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# Phytochemical screening and antimicrobial activity of plant leaf extract against enteric bacterial pathogens

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## ABSTRACT

*Punica granatum*, a fruit-bearing shrub belonging to the Lythraceae family, offers various benefits to humans, with both its fibers and seeds being valuable. The seeds contain important oil acids such as palmitic, punica, stearic, and oleic acids. In India, *P. granatum* (pomegranate) leaves have been investigated for their potential antibacterial activity against enteric pathogens. These pathogens are responsible for causing gastrointestinal infections and are often associated with antibiotic resistance. The study aims to explore whether pomegranate leaves possess properties that can combat these enteric pathogens effectively. This research holds promise in identifying a natural and alternative approach to address antibacterial challenges, potentially contributing to the development of novel antimicrobial agents. Pomegranate is commonly used as a traditional remedy for treating enteric bacterial pathogens. To investigate its antibacterial properties, researchers studied the aqueous extract of *P. granatum* leaf against enteric pathogens, conducting tests like biochemical examinations and microscopy on the isolates. The phytochemical analysis of *P. granatum* (pomegranate) leaf aqueous extract revealed the presence of various bioactive compounds. These compounds include amino acids, carbohydrates, alkaloids, steroids, flavonoids, terpenoids, saponins, tannins, and phenols. To evaluate its antimicrobial activity, the researchers used antibiotic discs, including chloramphenicol, vancomycin, and gentamicin, employing the standard Kirby Bauer method. Results showed that vancomycin had the highest zone of inhibition, followed by chloramphenicol and gentamicin, with respective measurements of 20 mm and 19 mm. Chloramphenicol exhibited sensitivity against *Escherichia coli* (19 mm), while gentamicin showed sensitivity against *Salmonella typhi* (20 mm), *Staphylococcus aureus* (19 mm), and *E. coli* (18 mm). Vancomycin demonstrated sensitivity against *S. aureus* (21 mm). The aqueous leaf extract displayed significant antibacterial activity at concentrations of 20, 40 mcg, and 60 µg, showing effective zones of inhibition ranging from 15 mm to 22 mm against *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, *Shigella dysenteriae*, *Proteus vulgaris*, and *S. typhi*. In conclusion, the study established that *P. granatum* leaf extracts possess remarkable antibacterial properties and contain valuable pharmaceutical bioactive compounds. Due to its efficacy in treating gastrointestinal disorders, it is utilized as a natural remedy in traditional medicine.

**KEYWORDS:** *Punica granatum* leaf, Phytochemicals constituents, Antimicrobial, Bacterial pathogens

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## INTRODUCTION

Since ancient times, humans have relied on medicinal plants and traditional herbal products for various therapeutic purposes.

India, with its rich and diverse flora, has been a hub of such natural remedies (Maghimaa & Palanisamy, 2019; Kabeerdass *et al.*, 2021a; Mathanmohun *et al.*, 2021; Pandeewari *et al.*, 2021a; Sankareswaran *et al.*, 2021; Maghimaa & Palanisamy,

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2016; Nashima & Palanisamy, 2016). Among the noteworthy natural power fruits in India is *P. granatum*, commonly known as Pomegranate, which has been traditionally used for treating various diseases. The fruit is used to prepare juice, as well as other products like jam, jelly, and beverages. Historical records show that ancient Egyptians used extracts from this plant, including its bark and fruits, to cure infections like dysentery and diarrhea. The traditional system of Ayurveda in India has utilized Pomegranate as a medicine for thousands of years (Vakayil et al., 2021a; Kabeerdass et al., 2022a). However, drug resistance has become a significant clinical challenge in treating microbial infections like dysentery and cholera (Kabeerdass et al., 2021b, c; Baburam et al., 2022; Nair et al., 2022; Sharmila et al., 2022; Vakayil et al., 2022a). Despite this, some antibiotics with antibacterial properties remain unexplored in the pharmacological context, supporting their use in traditional anti-diarrheal remedies. Peel and leaf extracts of Pomegranate have shown various pharmacological properties (Fan et al., 2021; Sun et al., 2021; Kabeerdass et al., 2022b, c), and the fruit itself has been reported to possess antiviral properties against influenza, pox, herpes, and HIV-1 virus. Its main components, hydrolyzable pigments like anthocyanins and tannins, offer valuable health benefits to humans, including antibacterial effects. The plant also contains flavonoids like punicalagin, ellagic acid, caffeic acid, and luteolin, among which punicalagin exhibits higher inhibitory effects on the influenza virus. The mature Pomegranate fruit comprises fleshy appendages or seeds covered by a white, membranous, edible layer of the pericarp.

*P. granatum* juice has been found to possess robust antioxidant activity, surpassing that of fruit juices from oranges and grapes. These natural antioxidants contribute to cell rejuvenation and anti-aging effects in humans. Additionally, Pomegranate shows potent anticancer and anti-inflammatory properties against various human malignancies. This research aims to evaluate the antimicrobial and phytochemical properties of *P. granatum* leaf extract against multidrug-resistant enteric pathogens in vitro. This investigation holds promise for uncovering new potential uses of *P. granatum* as a natural remedy in combating drug-resistant infections.

## MATERIALS AND METHODS

### Plant Material Collection

The fresh leaf of *Punica granatum* was obtained from the Kolli hills, Rasipuram, Namakkal District, Tamil Nadu, India. The taxonomic identities of the plant were verified (Ansari et al., 2019; Vakayil et al., 2021c; Kabeerdass et al., 2022d; Vakayil et al., 2022b).

### Preparation of Plant Extract

The leaves were carefully washed, dried in the shade, ground into a powder, and stored in airtight bottles at 4 °C. To prepare the leaf extract, 50 g of the powder was soaked in 250 mL of water for 8 hours using a Soxhlet apparatus at a temperature of 65 °C. After 8 hours, the leaf extract was collected, concentrated, and

stored in an airtight container at 4 °C for further use (Abirami & Maghima, 2019; Vakayil et al., 2019, 2020, 2021b,e,f; Abirami et al., 2021).

### Phytochemical Analysis

The concentrated aqueous extract was subjected to phytochemical analysis to screen for antimicrobial and phytochemical properties (Sivakumar et al., 2018).

### Clinical Sample Collection and Culture Preparation

The diarrheal stool samples were collected in sterile containers from hospitals and laboratories in Salem and transported to the laboratory. The samples were inoculated in MacConkey broth, and after 24 hours, pure cultures were obtained on MacConkey agar plates. The pure cultures were then maintained in nutrient agar slants.

### Physiological and Biochemical Analysis

The isolates were identified based on their biochemical and physiological profiles.

### Antimicrobial Sensitivity Test

The isolates were subjected to an antibiotic sensitivity test using the disc diffusion method, and the plates were incubated for 24 hours. The zone of inhibition was measured using a plate ruler, and the isolates were categorized as Resistant or Sensitive.

### Determination of Antibacterial Activity

The aqueous leaf extracts at different concentrations (20, 40, 60 µg) were evaluated against the isolated pathogens using the agar well diffusion method. The plates were incubated at 37°C overnight, and the zone of inhibition (ZOI) was measured.

## RESULTS

Table 1 displays the presence of various compounds, including amino acids, carbohydrates, alkaloids, steroids, flavonoids, terpenoids, saponins, tannins, and phenols in the aqueous leaf extract of *P. granatum*. The pathogenic bacteria were isolated from diarrheal stool samples and subjected to biochemical characterization and microscopy. Microscopic examination revealed the presence of both gram-negative and gram-positive bacteria. Biochemical tests, including IMViC, catalase, oxidase, urease, carbohydrate test, and TSI tests, were conducted. The identified pathogens included *E. coli*, *S. aureus*, *P. aeruginosa*, *S. dysenteriae*, *S. typhi*, and *P. vulgaris*. Table 2 presents the results of the antibiotic sensitivity test, indicating that Vancomycin had the highest zone of inhibition (21 mm), followed by Chloramphenicol and Gentamicin (20 mm and 19 mm). Chloramphenicol exhibited sensitivity against *E. coli* (19 mm), while Gentamicin showed sensitivity against *E. coli* (18 mm), *S. aureus* (19 mm), and *S. typhi* (20 mm). Vancomycin was found to be sensitive against *S. aureus* (21 mm).

**Table 1: Phytochemical compounds in *P. granatum* leaf extracts**

Phytochemical constituents	Acqueous extract
Alkaloids	+
Steroids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenols	+
Terpenoids	+
Amino acids	+
Carbohydrates	+

'+' presence and '-' absence of compound

**Table 2: Antibiotic sensitivity towards the isolated pathogens**

Isolates	Zone of inhibition (mm)		
	Gentamicin	Vancomycin	Chloramphenicol
<i>P. aeruginosa</i>	15	6	6
<i>S. aureus</i>	19	21	6
<i>S. dysenteriae</i>	16	6	6
<i>P. vulgaris</i>	14	6	6
<i>E. coli</i>	18	6	19
<i>S. typhi</i>	20	6	15

Table 3 demonstrates the significant antibacterial activity of the aqueous leaf extract at concentrations of 20, 40, and 60 µg, showing an effective zone of inhibition in the range of 15-22 mm against *P. aeruginosa*, *E. coli*, *S. aureus*, *S. dysenteriae*, *P. vulgaris*, and *S. typhi*. These findings highlight the potential of the leaf extract of *P. granatum* as an effective antimicrobial agent against the tested pathogens.

## DISCUSSION

There is a growing need for innovative antimicrobial agents derived from plant sources that can offer protection, safety, and cost-effectiveness while addressing antibiotic resistance (Maghimaa & Palanisamy, 2012; Maghimaa et al., 2012; Pandeewari et al., 2021b; Sivakumar et al., 2021a, b; Sabarinath et al., 2022a, b) and minimizing the adverse side effects associated with synthetic drugs. Pomegranate (*P. granatum*) peel has been widely utilized as an antimicrobial agent (Abirami & Maghimaa, 2019), and its antibacterial properties have been extensively studied in the past. Through phytochemical investigation, active inhibitors present in *P. granatum* leaves have been identified as potent components, making the leaf extract of *P. granatum* a readily available resource of natural antioxidants (Abirami & Maghimaa, 2019). This finding suggests that the peel extract could serve as an effective antibacterial agent. Such a diverse array of phytochemicals suggests that the *P. granatum* leaf extract possesses a wide range of potential health benefits. These bioactive constituents are known for their antioxidant, anti-inflammatory, and antimicrobial properties, which make the pomegranate leaf extract a valuable natural resource with potential therapeutic applications. For the scientific community involved in drug discovery and development, the key focus is on identifying novel active constituents responsible for antibacterial activity (Rajkumar et al., 2023a, b). This

**Table 3: Antimicrobial activity of *P. granatum* aqueous extract against the pathogens**

Pathogens	Zone of inhibition (mm)		
	20 µg	40 µg	60 µg
<i>Paeruginosa</i>	17	19	22
<i>S. aureus</i>	15	18	21
<i>S. dysenteriae</i>	17	20	22
<i>P.vulgaris</i>	16	18	20
<i>E. coli</i>	15	18	21
<i>S.typhi</i>	16	18	22

requires a multidisciplinary approach to recognize and understand the dynamic compounds that contribute to the antimicrobial properties of *P. granatum* leaf extract. By exploring and harnessing these natural compounds, we may discover valuable solutions to combat bacterial infections in a safer and more sustainable manner.

## CONCLUSION

The alarming rise in antibiotic resistance among microorganisms has led to a crucial demand for the development of new antimicrobial compounds. In this context, *P. granatum* emerges as a vital component, valued for its therapeutic properties in traditional medicine.

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