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Assessing the Effectiveness of Lemon Juice in Treating Infections and Alleviating Oxidative Stress

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ABSTRACT

Background: Numerous foods and environmental sources contain bacteria that are resistant to at least one antimicrobial agent used in medicine and agriculture. Resistance to antibiotics in gram positive bacteria like *Staphylococcus aureus* is of special concern. Lemon juice has been reported to act as an antioxidant in various biological systems. They were reported to be effective against a variety of bacterial strains.

Aim and Objective: The aim of this study was to determine the antibacterial activity of lemon juice and to compare with that of the commercially available antibiotic penicillin in inhibiting the growth of *Staphylococcus aureus*.

Materials and Methods: The Kirby-Bauer disc diffusion technique was employed to evaluate the effect of lemon juice and commonly used antibiotic Penicillin on bacterial infections caused by *Staphylococcus aureus*.

Result: The results indicate that penicillin was superior to lemon juice in inhibiting the growth of *Staphylococcus aureus*. However, lemon juice has also produced significant inhibitory results against the growth of gram-positive bacteria *Staphylococcus aureus* when compared to penicillin. One-way statistical analysis ANOVA followed by Dunnett's multiple comparison tests and the T test followed by the Mann Whitney test were used to conduct statistical analysis in GraphPad Prism 9.1.

Conclusion: Lemon juice extract was found to have remarkable activity against *Staphylococcus aureus* than commercially available antibiotic penicillin.

Keywords: Antimicrobial agent, *Staphylococcus aureus*; Lemon juice; Penicillin; Gram positive bacteria

INTRODUCTION

Throughout history, herbal medicines have played a significant role in treating numerous infectious diseases and have demonstrated their effectiveness in numerous instances [1]. These medicinal solutions are predominantly derived from plant components, encompassing leaves, flowers, fruits and stems. These natural extracts hold the potential for creating novel antimicrobial compounds characterized by distinct chemical structures and mechanisms of action. This innovation serves as a crucial defense against the emergence of multidrug-resistant microorganisms. The vast array of plant sources and their diverse components offers an inexhaustible reservoir of chemical diversity for the development of antimicrobial drugs [2, 3]. The advent of antibiotics revolutionized the field of medicine, providing powerful weapons against bacterial infections that once posed dire threats to human health. However, the indiscriminate use of antibiotics and the alarming emergence of antibiotic-resistant strains of bacteria have raised concerns about the long-term sustainability of this medical arsenal. As a result, researchers have been increasingly drawn to the exploration of alternative and supplementary approaches to traditional antibiotics, seeking more sustainable and potentially less resistant options [4]. One such avenue of investigation revolves around the antibacterial potential of natural substances and in this context, lemon juice has emerged as a

promising candidate. In this article, we delve into the comprehensive evaluation of the antibacterial efficacy of lemon juice in comparison to the time-honored conventional antibiotic, Penicillin. Medicinal plants hold a significant place in the traditions of virtually all cultures, with single and poly herbal preparations being employed extensively throughout history for treating various ailments. Among these natural remedies, citrus fruits, known for their acidic nature, offer a wealth of essential nutrients that confer numerous health benefits. They serve as abundant sources of vitamins and a broad spectrum of vital nutrients necessary for the body's well-being. Whether consumed as fresh fruits or processed into hand-squeezed or industrially produced juices, citrus fruits predominantly contain flavanones and flavones. Within the Citrus (*C.*) genus, several species, including *C. limon* (lemon), *C. aurantium* (bitter orange) and *C. paradisi* (grapefruit), have piqued the interest of researchers due to their potential antimicrobial properties [5-7]. Lemon juice, derived from the citrus fruit *Citrus limon*, has a long history of use for its numerous health benefits. Among these, its potential as an antibacterial agent has garnered significant attention. Lemons are renowned for their high content of citric acid, a compound recognized for its antimicrobial properties. Beyond citric acid, lemon juice also contains essential oils, flavonoids and a spectrum of bioactive compounds, all of which contribute to its multifaceted antibacterial potential. The interest in lemon juice's antibacterial properties arises from the growing need for sustainable and environmentally friendly alternatives to synthetic antibiotics. The overuse and misuse of antibiotics in healthcare, agriculture and livestock farming have contributed to the alarming rise of antibiotic-resistant bacteria. This phenomenon threatens our ability to treat infections effectively and poses a grave global health risk. Consequently, the exploration of natural remedies like lemon juice not only holds the promise of therapeutic innovation but also aligns with the imperative of preserving the effectiveness of antibiotics for future generations [8-10]. For instance, Hammers et al. (1999) conducted a study exploring the essential oils from various Citrus species, revealing Minimum Inhibitory Concentrations (MIC) ranging between 5-2% (v/v) for these oils and extracts [11]. Furthermore, AL-Jedah, et al., investigated a mixture of spices, including lemon and found that this combination exhibited a static effect on a spectrum of assayed bacteria. Hayes and Markovic delved into the antimicrobial attributes of lemon, identifying its significant efficacy against microorganisms such as *Staphylococcus aureus*, *Klebsiella*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* [12]. Another study highlighted the antibacterial and antifungal activity of grapefruit seed and pulp extracts [13]. Additionally, Adedeji, et al., (2007) reported antibacterial activity in Citrus extracts from lemon, lime and grapefruit against various bacterial strains, with lime and lemon juice demonstrating superior antibacterial efficacy compared to commercial antibiotic discs [14]. As we delve deeper into the evaluation of lemon juice's antibacterial efficacy, we aim to provide insights into its potential as a complementary or supplementary approach to conventional antibiotics. In the current era, the global surge in antibiotic-resistant pathogenic microorganisms has spurred researchers to investigate the potential of utilizing fruit peels as antimicrobial agents. The present study delves into the antimicrobial properties of yellow lemon (*Citrus limonia*) against specific microorganisms [15]. Lemon (*Citrus limon*) juice has been shown to suppress the growth of germs such as *Staphylococcus aureus*. Lemon juice's acid pH is one of the variables that can restrict bacterial development, as it can cause the internal pH of bacterial cells to fall, hence inhibiting bacterial cell growth. Lemon juice (*Citrus limon*) contains a variety of bioactive components, including flavonoids, carotenoids, limonoid, tannin and terpenoids. Each of the bioactive chemicals found in lemon (*Citrus limon*) has antibacterial properties.

Staphylococcus aureus (*S. aureus*) is a type of bacteria that can cause a range of different ailments (Figure 1). They are Gram-positive cocci with a diameter of between 0.5 and 1.0 μ m. They cluster together, form pairs and, on rare cases, create short chains. It is a facultative anaerobe that may grow in the absence of oxygen and frequently contains catalase and nitrate reduction enzymes. *Staphylococcus aureus* is an opportunistic organism that can cause abscesses on the skin, respiratory infections such as sinusitis and food poisoning. They cause infection by producing virulence factors like as protein toxins and a cell-surface protein that attracts and inactivates antibodies. This kind possesses a number of virulence traits that enable it to adhere to the host's surface, invade or avoid the immune system and cause harmful effects.

Staphylococcus aureus pathogenicity is multifactorial and is a result of the combined action of many virulence determinants. *Staphylococcus aureus* is notorious for developing antibiotic resistance.

Antibiotic-resistant strain infections frequently develop in epidemic waves starting by a single or a few viable clones. During these epidemics, Methicillin-Resistant (MRSA) is a significant pathogen. It can cause anything from minor skin infections such as pimples to life-threatening conditions such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia and sepsis (Figure 1).

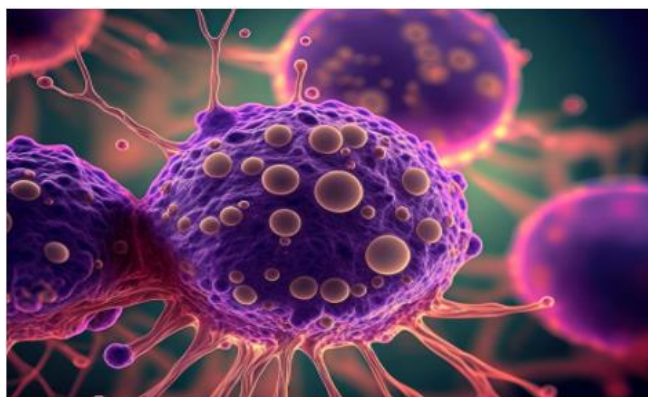


Figure 1: Structure of *Staphylococcus aureus* under microscope (generated in *Freepic*).

Antibiotics are antimicrobial substances produced by fungus, plants or synthetic molecules. These agents are bactericidal (kill all bacteria) or bacteriostatic (suppress bacterial growth). Penicillin acts as a bactericide. The beta-lactam ring, along with the amide, thioether and carboxylic acid groups, are all features present in these molecules. Penicillin works by impeding the transpeptidase enzyme, emulating a peptidoglycan chain and creating an ester bond with the enzyme. The large structure of the penicillin group hinders a nucleophile from targeting the ester carbonyl. This blockage stops the ester from engaging with another peptidoglycan chain. Ampicillin functions by disrupting cell wall formation. It does this by binding to Penicillin-Binding Proteins (PBPs), obstructing the synthesis of peptidoglycans in the cell wall and neutralizing inhibitors of autolytic enzymes. The antibiotics used in this study have their structures displayed in Figure 2.

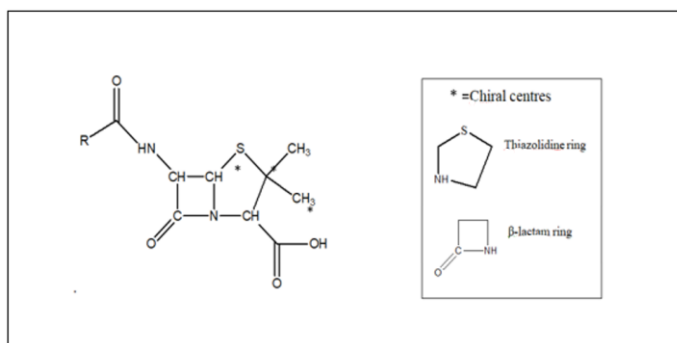


Figure 2: Structure of antibiotic Penicillin

Penicillin is the preferred treatment for *Streptococcus aureus* infections. Most *Streptococcus aureus* strains have achieved penicillin resistance due to the bacteria producing an enzyme called penicillinase. Penicillin inhibits the growth of bacteria by attaching to the beta-lactam ring of the enzyme DD-transpeptidase, stopping it from performing cross-linking activity and blocking the production of new cell walls. Without cell wall, bacteria are sensitive to the effects of outside water and molecular pressures, which causes the bacteria to die very quickly as a result. Exploring the effectiveness of lemon juice in contrast to Penicillin, one of the most celebrated antibiotics in medical history, can provide insights into the ability of the natural remedies to complement synthetic pharmaceuticals in the fight against bacterial infections. This penicillin was structurally modified after the growth in semi-synthetic pathways, to give amoxicillin with improved activity. It was found to be effective against lower and upper respiratory tract infection, urinary tract, skin and soft-tissue infections. Amoxicillin is the most frequently used antibiotics, designed to fight against resistance due to penicillin. Structurally, in amoxicillin an amino group is attached to penicillin. Amoxicillin is a medium-spectrum antibiotic, found active against a wide range of gram-positive bacteria (*Streptococcus* species, *Enterococcus* species, *Listeria monocytogenes*) and active against some of the gram-negative bacteria (*Escherichia coli*, *Haemophilus influenzae*). It has been marketed from 1972, being the most often used drug in the class of penicillin due to its better oral absorption ability as compared to other β -lactam antibiotics. However, over the decades the bacteria have well developed antibiotic resistance, making it a serious cause of health problem globally. Many of the gram-positive and gram-negative bacteria had been able to produce β -Lactamase essential in the cleavage of β -lactam ring, leading to the common problem of bacterial resistance.

The findings from this research offer valuable insights into the potential future use of lemon juice for therapeutic purposes in countering a broad spectrum of multidrug-resistant microorganisms. This investigation holds implications not only for the development of alternative antibacterial agents but also for promoting sustainable and holistic approaches to healthcare.

MATERIALS AND METHODS

Collection of material

Fresh fruits of *Citrus limonum* Burm were meticulously gathered from the campus situated in Dibrugarh. To ensure the authenticity of the specimens, they were cross-verified and confirmed at the Botanical Survey of India in Meghalaya. A total of five ripe lemon fruits were chosen for the subsequent procedures. Firstly, the lemon fruits were carefully peeled and their luscious juice was extracted using a plastic juice extractor. To ensure the purity of the extracted fruit juice, it underwent a dual filtration process. Initially, it was filtered through Whatman number one filter paper, followed by a secondary filtration through a 0.45 μm membrane filter (Sigma). This thorough filtration process was undertaken to remove any impurities or particulate matter. The resulting clear filtrate was then precisely measured and transferred into a sterilized Petri Dish, where it was allowed to naturally evaporate at room temperature. After the complete evaporation, the weight of the residue was meticulously determined and recorded as the yield (w/v) of the fruit juice extract. Subsequently, the fruit juice extract was subjected to sterilization using UV irradiation for a duration of 20 hours, ensuring the elimination of any potential microbial contaminants. To verify the sterility of the reconstituted extract, an aliquot was placed on nutrient agar plates and observed for any signs of microbial growth. This step was crucial to guarantee that the extract was devoid of any microbial contamination. Once confirmed sterile, the fruit juice extract was carefully preserved in sterile containers at a temperature of 4°C. It was used for subsequent phytochemical analysis and antimicrobial susceptibility testing within a time frame of 2-4 days from the date of its preparation to ensure the reliability of the results.

Preparation of lemon juice extracts

A volume of 100 milliliters of the raw lemon juice was meticulously extracted using a plastic juice extractor. The purpose of this extraction was to obtain the lemon juice in its purest form. To quantify the amount of extract obtained, the yield of each sample was measured in grams (g), providing a precise indicator of the quantity of extracted material. To ensure the preservation of the extract's integrity and active compounds, it was thoughtfully stored in opaque or dark bottles. These dark bottles were then placed inside a refrigerator set at a constant temperature of 4°C. Maintaining the extract in such a controlled and low-temperature environment is crucial for its long-term stability and to prevent degradation. For subsequent laboratory applications and experiments, stock solutions were meticulously prepared from each extract. This was accomplished by dissolving the extract in an appropriate amount of a solvent specific to the intended application, resulting in a concentration of 100 milligrams per milliliter (mg/mL). These concentrated stock solutions serve as the baseline for various experiments, ensuring that a known concentration of the extract is available for each test. During the course of antimicrobial studies or other experiments, dilutions of these stock solutions were thoughtfully and accurately prepared. The dilutions were made in accordance with the requirements of the specific study, thereby allowing for the examination of the extract's effects at different concentrations. This controlled and systematic approach ensures that the research is conducted with precision, making it possible to observe how the extract interacts with microorganisms or other test subjects at varying levels of concentration.

Phytochemical analysis

The reconstituted fruit juice extract was analyzed using standard phytochemical techniques to determine the presence of compounds such as glycosides, tannins, flavonoids, alkaloids, saponins, carbohydrates, proteins and water-soluble vitamins.

In vitro study

Antioxidant activity: The antioxidant activity of the lemon juice was evaluated using the 2,2'-Azino-bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and Hydrogen Peroxide (H₂O₂) assay method using ascorbic acid as a standard.

ABTS assay: The ABTS radical scavenging activity of different concentrations of lemon juice was estimated by the 6-method reported by Re, et al., with slight modification. In this method, a 7 mM ABTS stock solution was prepared by dissolving ABTS in double distilled waters and simultaneously, a 245 mM Potassium Persulfate (PPS) solution was prepared using double distilled water. The PPS solution was added to the ABTS solution, yielding a final PPS concentration of 2.45 mM. This mixture was left to incubate overnight under dark conditions at room temperature. To obtain the working solution, we carefully diluted the previously mentioned mixtures with methanol until it attained an absorbance value of approximately 0.75 ± 0.03 at 734 nm. Within 96-well plates, 200.0 μ l of the ABTS working solution was thoroughly combined with 10.0 μ l of various concentrations (5, 10, 15, 25, 50, 75 and 100 μ g/mL) of lemon juice. These mixtures were thoroughly shaken and allowed to stand at room temperature for 15 minutes under dark conditions. Subsequently, absorbance measurements at 734 nm were recorded using a Microplate spectrophotometer.

DPPH assay: A series of lemon juice and ascorbic acid solutions were prepared in methanol at concentrations of 5, 10, 15, 25, 50, 75 and 100 μ g/mL by diluting the respective stock solutions of lemon juice and ascorbic acid, which were originally at a concentration of 1 mg/mL. To establish a baseline for reference, a control sample was prepared using only DPPH reagent and methanol. Subsequently, 1 mL of each lemon juice solution was mixed with 2 mL of the DPPH solution. The resulting mixture underwent 30-minute incubation period in complete darkness at ambient room temperature. After incubation, the absorbance of the solution was measured at 517 nm using a UV-Vis.

H₂O₂ scavenging activity: A 40 mM H₂O₂ solution in phosphate buffer (pH 7.4) was prepared. Various concentrations of lemon juice (5, 10, 15, 25, 50, 75 and 100 μ g/mL) were added to 0.6 mL of 40 mM H₂O₂ solution. After setting it aside for 10 minutes, the absorbance of the H₂O₂ solution was measured at 230 nm. Ascorbic Acid was used as the reference standard.

To initiate the antimicrobial investigation, we specifically selected *Staphylococcus aureus*, a gram-positive bacterial strain, as the target organism for assessing the antimicrobial properties of the lemon juice.

Microorganisms and culture: We chose the gram-positive bacterium *Staphylococcus aureus* for our investigation. To prepare the bacterial cultures, we sub cultured them from solid media into Mueller Hinton broth. These cultures were then incubated for a period of 24 hours at a temperature of 37°C. Subsequently, the resulting bacterial cultures served as the inoculum for our assessment of antibacterial activity. Throughout the course of our study, the bacterial strains were meticulously preserved at a temperature of 4°C, housed within Mueller Hinton agar slants. This careful maintenance ensured the viability and stability of the bacterial strains for the duration of our research.

Antimicrobial studies of extracts: To assess the antimicrobial potential of the lemon juice under investigation, we employed the agar well diffusion method as described by Martin and Ernst in 2003. First, a sterile petri plate was utilized to pour molten Mueller Hinton agar medium, which was maintained at a temperature range of 40°C-45°C. This agar medium was then inoculated with a microbial culture. For the bacterial test strain, a standardized bacterial stock suspension with a concentration of 10^8 - 10^9 Colony-Forming Units Per Milliliter (CFU/ml) was used. Subsequently, the plates were allowed to stand for approximately 15-20 minutes to solidify. To facilitate the assessment, we used a sterile borer to create three evenly spaced wells, each measuring 8 mm in diameter, in each petri plate. These wells served as reservoirs for the test substances. Next, 50 microliters of each fruit peel solvent stock solution, which contained an extract concentration of 5 milligrams, were carefully loaded into their respective wells. As a positive control, 100 microliters of a broad-spectrum antibiotic, Amoxicillin, containing 50 micrograms, was loaded into a designated well. Negative controls were prepared by loading the respective solvents without any extract. The petri plates containing the bacterial cultures were then incubated for 24 hours at a temperature of 37°C, while yeast culture plates were maintained at 30°C for 48 hours. After this incubation period, we precisely measured the diameters of the inhibition zones, including the diameter of the well and recorded these values. These measurements were then tabulated to determine the effectiveness of the lemon juice extract against the test microorganism. Simultaneously, solvent controls were run alongside these experiments to ensure the accuracy and reliability of the results.

Minimum Inhibitory Concentration (MIC) determination

To determine the Minimum Inhibitory Concentration (MIC) of lemon juice extracts that exhibited substantial inhibition zones using the agar well diffusion method, the following procedures were carried out. For the bacterial strain, it was cultured in a 10-milliliter volume of aqueous SCD medium, which includes soybean, casein and digest components. This culture was incubated for 24 hours at a temperature of 35.5°C. Subsequently, dilutions of the bacterial culture were prepared and adjusted to reach a concentration range of 10^6 - 10^7 microorganisms per milliliter, which was later used as the inoculum for the MIC test. In the case of the MFF culture (presumably a fungal culture), a potato and dextrose agar slant medium were utilized, cultivated for a week at 27°C. After this cultivation period, it was washed with saltwater containing 0.05% Tween 80 as per the methodology described by Rabbani, et al. in 2016. The concentration of the spore suspension was adjusted to 10^6 microorganisms per milliliter for further use as the inoculum in the MIC test. The cultured fluid was subsequently diluted and adjusted to reach a concentration range of 10^7 - 10^8 microorganisms per milliliter and was used for inoculation in the MIC test. To assess the MIC, the lemon juice extracts were first suspended in water and then these solutions were further diluted. The dilution was carried out with an SCD medium for bacteria and a GP medium for the MFF and yeast. Various twofold diluted solutions with concentrations ranging from 1000 to 10 milligrams per milliliter (mg/mL) were prepared. In each case, 1 milliliter of the culture medium, containing different concentrations of the test material, was inoculated with 0.1 milliliter of the microorganism suspension, as prepared earlier. Following this inoculation, bacteria were cultured for one day at 35.5°C, the MFF culture for 7 days at 25°C and yeast for 2 days at 30°C, as per the protocol outlined by Rabbani et al. in 2016. The growth of the microorganisms was closely monitored during these incubation periods. When no growth of the microorganism was observed in the medium containing the lowest concentration of the test materials, that specific concentration was defined as the Minimum Inhibitory Concentration (MIC) of the test material.

RESULTS

Phytochemical analysis of lemon juice

The extraction process resulted in a fruit juice yield of 0.33% (w/v). Subsequent phyto chemical analysis revealed the presence of various compounds. These include alkaloids, cyanogenetic glycosides, cardiac glycosides and steroidal glycosides, as well as tannins, saponins, flavonoids and water-soluble vitamins, as detailed in Table 1.

Table 1: Phytochemical analysis of *Citrus limonum* fruit juice.

Test for chemical groups	Successive extracts
	Phytochemical compounds presence in juice
Alkaloids	+
Amino acids	+
Carbohydrates	+
Fats and oils	-
Flavonoids	+
Glycosides	+
Gums	-
Lignin	+
Mucilage	-
Proteins	+
Steroid	-
Saponin	+
Tannins and Phenolic compounds	+
Triterpene	+

Anti-Oxidant activity

In our assessment of antioxidant activity, we examined the performance of both ascorbic acid and lemon juice across three different radical scavenging assays: DPPH, hydrogen peroxide and ABTS assays. These assays serve as crucial indicators of antioxidant potential, with higher values signifying stronger antioxidant activity.

DPPH Free radical scavenging activity: The ability of silver nanoparticles to neutralize radicals was evaluated using the DPPH radical scavenging assay. DPPH consists of stable free radicals that undergo reduction when they encounter an antioxidant compound, subsequently diminishing the absorbance capacity of DPPH at 517 nm (Table 2) (Figure 3).

Table 2: DPPH Radical Scavenging Activity of Lemon Juice

SL. No.	Conc.	Ascorbic acid	Lemon juice
1	5	24.13 ± 0.78	17.10 ± 1.37
2	10	35.17 ± 0.96	29.34 ± 1.06
3	15	47.87 ± 0.61	42.87 ± 0.57
4	25	61.12 ± 1.21	51.08 ± 0.90
5	50	77.98 ± 0.62	68.25 ± 2.80
6	75	85.91 ± 1.06	77.34 ± 1.73
7	100	88.82 ± 0.81	81.98 ± 1.17

Note: Values are expressed as Mean ± SEM; (n=3).

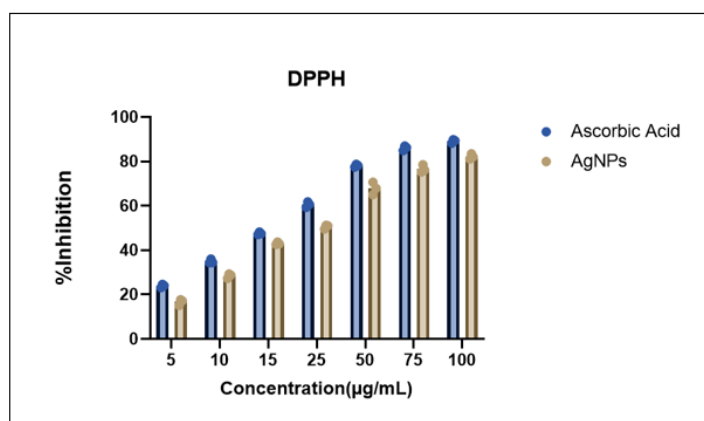


Figure 3: Comparative antioxidant effect of lemon juice and ascorbic acid on DPPH radical scavenging assay.

Beginning with the DPPH assay, our investigation revealed that ascorbic acid outperformed lemon juice in its capacity to neutralize DPPH radicals, indicating its superior effectiveness in this regard. The effectiveness of lemon juice and standard ascorbic acid in mitigating DPPH radicals was quantified through IC_{50} values. Our observations showed that lemon juice exhibited a concentration-dependent scavenging of free radicals, with maximum potency achieved at the specified concentration of 100 µg/ml. At this highest tested concentration, lemon juice demonstrated remarkable antioxidant activity, with a DPPH radical scavenging rate of 82.18%, albeit slightly lower than the standard ascorbic acid, which exhibited an inhibition percentage of 89.12%. In terms of IC_{50} values, lemon juice registered a value of 22.33 µg/ml, while ascorbic acid exhibited a slightly lower value of 17.33 µg/ml. These results reveal the potential of Lemon Juice as significant antioxidants, even though ascorbic acid displayed a marginally higher efficacy in neutralizing DPPH radicals.

Hydrogen Peroxide (H_2O_2) scavenging activity: The scavenging potential of both Lemon Juice and the reference standard ascorbic acid against Hydrogen Peroxide (H_2O_2) was quantified by determining their respective IC_{50} values. It was observed that the H_2O_2 scavenging effects of Lemon Juice and ascorbic acid exhibited dose-dependent behavior, with the highest inhibition achieved at the highest concentration, as summarized in Table 3 and illustrated in Figure 4. Table 3 presents the comparative analysis of ability of Lemon Juice to scavenge hydrogen peroxide in relation to that of ascorbic acid, the established reference standard. Notably, lemon juice displayed a dose-dependent pattern of H_2O_2 scavenging activity similar to that of ascorbic acid. In the H_2O_2 radical scavenging model, lemon juice exhibited a maximum percent inhibition of 86.73% at a concentration of 100 µg, while ascorbic acid, the reference standard, demonstrated a slightly higher inhibition rate of 91.94%. These results underscore the significant antioxidant capacity of Lemon Juice in mitigating H_2O_2 radicals, albeit with a slightly lower efficacy when compared to the established antioxidant agent, ascorbic acid (Table 3) (Figure 4).

Table 3: H_2O_2 Radical scavenging activity of lemon juice.

Sl. No.	Conc.	Ascorbic acid	Lemon juice
1	5	20.11 ± 1.45	13.46 ± 0.66
2	10	30.54 ± 0.65	23.14 ± 0.51
3	15	42.35 ± 0.78	35.61 ± 0.55
4	25	58.04 ± 0.75	52.44 ± 0.40
5	50	70.22 ± 1.11	65.10 ± 0.68
6	75	79.16 ± 1.34	72.35 ± 1.22
7	100	92.44 ± 0.65	85.32 ± 0.50

Note: Values are expressed as Mean ± SEM; (n = 3).

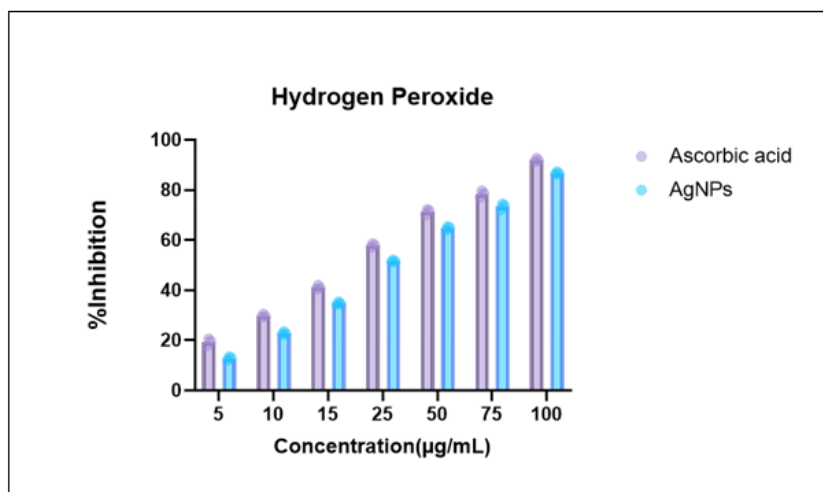


Figure 4: Comparative antioxidant effect of lemon juice and ascorbic acid on Hydrogen Peroxide (H₂O₂) scavenging activity.

Our study highlights the noteworthy antioxidant potential of Lemon Juice in scavenging hydrogen peroxide (H₂O₂) radicals. Although the Lemon Juice exhibited a slightly lower IC₅₀ value (19.69 µg/ml) compared to the reference standard ascorbic acid (24.05 µg/ml), their effectiveness in H₂O₂ radical inhibition is still of considerable significance. These findings underscore the promising role of Lemon Juice as alternative antioxidants and warrant further exploration of their diverse biological activities and applications.

ABTS radical scavenging assay

In the ABTS radical scavenging assay, Lemon Juice possess notable antioxidant capabilities, as evidenced by their significant inhibitory effects in the ABTS radical scavenging assay, particularly at a concentration of 100 µg. While lemon juice exhibited a substantial maximum percentage inhibition of 82.90%, it's noteworthy that the standard Ascorbic Acid achieved a slightly higher inhibition rate at 88.56% (Table 4 and Figure 5). Moreover, the dose-dependent nature of ABTS scavenging activity was observed for both Lemon Juice and Ascorbic Acid, with the highest inhibition attained at the highest concentration level. These findings highlight the potential of Lemon Juice as effective antioxidants and support their further exploration in various applications and research endeavors (Table 4) (Figure 5).

Table 4: ABTS radical scavenging activity of lemon juice.

Sl. No.	Conc.	Ascorbic acid	Lemon juice
1	5	15.86 ± 0.56	11.20 ± 0.87
2	10	21.30 ± 0.94	15.19 ± 0.57
3	15	26.21 ± 0.79	22.35 ± 0.16
4	25	67.95 ± 0.86	55.28 ± 2.73
5	50	74.11 ± 1.74	66.78 ± 1.85
6	75	79.48 ± 1.64	76.45 ± 1.17
7	100	89.16 ± 1.06	83.10 ± 1.77

Note: Values are expressed as Mean ± SEM; (n=3).

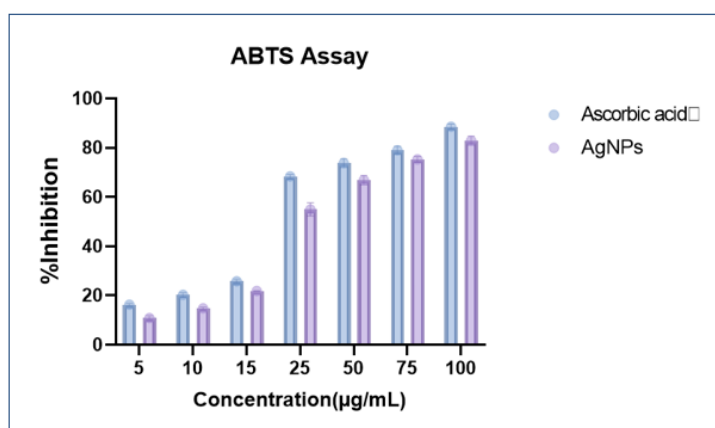


Figure 5: Comparative antioxidant effect of lemon juice and ascorbic acid on ABTS radical scavenging assay.

Our assessment of the IC₅₀ values for both the lemon juice and ascorbic acid revealed distinct antioxidant properties. The IC₅₀ value for Lemon Juice was measured at 27.01 µg/ml, while ascorbic acid exhibited a slightly lower IC₅₀ value of 20.49 µg/ml. These findings indicate that ascorbic acid displayed a comparatively stronger ability to neutralize the tested radicals in comparison to Lemon Juice. Nevertheless, both compounds demonstrated significant antioxidant potential, showcasing their relevance in scavenging free radicals and potentially contributing to various applications in the field of oxidative stress management and health promotion.

The results indicated that Lemon Juice exhibited substantial radical scavenging activity in all the three *in vitro* antioxidant models, which was comparable to that of ascorbic acid and even slightly lower than that of ascorbic acid. This enhanced antioxidant activity of biosynthesized silver nanoparticles was attributed to the presence of bioactive molecules on their surface.

Evaluation of antibacterial activity of lemon juice and penicillin on growth of *Staphylococcus aureus*

To evaluate the antimicrobial activity of lemon juice, conventional β-lactam antibiotic penicillin and amoxycillin on the growth of the gram-positive bacterium *Staphylococcus aureus*, a series of statistical tests were conducted. To analyze the zones of inhibition (mm) generated by lemon juice, penicillin, standard drug (Amoxycillin) and the control groups, a one-way ANOVA test was employed, allowing for multiple comparisons among these four distinct populations. The statistical analyses were carried out using GraphPad Prism 9.1 software and the resulting tables and graphs from the test are presented in Table 5. Notably, when dealing with gram-positive bacteria like *Staphylococcus aureus*, Streptococcus and Bacillus, β-lactams have consistently demonstrated superior performance compared to other classes of antibiotics (Figures 6 and 7). This phenomenon can be attributed to the β-lactam antibiotics' ability to inhibit peptidoglycan production in the bacterial cell wall. A one-way ANOVA test was employed to compare the zones of inhibition (measured in millimeters) resulting from lemon juice, penicillin, amoxycillin (standard drug) and the control (Table 5) (Figures 6 and 7).

Table 5: Zones of inhibition (in millimeters) by lemon juice, penicillin, amoxycillin (standard drug) and control.

Lemon juice	Average resistorzone (mm)		
	Penicillin	Amoxycillin (Standard drug)	Control (no antibiotic)
16.4 ± 0.1**	21.2 ± 0.4**	25.7 ± 0.4	5.2 ± 0.1
16.5 ± 0.9**	22.3 ± 0.7**	25.4 ± 0.2	5.6 ± 0.6
16.0 ± 0.1**	22.5 ± 0.5**	26.0 ± 0.5	5.2 ± 0.4

Note: P<0.05(significant**), which means that the mean values were significantly different between the groups.



Figure 6: MIC study of lemon juice, penicillin, amoxycillin (standard drug) and control against *Staphylococcus aureus*.

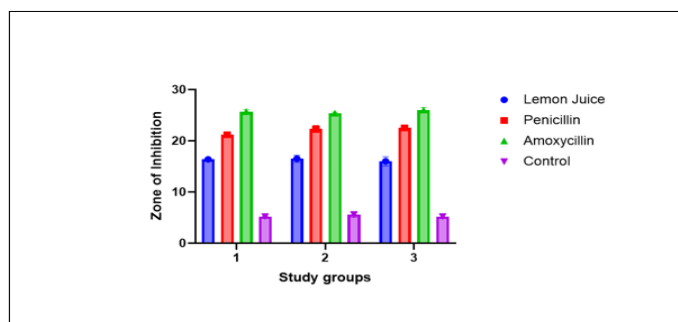


Figure 7: Effect of lemon juice and penicillin on *S. aureus*.

From the one-way ANOVA test, where multiple comparison was done among the control, penicillin, lemon juice and amoxicillin treated groups, it was observed that the zone of inhibition values exhibits the significance at $p < 0.05$ indicating that lemon juice though obtained from natural origin exhibit potential capacity to inhibit the growth of *S. aureus* when compared to penicillin. Amoxycillin demonstrates a significant inhibitory effect against *S. aureus*, with a 90% inhibition rate. In comparison, Penicillin exhibited a 60% inhibitory effect, while lemon juice showed a 45% inhibition. The control, on the other hand, had only a minimal inhibitory effect of 5%. From these results, Amoxycillin emerged as the most potent inhibitor of *S. aureus*, succeeded by Penicillin, then lemon juice and finally the control group. Lemon juice, while not as effective as some antibiotics, still demonstrated a notable inhibitory effect. This can be attributed to its rich content of secondary metabolites. Among these are vitamin C, flavonoids and polyphenols. These compounds have been recognized for their health benefits and therapeutic potential. Given the broad range of bioactive compounds in lemon juice, there exists an intriguing possibility for its constituents to be employed in the development of novel therapeutic formulations with antibacterial properties.

DISCUSSION

The antioxidant activity of lemon juice has been a subject of interest and research due to its potential health benefits. Lemons are rich in various bioactive compounds, including vitamin C, flavonoids and carotenoids, which contribute to their antioxidant properties. Antioxidants play a crucial role in neutralizing free radicals in the body, which are reactive molecules that can cause oxidative stress and damage to cells. Here, we'll discuss the key components of lemon juice that contribute to its antioxidant activity and their potential health implications. Lemon juice is renowned for its high vitamin C content, a powerful water-soluble antioxidant. Vitamin C is known to scavenge free radicals, protecting cells from oxidative damage. It also regenerates other antioxidants, such as vitamin E, enhancing the overall antioxidant defense system in the body. Lemons contain various flavonoids, including hesperidin and naringin, which possess antioxidant properties. These compounds have been studied for their potential anti-inflammatory, anti-cancer and cardiovascular protective effects. Flavonoids in lemon juice contribute to its ability to combat oxidative stress and inflammation. Although present in smaller amounts compared to other fruits and vegetables, lemons contain carotenoids like beta-carotene and lutein. Carotenoids are known for their antioxidant properties and can help protect the body from oxidative damage. Adequate hydration is essential for overall health and lemon juice can be a refreshing way to increase water intake. Additionally, lemon juice is often promoted as a natural detoxifier, assisting the body in eliminating toxins. While the scientific evidence on detoxification is limited, staying hydrated is crucial for supporting the body's natural detox processes. When evaluating the antibacterial efficacy of lemon juice in relation to Penicillin, it becomes imperative to use a benchmark such as the standard antibiotic, amoxycillin, for an objective comparison. Lemon juice exhibits inherent antibacterial properties, primarily due to its citric acid composition and the presence of various secondary metabolites and phytochemicals. These constituents can inhibit bacterial proliferation to a certain degree. Conversely, penicillin a well-established beta-lactam antibiotic, functions by specifically targeting the peptidoglycan layer in bacterial cell walls. This interaction interferes with the synthesis of the cell wall, compromising its structural integrity and subsequently leading to bacterial lysis. This targeted mode of action has been demonstrated to be effective against a broad spectrum of bacteria, including both gram-positive and Gram-negative species. In comparison, the antibacterial modality of lemon juice is more generalized and not as potent. The efficacy of lemon juice as an antibacterial agent may vary across bacterial species and its spectrum of activity is not as encompassing as that of specialized antibiotics like penicillin. Therefore, while lemon juice offers some level of antimicrobial activity, its potency and specificity are limited when juxtaposed with conventional antibiotics. Future research into the antimicrobial properties of lemon juice should focus on isolating and characterizing the specific active compounds using techniques like GC-MS and LC-MS. Investigating potential synergistic effects with existing antibiotics could help combat resistant strains. It's essential to understand the mechanism of action of these active compounds and, once established, initiate *in vivo* studies and clinical trials to assess their therapeutic efficacy. The development of formulations, such as topical antiseptics or oral medications, can be explored, while also conducting broad-spectrum evaluations against various pathogens. Concurrently, safety and toxicological assessments are crucial. On a broader scale, the sustainability of large-scale lemon sourcing and the socio-economic benefits in citrus-rich regions warrant consideration. Promoting awareness about lemon's potential health benefits will further bolster its incorporation in both diets and traditional medicine.

CONCLUSION

The findings from our study underscored the superiority of penicillin over lemon juice in inhibiting the proliferation of *S. aureus*, especially when benchmarked against Amoxycillin. Interestingly, despite this differential, lemon juice exhibited noteworthy inhibitory effects against the growth of the gram-positive bacterium, *S. aureus*, even when juxtaposed with penicillin. This observation suggests that while traditional antibiotics like penicillin remain more potent, natural alternatives like lemon juice shouldn't be dismissed outright. The efficacy demonstrated by lemon juice holds significant potential, positioning it as a possible precursor or complementary agent in the formulation of newer, more potent and safer antimicrobial therapeutics.

AUTHOR CONTRIBUTIONS

KP: Conceptualization, Methodology, Resources, Writing-review and editing, Supervision. RS, JJS, MZA, OAE, BAW and SK: Conceptualization, visualization and writing-review. AD: Conceptualization, methodology and supervision. SK, MAI, PP, JJS and PT: Writing, editing and figure preparation.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

ETHICAL APPROVAL

Not applicable.

CONSENT TO PARTICIPATE

Not applicable.

CONSENT TO PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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