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ANTIBACTERIAL POTENTIAL OF POMEGRANATE PEEL AND SEED EXTRACTS AGAINST FOOD BORNE PATHOGENS

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ABSTRACT

Aqueous extracts of pomegranate (*Punica granatum*) peel and seeds were investigated to evaluate their antibacterial activity against selected pathogenic microorganisms *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Enterococcus feacalis* using disc inhibition zone technique and the results were compared with commercial antibiotic. The studied extracts possessed strong antibacterial activity against the tested microorganisms. Maximum antibacterial activity was shown by the peel extract against *Escherichia coli* with average zone of inhibition 19.5 ± 0.93 mm followed by seeds 7.12 ± 0.99 mm. The antibacterial activities of peel extract were comparable with commercial antibiotic, Amoxicillin (21.12 ± 0.23 mm). *Enterococcus feacalis* was appeared to be the most resistant with inhibition induced by the extracts of peel 14.87 ± 0.13mm and seeds 7.12 ± 0.19 mm. The results revealed that the aqueous extracts of pomegranate peel and seed possessed significant antibacterial potential against food borne pathogens.
Keywords: Pomegranate fruit waste, extract, antibacterial activity

INTRODUCTION

Infectious illnesses is one of the most common sources of death throughout the globe. Food borne pathogens like *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Streptococcus feacalis* has implicated a number of health disorder (Uraih, 2004). Although, certain synthetic antibiotics are traditionally used for the curing these infections, but microbial pathogens became resistant to these synthetic antibiotics (Bax et al., 2000; Bhavnani and Ballow, 2000). Considering the negative impacts of regular use of antibiotics, there is a need of novel antibiotics which have minimum or no side effects and these types of antimicrobial can be obtain from natural source (Deepa et al., 2012). Medicinal plants are the root of conventional medicine systems that are functioning form thousands of years. Recently a vast variety of therapeutic plants has been the recent area of research because of their strong potential against pathogenic microbes (Martin and Ernst, 2003; Upadhyay et al., 2010).

Among these plants, pomegranate has noteworthy antimicrobial activity (Voravuthikunchai et al., 2004; cite more references here). Each parts of pomegranate fruit showed antimicrobial property. Various extarcts especially the methanolic extract of pomegranate peel has been stated for its significant antimicrobial activity against pathogens *Escherichia coli, Yersinia enterocolitica, Listeria monocytogenes,* and *Staphylococcus aureus* (Al-Zoreky 2009). The pomegranate extracts retard and slows the growth of *Staphylococcus aureus* and subsequent enterotoxin production at various concentrations (reference). The study under consideration was aimed to explore the antimicrobial potential of pomegranate waste against food borne pathogens.

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MATERIALS AND METHODS

Sample preparation

Pomegranate fruit were bought from a local market of Sargodha and were transported in plastic bags to the Food Microbiology Laboratory, Institute of Food science and Nutrition, University of Sargodha. The fruits were washed and then separation between peels and arils was carried out by hand. The peels and seeds (after juice extraction) were dried in an oven (BINDER) at 55°C for 3 days. Then these dried peels and seeds were crushed into powdered using a grinder (MOULINEX) and stored in plastic bags for of extraction purpose.

Extraction procedure

Weighed carefully five gram of each powdered samples (seeds or peels) were weighed on a

balance (SHIMADZU) and then transferred to measuring conical flask. Then 100 mL distilled water was poured in it and kept on hot plates (VELP SCIENTIFICA) for better extraction. To prevent the loss of essential components through evaporation, all flasks were covered with aluminium foil. After specific time period this mixture was filtered through muslin cloth. Further filtration was carried out through vacuum filtration assembly by using Whatman No. 1 filter paper. Then to evaporate and concentrate this supernatant rotary evaporator (HB DIGITAL, HEIDOLPH) was used with specifications 60°C temperature under vacuum. Then for the drying of extract hot air oven at 60°C was used until constant weight.

Microorganisms

The pathogenic microorganisms used for the study were *Enterococcus feacalis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. These cultures were preserved by sub-culturing on nutrient agar plate after 10 - 15 days.

Antimicrobial study

The antimicrobial potential of the pomegranate waste extracts was assessed by disc diffusion method against above mentioned pathogens according the suggestion of Sadeghian *et al*. (2011) with some modifications.

RESULTS AND DISCUSSION

Estimation of antimicrobial activity

Novel antimicrobial agents with more strength are compared with existing antimicrobial agents is required because of the increasing resistance of pathogens. Synthetic antimicrobial agents have serious side effects so that's why natural souses antimicrobial agents are becoming the most popular research center. In the current study, the resistance of food-borne pathogenic microbes which were assessed for their potential against aqueous extracts of pomegranate waste in comparison with standard antibiotic was showed graphically. The detail of the results is as under:

Pathogenic *Escherichia coli* is a Gram negative bacteria with respect to cell wall structure and food-borne microbe. Fig. 2 illustrated the inhibition done by the aqueous extracts of pomegranate and antibiotic. The aqueous extracts reduced the growth of *Escherichia coli* and the highest inhibition was done by peel (19.50mm). Lowest inhibition zone which was noted for the seed aqueous extract of pomegranate was of 7.13mm. In contrast with standard antibiotic, wider inhibitory potential (21.12mm zone of inhibition) was observed against all tested pathogens according the expected results. Reddy *et al*. (2011), also stated the same observations while conducting studies on antimicrobial potential of aqueous extract of pomegranate peel and antibiotic in comparison. The result of study verified the comparative inhibitory potential of aqueous extract of peel 22.5 mm and antibiotic was 26.5mm against *E. coli*.

The antimicrobial potential of extracts against *Pseudomonas aeruginosa* is shown in Fig. 2. Extract of peel induced highest inhibitory zone (15.88mm) as compared to seeds extract (6.13 mm) (Fig. 2). However, the activity of peel extract was comparable with the antibiotic (18.00 mm). These findings were correlated with the outcomes of Dahham *et al*. (2010) who confirmed that water extract of inner peel displayed effective activity in contrary to *Pseudomonas aeruginosa* with inhibitory zone of 20.00mm.Tetracycline showed inhibitory activity against *Pseudomonas aeruginosa* with inhibition zone of 22.00 mm (Khan et al., 2011).

Staphylococcus aureus is a Gram positive bacteria which is a causative agent of various common illnesses like skin and soft tissue infection, pneumonia and diabetic complications. Among all tested microbes, after *E. coli*, *Staphylococcus aureus* showed least resistance with inhibitory zone of 17.00mm (Fig. 7a) by peel extract. Dahham *et al*. (2010) also exposed that aqueous extract of inner peel showed significant antibacterial activity against *Staphylococcus aureus* having range of diameter 25mm. Week antimicrobial; activity (Fig. 7b) was shown against *Enterococcus feacalis* with range of 14.88mm. Duman *et al*. (2009) also reported the least activity of pomegranate extracts against *Enterococcus feacalis*.

The current study stated that extracts showed difference in their reaction against Gram-positive and Gram-negative microbes. Gram-positive microbiota has least resistance for antimicrobial agents as compared to Gram-negative microbe due to variances in the cell walls of their structures (Nikaido and Vaara, 1985). Present study showed different trend than general consideration or in other words

changed this concept. Although *Pseudomonas aeruginosa*, a Gram negative bacterium, showed highest strength as compared to all other microbes other Gram-negative bacterium, *Escherichia coli*, cannot behave likewise.

The antimicrobial activity of extracts of pomegranate are mostly linked to the availbilty of numerous biologically active components i.e flavonoides (Reddy et al., 2007). The well-intentioned effect of the pomegranate with orientation to antimicrobial activity in contrast many diseases that are allied to structure of polyphenolic components of pomegranate (Naz et al., 2007). KIM etal (2002) stated that *Punica granatum* could be utilized for the treatment of different types of diseases.

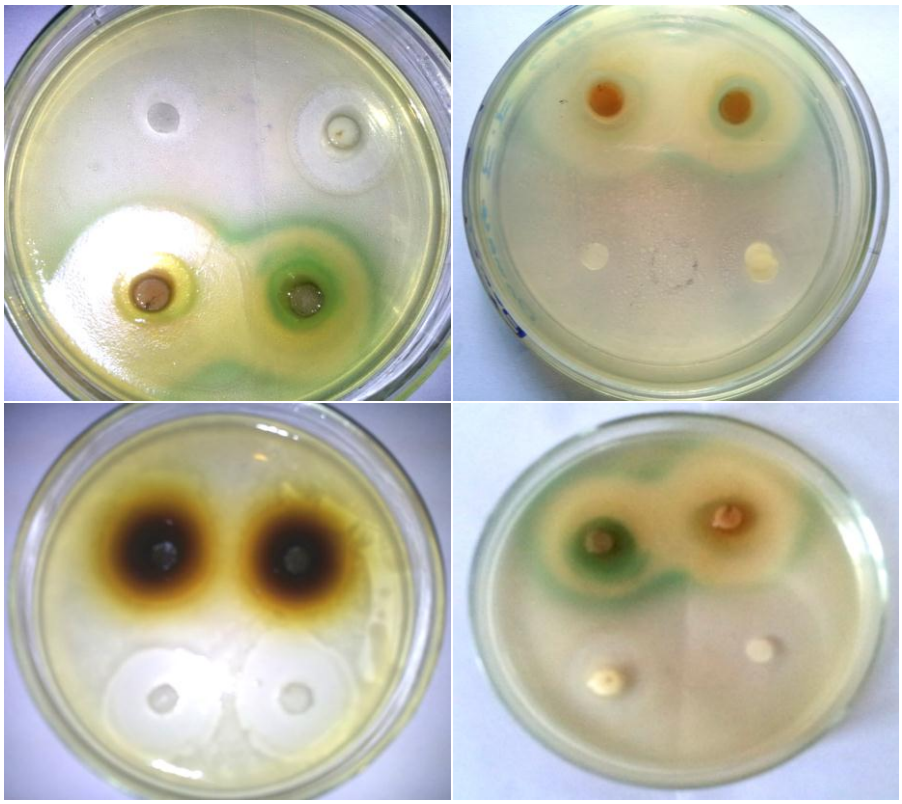

Fig. 1. Antibiogam of antimicrobial activity of peel and seeds extracts and antibiotics

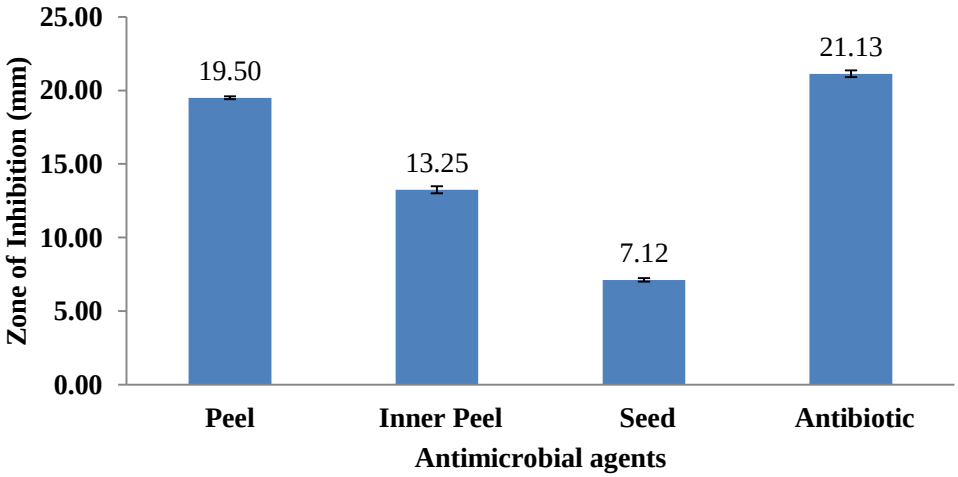

Fig. 2. Antimicrobial activity of pomegranate extracts against *Escherichia coli*

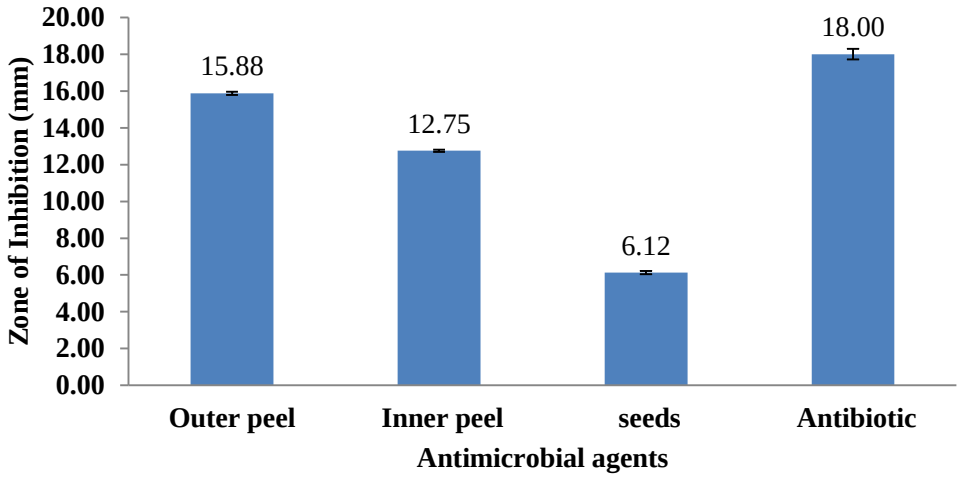

Fig. 3. Antimicrobial activity of pomegranate extracts against *Pseudomonas aeruginosa*.

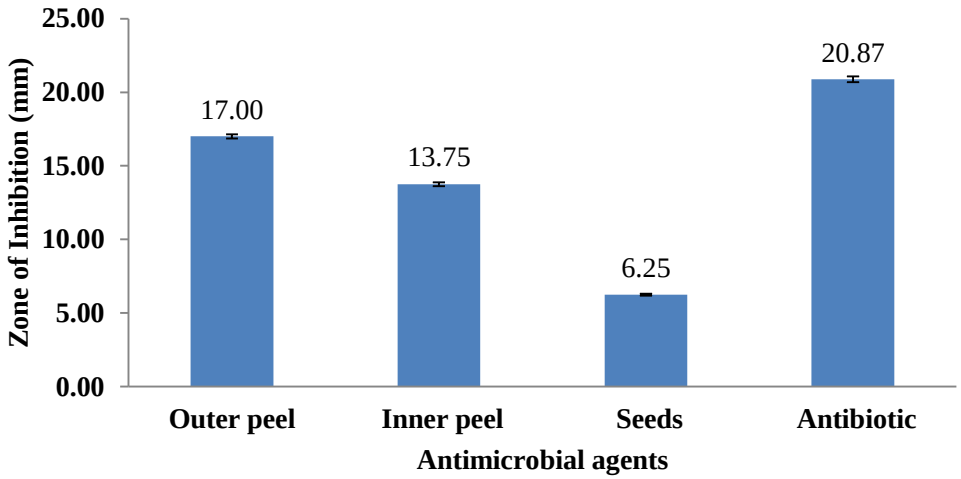

Fig. 4. Antimicrobial activity of pomegranate extracts against *Staphylococcus aureus*.

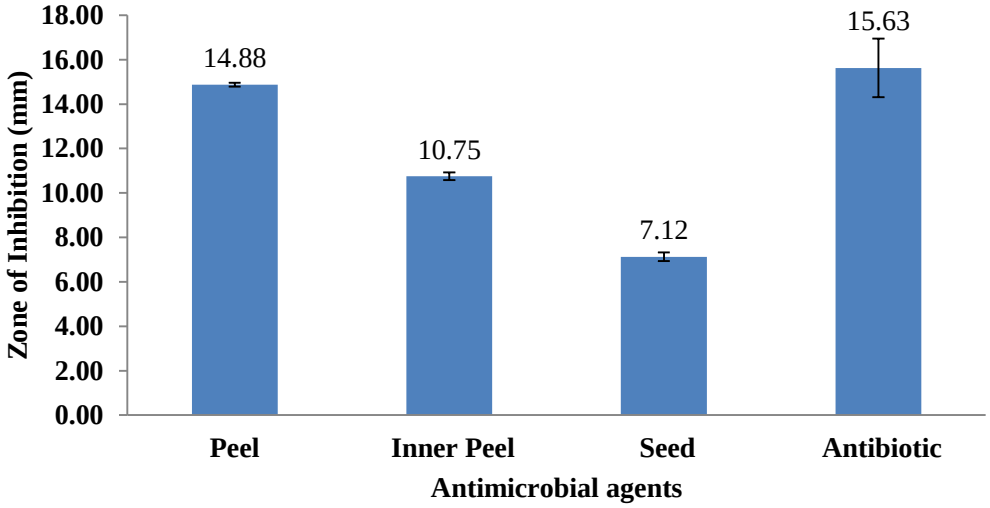

Fig. 5. Antimicrobial activity of pomegranate extracts against *Enterococcus faecalis*.

CONCLUSIONS

Pomegranate fruit is rich in compounds with notable therapeutic value and antimicrobial potential. Results showed that several water extracts of pomegranate have evidently exposed the wide spectrum of antimicrobial activity in contrast to food borne pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis and Pseudomonas aeruginosa*. Maximum activity was shown by Escherichia coli approximately diameter of 19.50mm. The antimicrobial results were comparable to the antibiotics present in the market. Add another take home message for readers.

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