

EVALUATION OF ANTIBACTERIAL ACTIVITY OF SELECTED FRUITS PEELS EXTRACT AGAINST DIFFERENT SPOILED MEAT ISOLATES

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ABSTRACT

The production of fruit waste from different food processing industries can cause adverse impact on environment. The efficient use of fruit waste to produce the valuable by-products can minimize carbon footprint and GHG emission. This study aimed to evaluate the antibacterial activities and phytochemical analysis of Banana, grapefruit, lemon, orange, papaya and watermelon peel extracts against spoilage causing bacterial isolate from chicken, fish and meat. The antibacterial activity of extracts were investigated by well diffusion method against ten bacterial isolates. The results showed that most of the ethanolic extracts have mild inhibitory activity at 20mg/ml concentration while the maximum inhibitory effect was observed at 100mg/mL conc. with 1 to 9 mm zone formation. Hence the lemon, orange, and water melon peel extract showed the best antibacterial activity as compared to other fruits peel extracts. The results of the phytochemical analysis of extracts, showed the absence of resins and steroids and the presence of glycosides, saponins, flavonoids and tannins in all extracts, while steroids were present in banana extract only. This study will be eco friendly and economical by using waste fruits peel for antibacterial purposes to protect the food from spoilage.

Keywords: Ethanolic extracts, Antibacterial activity, Phytochemicals, Food borne pathogens, well diffusion method

1. INTRODUCTION

The consumption of contaminated food by human causes the foodborne illness. The foodborne illnesses caused by pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Clostridium botulinus*, *Vibrio parahaemolyticus*) is a source of concern for worldwide public health organizations (El-Beltagi *et al.*, 2022; Yumnam *et al.*, 2023). All around the world approximate 600 million peoples affected by consuming bacterial contaminated food. There are about 250 different food borne diseases which have been discovered and reported (Abdel-Raouf *et al.*, 2014). One of the major symptoms of food borne diseases includes; fever, dehydration, abdominal cramps and diarrhea, depending on the presence of bacterial strains into contaminated food (Hasan *et al.*, 2022). The resistance of food borne pathogens against antibiotics is another concern to treat these illnesses by using same antibiotic (Stermitz *et al.*, 2000). The major cause of the development of antibiotic resistance in bacteria is the rapid use of antibiotics (Shetty *et al.*, 2016). The antibiotics resistance increases the demand to develop the new drugs for the treatment of human pathogens. Plants play important role for the extraction of potent compound which can be used for the different treatment of antibacterial infections (Hasan *et al.*, 2022). The extraction of potent compound from plants is low cost and more appealing source as compared to traditional pharmaceuticals compounds preparations (Roy *et al.*, 2023). It has been also reported that all parts of the plants such as; leaves, fruits, flowers, latex, bark, roots, flowers, pods, stalks, seeds, stems, hull and fruit rind consists antimicrobial constituents (Cebi and Erarslan, 2023; Zzaman *et al.*, 2021).

In present studies the focus was to find the antibacterial potential of fruits peel ethanolic extract against spoiled meat, chicken and fish bacteria. The phytochemical contents in the fruit peels were also investigated qualitatively. The use of fruits peel against bacterial growth inhibition aims to review the more scientific work for the production of new compounds. The extracted value added compounds from fruits peel might be valuable source in food preservation and therapeutic purposes for food borne illness (Ramadan *et al.*, 2015; Gao *et al.*, 2023).

There is an increasing interest to find out essential phytochemicals, which can be used as, an alternative to synthetic substances, which are usually utilized in different food, pharmaceutical and cosmetic industry. Previous studies, also showed that the peels and seeds of fruits contain phytochemicals in higher quantity in comparison of the edible part of the fruits (Siddique *et al.*, 2018). The total amount of phenolics and flavonoids are also shown to be higher in the by-products as compared to the final products of fruits (Rudra *et al.*, 2015; Tripathi *et al.*, 2023).

2. MATERIALS AND METHODS

2.1. Sample collection

Meat samples (chicken, fish and beef) were collected from local market of Karachi, Pakistan. The meat samples were left at room temperature for spoilage at room temperature.

2.2. Isolation of Bacteria from Spoiled Meat Samples.

For the isolation of bacteria, 1g of the spoiled meats (chicken, fish and beef) was taken into sterile test tubes containing 5ml saline water. The homogenate of the samples was prepared for the serial dilution of the samples.

The 100 μ l sample from the 10^{-4} dilution tube was taken and inoculated into nutrient agar containing petri plates. The inoculated plates were incubated at 37°C for 24 hours. The isolated colonies after pour plated method further streak on nutrient agar plates. The isolated colonies were obtained by streak plate method. The CFU/mL was also calculated for each sample by using the standard CFU formula (Ben-David and Davidson, 2014; Sieuwerts *et al.*, 2008).

2.3. Morphological and Biochemical Characterization of Isolates

The isolated strains were characterized on the basis of their colony and cell morphology. The ten strains were isolated from the spoiled meat samples and identified by IMViC, Urease, Catalase, Motility, Triple sugar Iron and Starch hydrolysis tests (Tshikhudo *et al.*, 2013).

2.4. Sample Collection of Fruit Peels

The fruit peels of *Citrus sinensis* (orange), *Citrus paradisi* (grapefruit), *Citrus Limon* (lemon), *Carica papaya* (papaya), *Citrullus lanatus* (watermelon), *Musa acuminata* (banana) were obtained from local fruit juice market, Karachi City, Pakistan. The peels were air-dried for two weeks and ground into powder in a mechanical blender. The powdered samples were stored in clean brown bottles at room temperature for further use.

2.5. Preparation of Ethanol Extract of Fruit peels

The powdered fruit peels were placed in a 500ml conical flask, each flask contained ethanol. The mixture of powdered peels and ethanol was stirred, and solution from each flask collected after 72 hours period. The collected extracts were placed in a water bath for the evaporation of ethanol. The solid form of each extract was stored in a refrigerator until required for further use (Ehiowemwenguan *et al.*, 2014).

2.6. Phytochemical Analysis of extracts:

The extracts of *Citrus sinensis* (orange), *Citrus paradisi* (grapefruit), *Citrus Limon* (lemon), *Carica papaya* (papaya), *Citrullus lanatus* (watermelon), *Musa acuminata* (banana) peels were analyzed for the presence of tannins, glycosides, steroids, flavonoids, saponins, and resins using standard procedures reported by Ehiowemwenguan *et al.* (2014).

2.7. Antibacterial Activity of Fruit Peel Extract by Well Diffusion Method

The determination of antibacterial activity was done by using well diffusion and disc diffusion technique as earlier reported (Balavijayalakshmi and Ramalakshmi, 2017). The different concentration (20, 50, 100mg/mL) of extracts were used.

The antibacterial activity of extracts was checked by well diffusion method as also previously reported by Klančnik *et al.* (2010). The 24 hours culture of isolates was inoculated on to agar petri plate by using sterile cotton swab. Wells onto the inoculated agar plate were made and different concentrations (20, 50, 100 mg/mL) of each extract was dispensed into the wells. The controls were set up with sterile distilled water and ethanol. The plates were incubated at 37°C for 24 hours and the zones of inhibition around the well were measured in millimeters (mm).

3. RESULTS

3.1. Isolation and identification of spoilage causing bacteria

The spoilage associated organisms three from Fish, three from Chicken and four from beef samples were isolated and selected for further studies. Among all these ten bacteria four species were identified as gram positive and other six were gram negative result shown in Table 1 and 2.

3.2. Phytochemical Analysis Of Fruit Peels

The different phytochemical components were observed to be present in the peels of *Citrus sinensis* (orange), *Citrus paradisi* (grapefruit), *Citrus Limon* (lemon), *Carica papaya* (papaya), *Citrullus lanatus* (watermelon), *Musa acuminata* (banana) as shown in Table 3. On the basis of polarity the phytochemicals were observed to be soluble in different types of solvent. The results of the phytochemical analysis of fruits peel extracts, showed the absence of resins and steroids and

the presence of glycosides, saponins and tannins in all extracts. While only banana peel extract showed presence of steroids and absent in all extracts. The flavonoids is also not present in grape fruit and papaya.

Codes	color	Size	opacity	elevation	margin	Gram Reaction	Shape	Growth pattern in nutrient broth
WSC-1	White	large	Translucent	flat	smooth	-ve	Short rods	Pellicle
WSC-2	Cream color	large	Opaque	Raised	Shiny smooth	-ve	rods	Sediment
WSC-6	White	small	dull	irregular	rough	+ve	Cocco bacilli	Uniform
WSF-1	Pinkish-brown	small	Opaque	flat	rough	-ve	cocci	Pelicle
WSF-2	cream	medium	Opaque	convex	smooth	-ve	rods	Pelicle
WSF-4	white	small	dull	convex	rough	-ve	rods	Uniform
WSB-1	Blue green	large		flat	rough	-ve	Rods	Uniform
WSB-3	white	small	Opaque	convex	smooth	+ve	cocci	Pelicle
WSB-4	Creamy white	small	Opaque	convex	Entire	+ve	Rods	Sediment
WSB-5	golden	Small	Translucent	flat	raised	+ve	rods	Sediment

Table 1. Colony and cell morphological characteristics of spoiled meat spoiled meat chicken and fish isolates.

Table:2 Biochemical Characterization of spoiled meat chicken and fish isolates.

Codes	Indole	MR	VP	Citrate	Glu	Gala	Fruc	Oxidase	Catalase
WSC-1	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
WSC-2	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
WSC-6	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve
WSF-1	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
WSF-2	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
WSF-4	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
WSB-1	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
WSB-3	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
WSB-4	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
WSB-5	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve

3.3. Antibacterial Activity of Fruits Peel Extract at different conc.

The potential of extracts against different isolates measure on the basis of clear zone formation around the inoculated sample in wells, data shown in Fig 1. The largest zone of inhibition against WSF-4 gram negative rods was observed with the ethanolic extract of lemon and banana, 6 and 5mm respectively at 20mg/mL conc. The lowest activity was measured in a range of 1mm zone of inhibition by water melon and orange peels extract against tested isolates.

The antibacterial activity of the ethanolic extracts of fruit peels increase with the increase in conc. At the conc. of 100mg/mL the largest zone upto 9mm was observed by the activity of different extract data presented in Fig.2. The ethanolic extracts of watermelon, lemon and orange have shown the highest antibacterial activity at 100mg/mL (Fig.4). The orange peel showed the highest activity with a zone of inhibition 8 mm against WSB-1, WSB-3 and WSC-6 isolates. The watermelon extract showed highest zone of inhibition 9mm against WSB-4 and 8mm against WSC-1. The lemon extract showed 9mm zone of inhibition against WSB-1 and WSB-3. The lowest activity was observed in a range of 2.5 mm zone of inhibition in Fig.3. These antibacterial activity results of fruit peel extracts are due to the presence of different soluble compounds into ethanol.

Table. 3. Biochemical contents of fruit peels extract in ethanol.

FRUIT PEELS	flavanoids (F)	glycosides (G)	resins (R)	saponins (S)	steroids (ST)	tannins (T)
Banana (B)	+ve	+ve	-ve	+ve	+ve	+ve
Grapefruit (GF)	-ve	+ve	-ve	+ve	-ve	+ve
Lemon (L)	+ve	+ve	-ve	+ve	-ve	+ve
Orange (OE)	+ve	+ve	-ve	+ve	-ve	+ve
Papaya (P)	+ve	+ve	-ve	+ve	+ve	+ve
Watermelon(WM)	+ve	+ve	-ve	+ve	-ve	+ve



Fig 1. Zones of inhibition by well diffusion method.
(B= Bana, GF=Grapre Fruit, L=Lemon, O=Orange and P=Pappaya)

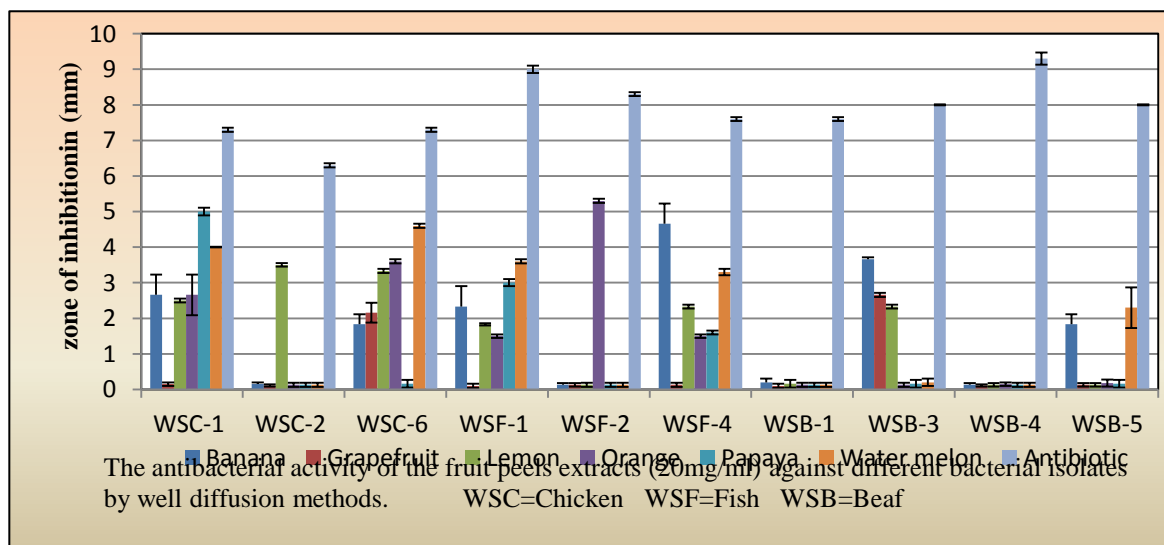


Fig 2. Antibacterial Activity By Well Diffusion Method (Zones of Inhibition at 20mg/mL).

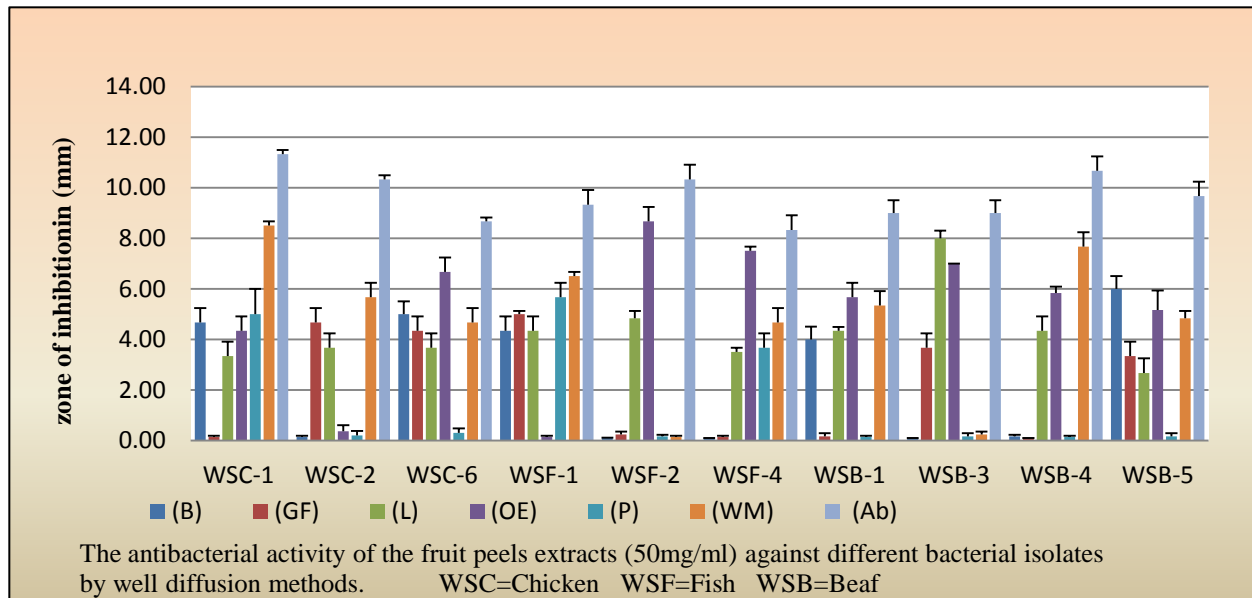


Fig 3. Antibacterial Activity By Well Diffusion Method (Zones of Inhibition at 50mg/mL).

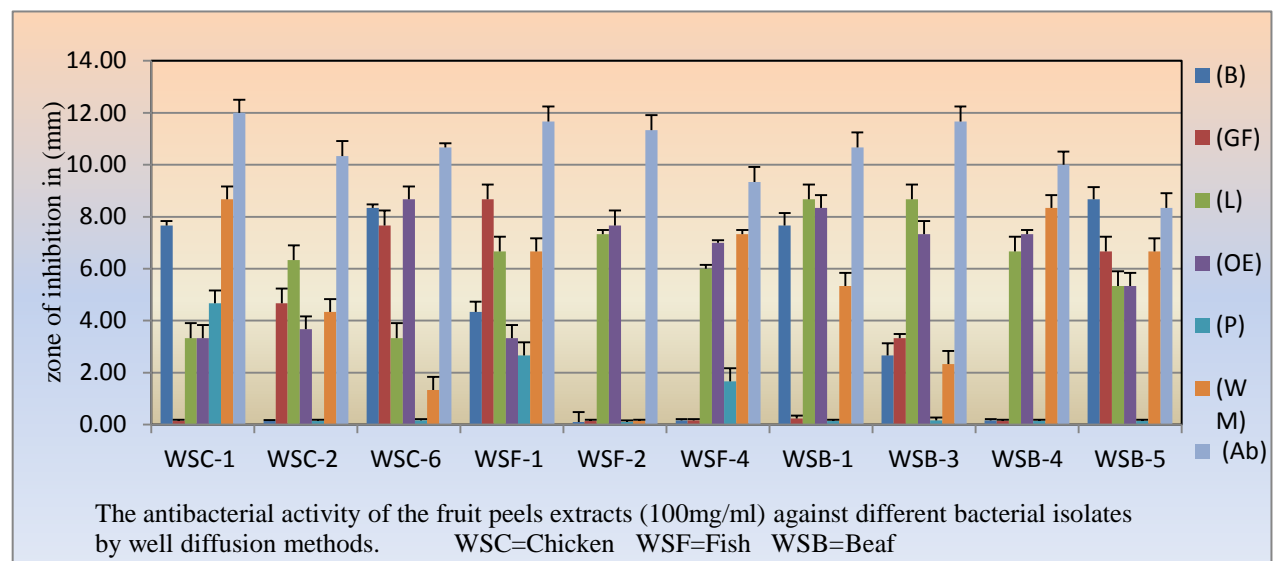


Fig 4. Antibacterial Activity By Well Diffusion Method (Zones of Inhibition at 100mg/mL).

DISCUSSION

The antibacterial activity result of the six tested fruits peel extract exhibited the potential against ten isolated gram positive and gram negative bacteria. The antibiotic (Amoxicillin) is used in order to observe the comparative analysis with the most effective fruit peel extracts. The highest activity of ethanolic fruits peel extract for isolates is in the order of Yellow lemon > Orange > water melon > Grape fruit > Banana > papaya peel. It was also reported in past studies that the plant extracts showed antibacterial activity against human pathogens (Nascimento *et al.*, 2000; Saad *et al.*, 2021).

The antibacterial activity results of all fruits peel extracts at 20mg/ml showed more potential against WS1 and WS6 isolates as compared to fish and beef isolates. The antibacterial activity of extracts showed the direct relation with the concentration of extracts as the concentration increases the activity is also increased (Hendi *et al.*, 2023; Kamel *et al.*, 2022). In the present studies the maximum activity of all fruits peel extracts was observed at 100mg/ml conc. in comparison of 20mg/mL conc. Lemon orange and water melon extract showed the highest potential of activity.

against all gram positive and gram negative bacteria at 100mg/mL concentration (Kamel *et al.*, 2022). The lemon peel extract is an effective antimicrobial agent against gram negative *Pseudomonas aeruginosa* (Dhanavade *et al.*, 201; Mandalari *et al.*, 2007). The antibacterial activity of lemon peel extract have been reported against gram negative *Klebsiellapneumonia* and other bacterial species (Dhanavade *et al.*, 2011; Saleem and Saeed, 2020)

In present work the water melon extract showed the antibacterial activity against all gram positive and negative bacteria except WSF-2 gram negative strain. It is also previously reported that watermelon has antibacterial effect against gram positive and negative bacteria in similar range of antibiotic (Chloramphenicol) (Neglo *et al.*, 2021). Banana peel extract also showed the potential activity against gram positive bacteria in comparison of gram negative bacteria at 100mg/mL conc. and exhibited lowest antimicrobial activity in comparison of lemon peel extract (Budiarti *et al.*, 2022).

In present studies The papaya extract showed the antibacterial activity only against two gram negative bacteria (WSC1 and WSF1) at 100mg/ml as the same effect was also reported in previous work (Abdel-Hameed *et al.*, 2023). The thin cell wall of gram negative bacteria also susceptible to the mechanical disruption. In previous studies it is reported that the antibacterial activity of fruits peel extracts also depend on the presence of phenolic and flavonoids compound (Remollo *et al.*, 2023). The highest antibacterial activity was observed at the 100mg/ml conc. of extracts. Previous work also reported that ethanolic and ethyl acetate papaya peels extract showed potent antibacterial activity against tested bacterial strains (Roshni and Lubaina, 2023).

In present studies the susceptibility of isolated strains with extracts based on the presence of different phytochemicals (flavonoids, glycosides, resins, saponins, steroids and tannins). The different clear zone formation around the inoculated extracts depend on the inhibition of isolates growth in presence of active compounds in ethanolic extracts. A study also suggested that ethanolic extract of fruits peel contains flavonoid, alkaloids, terpenoids, tannins, and saponins which showed the antibacterial activity (Mulia *et al.*, 2023). Previous studies suggested that the antibacterial activity of ethanolic extract is due to the solubility of active compounds into ethanolic solvent (Chabuck *et al.*, 2013; Hasan *et al.*, 2022; Meryem *et al.*, 2023)

CONCLUSION

In present studies it can be concluded that the fruits peel ethanolic extract might be used as a source of antibacterial agent for the preservation of meat, chicken and fish products and also for the treatment of food borne illnesses. The further purification is needed for the active phytochemical compounds which can be used in different food industries as an additives. The present study suggested that the use of six fruits peel extract for antibacterial activity can also reduce the addition of waste into the environment. This studies also indicated that the ethanolic extracts can be use as a precursor of antibacterial agent synthesis. Additionally the use of natural extracts showed more potential in place of the conventional compounds as additives and antibacterial agent. The extraction of phytochemicals from fruits peel by using ethanol showed potential antibacterial effect against gram positive and gram negative bacteria.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not Applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies.

CONSENT FOR PUBLICATION

Not Applicable.

AVAILABILITY OF DATA AND MATERIALS

Not Applicable.

FUNDING: None.

CONFLICT OF INTEREST

The authors declare no conflict of interest to disclose.

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