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Antimicrobial activity of ethanolic, methanolic and hot aqueous extract of *Punica granatum* peel

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Abstract
The main objective of the study is to evaluate antibacterial activity of *Punica granatum* pericarp (peel) extracts (hot aqueous, methanolic and ethanolic) against *E. coli* and *P. aeruginosa*, using Zone of Inhibition (ZOI) and Minimum Inhibitory Concentration (MIC) method. Hot aqueous, methanolic and ethanolic extracts of *P. granatum* pericap showed an average inhibitory zone diameter of 21.5, 22.5 and 23mm respectively which indicates that ethanolic extract showed best result having ZOI greater than that of the standard antibiotic Tetracycline (20.1mm) against *E. coli* while hot aqueous, methanolic and ethanolic extracts of *P. granatum* pericap showed an average inhibitory zone diameter of 21, 23.5 and 25.5mm respectively which indicates that ethanolic extract showed best result having ZOI greater than that of the standard antibiotic Tetracycline (22.5 mm) against *P. aeruginosa*. *P. granatum* peel has MIC of 0.312 µg/ml against *E. coli* and 0.421 µg/ml against *P. aeruginosa*. Data revealed that ethanolic extract of *P. granatum* peel has shown highest antimicrobial activity as compared to other extracts.

Key-Words: *Punica granatum*, Antimicrobial activity, Zone of Inhibition, Minimum inhibitory concentration

Introduction
Knowledge of herbs has been handed down from generation to generation for thousands of years. Herbal drugs constitute a major part in all traditional systems of medicines[1]. Plants have been used for thousands of years to conserve food and treat health diseases. Recently, natural products have been evaluated as sources of antimicrobial agents with efficacy against a variety of microorganisms. Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have antimicrobial properties[2]. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections[3]. The development of microbial resistance towards antibiotics makes it necessary to search for new potential effective compounds against pathogenic bacteria[4]. *Punica granatum*, commonly known as pomegranate, has been highlighted in some studies as having this property[5]. Extracts of all parts of the fruit appear to have therapeutic properties and some studies report that the bark, roots, and leaves of the tree have medicinal benefit as well [6]. The present investigation was to evaluate the antimicrobial property of *Punica granatum* peel.

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Material and Methods
Collection of plant part
The *Punica granatum* peel was collected from Indian Herb, Saharanpur and washed with water, saline and distilled water then cut into small pieces and dried at 60^0C for 48hours. The dried powdered was kept in air tight container at 4^0C for study.

Extraction of plant part
The plant part in extracted with hot aqueous, methanol and ethanol using soxhlet apparatus. All the solvent used in the study were analytical grade.

Screening of activity
Agar well diffusion method
Antibacterial activity was assessed by Agar well diffusion method of Kirby Bauer wherein Nutrient agar plates were prepared and were spreaded with 20µl of the available pathogenic cultures. Wells of 8 mm diameter were bored using sterile borer. Wells were loaded with antimicrobial, tetracycline as standard and distilled water as control and were incubated at 37^0C for 24 hours.

Minimum Inhibitory Concentration
Inoculated the Nutrient Broth (NB) with a loop full of isolated *E. coli* culture and incubated at 37°C for 24 hours. Fresh broth (50ml) was seeded with 0.5 ml of 24 hours old culture (lml in 100 ml NB). *P. granatum* rind peel extract was dissolved in solvent ["DMSO"] in a ratio of 1 mg/ml ". 0.2ml test solution was added to the

1st tube containing 1.8 ml seeded NB. 1ml of this dilution was transferred to the next tube to produce 2nd dilution and so on till the desired dilutions were obtained and incubated tubes at 37°C for 24 hours. The last tube with no visible growth of micro-organism (turbidity) represents the Minimum Inhibitory Concentration (MIC) of the sample. MIC was expressed in µg/ml.

Results and Discussion

The results of ZOI of the plant extract and its comparison with standard antibiotic Tetracycline is recorded in Table 1. The results of determination of MIC values of the *P. granatum* have been tabulated in Table 2. The following results show that the extract possessed antimicrobial activity against the tested organisms, depending upon the nature of the active ingredients present in the extracts and their capacity for diffusion into agar medium. ZOI activity of the ethanolic extract of the *P. granatum* found to significant activity against *E. coli* and *P. aeruginosa* respectively which has been showed in Table 1. *P. granatum* has a good antibacterial activity of MIC 0.312µg/ml is effective against *E. coli* and 0.421µg/ml is effective against *P. aeruginosa*. This sample was effective against 1ml of concentration. Results were shown in Table 2.

Hot aqueous, methanolic and ethanolic extracts of *P. granatum* pericap showed an average inhibitory zone diameter of 21.5, 22.5 and 23mm respectively which indicates that ethanolic extract showed best result having ZOI greater than that of the standard antibiotic Tetracycline (20.1mm) against *E. coli* while hot aqueous, methanolic and ethanolic extracts of *P. granatum* pericap showed an average inhibitory zone diameter of 21, 23.5 and 25.5mm respectively which indicates that ethanolic extract showed best result having ZOI greater than that of the standard antibiotic Tetracycline (22.5 mm) against *P. aeruginosa*.

Conclusion

From above study of three medicinal plants i.e. *Coriandrum sativum, Ocimum tenuiflorum and Phyllanthus emblica,* which are used in traditional medicine we got that they are active against bacterial strains but there were great variation in their antimicrobial activities. The results showed that *Ocimum tenuiflorum* and *Phyllanthus emblica* indicated maximum activity against *P. aeruginosa* with 20±0.32 and 25±0.25 mm zone of inhibition respectively and also exhibited potent antimicrobial action against all bacterial isolates tested. From current study, the data revealed that ethanolic extract of *P. granatum* peel showed better antimicrobial activity as compared to other extract. The potent antimicrobial activities of the extracts are as shown from the area of zone of inhibition on agar medium.

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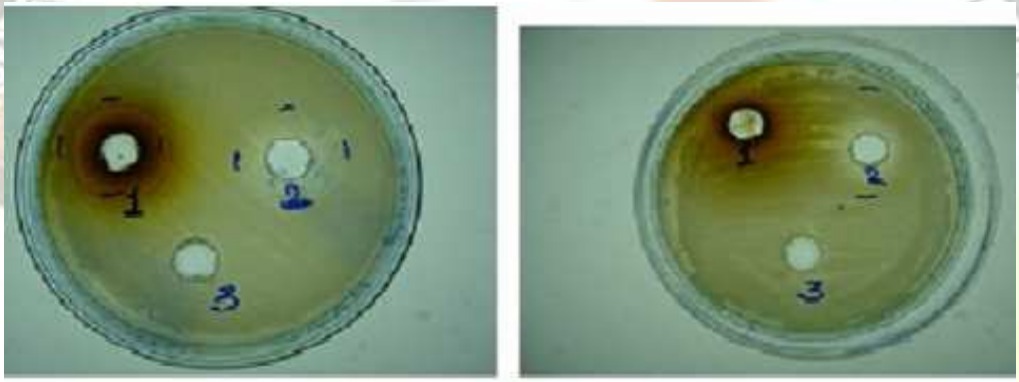
Table 1: Agar well diffusion method: Zone of Inhibition (ZOI)

Test organism	Hot Aqueous Extract		Methanolic Extract		Ethanolic Extract	
	Sample (In mm)	Tetracycline (In mm)	Sample (In mm)	Tetracycline (In mm)	Sample (In mm)	Tetracycline (In mm)
E. coli	21.5	24.5	22.5	25	23	20.5
P. aeruginosa	21	22	23.5	25	25.5	22.5

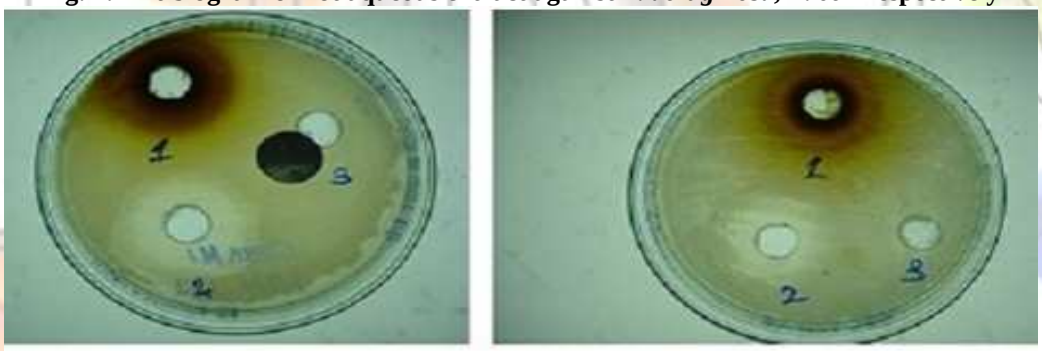
Table 2: Minimum Inhibitory Concentration (MIC)

Organism	MIC of Product µg/ml
E. coli	0.312
P. aeruginosa	0.421



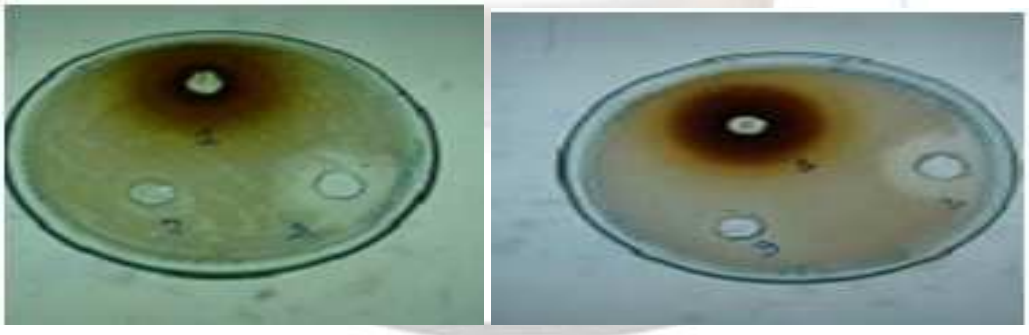
P. aeruginosa ***E. coli***

Fig. 1: Antibiogram of hot aqueous extract against *P. aeruginosa, E. coli* respectively



P. aeruginosa ***E. coli***

Fig. 2: Antibiogram of methanolic extract against *P. aeruginosa, E. coli* respectively



P. aeruginosa ***E. coli***

Fig. 3: Antibiogram of ethanolic extract against *P. aeruginosa, E. coli* respectively