

Evaluation of Peel, Pulp and Juices of Citrus Fruits for Vitamin C Content, Antioxidant and Antibacterial Activity

Pratiksha P. Shetty¹, Aanchal S. Singh², Aqeela A. S. Qureshi³

^{1,2,3}Department of Chemistry, Royal College of Arts, Science and Commerce, Mira Road, Thane, Maharashtra, India

ABSTRACT

Citrus fruits like Sweet Orange, Malta, and Lemon are extensively cultivated in India. This research aims to analyze the vitamin C, antioxidant, and antibacterial activities of these fruits. Aqueous extracts from the peel, pulp, and juice of these fruits, along with samples stored at 4°C, were prepared for analysis. The DPPH method was employed for antioxidant assessment, antibacterial properties were evaluated using the Agar well diffusion method, and vitamin C content in juices was estimated pH-metrically.

The results from the DPPH method indicated that storage at lower temperatures reduced antioxidant properties. All fruit juices exhibited higher antioxidant activity compared to their peel and pulp counterparts. Orange juice demonstrated the highest antioxidant activity, followed by Malta peel. Lemon juice was found to have the highest vitamin C content.

None of the extracts exhibited antibacterial properties against *Pseudomonas*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, and *Bacillus cereus*. The study suggests that orange juice is the most potent natural antioxidant among the selected citrus fruits. A mixture of all fruit juices exhibited synergistic effects, while combinations of any two juices showed antagonistic effects.

Keywords: Citrus fruits, Antioxidant, DPPH, Vitamin C.

1. Introduction

The foundation of medicinal preparations throughout history has been deeply rooted in the wealth of natural resources available to us. From spices and herbs to the diverse components of trees, these natural elements have served as the backbone of traditional medicine, highlighting the profound healing potential found in nature. In today's modern society, oxidative stress has become a significant concern due to factors like air pollution, smoking, UV radiation, and lifestyle choices, which contribute to cellular damage and various diseases [1]. Plant materials possess antioxidant properties attributed to active phytochemicals such as vitamins, flavonoids, terpenoids, carotenoids, cumarins, lignin, saponins, plant sterols, and more [2]. Therefore, a diet rich in antioxidants is vital for preventing a range of diseases in the human body.

Citrus fruits are recognized for their bioactive compounds, including phenolics, flavonoids, vitamins, and essential oils, which contribute to a variety of protective health benefits such as antioxidative, anti-inflammatory, antitumor, and antimicrobial activities [3].

While oranges dominate citrus fruit production, accounting for approximately 70% of output, the citrus group also encompasses smaller varieties like tangerines, mandarins, Clementines, and Satsuma, along with lemon, lime, and grapefruits [4].

India is home to several Citrus (C.) species, including C. limon (lemon), C. sinensis (sweet orange), and Malta (a hybrid of sweet lime and orange), which is commonly used as a substitute for traditional citrus varieties in the market.

This study aims to explore the synergistic and antagonistic effects of mixed citrus fruit juices, commonly consumed as beverages, with the goal of developing a beverage to enhance cardiovascular health and function. Additionally, considering the limited information on how storage temperature affects antioxidant properties, the study seeks to investigate this influence.

Given the rising concerns around antibiotic resistance and the use of synthetic antioxidant agents in our country [5], this study evaluates the vitamin C, antioxidant, and antibacterial properties of juice, peel, and pulp extracts from Sweet Orange, Malta, and Lemon.

The objectives of our research are to Compare the antioxidant properties of juice, peel, and pulp extracts from these citrus fruits; Analyse the impact of storage temperature on these selected properties; Investigate the synergistic and antagonistic effects of these juices on antioxidant activity; Estimate the vitamin C content in the juices of Sweet Orange, Malta, and Lemon; Assess antibacterial activity at lower and higher concentrations of these extracts.

2. Materials and Methods

2.1 Fruits

Sweet Orange, Malta and Lemon were brought from a local market and stored at both room temperature and 4°C in refrigerator.

2.2 Chemicals

2,2-diphenyl-1-picryl-hydrazyl (DPPH) (extra pure, make – SRL) was procured from Urmi Enterprises, Mumbai, India. Ascorbic acid, methanol and sodium hydroxide were purchased from Prerana enterprises.

2.3 Cultures

Bacillus cereus, *Pseudomonas*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* were procured from Microbiology department of Royal College of Science, Commerce and Arts.

2.4 Extract Preparation

The peel of the fruits was separated from the edible part, gently washed with distilled water. Aqueous extracts of peel or pulp were prepared by soaking 50 g of peel or flesh in 100 ml distilled water and boiling gradually for 15 minutes. The resulting solution was then filtered using Whatman filter paper. The extract was allowed to dry at 35°C on a thermostat. A known weight of residue was used to prepare solution for analysis. For juice extracts, 50 g of flesh was squeezed using a muslin cloth to obtain the juice. The juice extracts were then dried.

The solutions of varying concentrations (20, 40, 60, 80, 100, 200, 1000, and 10000 ppm) were prepared for analysis.

2.5 DPPH Assay

The extracts of concentrations of 20, 40, 60, 80, 100 and 200 ppm were evaluated for potential antioxidant activity using the free radical scavenging assay (DPPH). In cleaned and labelled glass tubes, 0.5 ml of sample extracts were thoroughly mixed with 3 ml of methanolic DPPH solution. The tubes

were then stored in dark for 30 minutes. A control solution was prepared by adding 0.5 ml methanol in 3ml of methanolic DPPH solution. The absorbance of the solutions were measured at 517 nm using Elico Spectrophotometer. Ascorbic acid was used as standard reference. The experiment was performed in duplicate and the potential scavenging activity was activity was calculated using the equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.6 Vitamin C Estimation

The content of vitamin C (ascorbic acid) was determined in peel, pulp, and juice extracts using pH metric methods. The pH meter was calibrated using ammonium hydroxide solution (pH=4), and the NaOH solution was standardized using succinic acid.

For the analysis, 5 ml of sample extracts was placed in a clean, labeled beaker, and distilled water was added until the glass rod was fully submerged. The resulting extract solution was titrated against NaOH. A first derivative graph ($\Delta \text{pH} / \Delta V$ vs. Volume of NaOH) was plotted, and the amount of vitamin C was calculated using the following equation:

$$\frac{V_x \times A \times 176.1}{1000} \text{ g of Vitamin C}$$

where V_x is Volume of NaOH to neutralize ascorbic acid and A is normality of NaOH.

2.7 Anti-Bacterial Property

The antibacterial activity was assessed using the agar well diffusion method. Nutrient agar was prepared according to standard procedures, poured into sterile plates, and allowed to solidify. The antibacterial properties were tested against *Bacillus cereus*, *Pseudomonas*, *Streptococcus pyogenes* and *Klebsiella pneumoniae*.

The inoculum suspension was spread evenly over the agar surface using a sterile cotton swab, and the plates were allowed to dry. After drying, wells were created in the agar, and sample extracts at concentrations of 1000 and 10000 ppm were dispensed into the wells. The inoculated plates were then incubated at 37°C for 24 hours, and the zone of inhibition was measured.

2.8 Synergistic and Antagonistic Effect

This study also investigated the synergistic and antagonistic effects of combinations of citrus fruit juices.

3. Results and Discussion

3.1 DPPH Assay

The experimental findings reveal that all the forms of all the three citrus fruits exhibit proton-donating ability but less than that of ascorbic acid. The radical scavenging activity increased with increase in concentration. Extracts of fruits stored at room temperature showed higher radical scavenging activity than those stored at 4°C in a refrigerator.

Among the citrus fruits, Malta extracts demonstrated superior antioxidant properties, followed by Sweet Orange extracts. Lemon extracts exhibited relatively poorer antioxidant activity. Additionally, the peel form of the fruits displayed the highest antioxidant ability, followed by the pulp form.

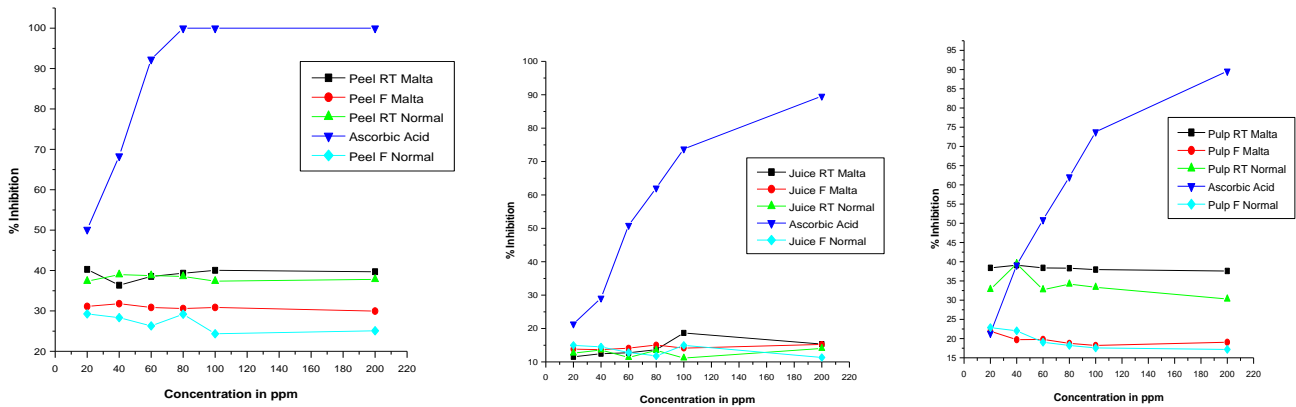
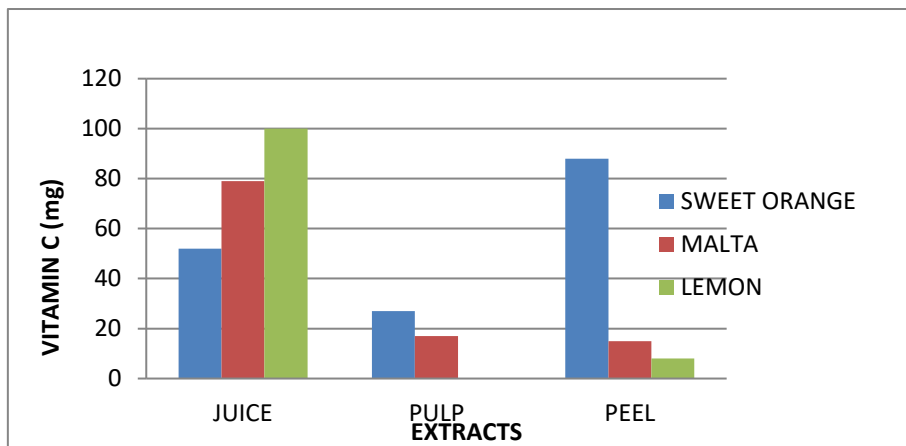


Fig. 1: % Inhibition VS Concentration in ppm of Peel, Juice and Pulp

3.2 Vitamin C Estimation

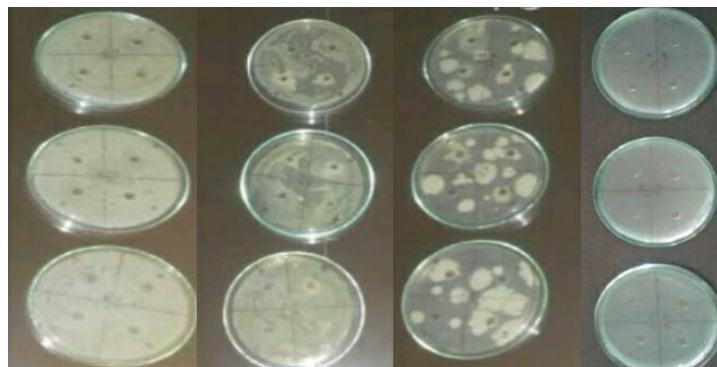
The highest Vitamin C content was observed in Lemon juice extract, followed by the peel extract of Sweet Orange. Conversely, Lemon peel extract displayed the lowest ascorbic acid content among the samples analyzed.



[Fig. 2: Vitamin C content]

3.3 Antibacterial Assay

The experiment revealed that the extracts were not effective against any of the bacterial cultures mentioned earlier, showing no antibacterial properties even at concentrations of 1000 ppm and 10000 ppm.



[Fig.3 : Zone of Inhibition - From left to right: *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Bacillus cereus* and *Pseudomonas*.]

3.4 Synergistic and Antagonistic effect

In this study, combining Sweet Orange, Malta, and Lemon juices resulted in a synergistic interaction, enhancing radical scavenging ability beyond that of the individual juices. However, when any two fruit juices were combined, antagonistic interactions were observed, leading to a reduction in radical scavenging ability compared to the individual juices.

4. Conclusion

Extracts of fruits stored at room temperature showed higher radical scavenging activity than those stored at 4°C in a refrigerator. Among the citrus fruits, Malta extracts demonstrated superior antioxidant properties, followed by Sweet Orange extracts. Lemon extracts exhibited relatively poorer antioxidant activity. Additionally, the peel form of the fruits displayed the highest antioxidant ability, followed by the pulp form. The highest Vitamin C content was observed in Lemon juice extract, followed by the peel extract of Sweet Orange. Synergistic effect was observed when all three juices were mixed and antagonistic effect was observed when two of them were combined. None of the extracts showed antibacterial activity against any organism. Increase in concentration showed no change in antibacterial activity.

References:

1. Diankov, Svetlomid & Karsheva, Maria & Hinkov, Ivaylo. (2011). Extraction of natural antioxidants from lemon peels. kinetics and antioxidant capacity. *Journal of the University of Chemical Technology and Metallurgy*. 46. 315-319.
2. Arora, Mamta & Kaur, Parminder. (2013). Antimicrobial & Antioxidant Activity of Orange Pulp and Peel. *International Journal of Science and Research*. 2. 412-415.
3. Oikeh EI, Omoregie ES, Oviasogie FE, Oriakhi K. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. *Food Sci Nutr*. 2015 Jul 30;4(1):103-9. doi: 10.1002/fsn3.268. PMID: 26788316; PMCID: PMC4708628.
4. Mathur, Abhishek & Verma, Satish & Purohit, Reena & Gupta, Vinay & Dua, V & Prasad, GBKS & Mathur, Deepika & Singh, Santosh & Singh, Shivsaran. (2011). Evaluation of in vitro antimicrobial and antioxidant activities of peel and pulp of some citrus fruits. *IJBTT*. 1.
5. John, Sheila & Monica, Sarah & S., Priyadarshini & Sivaraj, C. & Arumugam, Pramodh. (2017). Antioxidant and antimicrobial activity of lemon peel. 46. 115-118.