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Original Article

Evaluation of Phytochemicals and Antibacterial Usefulness of Citrus Limon & Cicer Arietinum in Synergistic Effect with Antibiotics Against Clinically Important Bacteria

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ABSTRACT

Background: The emergence of antibiotic resistance necessitates exploring alternative treatments, including natural plant extracts. Lemon juice (Citrus limon) and chickpea (Cicer arietinum) seed aqueous extract, known for their medicinal properties, are investigated for their potential antibacterial effects.

Objective: This study aimed to assess the antibacterial efficacy of lemon juice and chickpea seed aqueous extract, individually and in combination with antibiotics, against various pathogenic bacterial strains.

Methods: Aqueous and ethanolic extracts of C. limon and C. arietinum were prepared. Antibacterial activity against Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa, sourced from the University of Education, Lahore, was assessed using the disc diffusion method. Extract concentrations of 5%, 10%, 20%, and 40% were tested. Phytochemical analysis identified active compounds in the extracts. Antibacterial efficacy was quantified by measuring inhibition zones in millimeters.

Results: Lemon juice showed dose-dependent antibacterial activity with inhibition zones up to 19.8±0.088 mm at 40% concentration. Chickpea extract was effective against S. aureus with a maximum inhibition of 14±0.577 mm at 40%. Combined treatments enhanced antibacterial effects, particularly against S. aureus, with a combination of lemon juice, chickpea extract, and antibiotics yielding an inhibition zone of 20.1±0.058 mm at the highest concentration. In contrast, chickpea extract alone showed no activity against E. coli, K. pneumoniae, or P. aeruginosa.

Conclusion: The study confirms the significant antibacterial potential of lemon juice across multiple bacterial strains and the specific efficacy of chickpea extract against S. aureus. The combination of these extracts with antibiotics suggests a promising avenue for augmenting antibacterial treatment strategies.

Keywords: Antibacterial Activity, Citrus limon, Cicer arietinum, Plant Extracts, Antibiotic Resistance, Alternative Therapies.

INTRODUCTION

Antibiotics, a cornerstone in the battle against bacterial infections, were first discovered by Alexander Fleming in 1928, marking a revolution in medical science and saving countless lives (1). Gerhard Domagk's discovery of the antiseptic Prontosil in 1939 further advanced this field, earning him the Nobel Prize (2, 3). However, the initial triumph was soon tempered by the emergence of antibiotic resistance, a phenomenon first observed in E. coli and S. aureus, which showed resistance to penicillin shortly after its discovery (4, 5). Today, antibiotic resistance poses a significant challenge in modern medicine, with an increasing number of pathogens demonstrating reduced sensitivity to commonly used antibiotics (6). This alarming trend of harmful resistant microbes has been escalating over the years (7).

Parallel to the developments in synthetic medicine, the traditional use of plant extracts for disease treatment has been well-documented, with numerous plants identified for their medicinal properties (9). Among these, the Citrus genus, a member of the Rutaceae family, holds a prominent place in traditional medicine. Citrus limon, in particular, is celebrated not only for its delightful

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taste but also for its array of therapeutic properties. It offers a rich source of essential nutrients and bioactive compounds, including vitamins and secondary metabolites, which contribute to health enhancement and disease prevention (15). Citrus Limon is noted for its diverse pharmacological activities, including its antibacterial and anticancer properties, attributed to its content of alkaloids, vitamins C, flavonoids, phenols, steroids, reducing sugar, and other antioxidants (16, 17, 18, 26). Genomic studies reveal that C. limon originated as a hybrid between the acrid orange and lime, with its exact origins believed to be in India.

Cicer arietinum, commonly known as chickpea, is a staple in diets across various regions of the world and is recognized for its high content of proteins, fibers, minerals, and vitamins (10). Notable for having the highest oil content among dry pulses (3-10%), C. arietinum is also free from anti-diet elements and rich in carotenoids, such as beta-carotene (11, 12). Research by Dr. Maryam M. Elmi & Ozcelik et al. highlighted the antibacterial and antifungal properties of C. arietinum, suggesting its potential use in controlling microbial growth (13, 14).

The present study focuses on evaluating the antibacterial properties of aqueous and ethanolic extracts of C. limon and C. arietinum against commonly resistant pathogenic bacterial strains like E. coli, P. aeruginosa, S. aureus, and K. pneumoniae. Additionally, the study delves into analyzing the phytochemical constituents of these extracts, identifying the specific components responsible for their antimicrobial activity. This research contributes to the growing body of knowledge on alternative antimicrobial strategies, particularly in the context of increasing antibiotic resistance, and underscores the potential of these plant extracts in developing new therapeutic agents.

MATERIAL AND METHODS

In this study, we examined the antibacterial properties of Citrus limon (C. limon) and Cicer arietinum (C. arietinum) against several resistant bacterial strains. The methodology encompassed the preparation of plant extracts, their phytochemical analysis, and the assessment of their antibacterial activity.

Bacterial cultures of Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa were obtained from the Zoological Lab at the University of Education, Lower Mall Campus, Lahore, Pakistan. Fresh samples of C. limon were procured from a local market, and C. arietinum seeds were acquired from the same source.

The aqueous extract of C. arietinum was prepared by first cleaning the seeds to remove dirt and other materials, followed by air-drying at room temperature. The dried seeds were then ground into a fine powder. Following the method of Basri and Fan (21), a 10% solution was prepared by mixing 50 grams of the powdered seeds with 500 mL of distilled water. This mixture was agitated continuously for 24 hours using a magnetic stirrer and then filtered through Whatman filter paper no. 1. The extract was pasteurized using a 0.45μm filter paper to ensure sterility and the absence of germs.

For the preparation of C. limon pulp, fresh fruits were halved and squeezed to obtain the pulp. The antibacterial activity of both C. arietinum and C. limon extracts was assessed using the disc diffusion method. Filter paper discs of approximately 5 mm in diameter were placed on petri plates. Then, 10 µL of the fresh aqueous and ethanolic extracts of C. arietinum and C. limon pulp were applied to each of the bacterial species. Amoxicillin was used as a positive control, while the respective solvents (distilled water and ethanol) served as negative controls (21). The experimental conditions, including time gap, dosage concentration, temperature range, and environmental exposure, were meticulously controlled. The inhibition zones were measured after 24 hours using Vernier calipers.

Phytochemical analysis was conducted to identify the active constituents in the extracts. Various tests were performed to detect the presence of tannins, glycosides, proteins, cardiac glycosides, steroids, phytosterols, phenols, flavonoids, carbohydrates, alkaloids, and saponins using standard methods (1, 9, 10, 11, 15, 17, 18, 21). The specific procedures included heating, addition of reagents like ferric chloride, Fehling's solution, Millon's reagent, Ninhydrin, sulfuric acid, and observing color changes or precipitate formation. All methods were carried out following the relevant guidelines and regulations. Ethical approval was obtained from the institutional review board. Data collection was systematic, ensuring accuracy and reliability. The data analysis involved quantitative measurements of inhibition zones and qualitative assessments of phytochemical tests. The results were then statistically analyzed to determine the efficacy of the plant extracts against the bacterial strains. This comprehensive approach provided a thorough understanding of the antibacterial potential and chemical composition of C. limon and C. arietinum.

RESULTS

The results of this study, as presented in Tables 1 to 4, reveal insightful observations regarding the antibacterial effects of various treatments against different bacterial strains.

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Table 1 MEAN (mm) ±SEM values of antibacterial effect of various concentrations of (Anti) Positive control, (L.J) Lemon juice, (C.E) Chickpea aqueous extract (D.W) Negative control, on P. aeruginosa

Treatments		5%	10%	20%		40%
Anti		1.1±0.058	4.1±0.058	9.03±0.033		19.8±0.088
L.J		0.9±0.058	3.1±0.058	7.9±0.058		18.8±0.088
C.E		0.0±0.000	0.0±0.000	0.0±0.000		0.0±0.000
D.W		0.0±0.000	0.0±0.000	0.0±0.000		0.0±0.000
Anti + C.E		2.1±0.088	5.1±0.058	8.1±0.058		17.1±0.058
Anti + L.J		2.1±0.058	7.1±0.058	16.1±0.058		21.1±0.058
L.J + C.E		1.9±0.058	4.9±0.058	8.96±0.088		14.1±0.033
Anti + L.J+ C.E	2.1±0.058		6.1±0.058	11.1±0.058		18.1±0.058

In Table 1, the antibacterial effect of treatments against P. aeruginosa is detailed. The positive control (Anti) demonstrated a dose-dependent increase in efficacy, with mean inhibition zones ranging from 1.1±0.058 mm at 5% concentration to 19.8±0.088 mm at 40%. Lemon juice (L.J) also showed a similar trend but with slightly lower effectiveness, reaching an inhibition zone of 18.8±0.088 mm at the highest concentration. Notably, chickpea aqueous extract (C.E) and the negative control (D.W) exhibited no antibacterial activity across all concentrations. The combinations of treatments, particularly Anti + L.J and L.J + C.E, indicated enhanced antibacterial effects compared to individual treatments, with Anti + L.J reaching an inhibition zone of 21.1±0.058 mm at 40%.

Table 2 MEAN (mm) ±SEM values of antibacterial effect of various concentrations of (Anti) Positive control, (L.J) Lemon juice, (C.E) Chickpea aqueous extract and (D.W) Negative control, on K. pneumoniae.

Treatments		5%	10%	20%	40%
Anti		2.0±0.058	7.1±0.058	11.0±0.067	19.1±0.058
L.J		0.9±0.033	6.1±0.088	10.1±0.058	18.0±0.067
C.E		0.0±0.000	0.0±0.000	0.0±0.000	0.0±0.000
D.W		0.0±0.000	0.0±0.000	0.0±0.000	0.0±0.000
Anti + C.E		1.1±0.033	5.9±0.058	9.1±0.058	15.1±0.058
Anti + L.J		3.0±0.058	8.1±0.058	15.0±0.033	21.0±0.033
L.J + C.E		1.1±0.058	6.0±0.033	8.9±0.058	12.1±0.058
Anti+ L.J+ C.E		2.1±0.058	6.1±0.058	11.1±0.058	18.1±0.058

In Table 2, the focus shifts to K. pneumoniae. Here again, the positive control showed a consistent increase in antibacterial activity with increasing concentrations, peaking at 19.1±0.058 mm at 40%. Lemon juice's efficacy was notable, especially at higher concentrations, reaching 18.0±0.067 mm at 40%. The combination treatments, particularly Anti + L.J, demonstrated a synergistic effect, achieving an inhibition zone of 21.0±0.033 mm at 40%.

Table 3 MEAN (mm) ±SEM values of antibacterial effect of various concentrations of (Anti) Positive control, (L.J) Lemon juice, (C.E) Chickpea aqueous extract and (D.W) Negative control, on E.coli.

Treatments	5%	10%	20%	40%
Anti	1.0±0.066	5.3±0.033	7.6±0.33	18.3±0.333
L.J	1.1±0.057	3.9±0.067	7.3±0.33	17.5±0.280
C.E	0.0±0.000	0.0±0.000	0.0±0.000	0.0±0.000
D.W	0.0±0.000	0.0±0.000	0.0±0.000	0.0±0.000
Anti + C.E	1.0±0.033	4.1±0.057	7.76±0.14	14.5±0.288
Anti + L.J	3±0.057	6.4±0.200	14.0±0.033	19.8±0.155
L.J + C.E	0.9±0.033	5.0±0.057	9.0±0.033	14.1±0.057
Anti + L.J+ C.E	2.1±0.088	6.7±0.140	8.7±0.33	17.1±0.08

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Table 4 MEAN (mm) ±SEM values of antibacterial effect of various concentrations of (Anti) Positive control, (L.J) Lemon juice, (C.E) Chickpea aqueous extract (D.W) Negative control on S. aureus.

Treatments	5%	10%	20%	40%
Anti	3.0±0.0338.133±	8.1±0.088	12.5±0.289	17.4±0.200
L.J	2.1±0.888	7.0±0.033	11.6±0.33	15.73±0.144
C.E	0.0±0.000	2.8±0.088	7.8±0.133	14±0.577
D.W	0±0.00	0.0±0.000	0.0±0.000	0.0±0.000
Anti + C.E	3.9±0.067	8.93±0.067	14.3±0.153	18.1±0.058
Anti + L.J	1.0±0.000	6.86±0.133	11.9±0.033	19.03±0.033
L.J + C.E	0.0±0.000	7.1±0.588	13.1±0.053	19.1±0.058
Anti+ L.J+ C.E	2.0±0.033	7.2±0.100	12.1±0.53	20.1±0.058

The results against E. coli, presented in Table 3, mirrored the trends observed in previous tables. The positive control showed progressive effectiveness, with a maximum zone of inhibition of 18.3±0.333 mm at 40%. The combination of Anti + L.J was particularly effective, exhibiting an inhibition zone of 19.8±0.155 mm at the highest concentration. Interestingly, the combination of L.J + C.E also showed considerable activity, with an inhibition zone of 14.1±0.057 mm at 40%.

Table 4 presents the results against S. aureus. The positive control maintained its pattern of increased efficacy with higher concentrations, reaching an inhibition zone of 17.4±0.200 mm at 40%. The chickpea aqueous extract displayed activity at higher concentrations, with an inhibition zone of 14±0.577 mm at 40%, a notable finding given its lack of activity against other bacterial strains. The combinations, particularly L.J + C.E and Anti + L.J + C.E, exhibited enhanced antibacterial effects, with the latter achieving the largest zone of inhibition of 20.1±0.058 mm at 40%.

Overall, the results underscore the potential of lemon juice and chickpea aqueous extract, both individually and in combination with the positive control, in exerting antibacterial effects against various bacterial strains. The synergistic effects observed in the combination treatments are particularly promising, suggesting a potential avenue for enhancing antibacterial efficacy, especially in the context of increasing antibiotic resistance.

DISCUSSION

The study explored the antibacterial properties of lemon juice and chickpea seed aqueous extract, both individually and in combination with antibiotics, against various bacterial strains. The findings revealed that lemon juice, containing vital substances such as antioxidants, flavonoids, phenols, steroids, reducing sugars, and alkaloids, exhibited significant antimicrobial effects. This aligns with previous research indicating lemon's curative potential (16, 22), and its effectiveness against diarrhea-causing bacteria due to its rich content of potassium, vitamin C, alkaloids, and citric acid (16, 23, 24, 25). Mathai K. and colleagues also corroborated lemon juice's antibacterial potential, particularly against S. mutants (26).

The antibacterial mechanism of flavonoids, a key component in lemon, involves inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane functions, affecting energy metabolism, and impacting biofilm synthesis and membrane permeability (32, 33). Flavonols and phenolic acids, in particular, demonstrate substantial antibacterial action, inhibiting bacterial virulence factors such as toxins and enzymes, and enhancing the efficacy of antibiotics (34).

Conversely, chickpea seed aqueous extract displayed limited antibacterial activity. Previous studies by Mallikarjuna et al. (2007) found chickpea extract effective against fungi and identified antifungal peptides cicerin and arietin in chickpea seeds (27, 31). While legumes like chickpeas are known for their antibacterial effects against Bacillus cereus and antioxidant potential (28), the current study found chickpea extracts effective against S. aureus but not against K. pneumoniae, P. aeruginosa, or E. coli.

The study's distinctiveness lies in its exploration of the combined effects of antibiotics, lemon juice, and chickpea seed aqueous extracts. Notably, the synergistic combination of lemon juice and antibiotics showed enhanced antibacterial activity compared to antibiotics alone. In contrast, the combination of chickpea aqueous extract and antibiotics was more effective only against S. aureus. However, the study had limitations. The scope was confined to in vitro conditions, which may not fully replicate in vivo environments. Future research should consider in vivo studies to validate these findings. Additionally, the specific mechanisms through which these extracts exert their antibacterial effects, particularly in combination treatments, warrant further investigation.

CONCLUSION

In conclusion, lemon juice demonstrated broad-spectrum antibacterial properties, while chickpea seed aqueous extract showed limited activity. The combinations, especially of lemon juice and antibiotics, presented a promising avenue for enhancing

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antibacterial efficacy. This study underscores the potential of using plant sources in developing alternative or complementary antibacterial therapies.

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