

ORIGINAL ARTICLE

A Study on Antibacterial Activity of Crude Ethanolic Fruit Peel Extracts against Bacterial Isolates by well Diffusion Method and Analysis of Punica Granatum by Thin Layer Chromatography

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ABSTRACT

Milk has strong sensory qualities and has all the nutrients needed for the growth of all microorganisms. Additionally, milk is thought to prevent or lower the chances of certain illnesses linked to nutritional deficiencies. raw, unpasteurized milk contains a wide variety of microorganisms, including *Salmonella*, *Campylobacter jejuni*, *Escherichia coli*, and *Aeromonas*, all of which are linked to health problems. Citrus fruit contains sugar, organic acids, lipids, polysaccharides, vitamins, minerals, flavonoids, carotenoids, and volatile compounds. The aim of our study was to determine the antibacterial activity of crude ethanolic fruit peel extracts against bacterial isolates and to analyse components in the fruit peel extract by thin layer chromatography. The Orange, Sweet lime and pomegranate peels were collected from koyambedu market Chennai. The crude ethanolic extracts were prepared and antibacterial activity was performed by well diffusion technique. The methanol ethanolic and aqueous extracts of pomegranate exhibited antibacterial activity against *Listeria*, *S.aureus* and *salmonella* spp with zone of inhibition ranging from 19mm at concentration of 15 mg/ml and 25 mg/ml. The presence study showed lowest antibacterial activity against *salmonella typhi*, *Bacillus* species with zones of inhibition as 15mm and 14mm at a concentration of 500µl/ml and 250µl/ml by well diffusion method. The current study revealed RF values of two fractions a and b as 0.11 and 0.93.

KEYWORDS

• Fruit peel extract • Well diffusion technique • Antibacterial activity • Carotenoids

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INTRODUCTION

Milk has large number of nutrients, including carbohydrates, proteins, fats, and minerals, are found in dairy products and are thought to be beneficial for the growth of microbes and certain foodborne diseases. The entire community is concerned about the presence of bacteria in milk and milk products. Such developments in the dairy business are expected to continue in the future as the production, processing, and consumption of dairy products all continue to rise. The existence of pathogenic bacteria, which may have entered the milk from the source animal or may have entered during incorrect milking, storage, or processing, might compromise the quality of milk and dairy products. Diseases ranging from dyspepsia to more serious clinical problems may be caused by the intake of contaminated dairy products. Different kinds of microbes may have an impact on the product's nutritional value, which could result in major financial losses. (Dubey, Raj, Kumar, 2022)

Antibacterial activity of fruit peels

Citrus sinensis is the scientific name for orange, a sweet and juicy fruit from the Rutaceae family. *Citrus sinensis* is one of the most significant and commonly produced fruit crops, with worldwide output estimated to be approximately 120 million tons. Orange trees are frequently grown in tropical and subtropical regions for their delicious juice and therapeutic properties. (Shakthi Deve A, Sathish Kumar T, Kumaresan K, Rapheal, 2014).

Pomegranate has a broad spectrum of antibacterial properties and appears to inhibit Gram-negative and Gram-positive bacteria. distinct extracts from diverse sections of the pomegranate have distinct antibacterial properties. Many academics' studies revealed that PPE had stronger antibacterial action than other sections, and that antimicrobial activity was proportional to total flavonoids and tannins concentration (Jing Chen., *et.al.*, 2020).

Sweet lime peel extracts have shown considerable antibacterial activity against a variety of bacterial species. Studies have shown that the extracts have antibacterial properties, suppressing the development of harmful bacteria such *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. Sweet lime peel extracts' antibacterial properties have been

linked to the presence of bioactive components such as flavonoids, alkaloids, phenolics, and terpenoids. (Singh, *et.al.*, 2019).

METHODS

Collection of fruit peels

The Orange, Sweet lime and pomegranate peels were collected from koyambedu market Chennai.

Preparation of fruit peel extracts

The fruit peels of sweet lime, Orange and pomegranate were washed using sterile distilled water. The washed fruit peels were dried under shadow. The fruit peels were powdered. 20 grams of powdered fruit peels (Orange, Sweet lime and pomegranate) were soaked in 100 ml of ethanol. The fruit peels powders solvent were soaked overnight at freezing temperature The flasks were kept for thawing. After thawing the powdered solution of peels of different fruits were filtered by conventional methods. The filtrates were centrifuged at 1500 rpm for 30 minutes. The supernatant was kept in oven for 24 hours to remove excess of solvent, The dried powders of different fruit peels were kept in sterile container in dark for further use. (Neda jaur, reza, pany, and ali., 2022)

COLLECTION OF DAIRY PRODUCTS

The dairy products namely Butter, Milk and yoghurt were collected from the supermarket.

Spoilage of dairy product

The Butter, Milk, and Yogurt were subjected to spoilage by exposure of the mentioned dairy products to unfavourable conditions of growth.

Isolation of bacteria from Spoiled Dairy products

The spoiled Butter, Milk and Yogurt were inoculated into nutrient agar, Macconkey agar *Salmonella shigella* agar and Man Rogosa Sharpe media The plates were incubated at 37 degree celsius for 24 hours. The plates were observed for growth

Isolation of *Lactobacilli* from Milk, Butter and Yogurt

The dairy products were inoculated on MRS medium and incubated at 37 degree celsius for

24 hours The plates were observed for growth of lactobacilli and yeast

Microscopic observation

The colonies from basal media were subjected to gram staining and endospore staining based on cultural characteristics. The motility was observed based on gram staining.

Antibacterial activity of crude ethanolic fruit peels extracts by well diffusion agar method (Neda jaur, reza, pany, and ali., 2022).

Stock solutions were prepared by dissolving 1 mg of fruit peels extracts in 1 ml of ethanol and stored at refrigerator temperature for antibacterial activity. The bacterial isolates from spoiled Butter, Milk and Yogurt were transferred into nutrient broth and incubated at 37 degree celsius for 15 minutes. The turbidity of the broth cultures were checked with 0.5N Mcfarlandsolution. Mullerhinton agar was prepared and poured aseptically into sterile petri plates. The lawn was made using spoiled dairy product isolates aseptically. The wells were made using sterile puncture aseptically. The well were loaded with different concentrations using crude ethanolic peel extract of Orange, Sweet lime, Pomegranate and mixed fruit peel(the extract were mixed) The plates were incubated at 37 degree celsius for 24 hours. The plates were observed for zone formation. (Neda jaur, reza, pany, and ali., 2022)



Orange Pomegranate Sweet lime



Lawn preparation Well loading

Analysis of pomegranate peel extract - Thin layer chromatography

Ready made TLC plate was cut to the required size. A sample was spotted at one end of the TLC plate. The plate was kept in the beaker containing the mobile phase in such a way that the near the sample application should touch the mobile phase. Allow the chromatogram to run about 1-2 hours. (3/4th level for TLC plate). The plate was dried at RT and viewed under UV Light. Rf value of the sample can be determined.

DAIRY PRODUCTS AND THEIR SPOILAGE

The lactobacilli were isolated from raw milk and identified based on microscopic examination and inoculated on MRS media.

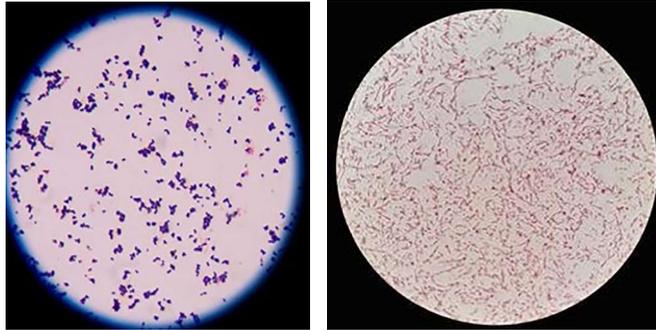
The spoiled Butter, Milk and yoghurt were observed by change in colour, Consistency, odour and formation of ropiness.

CONSOLIDATE CHART - SHOWING HIGHEST ANTIBACTERIAL ACTIVITY

Extract	Bacterial Isolate	Observation (Zone of Inhibition)	
		500 µl	250 µl
Orange	Butter		
	Milk		
	Yogurt		
Pomegranate	Butter <i>S. albicans (B5)</i>	16mm	12mm
	Milk <i>S. albus</i>	15mm	104mm
	Yogurt <i>S. typhi</i>	15mm	14mm
Sweet lime	Butter <i>Bacillus sps</i>	14mm	12mm
	Milk		
	Yogurt	-	
Mixed	Butter <i>Bacillus sps (B5)</i>	15mm	12mm
	Milk <i>S. albus (M4)</i>	14mm	12mm
	Yogurt		

Based on observation it was reported that *S. albicans* and *Bacillus* species isolated from spoiled butter were found to be sensitive to the Crude ethanolic extracts of Pomegranate peel at high concentration of 500 µl and 250 µl showing zone of inhibitory as 16mm and 12mm.

Gram staining



Gram positive cocci

Gram negative bacilli



Oxidase negative

Nutrient agar - gram negative bacilli

Endospore

Catalase positive



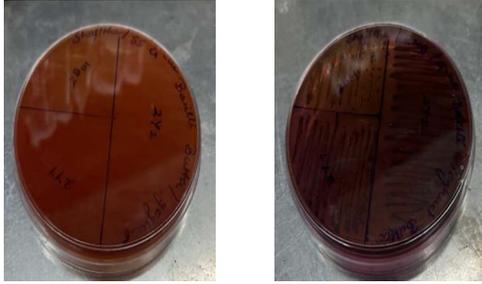
Gram positive cocci



Nutrient agar



Mac conkeyagar - gram negative bacilli



MSA- Gram positive cocci

SS Agar- gram negative bacilli



MRS - Lactobacilli

Biochemical test for gram negative bacilli



Antibacterial activity - butter - sweet lime, mixed and orange fruit peel extracts

Butter- Sweet lime and Mixed peels ext



Bacillus species



Salmonella spp

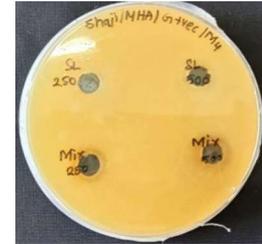


S.albus

Antibacterial activity - milk - Sweet lime, Mixed and Orange fruit peel extracts



Bacillus species



S.albus

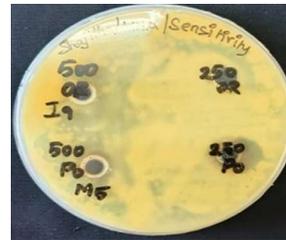
Antibacterial activity - yogurt - sweet lime and mixed fruit peel extracts



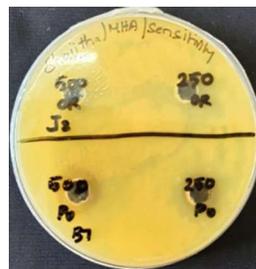
Bacillus species



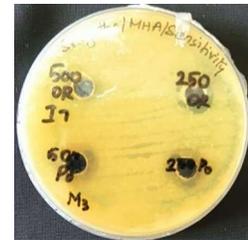
S.albus



Antibacterial activity - Butter - Orange and Pomegranate peel extracts

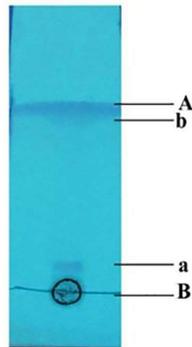


Bacillus species



S.albus

Thin layer chromatography of Pomegranate Peel extract



Sample	Distance Moved by the Solvent (A) in Cm	Distance Moved by the Solute (A-D) in Cm	Rf (A,B/A)
a	4.4	0.5	0.11
b	4.4	4.2	0.93

Thin layer chromatography analysis showed the distance moved by the solute of the pomegranate peel extract and found the Rf value of fractions a and b as 0.11 and 0.93 using ethanol as a mobile phase.

DISCUSSION

The current study showed lowest antibacterial activity against *Bacillus spp* using pomegranate, sweet lime and mixed crude ethanolic fruit peel extracts. The previous work conducted by Rizk stated that the ethanolic and aqueous extracts of banana, pomegranate and orange fruit peel extracts against *E. coli*, *Salmonella enterica*, *S. aureus* with highest zone formation in a range of 25mm, 22mm and 19mm. The current study showed highest activity with the zone of inhibition ranging from 16mm to 14mm using ethanolic extracts of pomegranate, sweet lime, orange and mixed fruit peel extracts against *Salmonella typhi*, *Bacillus* and *S. albicans* based on review of literature by Rokkaya sami., 2021. The methanol, ethanolic and aqueous extracts of pomegranate exhibited antibacterial activity against *Listeria*, *S. aureus* and *Salmonella spp* with zone of inhibition ranging from 19mm at concentration of 15 mg/ml and 25 mg/ml. The present study showed lowest antibacterial activity against *Salmonella typhi*, *Bacillus* species with zones of inhibition as 15mm and 14mm at a concentration of 500µl/ml and 250µl/ml by well diffusion method. The previous work

done by Lara caurpos *et al.*, 2022 explained the antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* by agar diffusion disc method. The current studies focused on evaluating antimicrobial activity using crude ethanolic extracts of pomegranate, orange, sweet lime and mixed fruit at a concentration of 500µl/ml and 250µl/ml against *Salmonella* species, *Bacillus* and *S. albicans*.

CONCLUSION

The authors of the current study evaluated the antimicrobial activity of crude ethanolic fruit peel extracts of Pomegranate, Sweet lime, Orange and Mixed fruit peel extract against *Salmonella spp*, *Staphylococcus albus* and *Bacillus spp* isolates namely butter, milk and yoghurt. The bacterial isolates were highly resistant to fruit peel extracts except pomegranate, sweet lime and mixed fruit peel extracts. The emergence of antibiotic drug resistance, multi drug extensive drug resistance is a major concern across the world. The drug resistance is due to mutation, frequent use of drugs by the patients, especially immunocompromised people. Hence, there is a need for alternative and complementary medicine against multi drug resistant bacterial isolates. Fruit peel extracts are considered as waste. The fruit peels are used as manure in agriculture and as animal feed. The fruit peels were found to contain many biological components which are active against microorganisms such as bacteria, fungi and viruses. Fruit peels are freely available and the current study focused on determining the antibacterial activity against bacterial isolates from spoiled dairy products. The authors of this study reported that crude ethanolic pomegranate, sweet lime and mixed fruit peel extracts are active against *Bacillus species*, *S. albicans* and *Salmonella spp*. Isolates from butter compared to milk and yogurt. The spoilage of dairy products may lead to foodborne diseases when consumed contaminated ones. The authors concluded this study which states that the fruit peel extracts are open to resolve foodborne infections caused by multidrug resistant bacteria. The authors of the current study concluded that the ethanolic crude fruit peel not only has nutritional value but also has antibacterial activities and analysed the separation of fractions a and b in Pomegranate fruit peel extract based on Rf values.

Ethical issues: No ethical issues were found.

Conflicts of interest: Nil

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