

Antibacterial Potentiality and Phytochemical Exploration of Indian Seasonal Fruitlets Epicarp



Microbiology

KEYWORDS : Epicarp, MIC, Antibacterial activity, Phytoconstituents

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ABSTRACT

Fruits are utilized by local people as the part of their meal. Peoples supposed to consume fruits and discard their seed and peel as waste. The present study was carried out to enhance the utilization of the waste products from the food processing industries. Fruit peels are the major by product of food processing industry. The fruit samples studied in our work were Punica granatum (Pomegranate), Citrus sinensis (Sweet Orange) and Musa balbisiana (Banana). The fruit peels were collected from Thanjavur district of Tamilnadu. The zone of inhibition is being studied for determination of antibacterial property of the fruit peel extracts and phytochemical analysis. In the present study the dried fruitlets epicarp was subjected for successive solvent extraction by using different solvents like ethanol and water. MICs were tested at concentrations ranging from 0.08-1.28 mg/ml. This study was concluded that the ethanol extract shows more significant activity then water extract.

INTRODUCTION

Many of the plants used today were known to the people of ancient cultures throughout the world for their preservative and medicinal power scientific experiments on the antibacterial properties of fruits and their components have been documented in the form of crude extract, infusions or plaster of treat common infection without scientific basis for the traditional use of these medicinal fruit [1]. Pomegranate is currently cultivated in variety of countries, including Spain, United States, Iran, Turkey, India, Israel, China and countries along the north coast of Africa, among others. The pomegranate used as the most famous usage worldwide has been as a Vermifungal or Taenicidal agent, i.e., a killer and expeller of intestinal worms. The alkaloids contained in the root, tree bark and to lesser extent, fruit rind , cause the "Tapeworm" to relax its grip on the weakened parasites to be easily expelled by a second herbal drug, one which is cathartic [2,3,4].

Citrus sinensis, orange or sweet orange is a small tree in the Rutaceae (citrus family) that originated in southern China, where it has been cultivated for millennia. Oranges are now grown commercially worldwide in tropical, semi-tropical, and some warm temperate regions, and have become the most widely planted fruit tree in the world. Oranges are the world's most popular fruit and are eaten fresh, used for juice. The total global commercial production of oranges in 2010 was 69.4 million metric tons (Mt), harvested from 4.1 million hectares. Many beauty products contain orange peel as an ingredient. Orange peels helps naturally cleaning the face along with helping in preventing pimples and acne. They have beneficial antioxidants that help in preventing your skin from premature aging. Thus, an orange peel face mask helps in providing a natural glow and radiance on skin. Addition of dry powder of orange peels to bathing water makes the skin to glow naturally.

In some countries, bananas used for cooking may be called plantains. The fruit is variable in size, color and firmness, but is usually elongated and curved, with soft flesh rich in starch covered with a rind which may be green, yellow, red, purple, or brown when ripe. The fruits grow in clusters hanging from the top of the plant. Almost all modern edible seedless bananas come from two wild species *Musa balbisiana*, *Musa paradisiaca* and *Musa acuminata* depending on their genomic constitution [5,6,7]. The flowers of banana cure bronchitis, dysentery and on ulcer; cooked flowers are given to diabetics. Young leaves are placed as partices on burns and other skin afflictions. Some of the specific diseases known to be cured by banana such as Anaemia, high in iron, banana are believed to stimulate the production of hae-

moglobin in the blood and so helps in cases of anaemia. Blood pressure, banana is extremely high in potassium yet low in salt, making it the perfect food for helping to beat blood pressure [8].

MATERIALS AND METHODS

The ethanolic extracts of the showed potential antibacterial property against *Staphylococcus aureus*. Flavonoids and Tannins found in the extract are responsible for the activity. The plants used in this study were *citrus sinensis* (sweet orange), *punica granatum* (pomegranate) and *Musa balbisiana* (banana). The peels were collected from the local fruit juice shops. After collection, the peels were shade dried at room temperature (32-35°C) to constant weight over a period of 5 days. 15g of each of the plant parts were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers.

Antibacterial activity of peels extracts were investigated on four human pathogens bacterial strains used in this study includes *Klebsiella*, *Pseudomonas*, *Staphylococcus* and *Escherichia coli*. The microorganisms were obtained from the Vaishnavi Laboratory, Kumbakonam, Thanjavur District, Tamilnadu.

Muller-Hinton Agar was prepared and sterilized by autoclaving at 121°C for 15 minutes and mixed well before pouring. The Nutrient broth was prepared and sterilized by autoclaving at 121°C for 15 minutes.

15g of the powdered plants such as pomegranate peel, sweet orange peel and banana peel were soaked separately in 10ml of distilled water at ambient temperature for 24 hour under shaking condition at 130 rpm. The peels extracts was then filtered using Whatmann filter paper No1. Each peels extracts were transferred to glass vials and kept at 4°C before use.

The dried and powdered peel (pomegranate and sweet orange) materials (15g) were extracted successively with 200ml of ethanol separately by using soxlet extractor. 90g of the powdered peel was dispensed in 900ml of ethanol in a 1L capacity conical flask. The mixture was stirred vigorously intermittently with a magnetic stirrer and then allowed to stand for 48 hours. It was stirred again and filtered through Whatmann filter paper lined funnel into a conical flask. The filtrate was evaporated with a rotary evaporator. All extracts obtained were stored in a refrigerator until required for use.

For antibacterial activity of extracts determination about 0.1% standardized inoculum suspension was swabbed uniformly in

Muller-Hinton Agar. Antibacterial activity at the extracts was tested using the disc diffusion method. Paper disc (6mm) were soaked in 20µl of each extracts diluted in 25% DMSO and dried at 37°C overnight. The loaded disc was placed on the surface of medium, the compound was allowed to diffuse and the plates were kept for incubation at 37°C for 24 hours.

The following concentrations were obtained: 0.08g-1.28g. Each volume of extract and Muller-Hinton broth (2ml) was dispensed into sterilized test tubes. A quantity (0.1ml) of standardized inoculum (1.25×10⁷cfu/ml) was added to each of the test tubes which were incubated aerobically at 37°C for each 24 hours. The lowest concentration of the extracts which inhibited microbial growth (no turbidity) was recorded as the minimum inhibitory concentration (MIC).

PHYTOCHEMICAL ANALYSIS

Phytochemical examinations were carried out for all the extracts as per the Standard methods.

TEST FOR ALKALOIDS:

To 3ml of the extract was added 1ml of 1% HCL. This resulting mixture was then treated with few drops of Mayer’s reagent. The appearance of a creamy white precipitate confirmed the presence of alkaloids [9].

TEST FOR SAPONINS:

Fruits peel extracts were diluted with distilled water to 2ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

TEST FOR TANNINS:

To the fruits peel extracts, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

TEST FOR FLAVONOIDS:

Fruits peel extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

TEST FOR STEROIDS:

About 0.5g of extract was dissolved in 3 ml of chloroform and filtered. Concentrated sulphuric acid was added to the filtrate to form a lower layer. Reddish brown color develop was considered as positive results for the presence of steroids ring.

TEST FOR RESINS:

To 5ml of the extract was added 5ml of copper acetate solution. The mixture was shaken vigorously and allowed to separate. The appearance of a reddish-brown precipitate indicated the presence of resins [10].

TEST FOR PHYTOSTEROL:

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drop of diluted acetic acid; 3 ml of acetic anhydride was added followed by few drops of Conc.H₂SO₄. Appearance of bluish green color showed the presence of phytosterol.

TEST FOR REDUCING SUGAR:

The residue was re-dissolved in water on the water bath. To 2ml of the solution, in the test tube was added, 1ml each of Fehling’s solutions A and B. The mixture was shaken and heated in a water bath for 10minutes. The color obtained was recorded. A brick- red color indicates reducing sugar.

TEST FOR FREE AMINOACIDS:

Few drops of Ninhydrin reagent was added to 1 ml of extract. The development of purple colour shows the presence of amino acids.

TEST FOR TERPENOIDS:

About 0.5g of extract was dissolved in 3 ml of chloroform and filtered. Concentrated sulphuric acid was added to the filtrate to form a lower layer. Reddish brown color develop was considered as positive results for the presence of steroids ring.

RESULTS:

The results of the antibacterial activity of aqueous and ethanol extracts of various fruit peel such as Pomegranate, Sweet orange and Banana against the human pathogens are shown in **table 1**. In ethanol extracts of pomegranate peel the highest zone of clearance was obtained against *Staphylococcus aureus* (25mm) and the lowest zone of clearance was obtained against *Klebsiella pneumonia* (19mm). In ethanol extracts of Sweet orange peel the highest zone of clearance was found against *Staphylococcus aureus* (11mm) and the lowest zone of clearance was obtained against *Klebsiella pneumonia* (7mm). In ethanol extracts of banana peel the highest zone of clearance was obtained against *Escherichia coli* (17mm) and the lowest zone of clearance was obtained against *Klebsiella pneumoniae* (15mm).

In aqueous extracts of pomegranate peel the highest zone of clearance was attained against *Staphylococcus aureus* (20mm) and the lowest zone of clearance was acquired against *Escherichia coli* and *Klebsiella pneumoniae* (17mm). In aqueous extracts of sweet orange peel the highest zone of clearance was obtained against *Klebsiella pneumoniae* (22mm) and the lowest zone of clearance was obtained against *Escherichia coli* (11mm). In aqueous extracts of banana peel the highest zone of clearance was obtained against *Staphylococcus aureus* (20mm) and the lowest zone of clearance was obtained against *Klebsiella pneumoniae* (12mm). **Figure1&2** illustrated the zone of inhibition of the various fruits peel from the aqueous and ethanol extracts.

Table 1 Antibacterial activity of various fruitlets epicarp from different solvent extraction

Organisms	Zone of inhibition (mm)					
	Pomegranate peel Extracts		Sweet orange peel Extracts		Banana peel Extracts	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
<i>E. coli</i>	17mm	18mm	11mm	10mm	18mm	17mm
<i>S. aureus</i>	20mm	25mm	16mm	11mm	20mm	14mm
<i>P. aeruginosa</i>	13mm	24mm	15mm	10mm	13mm	16mm
<i>K. pneumoniae</i>	17mm	19mm	22mm	7mm	12mm	15mm

Figure1: Antibacterial activity of fruitlets epicarp from aqueous extraction

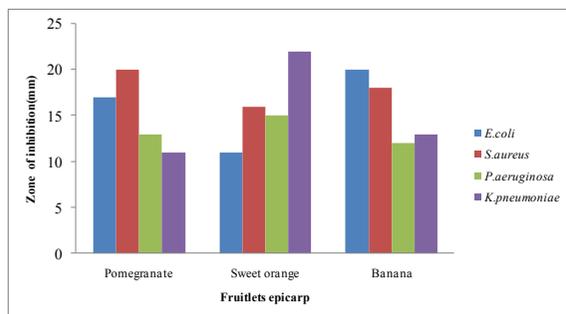


Figure 2: Antibacterial activity of various fruitlets epicarp from ethanol extraction

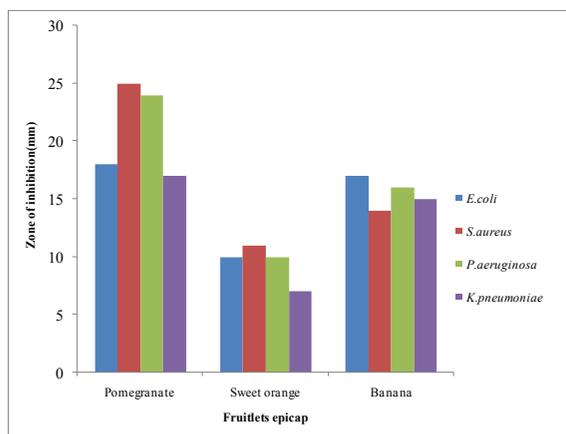


Table 2: Phytoconstituents of various fruitlet epicarp from different solvents

Phytochemical Compounds	Solvent systems					
	Pomegranate		Sweet orange		Banana	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
Alkaloids	-	+	-	+	+	+
Flavonoids	+	+	+	+	-	+
Glycosides	+	-	-	+	+	+
Steroids	+	+	-	+	-	-
Saponins	-	+	+	+	-	+
Tannins	-	+	+	+	-	+
Terpenoids	+	+	-	+	-	-
Resins	-	-	-	+	-	-
Free Aminoacids	-	+	+	+	-	-

Table 3: MIC values of various fruitlets epicarp extracts for different bacterial strains from the ethanol

Organisms	Pomegranate peel extract (g/ml)					Sweet orange peel extract (g/ml)					Banana peel extract (g/ml)				
	0.08	0.16	0.32	0.64	1.28	0.08	0.16	0.32	0.64	1.28	0.08	0.16	0.32	0.64	1.28
<i>E. coli</i>	1.23	1.07	1.02	0.93	0.82	0.99	0.86	0.82	0.79	0.62	0.83	0.78	0.62	0.54	0.51
<i>S. aureus</i>	0.57	0.50	0.62	0.67	0.69	0.54	0.50	0.65	0.62	0.59	0.75	0.75	0.84	0.92	0.95
<i>P. aeruginosa</i>	0.88	0.72	0.69	0.72	0.78	0.84	0.82	0.63	0.65	0.68	0.93	0.87	0.74	0.82	0.76
<i>K. pneumoniae</i>	0.62	0.59	0.57	0.53	0.69	0.79	0.73	0.69	0.62	0.69	0.96	0.85	0.78	0.68	0.71

Table-2 shown phytochemical examination of various fruit peels, different phytochemical constituents present in fruit peel and it has different degrees of solubility in different types of solvents depending on their polarity. In the ethanol extracts of each fruit peel, the phytoconstituents present include: Glycosides, tannins, triterpenoids, steroids, flavonoids, saponins, amino acids, reducing sugars, alkaloids and volatile oil, which is having various pharmacological activity. **Table-3** depicted the MICs values of seasonal fruit peel against human pathogens. In the ethanol extract of each fruit peel exhibited the least MIC of 1.28g/ml was *Escherichia.coli*, the least MIC of 0.32g/ml was *Pseudomonas aeruginosa*, the least MIC of 0.16g/ml was *Staphylococcus.aureus* and the least MIC of 0.64g/ml was *Klebsiella pneumoniae*.

DISCUSSION:

The fruit peel shows various therapeutics as antibacterial, anti-fungal, antioxidant, antitumor, antiviral, antimaterials and antimutagenic effects as reported by different author [11]. Much work has been carried out to demonstrate ethanomedicinal value of various plants in India because traditional natural products are widely used to cure certain diseases. The present investigation focused on the peel extract using various solvent systems indicated the antimicrobial activity of each extract. During antimicrobial screening of fruit peels namely pomegranate, sweet orange and banana with aqueous and ethanol extraction was displayed to be effective against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

In general secondary metabolites present in the plants have been responsible for their therapeutic activity. They reported that flavonoids are responsible for the antimicrobial activity associated with some ethanomedicinal plants [12,13]. The preliminary phytochemical screenings carried out showed these three fruit peel contain some secondary metabolites such as glycosides, alkaloids, saponins, volatile oils, flavonoids and tannins. According to literature all the fruit peel taken under evaluation expressed good antibacterial activity may be due to presence of flavonoids as secondary metabolic constituents.

A high MIC value indicates low activity and vice versa. In this study the gram negative organism had the lowest MICs. These suggest their susceptibility to the extracts of the peels [14]. On the basis of the results obtained in this investigations it can determine that the ethanol extract of each fruit peels had significant invitro broad spectrum of antimicrobial activity. Thus extract from the plants can be used to control infections caused by *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

CONCLUSION:

Recycling of fruit waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry. This work identified the antibacterial activity, phytochemical analysis and MIC values of the various

fruit peels from different solvents. Hence it strongly displays that fruit peels can be used for number of ailments and it opens a way to the scientific advancements of the pharma industries and the world.

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