

Nutritional, Antioxidant, And Antimicrobial Properties Of Citrus Peels: A Sustainable Valorization Approach

ABSTRACT

Background: Citrus peels are often discarded as waste despite being rich in bioactive compounds with potential health benefits. Traditional extraction methods for these compounds are not always sustainable and can lead to environmental harm. Using a sustainable vaporization approach, the bioactive compounds in citrus peels can be efficiently extracted and retained for various applications. Valorization strategies have been used for various waste streams, including agro-industrial by-products and olive mill wastewater.

Aims: Using a sustainable vaporization method, this study seeks to examine the antibacterial, antioxidant, and nutritional qualities of citrus peels.

Methods: waste valorization remains a promising approach for sustainable waste management and resource recovery. Quantitative studies and antimicrobial activities were carried out using peels of *C. aurantifolia* (Key Lime), *C. maxima* (Pomelo), *C. sinensis* (orange), *Citrus limetta* (Sweet Lemon), and *C. reticulata*. *Citrus aurantifolia* is believed to be a hybrid of *Citrus medica*, *Citrus grandis*, and a Micro-citrus species, with significant antibacterial activity.

Result: *Citrus limetta* Mosambi is gaining popularity due to its delicious taste and high vitamin-C content. *Citrus sinensis* is a small, spherical fruit rich in vitamin C and vitamin A. Pummelo, or *Citrus maxima*, is known for its large, sweet fruits with abortifacient and menstrual stimulant properties. Peels of *C. maxima* exhibit the highest amount of antioxidant activity equivalent to ascorbic acid and display significant antimicrobial activity against *Klebsiella species*. *Citrus reticulata*, found mainly in eastern India, has a maximum amount vitamin C among citrus species and exhibits high antimicrobial activity.

Keywords: Valorization, citrus fruits, Vitamin-C, antibacterial, agro-industrial waste, nutraceutical.

1. Introduction:

Citrus fruits play a common role in our daily lives, and their peels, pulps, and seeds offer various benefits. Since childhood, we've known that citrus fruits are rich in vitamins and are good for human health. The peels of fruits make up a significant portion of their weight and are often discarded as waste. While the pharmaceutical industry and synthetic medications are widely relied upon in the modern world, many people are unaware of the beneficial qualities of numerous herbs and vegetables. One of the main antioxidants in circulation, vitamin C, has immune-stimulating and anti-inflammatory properties and is necessary for optimal health at every stage of life. Scientific pathology may recommend synthetic pharmaceuticals to treat a variety of ailments, but it's important to focus on our natural healing process, which is something that Mother Nature always does her best to support [1]. However, it's worth noting that these fruit remnants can still be utilized in several ways rather than simply being thrown away. Citrus fruits are known for being a rich source of phytochemicals and biologically active compounds that contribute to their health-promoting properties. It's recommended to incorporate a variety of fruits and vegetables into your diet to ensure a well-rounded intake of

nutrients and bioactive compounds. Valorization is a concept aimed at sustainably managing industrial residues by recovering valuable components or producing useful products from waste materials [2]. It encompasses various approaches, including physical, chemical, and biological methods, to transform waste into economically valuable and environmentally friendly products [3]. The concept aligns with industrial ecology principles, promoting loop-closing and resource efficiency [4]. Valorization strategies have been applied to diverse waste streams, such as agro-industrial by-products and olive mill wastewaters, focusing on extracting antioxidants and producing enzymes. However, the valuation of knowledge in the valorization process presents challenges due to its subjective nature. Financial valuation methods, particularly the income approach, are commonly used but require assumptions that may be difficult to validate [5]. Despite these challenges, waste valorization remains a promising approach for sustainable waste management and resource recovery.

When citrus peel is used as a substrate in anaerobic digestion processes, it can inhibit the process. This is because the high content of certain compounds in citrus peel, such as d-limonene, can interfere with the activity of methanogenic bacteria responsible for methane production. As a result, this interference can lead to reduced biogas production and longer lag phases before methane production begins [6].

Citrus fruit peels contain high levels of flavonoids, polyphenols, and dietary fiber. These components have antioxidant, anti-inflammatory, and potential anti-cancer properties. Extracts from citrus peels have been studied for their cholesterol-lowering effects, skin health benefits, and as potential natural remedies for various health conditions. Fruit residues, often considered waste, have the potential to be valuable nutraceutical resources. Citrus peels, pulp, and seeds contain various beneficial compounds that offer health-promoting properties. By effectively utilizing these residues, we can reduce waste and harness their nutraceutical potential [7]. The value of citrus fruit residues as nutraceuticals can be recognized by finding innovative ways to use them, thus reducing waste and benefiting from their health-promoting properties. Proper processing and quality control are essential to maximize their safety and effectiveness as nutraceutical resources. The use of citrus peel waste in the medicine industry involves extraction techniques to isolate and concentrate the bioactive compounds. Ongoing research and development aim to explore their therapeutic potential, optimize extraction methods, and develop innovative drug delivery systems. While citrus peel and its bioactive compounds show promise in medicine, it is crucial to ensure appropriate quality control, standardization, and safety evaluation when using them for medicinal purposes [8].

Citrus peels were once considered waste, but they are now valued for their medicinal properties and are used in various applications, including food products. Citrus peels are rich in vitamins, fibers, and bioactive compounds, which contribute to their potential health benefits. Their antioxidant properties help to eliminate harmful free radicals in the body, providing protection against degenerative conditions. They also have antimicrobial properties that can inhibit the growth of certain bacteria and fungi. Additionally, researchers have taken note of their anti-inflammatory and anticancer properties. Whether in the form of powder or essential oils, citrus peels have been successfully incorporated into food products to enhance their nutritional value without compromising taste and sensory attributes when used in appropriate amounts. The journey of citrus peels, from being discarded as waste to being utilized in the laboratory and eventually making their way to the dining table, is a testament to their versatility and potential for both health and economic benefits[9].

The utilization of waste materials generated from citrus fruit processing, such as peels and pulp, for the extraction of cellulose and nanocellulose, is a fascinating area of research. Cellulose is a natural polymer found in plant cell walls and has various applications due to its unique properties. In the present study, the waste materials from citrus were treated with an alkaline solution to extract cellulose. The cellulose was then subjected to acid hydrolysis, resulting in the reduction of fiber size and the formation of nanocellulose. X-ray diffraction analysis revealed that the cellulose had an amorphous structure, indicating the breakdown of the crystalline regions during the extraction process. At high magnification, break points in the cellulose fibers were observed, resembling carbon nanotubes in appearance. This indicates that the acid treatment caused structural modifications to the cellulose fibers. Overall, this study highlights the potential of citrus peel waste as a valuable source of cellulose and nanocellulose. By extracting and modifying cellulose from citrus waste, researchers have discovered a novel application for this abundant agricultural byproduct. The utilization of citrus peel waste for cellulose extraction offers both environmental and economic benefits, as it reduces waste generation and provides a sustainable alternative to traditional cellulose sources. [10]. Citrus fruits generate significant waste during processing, including peels, seeds, and pomace. Proper management and utilization of this waste can minimize environmental impacts and optimize resource utilization for a more sustainable approach to citrus fruit processing.

2. Materials and Methods:

2.1. Samples Collection and preparation:

Peels of the citrus species that were taken for the experiments were *Citrus aurantifolia*, *Citrus limetta*, *Citrus reticulata* (Aroma King lemon/ gandhoraj), *Citrus sinensis*, and *Citrus maxima*



Citrus sinensis (Orange)



Citrus limetta (Sweet lemon)



Citrus maxima (pomelo)



Citrus reticulata (Gandhoraj lemon)



Citrus aurantifolia (Keylime)

Fig 1: These samples were taken.

Peels of every citrus species have been attached for a clear view. [fig 1]

2.2. Quantitative nutritional analysis:

Total vitamin C and protein estimation by UV spectrophotometric method, followed by vitamin C quantification assay using the potassium iodate and potassium iodine titration method [11]. Bradford assay, and Lowry assay [12]. Carbohydrate estimation was done through the Anthrone method [13].

2.3. In vitro antioxidant assay using DPPH:

The stability of the extracts' DPPH (2, 2-diphenyl-2-picrylhydrazyl) free radical scavenging activity was assessed using the usual method [14].

2.4. In vitro antimicrobial assay:

The antibacterial assay was performed using the disc diffusion method against Gram-negative *Klebsiellasp.&Enterobactersp.* and Gram-positive bacteria *Staphylococcus epidermis*, *Bacillus sp.* The bacteria strains were maintained in 100 mL of nutrient broth at 37°C and incubated overnight.

3. Results:

Quantitative nutritional analysis of peels of the samples

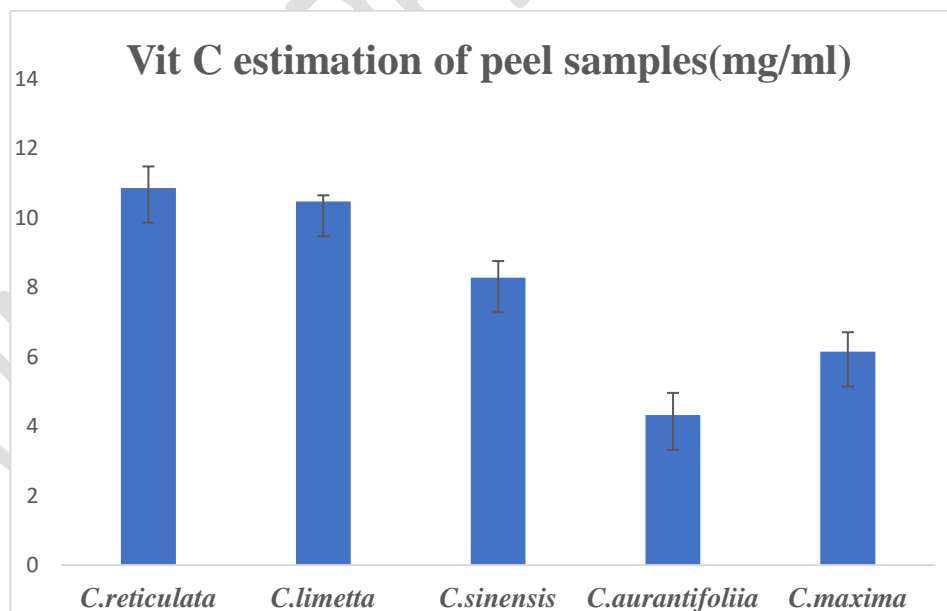


Fig. 2: Vitamin C amount in the peel samples

Out of five samples, *C.reticulata*(10.85±2.49 mg/ml), *C. lime* (10.46±2 mg/ml), and *C. sinensis*(8.96±1.6 mg/ml) have a higher amount of vitamin C than the other samples. Sd was measured ($n = 3$) for each of the samples.

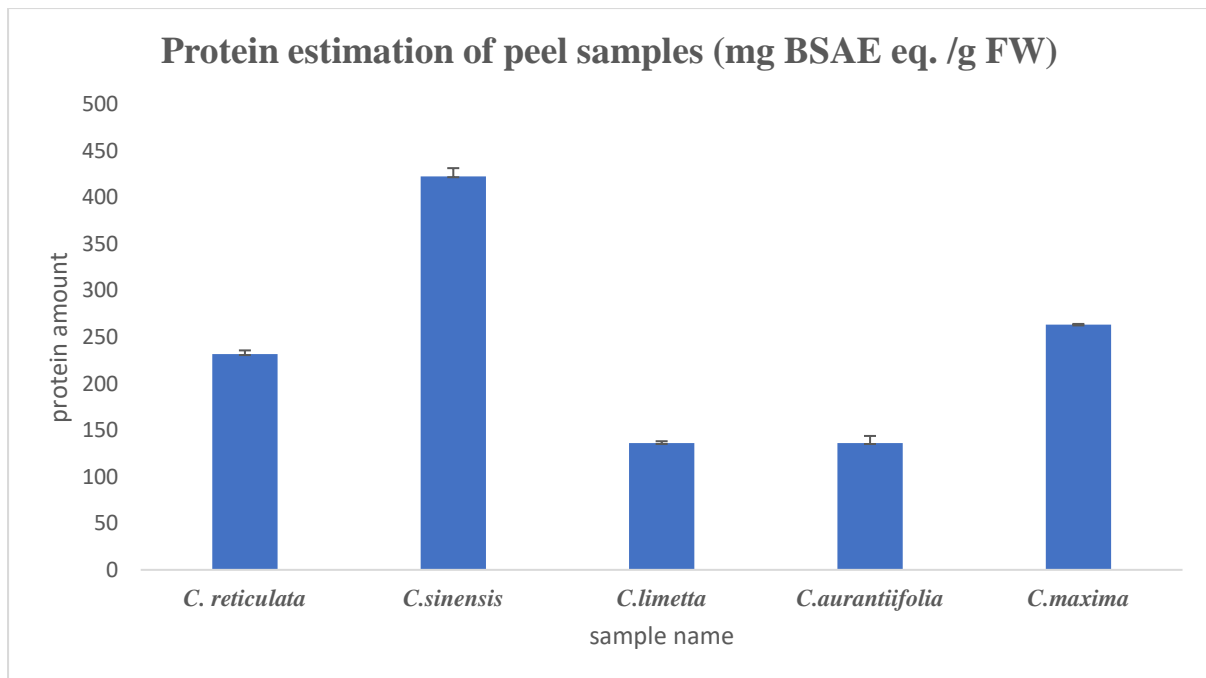


Fig. 3: Protein amount in the peel of the samples

Peels of the citrus fruits have enough protein, out of these *C. sinensis* has a maximum amount of protein 422.76±8.76 (mg BSAE eq. /g FW) median *C. reticulata* 231.7±4.0115 (n=3) for each of the samples [fig3]

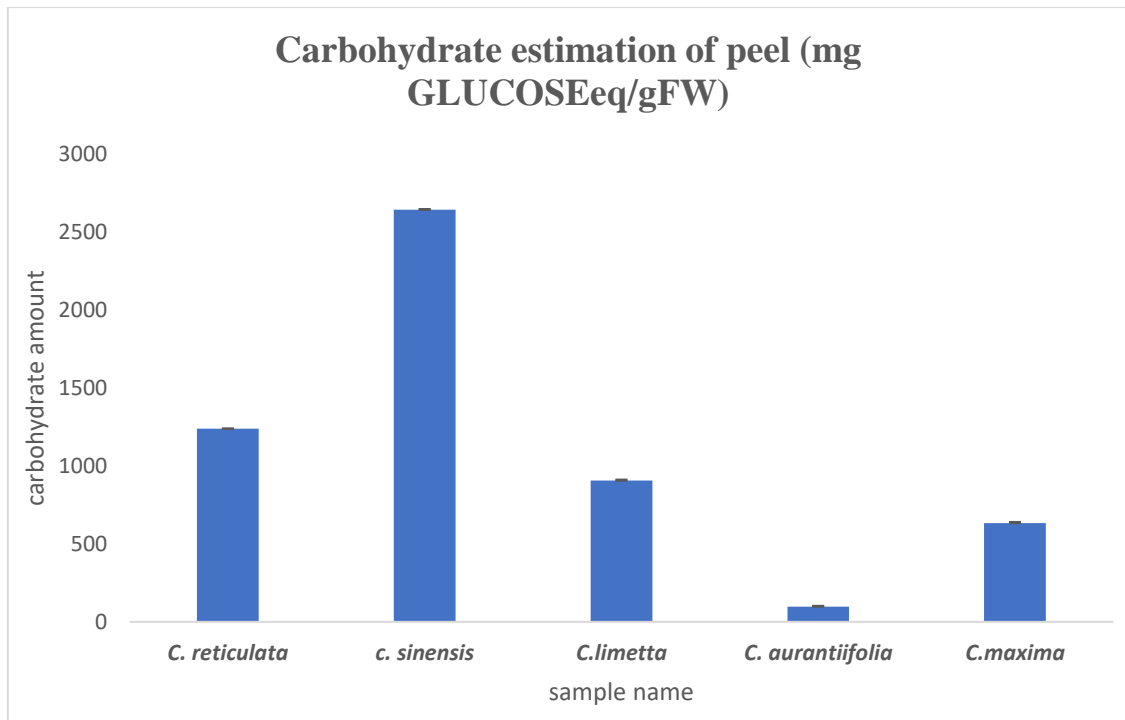


Fig. 4: Carbohydrate amount in the peel samples

The result clearly shows *C. sinensis* maximum carbohydrate of 2643.08 ± 4.98 equivalent to glucose, and a median of 905.95 ± 6.47 *C. limetta* Sd measured ($n=3$) for each of the samples [fig 4]

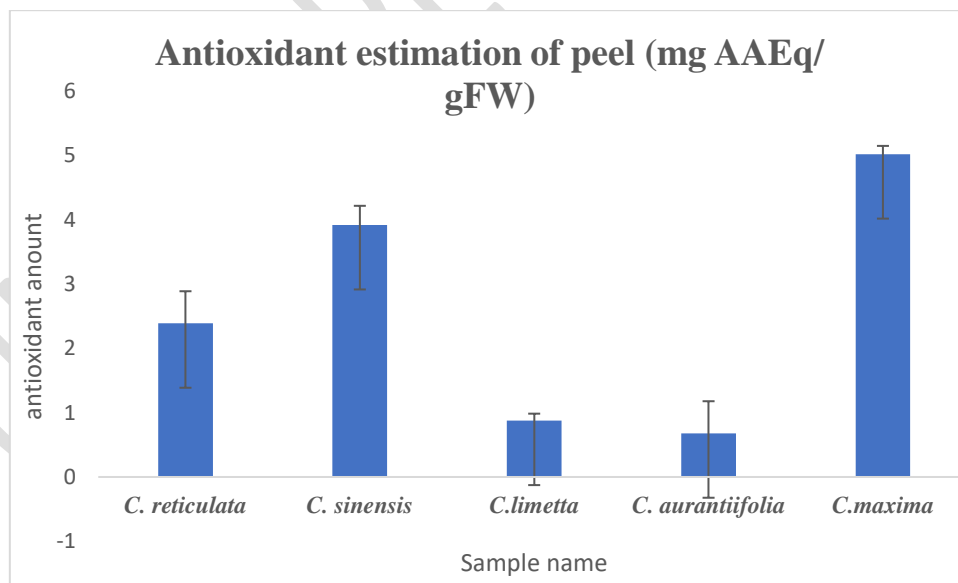


Fig. 5: Antioxidant activity of the peel samples

C. maxima and *C. sinensis* have maximum antioxidant activity equivalent to ascorbic acid than others. Sd measured ($n=3$) for each of the samples [fig 5]

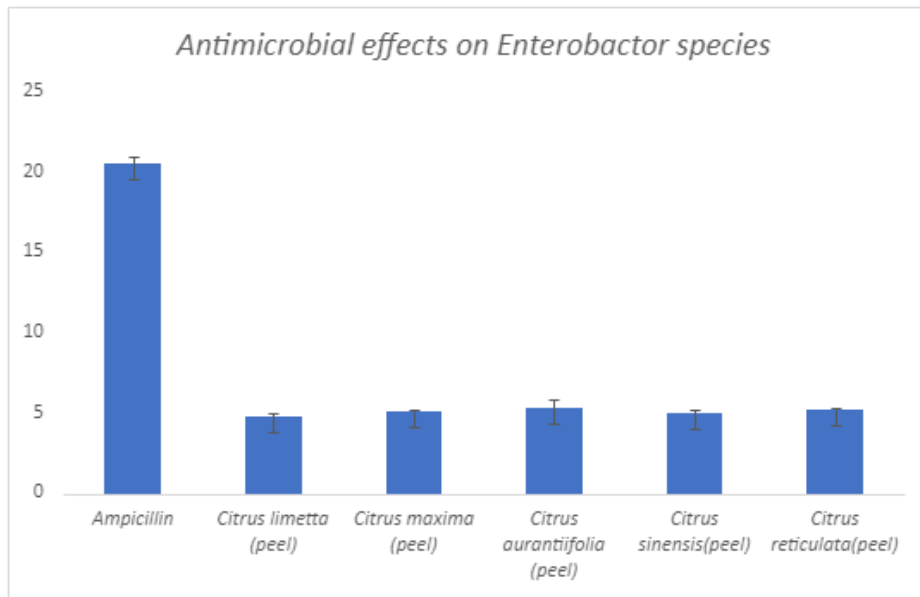


Fig 6a: The diagrammatic representation of the antimicrobial activity of the samples on *Enterobacter* species.

❖ "SD measured (n=3)" is mentioned throughout the manuscript and suggests that the experiment was conducted three times, it indicates that the standard deviation was calculated based on data obtained from three experimental runs.

Antimicrobial effects on *Enterobacter* species



Fig 6b: The measurement area is the disc diffusion results using the gram (-) bacteria *Enterobacter* sp. inhibitory zones (mm scale).

The result clearly shows that all of the samples have a similar amount of antimicrobial activity among other samples. ampicillin was the control, and Sd was measured (n=3) for each sample. [fig 6a]

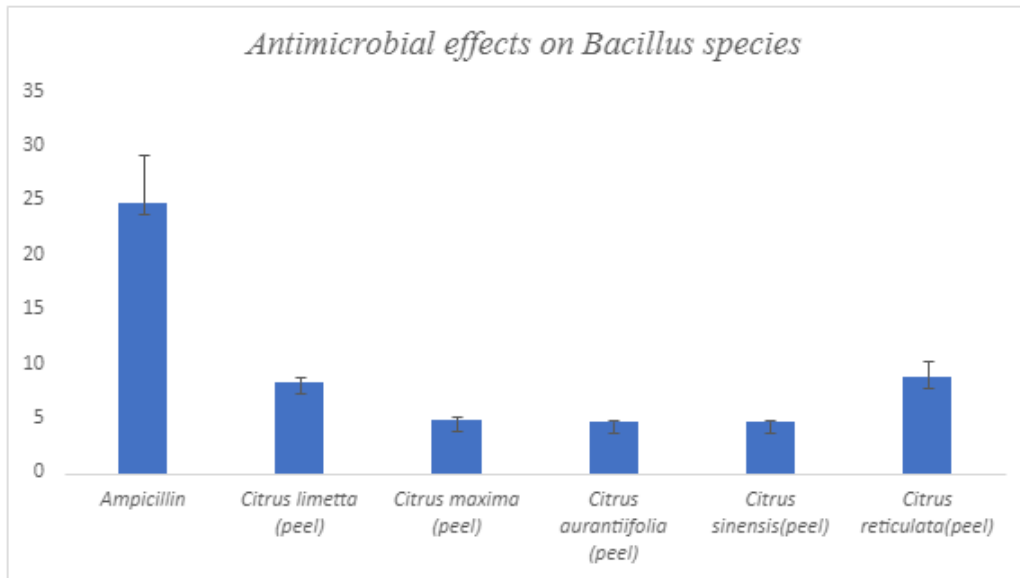


Fig 7a: The diagrammatic representation of the antimicrobial activity of the seed samples on *Bacillus* species. bacteria.



Fig 7b: shows the disc diffusion results using the gram (+) bacteria *Bacillus* sp. inhibitory zones (mm scale) are the area of measurement.

The results indicate that *C. limetta* and *C. reticulata* exhibit similar levels of antimicrobial activity compared to other samples. Ampicillin was used as the control, and each sample's standard deviation (SD) was measured (n=3). [Fig. 7a-7b]

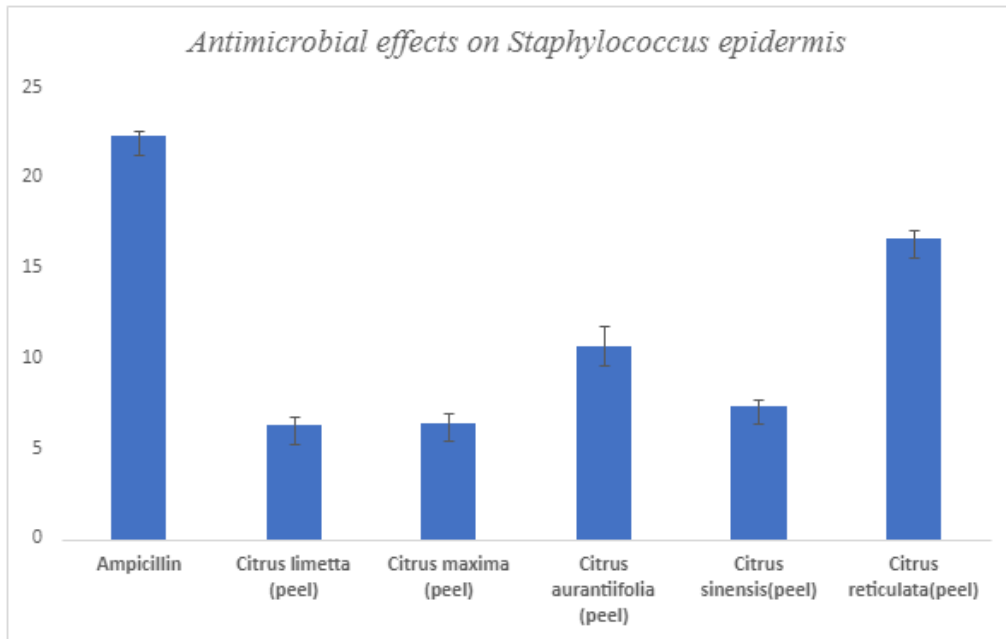


Fig 8a: The diagrammatic representation of the antimicrobial activity of the seed samples on *Staphylococcus epidermis* bacteria.

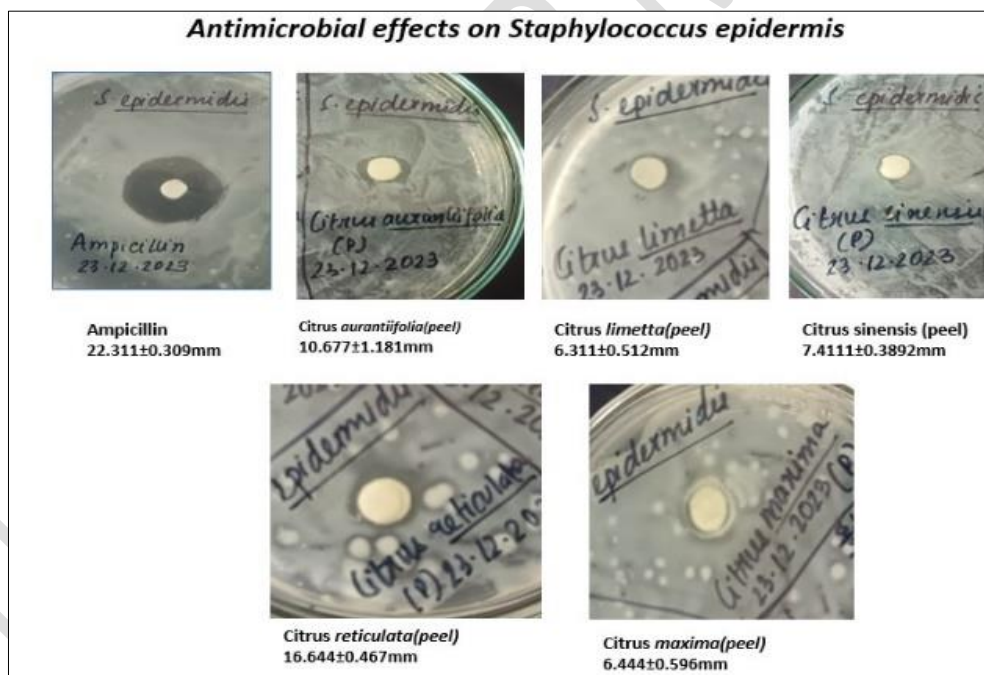


Fig 8b: shows the disc diffusion results using the gram (+) bacteria *Staphylococcus epidermis* inhibitory zones (mm scale) are the area of measurement.

The result clearly shows among all the samples *C. reticulata*, *C. aurantiifolia*, and *C. sinensis* showed maximum antimicrobial activity among other samples., ampicillin was the control, Sd measured (n=3) for each of the samples [Fig 8a-8b]

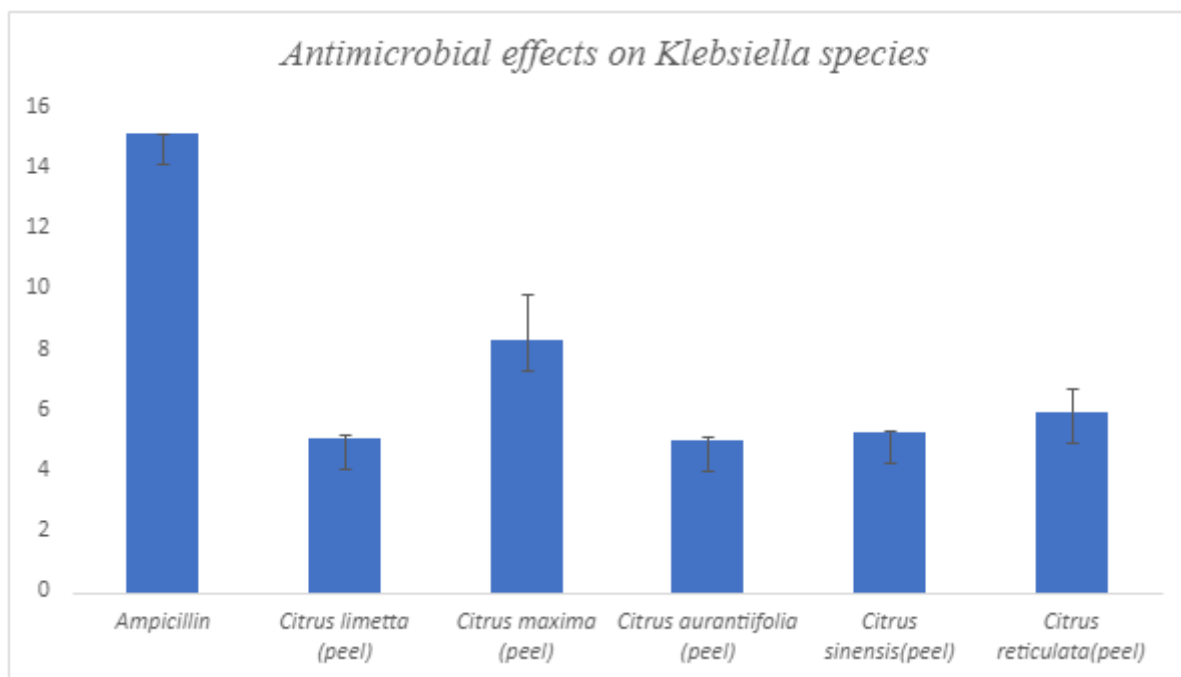


Fig 9a: shows the diagrammatic representation of the antimicrobial activity of the peel samples on *Klebsiella* bacteria.

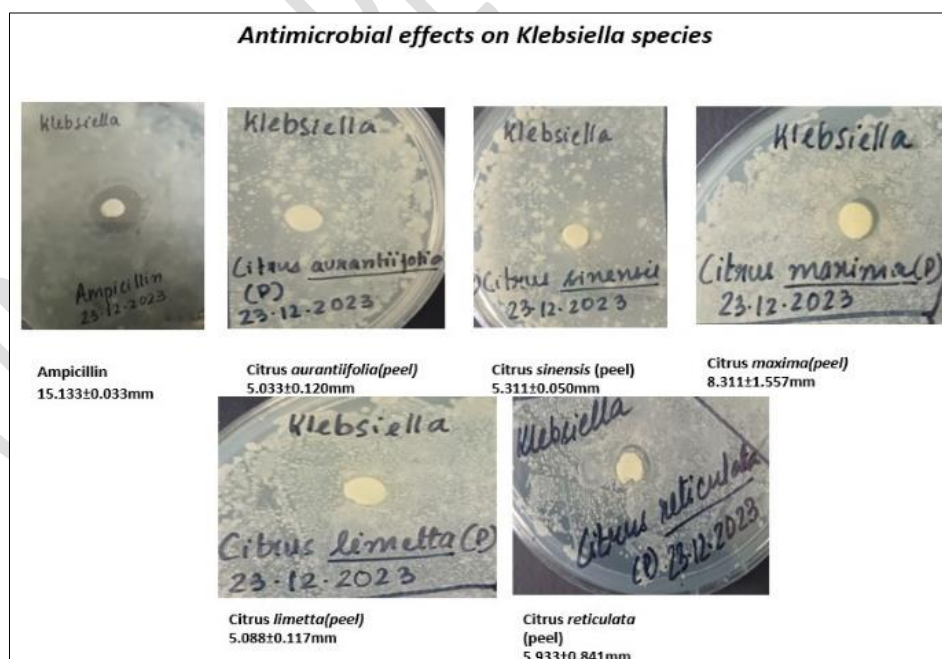


Fig 9b: shows the disc diffusion results using the gram (-) bacteria *Klebsiella* inhibitory zones (mm scale) are the area of measurement.

The result clearly shows that *C. limetta* and *C. maxima* showed maximum antimicrobial activity among other samples. ampicillin was the control, and Sd measured (n=3) for each of the samples [fig 9a-9b]

DISCUSSION

In recent years, research has demonstrated connections between oral microbiota and systemic illnesses, including head and neck cancer [15]. Ampicillin is a commonly used antibiotic that is effective against infections caused by various susceptible organisms, such as intestinal bacilli, salmonella, shigella, enterococci, listeria, and some forms of hemophilic bacilli. Citrus fruits are popular worldwide and offer various nutritional and medicinal benefits. For example, *Citrus aurantiifolia* is believed to be a hybrid of *Citrus medica*, *Citrus grandis*, and a Micro-citrus species. The spherical fruit, with a diameter of 25–50 mm, is typically harvested when green and turns yellow when ripened. *Citrus aurantiifolia* exhibits significant antibacterial activity. *Citrus aurantiifolia* and *C. maxima* show the highest antimicrobial activity. *Citrus reticulata*, also known as the Aroma King Lemon or Gandhoraj, combines the flavors of mandarin orange and lime. This species is found mainly in eastern India. The peel of *Citrus reticulata* has the highest amount of vitamin C among the five citrus species and exhibits high antimicrobial activity against *Staphylococcus epidermis* and *Bacillus* species. *Citrus limetta* Mosambi, a popular lime variety, is gaining worldwide popularity due to its delicious taste and high vitamin C content. *Citrus sinensis*, resembling a typical lime, is a small, spherical fruit rich in vitamin C and some vitamin A, making it highly nutritious. The peel and pulp of *Citrus sinensis* have the maximum amount of protein among all species, while the seeds and peel are good sources of carbohydrates.

Pummelo, or *Citrus maxima*, is a citrus tree producing large, sweet fruits with a history of use in herbal and traditional medicine due to its abortifacient and menstrual stimulant properties. Excessive use of these drugs can lead to severe liver damage, vomiting, and bleeding, which can be fatal. Contact with rue can also cause severe phytophotodermatitis and burn-like blisters after sun exposure. Peels of *C. maxima* exhibit the highest amount of antioxidant activity equivalent to ascorbic acid and display significant antimicrobial activity against *Klebsiella* species.

Citrus trash can provide nanocellulose, a flexible and biodegradable substance that provides an eco-friendly approach to waste management in the beverage sector [16,17]. The shape and characteristics of the final nanofibers are influenced by different extraction methods [17]. In comparison to conventional Hestrin-Schramm media, citrus peel and pomace enzymolysis (CPPE) medium has demonstrated a greater bacterial cellulose production [16]. The isolated nanocellulose has average diameters of 10–60 nm and crystallinity indices between 55% and 65% [16,17]. Cellulose fiber size can be decreased by alkaline treatment followed by acid hydrolysis, producing densely packed nanocellulose structures that resemble carbon nanotubes [18]. In paleobotanical investigations, nitrocellulose, another cellulose derivative, has been employed; the characteristics of the resulting film are influenced by different solvents and plasticizers. [19]

Extracts from citrus peels have shown strong antibacterial action against a range of infections. Lemon peel extract shown encouraging results against *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* [20]. Likewise, Turkish citrus peel oils demonstrated potent antibacterial properties, with bergamot and lemon oils showing the most efficacy [21]. According to a comparative analysis, lemon essential oil had the most inhibitory effects against *Candida albicans* and both Gram-positive and Gram-negative bacteria [22]. In citrus peel essential oils, limonene was found to be the main volatile component, although catechin and naringenin were important phenolic components [22]. *Citrus aurantium* peel extract had exceptional antifungal qualities against *Colletotrichum capsici*, while *Citrus sinensis* peel extract indicated strong antibacterial activity, especially against *Klebsiella pneumoniae*. [23]

Conclusion

Valuation techniques can have a significant impact on resource efficiency and waste reduction. For example, citrus peels can be repurposed to create valuable products such as animal feed, dietary supplements, and essential oils. The article effectively demonstrates how applying valuation methods to citrus fruit waste can highlight the financial and environmental benefits of implementing these strategies. Furthermore, it discusses the potential for scaling up these valuation methods and their support for environmentally friendly waste management techniques. This deeper investigation could enhance the overall impact of valuation and consider the potential for producing nutraceutical from citrus peels. These results demonstrate

citrus waste's potential as a useful resource for the synthesis and use of nanocellulose also suggests that citrus peel extracts have potential as natural antimicrobial agents.

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