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Original Research Article

Antimicrobial activities of sweet orange (*Citrus sinensis*) juice on isolated *Staphylococcus aureus*, *Escherichia coli, Pseudomonas spp*, and *Klebsiella spp*

J C Iwuji[1], S C Chukwuemeka[1], E C Ogbodo[2,*], E E Ogbonye[3], A K Amah[4], I O Uduchi[2], A O Okezie[5], A A Okebalama[6]

[1]*Dept. of Science Laboratory, Federal University of Technology, Owerri, Nigeria*
[2]*Dept. of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Awka, Nigeria*
[3]*Dept. of Human Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[4]*Dept. of Physiology, College of Medicine, Imo State University, Owerri, Nigeria*
[5]*Dept. of Chemical Pathology, Medical Laboratory Services, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria*
[6]*Alex Ekwueme Federal University, Ndufu Alike Ikwo, Ebonyi State, Nigeria*

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A B S T R A C T

Resistance to antibiotics is one of the greatest problems to the success of modern medication. Due to the emergence of multi-drug resistant bacteria, selection of effective antibiotics for suppression of microbial activity has been an incidental issue. The aim of this study is to determine the antimicrobial activities of sweet orange juice on isolated *S. aureus*, *E. coli, Pseudomonas spp, & Klebsiella spp*, which were subjected to different concentrations of sweet orange juice to determine their response to the juice concentrations. 25%, 50%, & 100% concentrations were used respectively using a well-in-agar diffusion method and it was discovered that all the microorganisms were sensitive to the sweet orange juice at 100% concentration only with zones of inhibitions as follows: *Staphylococcus aureus (4mm), E.coli (1mm), Klebsiella spp (1mm), & Pseudomonas spp (2mm).* This shows that the juice may not be recommended as alternative for antibiotics development since it only inhibited the microbial growth at 100% concentration only, and its respective zones of inhibition were minimal or less effective.

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1. Introduction

Medicinal plants can be transformed into a variety of new pharmaceuticals that have yet to be discovered because of their large chemical contents. Herbal medicine has been used by Africans and Asians since the dawn of humanity. Roots, barks, stems, leaves, and seed extracts and combinations were among the plant parts used as remedies.[1] Because of the various chemical ingredients contained in plants, they have been used as antibacterial agents. Aromatic chemicals such as phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, and tannins can be synthesized by plants, as well as nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and other endogenous metabolites.[2] These chemicals act as plant defense mechanisms or exudates that protect plants from predators such as microorganisms, pests, and herbivores.[3]

The variety citrus having a place with the Rutacea family, as per Ould-Yerou et al. contains around 140 genera and 1,300 species. A portion of the significant products of genus Citrus, incorporate *Citrus sinensis* (sweet orange), *Citrus Paradise* (Grape natural product), *Citrus limon* (Lemon), *Citrus reticulate* (tangerine), *Citrus grandis* (shaddock),

* *Corresponding author*.
E-mail address: augustinee442@gmail.com (E. C. Ogbodo).

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Citrus aurantium (acrid orange), *Citrus medica* (Citron), and *Citrus aurantifolia* (Lime).[4]

Citrus sinensis (sweet orange) contains several dynamic optional metabolites adding to the pharmacological exercises of the plant. In *Citrus sinensis* natural products, strip, leaves, squeeze, and roots, several kinds of chemical mixtures including flavonoids,[5,6] hydroxyamides, steroids, alkanes and unsaturated fats, coumarins, carbs, peptides, carbamates and alkylamines, carotenoids, unstable mixtures, and minerals like potassium, magnesium, calcium, and sodium have been recognized.[7] *Citrus sinensis* is high in vitamin C, which is a natural cell reinforcement that aids immune system function.[7,8] *Citrus sinensis* health effects can be summarized as anticancer, antibacterial, antioxidant, and anti-inflammatory.[9] Thus, the essence of the current study.

2. Materials and Methods

A variety of orange fruit (*Citrus sinensis*) was bought from Douglas market Owerri in the month of May. Four microorganisms were used in the study, comprising of one gram positive (*Staphylococcus aureus*) and three gram negatives (*Escherichia coli, Pseudomonas spp, & Klebsiella spp*). All microorganisms were obtained from New Concept Laboratory, Obinze. The study was conducted in the month of May, in the microbiology laboratory, New Concept Laboratory, Obinze.

The fruits were washed thoroughly, after collection, in distilled water. Afterwards the peels were removed and the orange juice was got by squeezing the orange fruit cut open at one end of the fruit, all these done in an aseptic condition.

The agar well diffusion method was performed to analyze the antibacterial activity of the orange juice against the test organisms. The orange juice diluted serially at 25%, 50%, & 100% dilution factors were inoculated into the agar well containing test organisms. The plates were incubated at 37°C for 18-24hours. Antibacterial activity was measured by measuring the diameter of zone of inhibition around the well. Synonymously, the double strength dilution factors (i.e 25%, 50%, & 100%) were inoculated with test organisms respectively, and were incubated at the same temperature and time interval. The minimum inhibitory concentrations were determined by the degree of cloudiness in the tubes. These assays were conducted twice for concurrent readings.

3. Results

Table 1 shows the diameter of zone of inhibition of the orange juice at different concentrations against the test organisms. The negative sign indicated that there was no inhibition, and the result showed that the gram positive organism was inhibited more than the gram negative test organisms.

Table 2 shows the minimum inhibitory concentrations of the orange juice. The negativesign in the table indicates that there was no growth, but the positive sign indicates that there was growth of test organisms at the said concentrations.

4. Discussion

The antimicrobial activity of sweet orange (*citrus sinensis)* juice against isolates consisting *Escherichia coli, Staphylococcus aureus, Klebsiella spp, and Pseudomonas spp* were studied. Since drug resistance and allergies have been the order of some patient's response, this plant material has been studied to find out how those challenges can be suppressed to effectively treat infections related with isolated organisms.

This study also revealed that all the organisms isolated were sensitive to the orange extract. This is in agreement with the review done by Oikeh et al. who stated that *Citrus sinensis L.* Osbeck is widely recognized globally because of its high Vitamin C contents which is implicated in providing essential health benefits,[10] and also Pepe et al. who studied these health benefits and discovered that it encompasses an antimicrobial effect in addition to others.[9] Moreover, the sensitivity of the isolated organisms to sweet orange juice suggests that it has a broad spectrum of activity since all the microorganisms subjected to it were sensitive to its inhibitory effect. This is synonymous to Sharma et al. who stated that some antibiotics have either a narrow spectrum of activity or a broader spectrum of activity.[11]

This present work revealed that *Invitro* (outside the body), the antibacterial activity of sweet orange juice on the aforementioned isolated organisms was minimal. The present results are in contrast Baba et al, who claimed that *Citrus sinensis* juice had a very high in vitro antibacterial action against isolated organisms in the 10-28mm inhibitory zone.[12] However, the findings are comparable to those of Okaka et al, who found that vitamin C in orange juice induces the synthesis of collagen (i.e. fibrous connective tissue), which is vital in wound repair and healing.[13] It also boosts the immune system's ability to attack foreign substances by increasing interferon levels and antibody responses.

Although the above authors have conflicting reports on the antibacterial impact of this sweet orange juice, it is preferable to go with the study offered by Cardenosa and Herath and their respective colleagues.[14] This minimal response of the isolated bacteria to the sweet orange juice might be as a result of the season at which the experiment was conducted. This is because, according to the report documented by Cardenosa et al., Vitamin C content and antioxidant property of sweet orange juice fluctuates with effect to season of harvesting.[14] In their report, it was recorded that oranges plucked in January had higher vitamin C content and antioxidant property when compared to that plucked in April. And coincidentally, this work was

Table 1: Susceptibility pattern of test organisms to sweet orange juice

Sample	Orange juice concentration (%)	Zones of inhibition (mm) of sweet orange juice against test organisms			
		E. coli	*S. aureus*	*Klebsiella spp*	*Pseudomonas spp*
Orange Juice	25	-	-	-	-
	50	-	-	-	-
	100	1.0	4.0	1.0	2.0

Table 2: Minimum inhibitory concentration (mic) of test organisms to sweet orange juice

Sample	Orange juice concentration (%)	Test organisms				
		E. coli	*S. aureus*	*Klebsiella spp*	*Pseudomonas spp*	MIC
Orange juice	25	+	+	+	+	
	50	+	+	+	+	100%
	100	-	-	-	-	

done in the month of May which by implication is not easily accessible to get January stock. In addition to the effect of the season of harvest, the cultivar of the sweet orange used might have contributed to the low antibacterial effect exhibited in the result recorded above. This is in agreement with Herath and colleagues who observed that the antibacterial effect of sweet oranges varies with the cultivars.[15] In their study, they enlisted about six different cultivars of sweet orange with the cultivar containing the highest antibacterial effect as Bibila sweet having a minimum of 10.0mm zone of inhibition.

5. Conclusion

Sweet orange juice is not suggested for use in the creation of novel drugs with a broad spectrum of activity since its antibacterial efficacy against isolated bacteria was low, despite the fact that it inhibited all of the bacterium isolates to some extent. However, further research should be undertaken to determine the optimal season to gather sweet Orange juice for a more efficient antibacterial impact. It is also important to identify the cultivar(s) that are accessible in our area and how selecting the optimal cultivar will improve the antibacterial effect.

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Author biography

J C Iwuji, Lecturer

S C Chukwuemeka, Graduate Assistant

E C Ogbodo, Medical Lab. Scientist

E E Ogbonye, Anatomist

A K Amah, Associate Professor

A O Okezie, Principal Medical Lab Scientist

A A Okebalama, Lecturer

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