



ANTIBACTERIAL ACTIVITY OF FRUIT PEELS AGAINST BACTERIAL ISOLATES

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ABSTRACT

The present study was performed to isolate thirteen bacterial isolates from contaminated fruit and water. These bacterial isolates were morphologically and biochemically characterised. The fruit peels of various fruits *i.e.* pomegranate (*Punica granatum*), pineapple (*Ananas comosus*), lemon (*Citrus limon*), orange (*Citrus sinensis*) and sweet lime (*Citrus limetta*) were collected to carry out phytochemical analysis. Peel extract of pomegranate gave maximum result for the presence of phytochemicals *i.e.* flavonoids and tannins along with orange and sweet lime. An attempt was also made to evaluate the antibacterial assay for the aqueous extracts of fruit peels of pomegranate, pineapple, lemon, orange, sweet lime against the six bacterial isolates *i.e.* *Streptococcus lactis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter aerogenes* by agar well diffusion method. The maximum zones of inhibition were shown by *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. The present study concluded that fruit peels can be used as effective antibacterial agents.

KEYWORDS: Phytochemical analysis, Antibacterial activity, Zone of inhibition

INTRODUCTION

India is the largest producer of medicinal herbs and is also called the botanical garden of world. A plant extracts having a biological origin, is used for medicinal purposes, and demonstrates a positive impact on human health and environment (Bravo *et al.*, 2000). In modern times, plants have been a source of analgesics and anti-inflammatory drugs and medicines for asthma, anti-arrhythmic agents and anti-hypertensive (Gupta *et al.*, 2017). These natural occurring substances have great potential for producing new drug. The use of antimicrobial substances is gaining more importance these days and has higher significant values in plant chemotherapy (Reyes-Munguía *et al.*, 2016). Fruits are an integral part of diet which can be consumed fresh, as juices, concentrates, jams or fruit based drinks. In the juice processing industries, peels and seeds are generally wasted. Peels waste are highly degradable and is a problem to various fruit processing industries and pollution monitoring agencies. The fruits and vegetables solid wastes contain mainly soluble sugar and other hydrolysable materials and fibre. Disposal of such wastes may increase the cost to processors and their direct disposal to soil or landfill may cause serious environmental problems. The recovery of by products from fruit wastes can improve the overall economics of processing units and will also reduce the problem of environmental pollution. Besides reducing environmental pollution, these by products are attractive source of dietary fibres and antioxidants such as vitamins, phenols and carotenoids. Also, due to increase in use of chemotherapeutics and resistance of various pathogenic microbial infectious agents against various antibiotics has led to the screening of several medicinal plants for potential antimicrobial activity. Plant based antimicrobial agents are attractive as they are often devoid of many side effects. The major bioactive compounds known for health

benefits are phytochemicals, especially phenolics in fruits and vegetables.

Various fruit peels like that of pomegranate (*Punica granatum*), pineapple (*Ananas comosus*), lemon (*Citrus limon*), orange (*Citrus sinensis*), sweet lime (*Citrus limetta*) exert various phytochemical and antimicrobial properties which can be used for the benefits of human health. Pomegranate is a potential source of antioxidants, phenols, flavonoids, alkaloids and organic acids. It is a native shrub from Western Asia and Mediterranean Europe that has a rich history of traditional use in medicine (Reguieg *et al.*, 2017). Not only the fruit, the flower, seed oil, seed extract but also peel extract of pomegranate have potent antioxidant activity. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruits and seeds are also investigated by Bhandary *et al.*, 2012. The peels (pericarp, rind or hull) amounts to approximately 60 % of the weight of the pomegranate fruit (Lansky and Newman 2007). Pineapple belongs to the family Bromeliaceae which is grown in several tropical and subtropical countries including Philippines, Thailand, Indonesia, Malaysia, Kenya, India, China and South America. Pineapple wastes are recommended as tremendous sources of organic raw materials and are potentially available for conversion into useful products through solid waste bioprocessing (Rashad *et al.*, 2015). Citrus fruits (lemon, orange, sweet lime) belong to the family Rutaceae are source of wide range of nutrients such as vitamin C, carotenoids, limonoids, minerals, essential oil, folic acid, alkaloids, potassium, flavonoids and dietary fibres that help to prevent the development of several diseases. The orange peel extract has many medicinal properties such as it maintains cholesterol level, reduces stomach acid, relieves digestive problems, and also prevents respiratory diseases. High content of vitamin C present in citrus fruits reduces the presence of spots caused

by premature aging. It is also used to treat and prevent vitamin deficiencies, colds, flu and scurvy and helping to fight viral and bacterial infections (Shetty *et al.*, 2016). The presence of phytoconstituents present in the plants has a potential of providing useful drugs for benefits of human welfare (Bhandary *et al.*, 2012). Major groups of compounds that are responsible for antimicrobial activity from plants include phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids (Ciocan & Bara, 2007). In another study, Lai & Ray (2004) demonstrated that some commonly used herbs and spices possess antimicrobial properties and also contain various phytochemicals.

The aim of the present study was to compare the antimicrobial activities of pomegranate, pineapple, lemon, orange and sweet lime against food and water borne pathogens and to study their potential as food bio-preservatives. The present study is also conducted to check the presence of various phytochemicals like tannins, alkaloids, flavonoids, terpenoids, cardiac glycosides and reducing sugars in the aqueous and ethanolic extracts of fruit peels.

MATERIALS AND METHODS

Isolation of bacterial isolates

In the present study, the bacterial isolates were isolated from pond water, rotten apple and sewage water by the serial dilution method. These bacterial isolates were morphologically and biochemically characterized by Gram's staining and various biochemical tests. Peels of various fruits like pomegranate, pineapple, lemon, orange and sweet lime were collected from a juice shop situated in local market, Chandigarh.

Preparation of aqueous, ethanolic and methanolic extracts

Collected peels were washed with 95% ethyl alcohol and then were cut into small pieces. These peels were kept in hot air oven for 3-4 days so that it could get dried. Dried peels were then grinded with the help of mixer grinder. The powdered peels were dissolved in polar (water) as well as non-polar (methanol and ethanol) solvents for the preparation of aqueous and ethanolic extracts respectively. After dissolving the extracts in water and ethanol, these were left on shaker at 90rpm for 24 hours at 37°C. After 24 hours, the extracts were then filtered using Whatman paper. The filtered solution was then stored at low temperature (4°C) in air tight container to avoid contamination.

Test for phytochemical analysis: (Ehiowemwenguan *et al.*, 2014)

The following tests were performed for phytochemical analysis.

Tannins: 1ml of the extract was treated with few drops of 0.1% ferric chloride and observed for brownish green and a blue black coloration.

Flavonoids: To 1ml extract 3 drops of ammonia solution (NH₃⁺) was added followed by 0.5ml of concentrated HCl acid. The resultant pale brown coloration of the entire mixture was observed.

Terpenoids: 0.5ml extract was treated with 2ml of chloroform and then 3ml H₂SO₄ was added, the yellow colour ring formation was observed.

Reducing sugar: 1ml of extract was treated with 1ml of Fehling's solution A & B. The mixture was shaken and

then heated in water bath for 10 minutes. The resultant brick red precipitates were observed.

Alkaloids: 1ml of extract was mixed with 1ml of Marqui's reagent [3ml conc. H₂SO₄ + 2 drops of 40% formaldehyde]. The dark orange or purple coloration appeared.

Cardiac glycosides: 5ml of extract was treated with 2ml of glacial acetic acid to which few drops of ferric chloride solution were added and then 1ml of conc. sulphuric acid. The brown ring was formed at interface, the violet ring below brown and the greenish ring in acetic acid layer.

Antibacterial Activity Assay (Girish and Satish, 2008):

The antibacterial activity of different extracts of fruit peels were assayed using agar well diffusion assay. In this procedure, nutrient agar media was prepared and autoclaved at 15 psi at 121°C for 15 minutes. Media was then poured into the petri plates and left undisturbed till solidified. Now put 50 µl of inoculum onto the agar medium and using sterile bent spreader, spread the inoculum onto the plates. The plates were left undisturbed for about half an hour. Then wells were prepared in the nutrient agar medium by using sterile tip or well puncher. Add 40 µl (30 µl extract and 70µl sterile water) of peel extract in the wells. The plates were kept in the incubator at 37 °C. After 24 hours, results for zone of inhibition were observed.

RESULTS AND DISCUSSION

Morphological Characterization

Various bacterial colonies were found from different sources like pond water and spoiled fruit by serial dilution technique. These bacterial isolates were allowed to grow on nutrient media and observed for their morphological characteristics. Different microorganisms showed different types of growth, arrangement, elevation, density etc. Different colors of colonies were also observed like white, yellow, rose pink and green on nutrient agar and Eosin Methylene Blue agar and maintained as pure culture in nutrient broth and nutrient agar at 4°C. Total thirteen bacterial isolates, out of which seven Gram positive and six Gram negative bacteria were observed. On the basis of Gram-staining and various biochemical tests, isolated bacterial isolates were morphologically and biochemically characterized (Table 1). These were identified as *Streptococcus lactis*, *Bacillus* sp., *Bacillus cereus*, *Escherichia coli*, *Bacillus* sp., *Bacillus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterobacter* sp., *Enterobacter* sp., *Escherichia coli*, *Enterobacter aerogenes*.

Phytochemical Analysis

The phytochemical analysis carried out with aqueous extracts of different fruit peels (orange, sweet lime, lemon, pineapple, and pomegranate) showed the presence of tannins, alkaloids, flavonoids, terpenoids, cardiac glycosides and reducing sugars. The abundance of these is different in different fruit peel extracts and the detection involves using chemicals and reagents and observing change in colour, ring formation *etc.* Table 2 showed phytochemical analysis of different fruit peels. The aqueous extract of pomegranate gave the best result for the presence of tannins as compared to orange and pineapple whereas citrus fruits extract of sweet lime and lemon gave negative result for presence of tannins. The extract tested for the presence of alkaloids reveals more amounts in sweet lime and pomegranate than other peels studied.

Flavonoids were present in good amount in extract of orange and pomegranate peels but showed negative results for all others. Terpenoids were mainly present in sweet lime in more amount than others. Among different phytochemicals it was observed that tannins had highest percentage ratio i.e. 30.07% (Mishra *et al.*, 2012). All the extracts showed average presence of cardiac glycosides, but pomegranate and orange had more amount of reducing sugars. Pineapple mainly showed negative results for presence of different phytochemicals. Aqueous extract of pomegranate and orange showed maximum flavonoids as compared to other fruit peel extracts. (Khan *et al.*, 2011) suggested that the tannin and phenolic acids of *Punica granatum* have antibacterial activity. *P. granatum* contains

large amount of tannins (25%) and antibacterial activity may be indicative of presence of secondary metabolites. The ethanolic extracts of different fruit peels were also tested for the presence of different phytochemicals. Ethanolic extracts of sweet lime and pomegranate gave best results for the presence of tannins followed by pineapple whereas orange have more amount of alkaloids as compared to terpenoids and reducing sugar. Alkaloids are present in average amount in sweet lime. Cardiac glycosides are only present in pomegranate extract and in minute amount in pineapple, whereas it is absent in others, but flavonoids were absent in pomegranate, pineapple and lemon but present in orange and sweet lime. Reducing sugars are present in all extracts but in nominal amount.

TABLE 1: Biochemical characterization of bacterial isolates

Organism	Amylase Prod.	H ₂ S	IMVIC				U	Catalase	Caesin Hydro.	Gelatin Liq
			IP	MR	VP	C				
<i>Streptococcus lactis</i>	+	-	-	+	-	-	-	-	+	-
<i>Bacillus sp.</i>	-	-	-	+	+	+	-	+	-	-
<i>Bacillus Cereus</i>	+	-	-	+	+	-	-	+	-	+
<i>Escherichia coli</i>	+	-	-	+	-	-	-	-	-	-
<i>Bacillus sp.</i>	+	+	+	+	-	+	-	+	-	-
<i>Bacillus sp.</i>	+	-	-	+	-	-	-	-	+	-
<i>Pseudomonas aeruginosa</i>	+	+	-	-	-	-	-	+	-	+
<i>Staphylococcus aureus</i>	+	+	-	+	-	-	+	+	+	+
<i>Bacillus Cereus</i>	+	-	-	-	+	-	-	+	+	+
<i>Enterobacter sp.</i>	-	+	-	-	+	-	-	+	+	-
<i>Enterobacter sp.</i>	-	-	-	-	+	-	-	-	+	-
<i>Escherichia coli</i>	+	-	+	+	-	-	-	-	-	-
<i>Enterobacter aerogenes</i>	-	-	-	-	+	+	-	+	+	-

IP = indole production, MR= methyl-red, VP= voges-proskauer, C= citrate test U= urease test, Prod. = production, Hydro=hydrolysis, Liq. = liquefaction

TABLE 2: Phytochemical analysis of different aqueous and ethanolic extract of different fruit peels

Test	Reagents used	Observation	Pomegranate		Pineapple		Lemon		Orange		Sweet lime	
			Aq.	Et.	Aq.	Et.	Aq.	Et.	Aq.	Et.	Aq.	Et.
Tannins	0.1% ferric chloride	Brownish green or blue black color	5+	4+	+	3+	-	2+	3+	2+	-	5+
Alkaloids	Marqui's reagent	Orange or purple color	4+	2+	-	-	2+	-	3+	5+	5+	4+
Cardiac glycosides	Glacial acetic acid and FeCl ₃	Brownish green ring	3+	4+	-	+	-	-	4+	-	2+	-
Terpenoids	Chloroform and H ₂ SO ₄	Yellow color ring	3+	3+	2+	+	4+	3+	2+	2+	5+	2+
Reducing sugar	Fehling's solution A and B	Brick red ppt	4+	3+	2+	3+	-	+	3+	2+	2+	3+
Flavonoids	Ammonia solution + conc. HCl	Pale brown color	4+	-	-	-	-	-	5+	3+	-	2+

Aq: Aqueous extract

Et: Ethanolic extract

+: sign indicates the positive test for that compound

-: sign indicates the negative test for the compound.

Antibacterial Assay

Out of the thirteen bacterial isolates, the six bacterial isolates (*Streptococcus lactis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter aerogenes*) were selected for antibacterial assay. Figure 1 showed antibacterial activity of different fruit peels extracts against the bacterial isolates. One-way ANOVA was performed for different microorganisms against different fruit peel extracts. P < 0.05 gave significant result and P > 0.05 gave non-significant results.

The maximum zone of inhibition was given by *Bacillus cereus* (20mm) followed by *Enterobacter aerogenes* (17.33mm) > *Escherichia coli* (15.67mm) > *Pseudomonas aeruginosa* (15.3mm) > *Staphylococcus aureus* (15mm) > *Streptococcus lactis* (14mm) for aqueous extract of pomegranate. Statistically, *Streptococcus lactis*, *Bacillus cereus* (P=0.0007) and *Pseudomonas aeruginosa* (P=0.0055) gave significant results for pomegranate extract. The results for aqueous pomegranate extracts showed high antibacterial effect on *E. coli* followed by *P.*

aeruginosa and less effect was recorded on *K. pneumonia* (Dahham *et al.*, 2010).

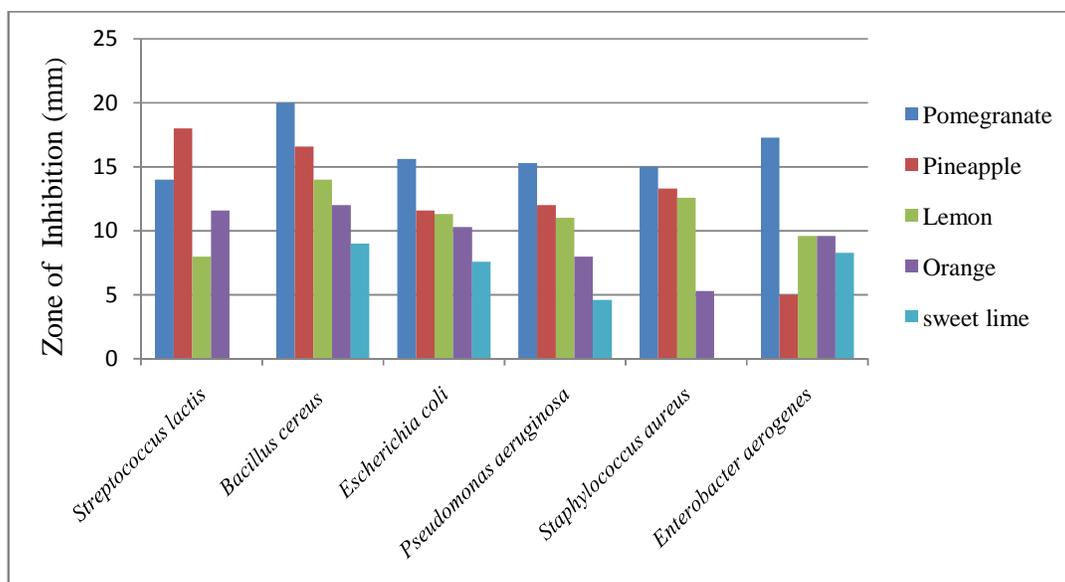


FIGURE 1: Graphical representation for zone of inhibition in (mm) of fruit peels extract against different microorganism.

For pineapple extract, maximum zone of inhibition was observed by *streptococcus lactis* (18mm) > *Bacillus cereus* (16.67mm) > *Staphylococcus aureus* (13.33mm) > *Pseudomonas aeruginosa* (12mm) > *Escherichia coli* (11.67 mm) > *Enterobacter aerogenes* (5mm). Statistically, *Streptococcus lactis*, *Escherichia coli*, *Pseudomonas aeruginosa* (P=0.0001) and *Staphylococcus aureus* (P=0.0010) gave significant results. In another study, it was reported that pineapple extract was more effective against *E. coli* with a zone of inhibition of 26 mm diameter (at conc. 1000 µg/ml.) and with *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* it was found to be 20mm, 22mm and 23mm respectively (Ahamed *et al.*, 2016). The maximum zone of inhibition was given by *Bacillus cereus* (14 mm) > *Staphylococcus aureus* (12.67mm) > *Escherichia coli* (11.33mm) > *Pseudomonas aeruginosa* (11mm) > *Enterobacter aerogenes* (9.6 mm) > *Streptococcus lactis* in case of lemon extract (8mm). Statistically, *Streptococcus lactis* and *Staphylococcus aureus* (P=0.0001) and *Enterobacter aerogenes* (0.0020) gave significant results. *Citrus sinensis* peel extracts has appropriate antimicrobial effects on gram positive and gram negative bacteria. Nisha *et al.* (2013) studied that the essential oils from *C. sinensis* peels showed antibacterial activity against *S. aureus*, *S. typhimurium*, *Enterobacter aerogenes*, *B. subtilis* and *E. coli*. For orange peel extract *Bacillus cereus* (12mm) > *Streptococcus lactis* (11.67mm) > *Escherichia coli* (10.33mm) > *Enterobacter aerogenes* (9.67mm) > *Pseudomonas aeruginosa* (8mm) and *Staphylococcus aureus* (5.5mm) gave maximum zone of inhibition. Statistically, *Bacillus cereus* and *Staphylococcus aureus* (P= 0.0001) gave significant results whereas other gave non-significant results. Extract of orange peel exhibited antibacterial against *E. coli* 10-16mm, *K. pneumoniae* 11-14mm, *P. aeruginosa* 9-21mm, *S. typhi* 9-18mm, *S. paratyphi*- A 11-18mm, *S. paratyphi*- B 9-16mm, *S. flexneri* and *V. cholerae* 9-19mm. (Nisha *et al.*, 2013). For sweet lime maximum zone of inhibition

was given by *Bacillus cereus* (9mm) > *Escherichia coli* > (7.67mm) *Enterobacter aerogenes* (8.33mm) > *Pseudomonas aeruginosa* (4.67mm). Statistically, *Bacillus cereus* and *Pseudomonas aeruginosa* (P=0.0032) gave significant results. Best results of screening were found in *P. aeruginosa* 13mm in comparison with *S. aureus* and *E. coli* (Mishra *et al.*, 2012). The citrus fruit peel extract used *Citrus sinensis* (orange), *Citrus limon* (lemon), and *Citrus limetta* (sweet lime) against the common gastrointestinal pathogens showed high antimicrobial activity (Nisha *et al.*, 2013). Natural substances have demonstrated antibacterial action mainly because most plants used in alternative medicine are composed of flavonoids, which act on bacterial cells disrupting the cytoplasmic membrane and inhibiting the enzymatic activity (Ahamed *et al.*, 2016).

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