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Research Article

Evaluation of the Antimicrobial Activity of the *Punica granatum* Peel Extract Against Isolated *MDR salmonella typhimurium*

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Abstract

Objectives: To produce broad-spectrum antimicrobial compounds, it is still important to assess the antibacterial potential of medicinal plants. Traditional medicine is one of the most generally available types of treatment in disadvantaged countries. The current study's goal was to assess the antibacterial activity of *Punica granatum* peel extract against MDR *salmonella typhimurium*.

Methods: This study tested *Punica granatum's* antibacterial efficacy using the disk diffusion method. Amoxicillin and *Punica granatum* peel extract are effective against *MDR Salmonella typhimurium*.

Results: It was discovered that *Punica granatum* crude extract worked well against *MDR salmonella typhimurium.* The minimum inhibitory concentration (MIC) and zone of inhibition values for the crude *Punica granatum* Peel Extract were found to be 1 mg/ml and 09 ± 0.50 mm to 19.50 ± 0.00 mm, respectively.

Conclusions: According to the study's findings, *Punica granatum* crude Peel Extract has *in-vitro* antibacterial activity, providing early evidence that the plant may be used to treat MDR infections.

Keywords: *Salmonella Typhimurium*; Multidrug Resistance; *Punica granatum*; Zones of Inhibition; Minimum Inhibitory Concentration (MIC)

Introduction

Salmonella Typhimurium is one of the most frequently isolated food-borne diseases. There are around 93.8 million patient of foodborne illness and 155,000 fatalities annually as a result of this widespread public health issue. The subspecies Salmonella Enterica is home to more than half of the more than 2500 Salmonella serotypes that have been reported to date and are responsible for the bulk of human Salmonella infections [10]. Fluoroquinolones, ampicillin, and chloramphenicol are some of the medications used to treat enteric fever since they are antimicrobials and have been demonstrated to be the most effective therapies [30]. Without antibiotics, case fatality rates are anticipated to range from 20% to 30%, but with the appropriate course of therapy, they fall to 2% to 4% [23].

The global prevalence of *Salmonella typhimurium* which is frequently resistant to five or more antimicrobial drugs has been one of the biggest concerns for public health [11,27]. New treatment compounds that are effective against these bacteria have been developed as a result of the growth in pathogens that are resistant to antibiotics. Plant materials have recently garnered a lot of attention as an alternative method to manage pathogenic germs [2], and various compounds contained in plant products have been proved to be especially targeted against pathogenic bacteria with a high level of resistance [19].

Several plants have been utilized as folk medicines since the dawn of human civilization. Due to its higher compatibility with the human body and lesser danger of adverse effects, herbal medicine continues to be the primary source of primary healthcare for 70-80% of the world's population, primarily in underdeveloped nations [5]. Plants include a wide range of complex chemical elements that aid the body's natural healing processes. Plants have been shown to provide therapeutic effects for a range of diseases, including cancer and infectious diseases like malaria and tuberculosis. However, the rise in diseases that are multidrug resistant and the well-documented side effects of some common medications has brought attention to the necessity for searching for alternatives in medicine.

One of the *Punicaceae* family's member is *Punica granatum,* is also referred to as pomegranate, grenade, and granats [31]. Many countries have long used *punica granatum* as a traditional remedy [25] to treat respiratory diseases, dysentery, diarrhoea, helminthiasis, acidosis, and bleeding. Additionally, it has been suggested that *Punica granatum* has anti-inflammatory [1,24], anti-atherosclerotic [3,22], antibacterial [7,18], and antiviral [32] characteristics. Gallocatechins, cyanidin, delphinidin, gallic acid, ellagic acid, pelargonidin, and sitosterol are among the components of P. granatum, and they are well known for their medicinal qualities [13]. Furthermore, Salmonella is known to be resistant to the antibacterial effects of Punica granatum extracts [18]. However, no research has been done to yet on the antibacterial properties of *Punica granatum* peels. As a result, the objective of this work is to assess the antibacterial activity of the peel of *Punica granatum* extracted using Ethanol.

Material and Methods

Materials and bacterial sample

The plant samples were collected from a local fruit vendor. The taxonomic identity of plant components was confirmed by the Department of Pharmacognosy, PWCOP in Yavatmal. For samples of chicken flesh, roadside dirt, and water, test bacteria were collected across the state of Maharashtra's five different regions. PWCOP Yavatmal conducted biochemical, microbiological, multidrug resistance pattern studied with antibiotics susceptibility testing and molecular identification of *salmonella typhimurium* analyses. The Peels of *Punica granatum* was first separated and air dried then finely powdered using a grinder. Nutrient agar, Nutrient Broth, and as dehydrated media, Muller Hinton Agar were acquired commercially from local vendors and used by Hi-Media Laboratories Limited in India used as the plating medium in this experiment. Additionally, chemicals and reagents of analytical quality were used.

Extraction of antibacterial compounds in ethanol:

A grinder was used to turn the *Punica granatum* peel into powder. The powdered components were then boiled for 4 hours in 1500 mL of ethanol using about 500 g of the materials. The solvent was subsequently extracted using a rotary evaporator at a decreased pressure, and it was then either dissolved in water or 50% dimethyl sulfoxide (DMSO, Himedia) before being used [9].

HPLC analysis

With a column of (4.6 x 250 mm, 5 m) and an detector with a sensitivity of 0.04 AUFS and a wavelength of 254 nm, high-performance liquid chromatography (HPLC) was used. The material was eluted at a rate of 0.8 mL/min over a 50-minute period using a linear gradient of acetonitrile (solvent A) and water with 1% formic acid (solvent B). 20 µl of the *Punica granatum* peel ethanol extract was loaded into the HPLC after being dissolved in a methanol and water mixture. (6: 4 v /v-1) [12].

Determination of the antimicrobial activity

The antibacterial activity of the isolates on the various extracts was evaluated using the Clinical and Laboratory Standards Institute Standards disk-agar method [21]. Briefly, 20 µl of *Punica granatum* peel extract (different concentrations: 100, 200, and 500 µg) dissolved in 50% DMSO were loaded onto sterile paper discs, and they were then allowed to dry for 18 hours at 37 °C in a aseptic atmosphere. The bacterial cultures were then further diluted to achieve the final inoculum, which had a turbidity of roughly 0.5 McFarland ($1.5x10^8$ CFU mL^{-1}). The Mueller Hinton Agar was then added to Petri dishes, and 100 µl of the solution containing $1x10^{-5}$CFU mL-1 of bacteria was added.

Determination of MIC

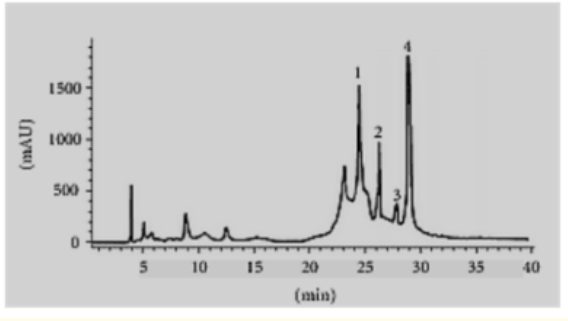
The raw extract was produced as stock solutions containing 0.5 mg/ml. In sterile nutritional broth, the comparable crude extract was serially diluted. The first tube in the series received 2.5ml after

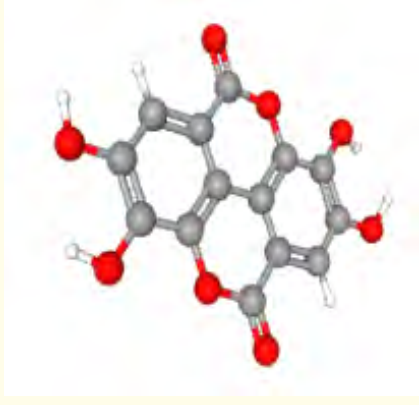
each serial dilution was completed in 5ml of broth. To challenge approximately $5x10^{-5}$ CFU/ml of test bacteria, standard inoculum (0.5 McFarland Standard) was prepared and 100 micro liter of the culture was added to each tube. After that, the tubes were kept at 37°C for the night. The evolution of the tubes was observed. For the purpose of measuring fine growth, On new nutrient agar plates, 50 micro liter of the antimicrobial-treated culture broth were sub cultured, and the outcomes were evaluated [28].

Results and Analysis

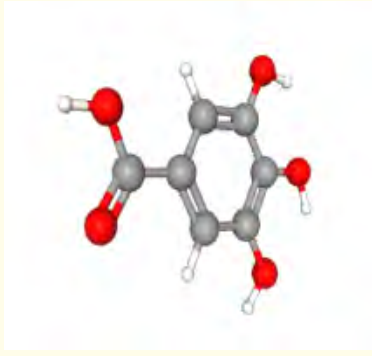
HPLC analysis

The same retention duration of their standards indicated that it is present as of gallic acid, ellagic acid and Punicalagin [15-17]. Figure 1 displays the chromatogram that was obtained.

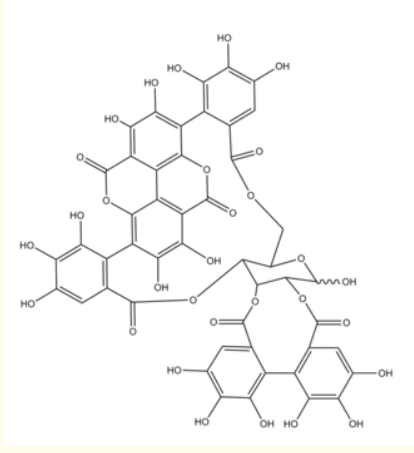




A)



B)



C)

Figure 1: Chromatogram of *Punica granatum* with structures of phytochemicals A) Ellagic acid B) Gallic acid C) Punicalagin.

Antimicrobial activity of *Punica granatum* peel ethanol extract

By measuring the surrounding zones of inhibition and determining the minimum inhibitory concentrations (MIC) using the agar dilution method, the antibacterial effectiveness of *Punica granatum* peel ethanol extract against *Salmonella Typhimurium* was assessed. Table 1 displays the disc diffusion method-determined antimicrobial activity of *Punica granatum* peel ethanol extract. At 500 g per disc, each of the tested strains' growth was inhibited, and the zone of inhibition grew dose-dependently. The zones of inhibition obtained against the investigated microorganisms ranged in mean value from 09.00 ± 0.50 to 19.50 ± 0.00 mm (Figure 2).

Test organisms	Concentration (mg/mL)	Mean diameter zones of inhibition (mm)	*Punica granatum* peel ethanol extract, (MIC)	Control (Amoxicillin) (MIC)
Salmonella typhimurium	0.5	19.50 ± 0.00	1 mg/mL	0.002 mg/mL
	0.2	16.03 ± 0.50		
	0.1	09.00 ± 0.50		

Table 1: Mean diameter zones of inhibition and Minimum inhibitory concentration of *Punica granatum* peel ethanol extract.

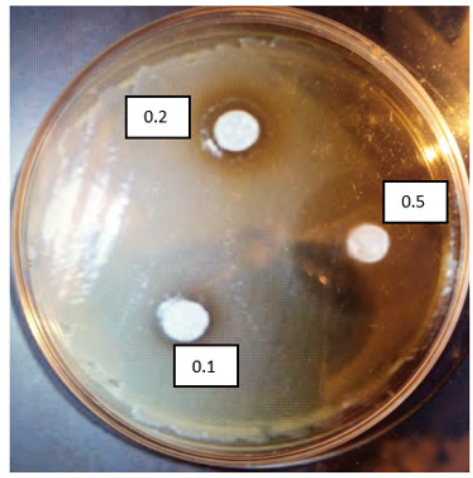


Figure 2: Antimicrobial activity of *Punica granatum* peel ethanol extract.

The outcomes achieved using the disc diffusion method was validated by the MICs calculated with the broth dilution method. The studied strain was susceptible to the antibacterial effects of *Punica granatum* peel ethanol extract, with values ranging from 0.09 to 1 mg/mL with that of Amoxicillin standard.

Discussion

According to numerous research, medicinal plants form the foundation of conventional medicine [6,29]. Humans can benefit from plants' anti-inflammatory, antibacterial, anti-oxidant and anthelmintic properties. Finding novel and affordable methods to control infectious diseases has become necessary due to the rise in bacterial resistance to current medicines. It were demonstrated that medicinal plants are an excellent source for isolating active antimicrobials [29].

Figures 1 illustrate how the HPLC analysis was carried out to study components from the *Punica granatum* peel ethanol extract (A, B, C). Along with a few other minor ingredients, this HPLC analysis mostly reveals some significant phenolic compounds [26], including punicalagin as a major ellagitannin, gallic acid, and ellagic acids. The retention time reveals gallic acid and ellagic acid [4], and punicalagin isomers are also found, which may be extrapolated to be one of the important components from the previously stated literature [27]. Punicalagin has anti-food-borne pathogen activity [29], ellagic acid has anti-microbial activity, and gallic acid has antibacterial activity against some intestinal bacteria [20].

The antibacterial properties of the *Punica granatum* peel ethanol extract was examined in this work. Based on information from ethnobotany, *Punica granatum* was chosen for this investigation; the plant has a long history of use in ethnomedicine [8]. The isolated or combined effects of the phytochemicals discovered in the peels may be related to the antibacterial capabilities demonstrated by the *Punica granatum* extract [4]. The concentration of *Punica granatum* extract affected its antibacterial action. This is consistent with a study that discovered concentration-dependent efficacy against *Salmonella typhimurium* in ethanol extracts of *Punica granatum* [26]. This result is consistent with a study that looked into *Punica granatum's* ability to modulate drug resistance [20]. This study used the disk diffusion method to test extracts of the *Punica granatum* peel ethanol extract and amoxicillin for antibacterial activity against strains of *Salmonella typhimurium*, which is consistent with previous studies [14].

Conclusion

Punica granatum extract's antibacterial properties could be attributed to the phytochemicals found in it. These findings support the traditional use of *Punica granatum* extract as an antimicrobial agent. More research into the development of natural alternative treatments for MDR *Salmonella Typhimurium* was required. It is important to conduct more research on a variety of issues, including toxicology against human or animal cells, mechanisms of action, *in vivo* effects, positive and negative interactions with typical antibiotics, and other subjects.

Data Availability Statement

All data generated or analyzed during this study are included in this article.

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For the submission this study, it does not include any research funding.

Competing Interests

The authors declare no conflict of interest with this research.

Ethical Approval

Since no animals were used in this study, ethical approval was not needed.

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This study was conducted at Pataldhamal Wadhwani College of Pharmacy, Yavatmal (M.S), 445001.

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