The Pharma Innovation Journal 2018; 7(4): 176-178

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(4): 176-178
© 2018 TPI
www.thepharmajournal.com
Received: 20-02-2018
Accepted: 25-03-2018

Jag Pal
College of Fisheries NDUA&T Kumarganj Faizabad Uttar Pradesh, India

CV Raju
Department of Fish Processing Technology; College of Fisheries Mangaloure Karnataka, India

Gayatri Pandey
College of Fisheries CSAUA&T; Etawah Campus, Uttar Pradesh, India

Amitha
Department of Fish Processing Technology; College of Fisheries Mangaloure Karnataka, India

BN Shukla
College of Fisheries NDUA&T Kumarganj Faizabad Uttar Pradesh, India

Correspondence
Jag Pal
College of Fisheries NDUA&T Kumarganj Faizabad Uttar Pradesh, India

Antimicrobial activity of pomegranate and orange peels extracts against selected food borne pathogens

Jag Pal, CV Raju, Gayatri Pandey, Amitha and BN Shukla

Abstract
Antimicrobial compounds were extracted from pomegranate (PPE) and orange peels (OPE) using the aqueous ethanol. The *in vitro* antimicrobial activities of pomegranate and orange extracts were investigated by agar-disc diffusion methods against five food borne pathogens *Bacillus. subtilus, Staphylococcus aureus, Escherichia coli, Pseudomonas fluroscens* and *Salmonella typhi.* The concentration of PPE and OPE used was 10000 µg/mL. Both of the extracts were found to be the most effective antibacterial fraction against Gram-positive bacteria when compared to Gram-negative bacteria. Highest zone of inhibition (3.55mm) was measured in case of *B. subtilus* treated with OPE extract where as for the same species PPE showed the 1.55 mm diameters of inhibition. The OPE did not inhibit the growth of *S. aureus and E coli* where as the PPE showed the diameters of inhibition against all the tested food borne pathogens. The present investigation suggests that the PPE is good inhibiter of food borne pathogen and can be utilised as a natural antimicrobial agent for controlling the microbial activity.

Keywords: antimicrobial, pomegranate peel, *escherichia coli and* zone of inhibition

Introduction
The microbial activity is a major mode of deterioration of several foods and is often responsible for the loss of quality and safety. The concern pathogenic and spoilage microorganisms in foods are increasing due to the increase in outbreaks of food borne disease (Tauxe, 1997) [1]. At presently there is a growing interest to use natural antimicrobial compounds, like plant extracts of herbs and spices for the preservation of foods, as these possess a characteristic flavour and sometimes show antioxidant activity as well as antimicrobial activity. An antimicrobial is agents that kills microorganisms or inhibit their growth in food system or any other systems. Several food-preservation techniques have traditionally been utilized to control microorganisms in food. These preservation techniques include chilling, freezing, drying salting smoking fermentation, and non thermal physical treatments or the addition of synthetic antimicrobials and antioxidants (Davidson and Naidu 2001) [2]. Preservation methods that utilize synthetic preservatives had wide applications in the food industry. However, in recent years, increasing concerns over negative impacts on health issues associated with the use of synthetic preservatives were noticed (Sofos and Georgnaras, 2010) [3]. These issues have motivated the scientific community to investigate natural antimicrobial products as an alternative antimicrobial agent. Plants are rich in natural substances with antimicrobial properties, and act as antioxidants and flavor and color enhancing agents that can increase organoleptic acceptability, extend the shelf life of food and prevent the growth of food borne pathogens. The present investigation was under taken to assess the antimicrobial activity of pomegranate and orange peel.

Material and methods
Preparation of extracts
The pomegranate and orange peels were collected from different juice vendors in Mangalore city Karnataka India. The pulp was separated manually from the peel and washed to remove the unwanted materials. The washed peels were cut into small pieces and both peels were dried in hot air oven at 60 ºC for 12 h. The dried peels were ground in the kitchen grinder to make the fine powder to pass through 1 mm sieve. The extraction was carried out according to the methods described by Iqbal *et al*. (2008) [4] with slight modification. About 25 g of peel powder was mixed with 150 mL of ethanol. The mixture of peel powder and ethanol subjected to shaking at ambient temperature for 12 h at the speed of 190 rpm. The mixture was filtered and residue was re extracted with same solvent. The filtrates of the mixture were placed under

a hood in the rotavapor to remove the residual ethanol under vacuum at 40 ºC. The extract was obtained from both the peels powder were weighed to calculate the yield. The both the extracts were stored at -20 ºC in a sample container for further analysis.

Bacterial cultures

Bacterial cultures namely *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2688), *Bacillus subtilis* (NCIM 2063), *Salmonella typhi* (NCIM 2501) *and Pseudomonas fluroscens* (NCIM 2099) were procured from National chemical laboratory, Pune, India.

Testing of Antimicrobial activity

The antimicrobial activity of PPE and OPE were performed by using agar disc diffusion method as given by (Bauer *et al*., 1966; Nair and Chanda, 2005) [5, 6]. The concentration of the PPE and OPE extracts were used 8000-1000 ppm whereas, the concentration of the ampicillian was used as the 1 mg/L as the positive control. Tested bacterial strains were first grown on Muller Hinton agar (MHE) medium for 18 to 24 h at 37 °C. A sterile 10 mm-diameter filter disc impregnated with respective extracts and ampicillin were placed on the infusion agar seeded with bacteria. Then, Petri dishes were kept at 4 °C for 1 h and subsequently incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. All experiments were carried out in Triplicate. The antimicrobial activities were expressed as the mean diameter of inhibition zone produced.

Antimicrobial activity of the PPE and OPE

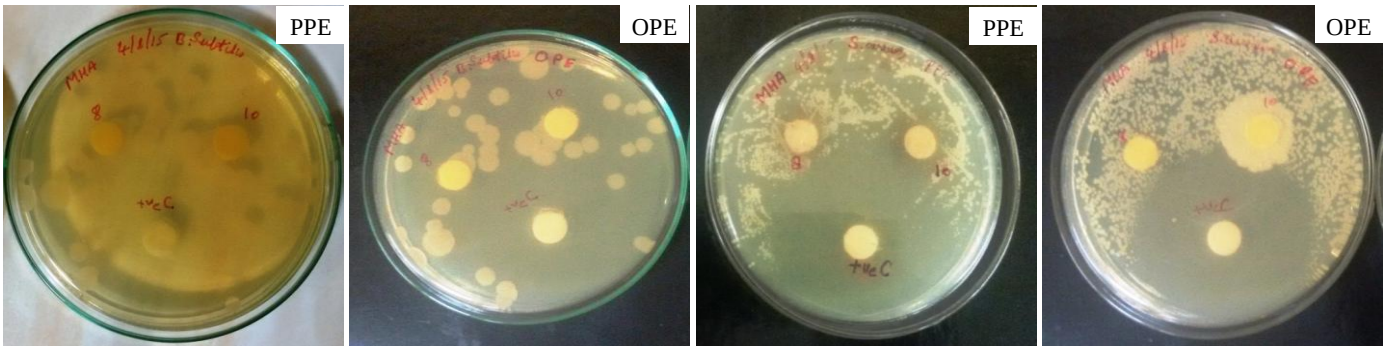
Antimicrobials are the substance that kills or inhibits the growth of micro-organisms such as bacteria, fungi and protozoans. The antimicrobial agents have long been researched for their effectiveness to inhibit growth of microorganisms in the foods. This has been done in an effort to increase food safety for the consumer, as well as to increase the shelf life of food products (Khan and Hanee, 2011) [7]. The antimicrobial activity of the PPE and OPE were tested for their sensitivity against five food borne pathogens namely *B. subtillus, S aureus, E coli P. flavine* and *S. typhi* by the disc diffusion method and the results are presented in the Table, 1 and fig. 1. The concentration of PPE and OPE used was 10000 µg/mL. Highest zone of inhibition (3.55mm) was measured in case of *B. subtilus* treated with OPE extract where as for the same species PPE showed the 1.55 mm diameters of inhibition. Both extracts PPE and OPE were found to be the most effective antibacterial fraction against Gram-positive bacteria when compared to Gram-negative bacteria. Similar kind of report was given by Oliveira *et al.* (2008) [8] and showed that most of the plant extracts are ineffective against Gram-negative organisms. The higher antimicrobial activity against gram positive bacteria was because the gram positive bacteria has less stable cell wall which allow the permeation of some antimicrobial agents. The lowest antimicrobial activity against gram negative bacteria was due to the presence of outer cell membrane of bacterium composed of phospholipids bilayer and proteins, avoids the permeation of antimicrobial agents inside the cell wall.

Table 1: Antimicrobial activity pomegranate and orange peel extracts

Species	Zone of inhibition (mm)		
	PPE	OPE	Ampicillin
B. subtilus	1.55±0.35	3.50±0.71	8.25±0.35
S. aureus	2.15±0.21	NI	7.40±0.14
E. coli	2.25±0.35	NI	9.50±0.71
P. fluroscens	1.90±0.14	1.25±0.35	5.50±1.41
S. typhi	1.25±0.35	1.05±0.78	4.15±0.92

Values are Mean ±SD n= 3, NI- Not inhabited

The OPE did not inhibit the growth of *S. aureus and E coli* where as the PPE showed the diameters of inhibition against all the tested food borne pathogens. However, in case of Gram-negative bacteria PPE and OPE were effective against *E coli, P. flavine* and *S. Typhi* but the diameter of inhibition zone was lower when compared to the Gram positive bacteria. Our results are also in support with the findings of Melendez and Capriles (2006) [9] who found that methanol extract of pomegranate fruit was active against *E. coli*, *S. aureus*, and *B. subtilis* with the diameter of inhibition zone of 12, 22, and 12 mm, respectively. The results of present investigation were quite lower than the results of previous study. According to Kanatt *et al.* (2009) [10] the pomegranate peel extract showed good antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas* and *E. coli*. The difference in inhibition zone of peel extracts against tested food borne pathogens may be due to the different extraction methods fallowed, freshness of fruits peel used and the variations in the season and region of growth (Mccarrell *et al.*, 2008)



A **B** **C** **D**

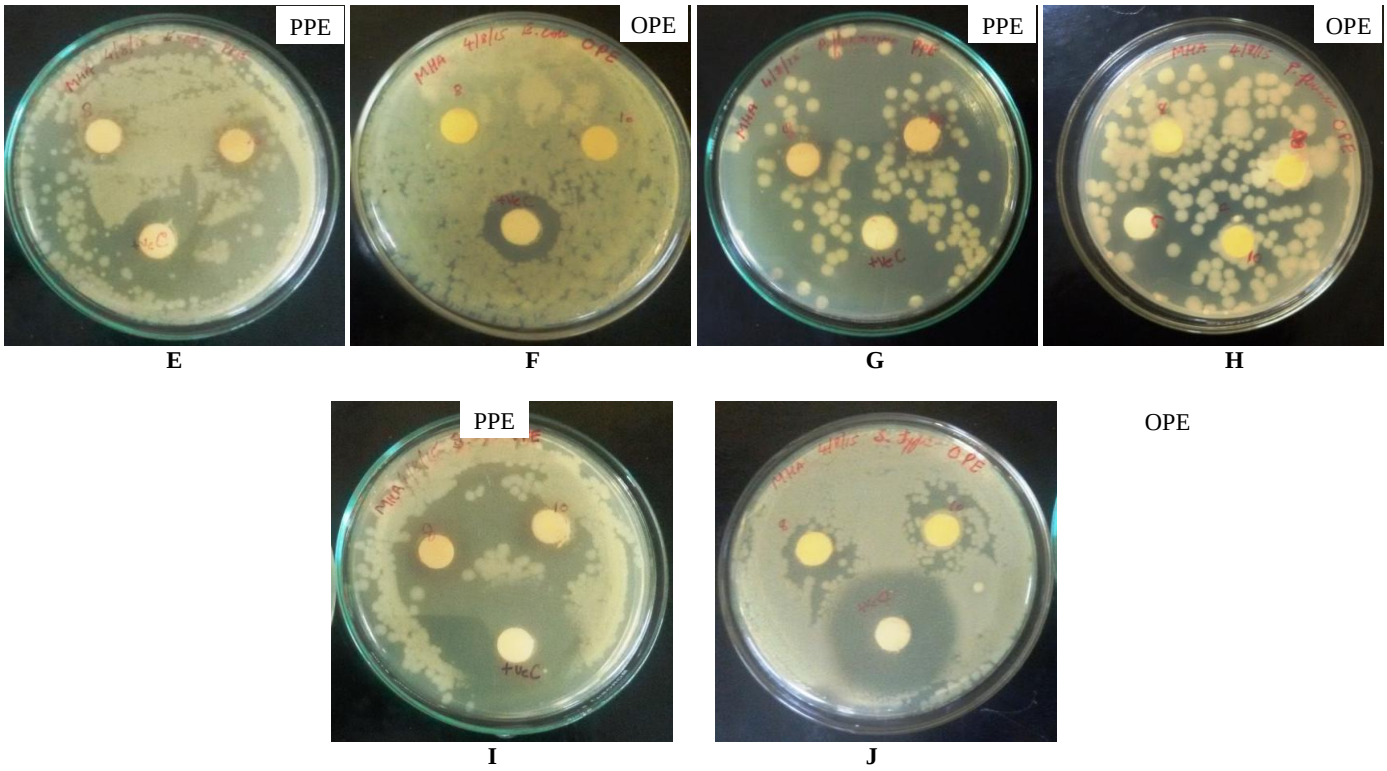


Fig 1: Diameter zone of inhibition (mm) of forborne pathogens **(A)** *B. subtilus* PPE **(B)** *B. subtilus* OPE **(C)** *S. aureus* PPE **(D)** *S. aureus* OPE **(E)** *E. coli* PPE **(F)** *E. coli* OPE **(G)** *P. fluroscens* PPE **(H)** *P. fluroscens* OPE **(I)** *S. typhi* PPE **(J)** *S. typhi* OPE

Conclusion

Natural antimicrobials compounds can improve shelf life of food products, and due to absence of synthetic agents, these compounds are safe without any side effects on human health. The results of present investigation revealed that the extracts of pomegranate and orange is the good source natural antimicrobials compounds and it could used as a antimicrobials agents in the food and food products to inhibit spoilage microorganism.

Acknowledgements

The authors wish to express their sincere thanks to All India Coordinated Research Project on Post-Harvest Engineering and Technology, CIPHET (ICAR) Ludhiana and Rajiv Gandhi National Fellowship by University Grant commission (UGC) New Delhi, India in carrying out the work successfully.

References

1. Tauxe RV. Emerging foodborne diseases: an evolving public health challenge. Dairy, Food Environmental Sanitation. 1997; 17:788-795.
2. Davidson PM, Naidu AS. Phyto-phenols. In: Naidu AS, editor. Natural Food Antimicrobial Systems. Boca Raton: CRC Press. 2000; 265-294.
3. Sofos JN, Geornaras I. Overview of current meat hygiene and safety risks and summary of recent studies on bio films, and control of Escherichia coli O157:H7 in nonintact, and *Listeria monocytogenes* in ready-to-eat, meat products. Meat Sci. 2010; 86(1):532-539.
4. Iqbal S, Haleem S, Akhtar M, Zia-ul-haq M, Akbar J. Efficiency of pomegranate peel extracts in stabilization of sunflower oil under accelerated conditions. Food Res. Int. 2008; 41:194-200.
5. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J Clin. Pathol. 1966; 45:493-496.
6. Nair R, Chanda S. Anticandidal activity of *Punica granatum* exhibited in different solvents. Pharm Biol. 2005; 43:21-25.
7. Khan JA, Hanee S. Antibacterial properties of *punica granatum* peels. Int. J App. Biol. Pharma. Tech. 2011; 2(3):23-27.
8. Oliveira I, Sousa A, Morais JS. Chemical composition and antioxidant and antimicrobial activities of three hazelnut (*Corylus avellana* L) cultivars. Food Chem. Toxicol. 2008; 46:1801-1807.
9. Melendez PA, Capriles VA. Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine. 2006; 13:272-279.
10. Kanatt SR, Chander R, Sharma A. Chitosan and mint mixture: A new preservative for meat and meat products. Food Chem. 2008; 107:845-852.
11. Mccarrell E, Gould S, Fielder M, Kelly A, El-sankary W, Naughton D. Antimicrobial activities of pomegranate rind extracts: enhancement by addition of metal salts and vitamin C. BMC Complem. Altern. Med. 2008; 8:64-70.