Evaluation of the antimicrobial activity using phytoextract against the bacterial pathogens

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Abstract--*Punica granatum* is a fruit-bearing shrub, that belongs to the family of Lythraceae. Every part of this plant is useful for humans like fibers and seeds. Oil acids of palmitic, punica, stearic, and oleic acids are present in the seeds. Pomegranate is commonly used as a conventional medication in India for the treatment of enteric bacterial pathogens. Antibacterial activities and Phytochemicals of hydroethanolic extract of *P. granatum* peel were studied against enteric pathogens. The isolates were examined by biochemical tests and microscopy. The phytochemical study analyzed the existence of carbohydrates, amino acids, alkaloids, flavonoids, terpenoids, steroids, tannins, phenols, and saponins. Chloramphenicol, vancomycin and gentamycin is the antibiotic disc used in the disc diffusion assay. Antimicrobial activity against the enteric pathogens was performed by the standard procedure (agar well diffusion method). The highest zone of inhibition for Vancomycin, followed by Chloramphenicol, and Gentamycin 19mm, and 18mm. Chloramphenicol displayed sensitivity to *E.coli* (18mm), Gentamycin explored sensitivity to *S.typhi* (19mm), *S. aureus* (18mm), *E. coli* (17mm), and Vancomycin was sensitive to *S. aureus* (19mm). The considerable activity of hydroethanolic peel extract in 20, 40mcg, and 60µg concentrations exhibits an effective zone of inhibition in the range of 14-21mm was noted on *P.aeruginosa, E. coli, S. aureus, S. dysentriae, Proteus vulgaris,* and *Salmonella typhi.* The end result reached that the peel extracts of *Punica granatum* has remarkable antibacterial property and pharmaceutical bioactive compounds. It is used as a natural medicine by itself to cure gastrointestinal disorders.

Key words*: Punica granatum; phytochemicals;antibacterial; enteric pathogens; antioxidant*

I. Introduction

Medicinal plants and traditional herbal products have been used by humans from the beginning of the human race. The green land of India has an affluent flora of diverse plants and natural products, and it's used in habitual therapeutics [1,2,3,4,5,6,7]. Recently epitomized *P. granatum* is a natural power fruit, generally named Pomegranate, abundant in India and herbal remedies it is used for the therapy of various kinds of diseases. The fruits are used for the preparation of juice, divergent value-additions of jam, jelly, and beverages. Egyptians used the extract of this plant akin to bark and fruits for the cure of numeral infections like dysentery and diarrhea. It is used as a traditional medicine of Ayurveda for thousands of years [8, 9]. Drug resistance [10, 11, 12, 13,14,15] is a chief clinical problem to treat microbial infections of dysentery and cholera. Nevertheless, few antibiotics are unexplored with antibacterial properties in the pharmacological method to support their use in traditional anti-diarrhoeal remedies. Peel extracts of this fruit have various pharmacological properties [16,17,18,19]. The antiviral property of these fruits was stated against influenza, pox, herpes, and HIV-1 virus. The main composition of this plant is hydrolyzable pigments of anthocyanins and tannins have valuable properties on the healthiness of mankind as well as antibacterial. The flavonoids of this plant are punicalagin, ellagic,caffeic acid, and luteolin, between them punicalagin only has higher inhibitory effects on the influenza virus. The mature pomegranate fruit comprises various fleshy appendages or covering of seeds detached with a white, membranous edible layer of the pericarp.

Robust antioxidant activity is found in *P. granatum* juice and it's considerably more than the fruit drinks of oranges and grapes etc. The antioxidants naturally found in the fruit will give nonaging of cells in humankind. Furthermore, it has brawny anticancer and anti-inflammatory properties against various anthropoid malignancies.

The objective of this research is to evaluate the antimicrobial and phytochemicals property of *Punica granatum* peel extract against multidrug-resistant enteric pathogens in in-vitro.

II. Materials and Methods

Collection of plant materials

The fresh peel of *P. granatum* was gathered from the market of Rasipuram, Namakkal District, Tamilnadu, India during the start of the winter season. The taxonomic identities of the plant were confirmed [20,21, 22, 23, 24].

Preparation of Plant Extract

The fruit peels were meticulously washed, shadow-dried, pulverized, and stored in air-tight bottles at 4ºC. Fruit peel extract was prepared by soaking 50gm of powder in 250ml of Ethanol for 8 hours using the soxhlet apparatus. The temperature of extracts is 65ºC for ethanol. After 8 hours peel extract was collected, concentrated, and stored at 4ºC in airtight container for further use [25, 26, 27, 28, 29, 30, 31].

Phytochemical analysis

The concentrated hydroethanolic extract subjected to phytochemical analysis for screening antimicrobial and phytochemical properties [32].

Clinical sample collection and culture preparation

Ten diarrhoeal stool samples were collected in sterile containers from hospitals, and laboratories at Salem and transported to the laboratory. Samples were inoculated in MacConkey broth and after 24 hours pure culture was obtained in the MacConkey agar plate. The pure cultures were maintained in nutrient agar slants.

Physiological and biochemical analysis

Isolates were recognized according to their biochemical and Physiological profilings.

Antimicrobial sensitivity test

The isolates were analyzed for an antibiotic sensitivity test which was carried out via a disc diffusion process and incubated for 24 h. The zone of inhibition via plate ruler was deliberated and expounded the isolates as Resistant, or Sensitive.

Determination of antibacterial activity

The hydroethanolic peel extracts with diverse concentrations (20, 40, 60µg) were assessed in opposition to the isolated pathogens using the agar well diffusion method and the plates were incubated at 37ºC overnight. The zone of inhibition (ZOI) was measured.

III. Results

The presence of amino acids, carbohydrates, alkaloids, steroids, flavonoids, terpenoids, saponins, tannins, and phenols in the hydroethanolic peel extract of *P. granatum* (Table -1). The pathogenic bacteria were isolated from diarrhoeal stool samples and examined for biochemical characterization and microscopy. Under the microscopic examination, gram-negative and gram-positive cocci were also observed. Biochemical analyses like IMViC, catalase, oxidase, urease, carbohydrate test, and TSI tests were performed. The identified pathogens were *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella dysentriae, Salmonella typhi*, and *Proteus vulgaris*. The highest zone of inhibition for Vancomycin, followed by Chloramphenicol, and Gentamycin 19mm, and 18mm. Chloramphenicol showed sensitivity to *E.coli* (18mm), Gentamycin explored sensitive to *E. coli* (17mm), *S. aureus* (18mm), *S.typhi* (19mm) and Vancomycin was sensitive to *S. aureus* (19mm) (Table 2). A significant activity of hydroethanolic peel extract in 20, 40, and 60µg concentrations show the effective zone of inhibition in the range of 14-21mm was observed on *P.aeruginosa, E. coli, S. aureus, S. dysentriae, P. vulgaris* and *S.typhi* (Table 3).

Table -1 Phytochemical compounds in *P. granatum* peel extracts

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

+ presence and '-'absence of compound

Table 2 - Antibiotic sensitivity towards the isolated pathogens (ZOI in mm)

Isolates	Chloramphenicol	Gentamycin	Vancomycin
P.aeruginosa	Resistant	14mm	Resistant
S. aureus	Resistant	18mm	19mm
S. dysentriae	Resistant	15mm	-
P.vulgaris	Resistant	13mm	-
E. coli	18	17mm	-
S.typhi	14	19mm	-

ZOI – Zone of Inhibition

Table 3 - Antimicrobial activity of *P. granatum* hydroethanolic extract against the pathogens

Pathogens	Concentration (in µg)		
	20 µg	**40 µg**	**60 µg**
P.aeruginosa	16mm	18mm	21mm
S. aureus	15mm	17mm	20mm
S. dysentriae	17mm	19mm	21mm
P.vulgaris	15mm	17mm	19mm
E. coli	14mm	17mm	20mm
S.typhi	15mm	17mm	21mm

IV. Discussion

Require innovative antimicrobial agents of plant source which is protective, secure, and economical for enhancing antibiotic resistance [34,35,36,37, 38, 39, 40] as well as the adverse side effects of man-made drugs. The peel of *P.granatum* is extensively used as an antimicrobial agent [30] and its antibacterial properties were studied in the past. Dynamic inhibitors in pomegranate fruit peels are exposed via phytochemical investigation as persuasive constituents and phytochemicals in the peel extract of *P. granatum* implies an anti-bacterial agent effortlessly reachable resource of natural antioxidant, this analogous interpretation was reported [30]. The pre-eminent resolution for the systematic society engaged in drug invention and progress is the new dynamic constitutes which are explicitly conscientious for the antibacterial activity, recognized by a multidisciplinary approach.

V. Conclusion

The necessity for the development of novel antimicrobial compounds due to the frightening increase in antibiotic resistance among microorganisms, these *P.granatum* is an imperative component and is relied on as a healing agent in the traditional medicinal system.

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