 9.81±0.41 mm* | 13.96±0.10 mm |
| Juice | *C. sinensis* | 11.48±0.66 mm | 13.98±0.17 mm |
| | *C. limon* | 7.95±0.13 mm* | 9.81±0.43 mm* |
| | *C. limetta* | 11.80±0.49 mm | 14.03±0.10 mm |
| CHX | | 11.98±0.09 mm | 13.95 ±0.08 mm |
| DMSO | | No activity | No activity |

**SD:** Standard deviation, **CHX:** Chlorhexidine, **DMSO:** Dimethyl sulfoxide.
***In comparison to CHX (p= 0.001).**
**# In comparison to CHX (p=0.016).**

Multiple independent t-tests were employed to compare the antimicrobial efficacy of each of the fruit extracts with CHX. CHX exhibited significantly higher ZOI in comparison to all other extracts (p≤0.05) except for *C. Sinensis* (peel), *C. Sinensis* (juice), and *C. limetta* (juice) (p>0.05) against *S. mutans*. In the case of *L. acidophilus*, *C. Sinensis* (peel) showed significantly higher mean ZOI (p≤0.05) as compared to CHX.

According to MIC and MBC values (table 2) in this study, out of all the fruit extracts, only *C. limetta* (peel) was comparable to CHX at MIC: 25 mg/mL, MBC: 50 mg/mL against *S. mutans* and the highest concentration was shown by *C. Limon* (peel, juice) (MIC: 50 mg/mL, MBC: 125 mg/mL). Against *L. acidophilus*, all the extracts except *C. Limon* were comparable to that of CHX at MIC: 25 mg/mL and MBC: 50 mg/mL.

**Table (2):** MICs and MBCs of *C. Sinensis*, *C. Limon*, and *C. limetta* extracts on *S. mutans* and *L. acidophilus*.

| **Extracts** | **MIC and MBC values (mg/mL)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***C. sinensis pulp*** | | ***C. sinensis peel*** | | ***C. sinensis juice*** | | **2% CHX (Positive control)** | |
| ***S. mutans*** | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| | 50 | 100 | 25 | 75 | 50 | 75 | 25 | 50 |
| | ***C. Limon pulp*** | | ***C. Limon peel*** | | ***C. Limon juice*** | | | |
| | MIC | MBC | MIC | MBC | MIC | MBC | | |
| | 25 | 75 | 50 | 125 | 50 | 125 | | |
| | ***C. limetta pulp*** | | ***C. limetta peel*** | | ***C. limetta juice*** | | | |
| | MIC | MBC | MIC | MBC | MIC | MBC | | |
| | 25 | 75 | 25 | 50 | 25 | 75 | | |
| ***L. acidophilus*** | ***C. Sinensis pulp*** | | ***C. Sinensis peel*** | | ***C. Sinensis juice*** | | **2% CHX (Positive control)** | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| | 25 | 50 | 25 | 50 | 25 | 50 | 25 | 50 |
| | ***C. Limon pulp*** | | ***C. Limon peel*** | | ***C. Limon juice*** | | | |
| | MIC | MBC | MIC | MBC | MIC | MBC | | |
| | 25 | 50 | 50 | 100 | 25 | 100 | | |
| | ***C. limetta pulp*** | | ***C. limetta peel*** | | ***C. limetta juice*** | | | |
| | MIC | MBC | MIC | MBC | MIC | MBC | | |
| | 25 | 50 | 25 | 50 | 25 | 50 | | |

**MIC:** Minimum Inhibitory Concentration, **MBC:** Minimum Bactericidal Concentration, **CHX:** Chlorhexidine.

Comparatively, almost all the extracts showed a greater mean diameter of ZOI against *L. acidophilus* than *S. mutans* (Figure 1-3). Besides, compared to CHX, only *C. Sinensis* (peel) extract has shown a significantly greater mean diameter of ZOI against *L. acidophilus* (Figure 2). Results of phytochemical analysis (table 3) revealed that alkaloid is found in all three citrus fruits' pulp, peel, and juice extracts, followed by tannin, which is absent only in *C. Sinensis* (juice) whereas terpenoids were not found in a maximum of the extracts.

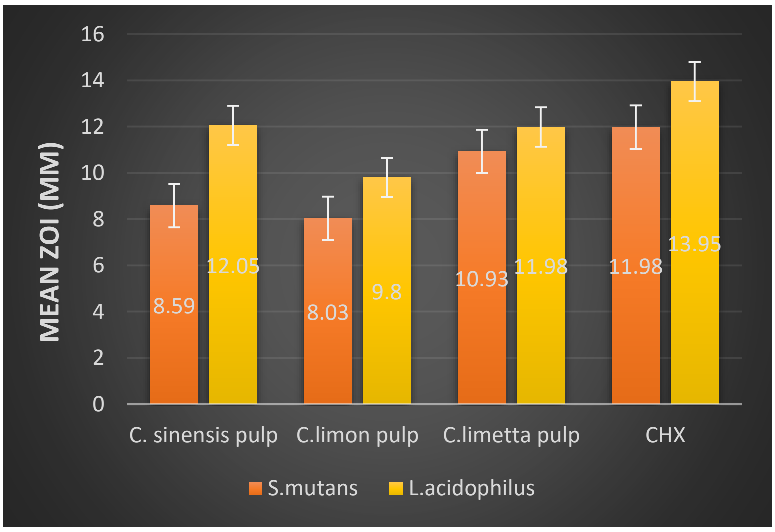


**Figure (1):** Comparison of the mean diameter of ZOI of citrus fruit pulp extracts and CHX against *S. mutans and L. acidophilus*.

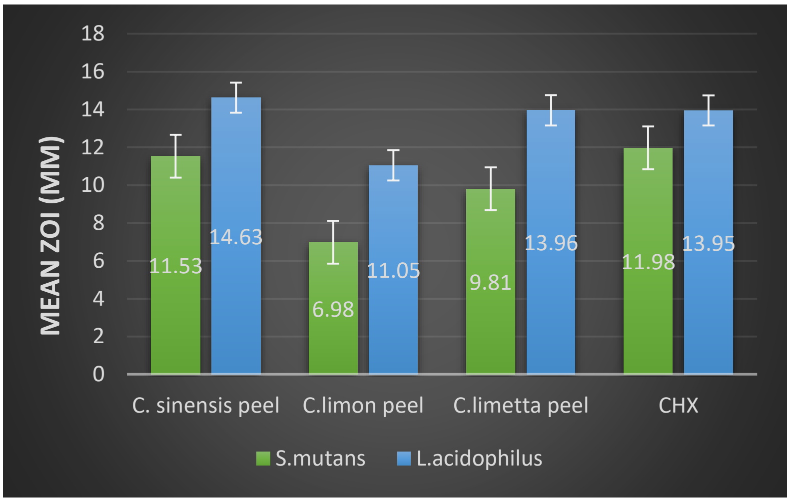


**Figure (2):** Comparison of the mean diameter of ZOI of citrus fruit peel extracts and CHX against *S. mutans and L. acidophilus.*

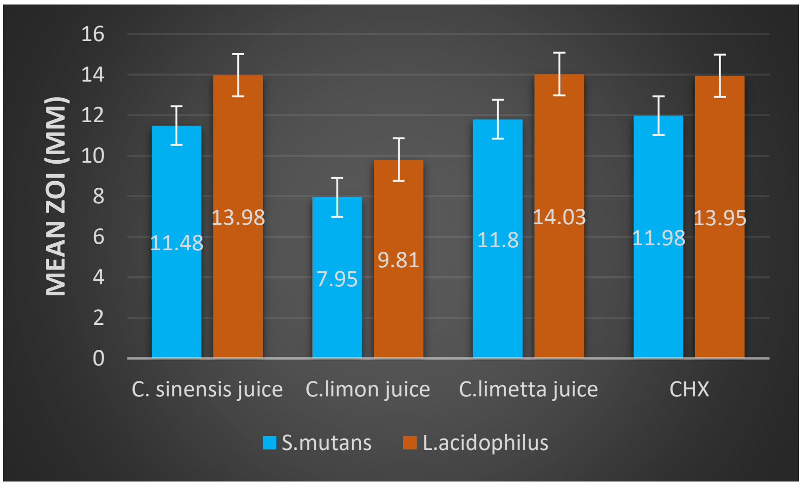


**Figure (3):** Comparison of the mean diameter of ZOI of citrus fruit juice extracts and CHX against *S. mutans and L. acidophilus.*

**Table (3):** Phytochemical screening of three citrus fruit extracts.

| | Alkaloids | Glycosides | Terpenoids | Reducing sugars | Saponins | Tannins | Flavonoids | Steroids |
|---|---|---|---|---|---|---|---|---|
| ***C.sinensis* pulp** | + | + | + | + | - | + | + | + |
| ***C.sinensis* peel** | + | + | + | - | + | + | + | - |
| ***C.sinensis* juice** | + | - | - | - | - | - | + | - |
| ***C.limon* pulp** | + | + | - | + | + | + | + | + |
| ***C.limon* peel** | + | - | + | + | - | + | - | - |
| ***C.limon* juice** | + | - | - | + | + | + | + | + |
| ***C.limetta* pulp** | + | + | + | + | + | + | + | + |

| | Alka-loids | Glyco-sides | Terpe-noids | Reducing sugars | Sapo-nins | Tannins | Flavo-noids | Ster-oids |
|---|---|---|---|---|---|---|---|---|
| ***C.limetta* peel** | + | + | - | + | + | + | - | - |
| ***C.limetta* juice** | + | - | - | - | - | + | + | + |

+ = Present, - = Absent.

## DISCUSSION

Out of all the oral health issues, dental caries is considered to be the most commonly occurring dental disease affecting people worldwide to a great extent (24). The effectiveness of different herbal and plant extracts against different micro-organisms, including oral pathogens, has already been demonstrated in the literature. However, the effectiveness of different citrus fruits' pulp, peel, and juice extracts against dental caries pathogens is yet to be investigated. So, the current study was conducted to assess and compare the effectiveness of pulp, peel, and juice extracts of *C. Sinensis, C. Limon, and C. limetta* against *S. mutans* and *L. acidophilus.*

In the current study, the highest mean ZOI against *S. mutans* among all the fruit extracts was shown by *C. limetta* (juice) followed by *C. limetta* (peel) and lowest by *C. Limon* (peel), which is not similar to the findings of the study conducted by Shakya A et al. who reported that *C. limetta* (peel) extracts showed better ZOI against gram-positive bacteria than that of juice extracts (25). In the current study, *C. Limon* (juice) showed better ZOI than its peel extracts which are similar to the findings of the study conducted by Shakya A et al. (25). Saponins and flavonoids were not found in the *C. Limon* (peel) extracts which were present in juice extracts. Because of this, *C. Limon* (juice) showed better ZOI than peel extracts. Shetty et al. revealed that ethanolic extracts of *C. Sinensis* (peel) showed mean ZOI within the range of 8 mm to 13 mm at all concentrations *against S. mutans and Lactobacillus,* which is nearer to the findings of the present study (18). Goudarzi et al. concluded that C. Limon (peel) extracts showed good antibacterial properties against *S. mutans* with mean ZOI of 28 mm and MIC and MBC within the range of 6.25 mg/mL to 25 mg/mL, which contradicts the findings of the current study (26). This may be due to the use of ethanol extract in the previous study, which showed better antimicrobial activity than the methanol extract used in the current study. Tawfik et al. used three different citrus fruit juice, i.e., *C. Limon, C. Aurantium, and C. paradise,* to examine the antimicrobial efficacy of these fruit juice against gram-positive micro-organisms like *S. aureus, P. Vulgaris and P. aeruginosa* and concluded that these fruit juices have a strong antibacterial effect against these pathogens (27).

Hasan et al. performed a study to assess the antimicrobial activity of citrus fruits such as citron *(C. medica),* satkora *(C. macroptera),* and adajamir *(C. assamensis)* against *Bacillus spp.* and *E. coli* and found that *C. macroptera* showed highest ZOI 2.2 cm against *Bacillus spp.* and 2.6 cm against *E. coli* whereas *C. assamensis* showed lowest ZOI 1.7 cm against *Bacillus spp.* and 2.1 cm against *E. coli* (28). Both fresh and dried *C. Sinensis* peel extracts were examined in a study conducted in Nigeria for their phenolic content and antibacterial activity. According to the study's findings, fresh *C. sinensis* peel extract has higher phenolic content and more potent antibacterial properties against the strains of bacteria under study than dry peel extract. The study's conclusions suggest that drying plant parts before extracting their phytonutrients may result in the loss of active components (29). Similar to this, the phytochemical examination of *C. Sinensis*, *C. reticulata*, *C. limetta*, and *C. maxima* peel extracts revealed the presence of flavonoids, saponins, steroids, terpenoids, alkaloids, and tannins, and they were effective against *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, and *E. coli*. Both studies corroborated our study, which had similar results (30).

Upadhyay and Shah compared the antimicrobial efficacy of *C. Sinensis* (peel) extract with CHX against some periodontal micro-organisms. They found that against *P. gingivalis,* the MIC and MBC value was 50 mg/mL, while against *A. actinomycetemcomitans* and

*P. intermedia*, it was 100 mg/mL. MIC and MBC value of CHX for *P. gingivalis* was within the range of 0.2 ug/mL to 1.6 ug/mL, while for *A. actinomycetemcomitans* and *P. intermedia*, MIC and MBC value was 12.5 mg/mL (31). These findings agree with the present study's findings that though the citrus fruit extracts possess antibacterial activity, in comparison to CHX, they possess low antibacterial activity for which they can be used as an adjunct to CHX for the treatment against periodontal pathogens. Even Hussain et al. suggested that hot ethanolic extracts of *C. Sinensis* (peel) showed MIC within the range of 12-15 mg/mL against periodontal pathogens, similar to the current study's findings (23).

Mishra RP et al. evaluated the antimicrobial efficacy of peel, seed, juice, and pomace extract of *C. limetta* (Mosambi) against *P. aeruginosa, E. coli, and S. aureus*. They demonstrated that peel and juice extracts were more effective against these pathogens than seed and pomace extracts (13). C. limetta showed the lowest MIC (juice), i.e., 22.85 mg/mL against *S. aureus*, and the maximum was shown by pomace column extract, i.e., 42.42 mg/mL against E. coli, but no MIC was seen in seeds. Pandey et al. found that methanol extracts prepared from the juice of *C. Limon* showed better ZOI against *P. aeruginosa* than seed and peeled extracts (32). According to a study done by Denkova-Kostova et al. on evaluating the antimicrobial activity of tangerine *(Citrus reticulata L.)*, grapefruit *(Citrus paradisi L.)*, lemon *(Citrus lemon L.)* and cinnamon *(Cinnamomum zeylanicum Blume)* essential oils against saprophytic and pathogenic micro-organisms found that cinnamon oil demonstrated the highest antimicrobial activity, followed by grapefruit, tangerine and lemon zest oil with MIC ranging from 6 to 60 ppm (33).

## CONCLUSION

The current study revealed that all the extracts of citrus fruits, i.e., pulp, peel, and juice have antibacterial activity against both microorganisms. However, they are more effective against *L. acidophilus.* Besides this, among all the extracts, *C. Sinensis* (peel) showed a significantly higher mean diameter of ZOI against *L. acidophilus* than CHX. Further in-vivo trials could be conducted to assess the antimicrobial efficacy of these fruit extracts in the oral cavity. Studies can be conducted using different concentrations of the extracts so that the correct dosage of the extract can be determined, which can be added to the different formulations to work effectively. Moreover, various clinical trials should be performed using different natural extracts, which can act as an alternative to commercial antimicrobials, thereby reducing cost and side effects. In all cases, clinical trials should be performed before using these plants in human therapy.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

All the authors have consented to publish the manuscript.

### Availability of data and materials

All data are contained within the article.

### Author's contribution:

**Dharmashree Satyarup:** conceptualization, writing-original draft, formal analysis, investigation, supervision, validation, visualization, and writing review & editing. **Sailaja Panda:** conceptualization, writing-original draft, data curation, formal analysis, investigation, methodology, project administration, software, supervision, validation, visualization, and writing review & editing. **Ramesh Nagarajappa:** formal analysis, software, supervision, writing review & editing. **Shakti Rath:** conceptualization, data curation, investigation, methodology, project administration, supervision**. Upasana Mohapatra:** formal analysis, visualization, and writing review & editing.


## FUNDING

None.


## CONFLICTS OF INTEREST

The authors declare no conflict of interest.


## ACKNOWLEDGMENTS

We thank the Central Research Lab of the Institute of Dental Sciences, Bhubaneswar, for providing the necessary equipment and bacterial specimens for the research.

## REFERENCES


1] Rahhal BM, Jaradat N, Hawash M, Qadi M, Issa L, Yahya A, Sanyora S, Saed M, Al-Rimawi F. Phytochemical Screening, Antioxidative, Antiobesity, Antidiabetic and Antimicrobial Investigations of Artemisia scoparia Grown in Palestine. Processes. 2022 Oct;10(10):2050.

2] Rath S, Padhy RN. Antibacterial efficacy of five medicinal plants against multidrug-resistant enteropathogenic bacteria infecting under-5 hospitalized children. J Integr Med. 2015 Jan;13(1):45–57.

3] Jaradat N, Hawash M, Qadi M, Abualhasan M, Odetallah A, Qasim G, Awayssa R, Akkawi A, Abdullah I, Al-Maharik N. Chemical Markers and Pharmacological Characters of Pelargonium graveolens Essential Oil from Palestine. Molecules. 2022 Jan;27(17):5721.

4] Akdemir Evrendilek G. Empirical prediction and validation of antibacterial inhibitory effects of various plant essential oils on common pathogenic bacteria. Int J Food Microbiol. 2015 Jun 2;202:35–41.

5] Cerrillo I, Escudero-López B, Hornero-Méndez D, Martín F, Fernández-Pachón MS. Effect of alcoholic fermentation on the carotenoid composition and provitamin A content of orange juice. J Agric Food Chem. 2014 Jan 29;62(4):842–9.

6] Rafiq S, Kaul R, Sofi SA, Bashir N, Nazir F, Nayik GA. Citrus peel as a source of functional ingredient: A review. Journal of the Saudi Society of Agricultural Sciences. 2018;17(4):351–8.

7] Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN, Boskou D. Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis). Food Chemistry. 2006;94(1):19-25.

8] Gorinstein S, Cvikrová M, Machackova I, Haruenkit R, Park YS, Jung ST, Yamamoto K, Ayala AL, Katrich E, Trakhtenberg S. Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. Food Chemistry. 2004 Mar;84(4):503–10.

9] Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999 Oct;12(4):564–82.

10] De Ancos B, Rodrigo MJ, Sánchez-Moreno C, Pilar Cano M, Zacarías L. Effect of high-pressure processing applied as pretreatment on carotenoids, flavonoids and vitamin C in juice of the sweet oranges "Navel" and the red-fleshed "Cara Cara." Food Res Int. 2020 Jun;132:109105.

11] Arafat Y, Altemimi A, Pratap-Singh A, Badwaik LS. Active Biodegradable Films Based on Sweet Lime Peel Residue and Its Effect on Quality of Fish Fillets. Polymers (Basel). 2021 Apr 12;13(8):1240.

12] Jeffrey J, Satari MH, Kurnia D, Sudigdoadi S. Inhibition of Streptococcus mutans growth induced by the extract of citrus aurantifolia peel. Journal of International Dental and Medical Research. 2020;13(1):122–7.

13] Mishra RP, Yadav S. Anjali. Study of antimicrobial activities of Citrus limetta. J Pharm Biomed Sci. 2012;19(15):1–4.

14] Shimada A, Noda M, Matoba Y, Kumagai T, Kozai K, Sugiyama M. Oral lactic acid bacteria related to the occurrence and/or progression of dental caries in Japanese preschool children. Biosci Microbiota Food Health. 2015;34(2):29–36.

15] Oikeh EI, Oviasogie FE, Omoregie ES. Evaluation of antimicrobial efficacy of ethanol extracts of fresh Citrus sinensis (sweet Orange) seeds against selected bacterial strains. Journal of Applied Sciences and Environmental Management. 2020;24(2):249–52.

16] Moulehi I, Bourgou S, Ourghemmi I, Tounsi MS. Variety and ripening impact on phenolic composition and antioxidant activity of mandarin (Citrus reticulate Blanco) and bitter Orange (Citrus aurantium L.) seeds extracts. Industrial Crops and Products. 2012;39:74–80.

17] Nkere CK, Iroegbu CU. Antibacterial screening of the root, seed and stembark extracts of Picralima nitida. African Journal of Biotechnology. 2005;4(6):522–6.

18] Shetty SB, Mahin-Syed-Ismail P, Varghese S, Thomas-George B, Kandathil-Thajuraj P, Baby D, . Haleem S, Sreedhar S, Devang-Divakar D. Antimicrobial effects of Citrus sinensis peel extracts against dental caries bacteria: An in vitro study. Journal of clinical and experimental dentistry. 2016;8(1):e71.

19] Khalil A, Jaradat N, Hawash M, Issa L. In Vitro Biological Evaluation of Benzodioxol Derivatives as Antimicrobial and Antioxidant Agents. Arab J Sci Eng. 2021 Jun;46(6):5447–53.

20] Hassim N, Markom M, Anuar N, Baharum SN. Solvent selection in extraction of essential oil and bioactive compounds from Polygonum minus. Journal of Applied Sciences. 2014;14(13):1440–4.

21] Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. Journal of Food Quality. 2019 Feb 3;2019:e8178294.

22] Andrews J. BSAC Working Party Report on Susceptibility Testing. 2001. Determination of inhibitory concentrations. J Antimicrob Chemother. 48:48–71.

23] Hussain KA, Tarakji B, Kandy BPP, John J, Mathews J, Ramphul V, Divakar DD. Antimicrobial effects of citrus sinensis peel extracts against periodontopathic bacteria: an in vitro study. Roczniki państwowego zak\ladu higieny. 2015;66(2).

24] Selwitz RH, Ismail AI, Pitts NB. Dental caries. The Lancet. 2007;369(9555):51–9.

25] Shakya A, Luitel B, Kumari P, Devkota R, Dahal PR, Chaudhary R. Comparative study of antibacterial activity of juice and peel extract of citrus fruits. Tribhuvan University Journal of Microbiology. 2019;6:82–8.

26] Goudarzi M, Mehdipour M, Hajikhani B, Sadeghinejad S, Sadeghi-Nejad B. Antibacterial Properties of Citrus limon and Pineapple Extracts on Oral Pathogenic Bacteria (Streptococcus mutans and Streptococcus sanguis). Int J Enteric Pathog. 2019;7(3):99–103.

27] Tawfik NO, Al-Haliem SM, Al-Ani WN. Evaluation of the antibacterial activity of citrus juices: An in vitro study. Al-Rafidain Dental Journal. 2010;10(2):376–82.

28] Hasan MdM, Roy P, Alam M, Hoque MdM, Zaman W. Antimicrobial activity of peels and physicochemical properties of juice prepared from indigenous citrus fruits of Sylhet region, Bangladesh. Heliyon. 2022 Jul 1;8(7):e09948.

29] Oikeh EI, Oviasogie FE, Omoregie ES. Quantitative phytochemical analysis and antimicrobial activities of fresh and dry ethanol extracts of Citrus sinensis (L.) Osbeck (sweet Orange) peels. Clin Phytosci. 2020 Dec;6(1):46.

30] Gupta S, Nath A, Gupta MK and Sundaram S: Phytochemical analysis and antibacterial activity of different citrus fruit peels. Int J Pharm Sci & Res 2021; 12(11): 5820-26.

31] Upadhyay ND, Shah MA. Comparative Evaluation of citrus sinensis extract on Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Prevotella intermedia with chlorhexidine: an in vitro study. IOSR J Dental Med Sci. 2021; 20(06): 19-23.

32] Pandey P, Nandkeoliar T, Tikku AP, Singh D, Singh MK. Prevalence of Dental Caries in the Indian Population: A Systematic Review and Meta-analysis. J Int Soc Prev Community Dent. 2021 Jun 10;11(3):256–65.

33] Denkova-Kostova R, Teneva D, Tomova T, Goranov B, Denkova Z, Shopska V, Slavchev A, Hristova-Ivanova Y. Chemical composition, antioxidant and antimicrobial activity of essential oils from tangerine (Citrus reticulata L.), grapefruit (Citrus paradisi L.), lemon (Citrus lemon L.) and cinnamon (Cinnamomum zeylanicum Blume). Zeitschrift für Naturforschung C. 2021 May 26;76(5–6):175–85.