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# *In vitro* assessment of antimicrobial activity of citrus lemon against selected clinical isolates from Shendi city, Sudan

## Abstract

***Background:*** The high prevalence of microorganisms resistant to antimicrobials commonly used in clinical practice has impacted healthcare services due to reduced patient treatment options. Medicinal plants are thought to provide new sources of compounds that potentially replace antibiotics in the treatment of bacteria that are resistant to antibiotics.

***Objective:*** This work aimed at determining the antibacterial activity of Citrus lemon juice against gram-negative and gram-positive bacteria.

***Aims:*** This is a cross-sectional study in Shendi City, Sudan, from March to December 2023, at the microbiology laboratory, Faculty of Medical Laboratory Sciences, Shendi University. A total of 50 samples were collected from different clinical specimens. Seven pathogenic gram-negative and two gram-positive bacteria strains were isolated and identified using gram stain and biochemical tests. We tested the Citrus Lemon extract at concentrations of (100%, 50%, 25, and 12.5% v/v) of lemon juice by the agar diffusion method with measurement of the diameter of the zone of inhibition around the extracts.

***Result:*** Out of a total of 50 clinical specimens, *Escherichia coli*, *Pseudomonas aeruginosa, Proteus vulgaris*, *Citrobacter*, *Salmonella Paratyphi B*, *Salmonella paratyphi A*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella oxytoca* were identified. These results showed the activity of antimicrobial Citrus Lemon pronounced dose-dependent on standard strains and clinical isolates. The data from this work highlight the potential of these Essential Oils (Eos) against pathogens important to human health, suggest new studies against other emerging pathogens for public health, and better understand their applicability as a natural and safe alternative for controlling infections.

***Conclusion:*** This study demonstrated that citrus lemon juice might have antimicrobial activity against different Gram-positive and Gram-negative pathogens and could be used to prevent various diseases caused by these organisms of multiple diseases caused by these organisms.

**Keywords**: citrus lemon, gram positive, gram-negative, antimicrobial activity, herbal medicine.

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## Introduction

Biological processes benefit human health and provide a lot of potential for creating novel medications. Applying naturally occurring antimicrobial compounds in plant chemotherapy is becoming more substantial and important.[1] The World Health Organization (WHO) states that medicinal plants can be a groundbreaking method of synthesizing semi-synthetic chemical compounds for medical use.[2] Given its complex chemical makeup, C. limon's medicinal potential is an intriguing field of investigation. Most of the fruit's secondary metabolites are flavonoids and additional substances like vitamins, phenolic acids, coumarins, carboxylic acids, and amino acids. Particularly D-limonene, monoterpenoids make up the majority of the constituents in essential oils. Because of these priceless chemical components, C. lemon plays a significant and indispensable role in the food and cosmetics sectors, keeping the audience informed and aware of its importance.[3–5] The primary constituents of lemon fruit, a plant used in herbal medicine, are alkaloid chemicals, which have antiviral, antifungal, anticancer, and antidiabetic properties. Saponin is an alkaloid chemical with antibacterial properties.[6] Numerous bioactive substances, including flavonoids, carotenoids, limonoids, tannins, and terpenoids, are found in lemon (Citrus limon) juice. Each lemons (Citrus limon) bioactive substance has an antibacterial property.[7] Lemon juice (Citrus aurantifolia) has additional benefits as an antioxidant and antibacterial properties. Citric acid and vitamin C comprise most lemon (Citrus limon) juice content. The acidity (pH) of lemon (Citrus limon) juice is increased by vitamin C and citric acid.[8] Around eighty percent of people worldwide treat their illnesses with herbal remedies. The data shows it is lower in industrialised nations and higher in less developed nations.[9] Among many underdeveloped nations today, bacteria are thought to be the primary cause of disease and mortality, particularly among children. *Salmonella spp*, *Staphylococcus aureus,* and *Escherichia coli* are the most common bacterial pathogens.[10] Despite the pharmaceutical firms' efforts to produce various antibiotics in recent decades, antibiotic resistance has dramatically grown in several bacterial infections. Typically, bacteria can spread and develop antibiotic resistance through various genetic mechanisms.[11–13] The limonene nano-emulsion impacted Gram-negative bacteria like *Escherichia coli* and had a comparable effect on Gram-positive isolates like *Bacillus subtilis* and *Staphylococcus aureus*.[14] Limonene nano-emulsion was also found to have a similar impact on *Escherichia coli, Staphylococcus*

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*aureus,* and *Bacillus subtilis*, causing their cell membranes to change and causing intracellular materials to leak out of the treated bacterial cells in each isolate.[15] The bacterial cells (*Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli*) displayed incomplete and malformed forms. Our research has demonstrated that limonene plays a crucial role in damaging the bacterial cell membranes of both Gram-positive and Gram-negative species. This damage leads to the release of internal components and eventual cell death. Importantly, the effectiveness of limonene, or any other antibacterial drug, is influenced by the cell type, with Gram-positive bacteria primarily targeted at the cytoplasmic membrane, and Gram-negative bacteria at the outer membrane.[16] Bactericidal activity and antimicrobial drugs differ because Gram-negative bacteria preserve their membrane homeostasis better than Gram-positive bacteria.[17] Additionally, it was noted that the lipopolysaccharide-based outer membrane of Gram-negative bacteria creates a hydrophilic permeability barrier that guards against the effects of highly hydrophobic medications.[18] The successful treatment of infectious diseases is seriously threatened by the fast-developing antibiotic resistance in bacterial populations[10,19] However, adverse effects associated with commercial antibiotics are commonly recognized.[20] Therefore, searching for and investigating new antimicrobial agents is crucial and continuous. Microorganisms have been used to develop the majority of antibiotics. Furthermore, plant materials are valuable for developing new antibacterial chemicals.[10,21] Several recent publications have been on traditional herbs' antibacterial activity against gram-positive and gram-negative bacteria.[10] Plant-derived compounds damage microbial cells by attacking the phospholipid bilayer of the cell membrane and interfering with enzyme processes, among other methods of action. Medicinal herbs can denaturize proteins, alter the permeability of cell walls, or result in the loss of macromolecules. They can also have bacteriostatic effects on the enzymatic activity linked to energy production. As a result, it is difficult for microbes to become resistant to these therapeutic herbs.[22] Acidic citrus fruits are rich in nutrients that are good for the body. Vitamins and other vital nutrients the body needs can be abundant in fresh fruits and their hand-squeezed or industrially processed juices. Fresh fruits are primarily composed of flavanones and flavones (Figure 1).[23,24]

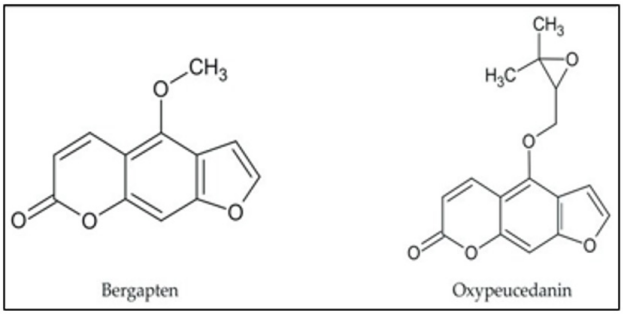


**Figure 1** The chemical structure of selected linear furano coumarins determines the photosensitizing effect of *C. limon.*

## Materials and methods

### Study design

This study was an analytical, cross-sectional, laboratory-based study. The samples have been spread across clinical facilities and hospitals across Shendi River Nile State, Sudan region. Situated 150 kilometres northeast of Khartoum on the east bank of the Nile, Shendi is a town in northern Sudan. Moreover, Shendi is located 250 km northwest and 45 km southwest of the historic cities of Meroawi and Napata.

### Samples collection

Fifty randomly collected samples (n=50) were taken in Shendi town, River Nile, Sudan.

### Collection of the specimens

Urine and stool were collected in sterile screw-capped universal containers, and wound swabs were obtained using sterile cotton swabs soaked with sterile normal saline under aseptic conditions.

### Isolation and identification of organisms

Several culture mediums were employed to identify and isolate clinical isolates, including Blood Agar, Macconckey Agar, CLED Agar, and Chocolate Blood Agar. We observed the plates to see if bacterial colonies developed into larger ones. After thoroughly isolating the bacteria, we identified them using biochemical assays, colony morphology, and Gram stain. Biochemical tests were performed after the Gram stain, and the relevant method was used to determine the isolated organisms fully. Maintaining Living Things Pure cultures were inoculated with organisms, incubated at 37°C for 24h, and then stored at 4°C in a refrigerator. This meticulous storage process ensures the preservation of the isolates for further analysis, providing a secure foundation for our work.

### Preparation of the aqueous extract of lemon

The fresh C. lemon fruits used in this investigation came from Shendi City, Sudan's local market. The citrus fruits were cut open with a sterile knife, cleaned in the lab with running tap water, surface sterilized with 70% alcohol, and their juice was squeezed out into a sterile universal container before being filtered into another sterile container to get rid of the seeds and other tissues. Bottles were aseptically opened, and one millilitre (1ml) of each crude juice sample was placed into one millilitre (1 ml) of sterile distilled water, which was employed as a different concentration (100%, 50%, 25, and 12.5%) against isolated organisms. When not in use, all extracts were kept at 4 °C.[25] To maintain the ascorbic acid levels and antioxidant capacity of the lemon extract, they were distributed at 0 and 5 ◦C, with appropriate packaging to prevent water loss and high relative humidity. Products made with lemons exhibit exceptional antioxidant and vitamin C retention in these circumstances.

### Preparation of bacterial suspension

Different specimens and subcultures provided clinical isolates. Test tubes containing 10 millilitres of normal saline were filled and autoclaved for 15 minutes at 121 degrees Celsius to sterilize them. Sterile normal saline was used as the inoculant for a loopful of pure bacteria. The McFarland standard solution was compared to the inoculum density.

### Testing of lemon extract for antimicrobial activity against standard organisms and clinical isolates

The agar well diffusion method is widely used to assess a plant's or microbial extract's antibacterial activity.[26] Touch between three and five well-isolated colonies that resemble the test organism with a sterile wire loop, then emulsify in three to four millilitres of sterile physiological saline or nutritional broth. Match the suspension's turbidity to the turbidity standard under adequate lighting conditions (mix the standard immediately before use). It is simpler to view turbid than a handwritten card or piece of paper. Inoculate a Mueller Hinton agar plate with a sterilized swab. Press and rotate the swab against the tube's wall above the suspension level to remove any extra fluid.

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To ensure uniform dispersion, rotate the plate for about a minute after swabbing the medium in three directions. After placing the petri dish lid on, dry the agar's surface for three to five minutes. Next, using a sterile cork borer, a 6 to 8 (mm) diameter hole is aseptically punched; the well is then filled with the necessary concentration of lemon extract.[26] Agar plates are then incubated under appropriate conditions. The diameter of each zone of inhibition, a meticulous process, can be measured in millimetres using a ruler on the plate's underside. The endpoint of inhibition is where growth starts.[27]

### Data collection and analysis

A self-administered questionnaire was used, supported by coding numbers to facilitate data sorting. Data were entered, checked, and analyzed using Microsoft Excel 2007. The final results were presented as frequencies and percentages.

## Results

From March to December 2023, a total of 50 patients exhibiting symptoms of respiratory tract infection, wound infection, and urinary tract infection were included in this study. Results demonstrating citrus extracts' comparative antibacterial activity against a standard control strain. At 100%, 50%, and 25% (v/v) concentrations, antibacterial activity was observed; at 12.5%, no antimicrobial activity could be observed. (Figure 2 & Tables 1–5).

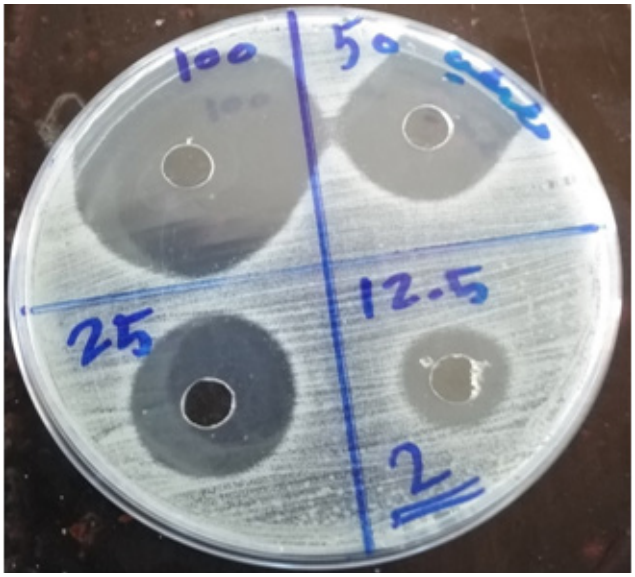


**Figure 2** Show the activity of citrus lemon against *Escherichia coli.*

**Table 1** The distribution of clinical specimens according to the gender

| Gender | Frequency | Percentage |
|---|---|---|
| Male | 15 | 30% |
| Female | 35 | 70% |
| Total | 50 | 100% |

**Table 2** The distribution of clinical specimens according to the sample type

| Sample | Frequency | Percentage |
|---|---|---|
| Urine | 37 | 74% |
| Wound swab | 10 | 20% |
| Sputum | 1 | 2% |
| Stool | 2 | 4% |
| Total | 50 | 100% |

**Table 3** The distribution of clinical specimens according to age

| Age | Frequency | Percentage |
|---|---|---|
| 15 - 24 years | 12 | 24% |
| 25 - 34 years | 6 | 12% |
| 35-45 years | 17 | 34% |
| More than 46 years | 15 | 30% |
| Total | 50 | 100% |

**Table 4** The frequency and percentage of isolated organisms

| Isolate | Frequency | Percentage |
|---|---|---|
| *E. coli* | 12 | 24% |
| *P. aeruginosa* | 9 | 18% |
| *P. vulgaris* | 6 | 12% |
| *Citrobacter* | 1 | 2% |
| *S. para typhi B* | 1 | 2% |
| *S. para typhi A* | 1 | 2% |
| *S. aureus* | 18 | 36% |
| *S. pyogenes* | 1 | 2% |
| *K. oxytoca* | 1 | 2% |
| Total | 50 | 100% |

**Table 5** The sensitivity of selected bacteria to different concentrations of lemon juice

| Bacteria | Concentration of juice lemon extract | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 100% | | 50% | | 25% | | 12.50% | |
| | S | R | S | R | S | R | S | R |
| *E. coli* | 12 | 0 | 12 | 0 | 12 | 0 | 0 | 12 |
| *P. aeruginosa* | 9 | 0 | 9 | 0 | 9 | 0 | 9 | 0 |
| *P. vulgaris* | 6 | 0 | 6 | 0 | 6 | 0 | 1 | 5 |
| *Citrobacter* | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| *S. para typhi B* | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| *S. para typhi A* | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| *S. aureus* | 18 | 0 | 18 | 0 | 18 | 0 | 10 | 8 |
| *S. pyogenes* | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| *K. oxytoca* | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| Percentage | 100% | 0.00% | 100% | 0.00% | 100% | 0.00% | 42% | 58% |

## Discussion

Resistance strains have emerged and spread due to the widespread use of antibiotics to treat bacterial infections, which has become a primary reason why treatments for infectious diseases have failed.[26] As a result, scientists have researched and developed novel chemicals to be employed in settings outside of traditional antibiotic therapy.[28] Nowadays, several businesses are searching for more natural, eco-friendly, and alternative sources of crop protection agents, antibiotics, antimicrobials, and antioxidants.[29] Citrus is one of the most significant commercial fruit crops produced on every continent.[30] Citrus lemons include Flavonoid chemicals that successfully inhibit the growth of bacteria, fungi, and viruses because they include groups of phenol compounds that can denaturize bacterial cell proteins and damage bacterial cell membranes.[31] In this investigation, the Lemon (Citrus limon) juices demonstrated antibacterial solid properties against gram-positive and gram-negative pathogens. The antibacterial activity was investigated using the suitable diffusion assay and the lemon juice extract; samples were tested at 100%, 50%, 25%, and 12.5% (v/v) concentrations. Fifty urine, wound, stool, and sputum samples were collected. The isolated bacteria are *Staphylococcus aureus* (36%) *Escherichia coli* (24%), *Pseudomonas aeruginosa*

(18%), *Proteus vulgaris* (12%), *Citrobactoer* (2%), *Klebsiella oxytoca* (2%), *Streptococcus pyogenes* (2%) *S. para typhi B* (2%) *S. para typhi A* (2%). This organism was compared with the standard control strain, and the result was approximately at the same level as the indicator for the activity of citrus lemon against isolation organisms. This study found the aqueous extract of Lemon showed the highest activity against *Staphylococcus aureus*, followed by *Escherichia coli*, *Pseudomonas aeruginosa Proteus vulgaris,* and the lowest activity against *S. para typhi B* followed by *S. para typhi A* and *Klebsiella oxytoca*. Moreover, the results indicated that the most effective concentration was 100%, and the effect of Lemon decreased dramatically when the concentration of lemon juice was reduced and the vase reversed. Additionally, it was consistent with the 2010 results of AL-Haliem and associates, who demonstrated that when tested *in vitro*, Lemon had vigorous antibacterial activity against *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Staphylococcus aureus*.[25] Also, research conducted in India in 2017 by Junab Ali and his colleagues shows that citrus lemons effectively achieved the intended outcome against most Gram-positive, Gram-negative, and fungal strains when used in the agar well diffusion method.[32] The highest level of antimicrobial activity was found by Nada et al. in a different study published in Iraq. The extract from the juice of C. lemon was found to inhibit 13 isolates out of the 15 isolates used in the study, all of which were Gram-positive or negative, with an inhibition zone ranging from 10 to 30 mm. *Pseudomonas aeruginosa* and staphylococcus *epidermidis* were the only two species that did not exhibit any susceptibility to this extract.[33] Clinical studies have shown that using pure hand gel with C. lemon as one of the ingredients daily reduces the microbial load, which is crucial to lowering the risk of healthcare workers spreading nosocomial illnesses.[34] In general, Rahman and his colleagues (2011) found that the size of the inhibition zone to represent relative antibacterial activity needs to be increased when microbial sensitivity to various plant extracts is determined. The agar medium's solubility, diffusion rate, and volatilization must impact the zone, which could affect the outcomes.[35]

## Conclusion

Within the kingdom of plants, medicinal plants are an essential source of medications and significantly impact the well-being of individuals and communities. Many plants have been investigated for the presence of potentially medicinal chemicals. As a result, it's essential to assess the citrus lemon fruit's antibacterial activity. According to the study, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Citrobactoer, Klebsiella oxytoca, Streptococcus pyogenes, S. para typhi B and S. para typhi A* exhibit practical antibacterial activities when used at different concentrations. The outcomes showed that citrus lemon juice has a significant function as an antibacterial agent against microorganisms. Some of the extracts exhibited activities that were likely beneficial in a therapeutic setting, and some of the extracts may have clinical applications in treating specific infections.

## Recommendation

Based on our findings, we advise using citrus lemon as an herbal remedy for individuals who are contraindicated from antibiotics or have illnesses that are resistant to them. More research is necessary to distinguish between the many phytochemical components and identify which possesses antibacterial qualities.

## Consent

The patient's written consent has been collected.

## Ethical approval

The study was approved by the Department of Medical Microbiology in Medical Laboratory Sciences at Shendi University, the study was matched to the ethical review committee board. Sample collection was done after signing a written agreement with the participants. Permission for this study was obtained from the local authorities in the study area. This study's aims and benefits were explained with the assurance of confidentiality. All protocols in this study were done according to the Declaration of Helsinki (1964).

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## Conflicts of interest

The authors declare that there are no conflicts of interest.

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