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# Phytochemical analysis, antibacterial and antibiogram activities of fruits peels against human pathogenic bacteria

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**Abstract**

Fruits are great sources of phytochemical compounds and multiple essential nutrients. Fruit peels can be exploited to reduce agro-waste as natural bioactive compound owning to the antimicrobial properties. The present work envisaged to explore the phytochemical compounds in aqueous and ethanol extracts of three fruit peels from pomegranate (*Punica granatum*), banana (*Musa acuminata*) and orange (*Citrus reticulata*). Additionally, it was aimed to ascertain the antibacterial activities of fruit peel extracts against six human bacterial pathogens; *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. Assessment of phytochemical properties of pomegranate, banana, and orange peels revealed that these peels were rich in many bioactive compounds especially alkaloid, flavonoid, phlobatannins, saponins, terpenoids, glycosides, anthocyanosides, steroids, phenols, proteins, and carbohydrates. The antibiogram analysis was performed using four different antibiotics including cepharadin, ceftriaxone, pipemidic acid, and ofloxacin. Antimicrobial profiling indicated that the studied microorganisms were resistant to cepharadin and ceftriaxone, but susceptible to pipemidic acid and ofloxacin. *S. aureus* was found to be resistant against all applied antibiotics. On the other hand, ethanol extracts of pomegranate peels showed maximum inhibitory activities (29.66 ± 1.63 mm) against *M. luteus*, banana peels (27.00 ± 0.33 mm) against *M. luteus*, and orange peels (29.00 ± 0.57 mm) against *K. pneumonia*. Collectively, similar to the whole fruit, the fruit peels are carrier of crucial nutrients and can be exploited for the production of novel and natural therapeutics to substitute the synthetic drugs and leveraging the development of potent novel antibiotics.



## Introduction

The use of naturally occurring nutrients and organic foods are gaining interest in recent times. These constituents are usually recognised as bioactive phytochemicals. It has previously been reported that several synthetic drugs carry multiple toxicities and side effects (Luciano and Perazella, 2014; Cohen and Weinstein, 2018); therefore, development of novel therapeutics as well as antimicrobials especially against infectious diseases caused by multidrug-resistant pathogens are inevitable. Phytochemicals are loosely defined as polyphenols, flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, and fibres (Pagliarulo *et al.,* 2016). These phytochemicals may individually or synergistically facilitate to reduce the risk for a range of chronic and inflammatory disorders (Hossen *et al.,* 2019), which include arterial sclerosis, disseminated multiple sclerosis, stroke, several types of cancers, diabetes mellitus, allergy, asthma, arthritis, Crohn's disease, Alzheimer's disease, psoriasis, septic shock, Acquired Immunodeficiency Syndrome (AIDS), and neurodegeneration (Cseke *et al.,* 2016). As a consequence, phytochemicals carry tremendous impact on the healthcare system and have potentials to prevent diseases and physiological disorders.

Majority of plant-based foods such as whole grains, beans, fruits, vegetables, and herbs contain phytochemicals (Septembre-Malaterre *et al.,* 2018). The utilization of plant extracts and phytochemicals is of great importance in therapeutics. During recent years, multiple studies have been conducted in several countries to prove such potencies (Islam *et al.,* 2018). However, most of the plant peels are largely dumped as solid waste and not utilized for other applications. For example, 40% of the whole weight of banana is generated as waste material in banana-based products in manufacturing industries (Nagarajaiah and Prakash, 2011). The banana peel should and could be exploited

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as a natural, eco-friendly, and economic supply of valuable elements while solving the environmental issues.

The present work focuses on the utilization of fruit peel to leverage waste management and antibiotic resistance which appears as an emerging challenge around the globe.

## Materials and methods

*Collection and preparation of dried plant material*

Fresh fruits i.e., pomegranates (*Punica granatum*), bananas (*Musa acuminate*), and oranges (*Citrus reticulata*) were collected from the fruit market of Azad Jammu and Kashmir, Pakistan. Fruits were thoroughly washed with running tap water and then with distilled water. The peels of each of fruits were separated and dried at room temperature (25 - 30°C) for 2 – 3 h. The dried peels were ground using a grinder. Fine powder was obtained and stored in polybags as described by Sharma *et al.* (2017) until further use.

*Aqueous extraction*

For the preparation of aqueous extraction, 1 g of powder from each of fruit peels was added to 100 mL of distilled water individually in conical flasks and left for 1 day in a magnetic stirrer. Following this, the resulting aqueous extract was filtered through Whatman filter paper I.

*Ethanolic extraction*

For the preparation of ethanolic extract, powder of each peels was subjected to Soxhlet extraction with 99% ethanol for 24 h. The mixture was evaporated to dryness in a rotary evaporator, and refrigerated. The condensed extracts were used for preliminary screening of phytochemicals as reported by Velumani (2016).

*Phytochemical analyses*

*Detection of alkaloids*

The preliminary phytochemical analysis of orange, banana, and pomegranate peels was performed on aqueous extract for the presence of alkaloids, flavonoids, tannins, phlorotannins, anthraquinones, saponins, terpenoids, anthocyanosides, steroids, glycosides, phenols, carbohydrates, and proteins by adopting the phytochemical procedure defined by Harborne (1998). Briefly, aqueous extract (1 mL) of each of the orange, banana, and pomegranate peel powder was added in a test tube with several drops of Wagner's reagents. The presence of alkaloids was indicated by the formation of reddish-brown precipitation.

*Detection of flavonoids*

The presence of flavonoids in the peels of orange, banana, and pomegranate was confirmed using 10% ammonium hydroxide in 1 mL of aqueous extract of the powdered peel of each of three fruits separately, and the formation of yellow colour confirmed their presence.

*Detection of tannins*

The formation of brownish green or bluish-black colour after treating aqueous extract of peels with 0.1% $FeCl_2$ indicated the presence of tannins in the extract.

*Detection of phlorotannins*

The formation of reddish precipitation after boiling 1 mL of aqueous extract of peels with 1% $HCl_2$ indicated the presence of phlorotannins in the extract.

*Detection of anthraquinones*

The presence of anthraquinones was examined by performing Borntrager's test. Briefly, 1 mL of the aqueous extract of peels was hydrolysed with diluted $H_2SO_4$ and later removed with benzene. Then, 1 mL of diluted ammonia was added to the extract. Rose pink colour formation indicated the presence of anthraquinones in the extract.

*Detection of saponins*

Foam test was used for the detection of saponins. Briefly, 10 mL of sterile distilled water was added to 2.5 mL aqueous extract of peels in a test tube and was shaken thoroughly for 30 s. Following shaking, it was allowed to stand for 30 min. Formation of honeycomb froth / foam indicated the presence of saponins in the extract.

*Detection of terpenoids*

Salkowski's test was used to confirm the presence of terpenoids in the extract by mixing 1 mL of aqueous extract of peels in 0.5 mL of concentrated $H_2SO_4$ and chloroform. The red-brown colour in the interface formed indicated the presence of terpenoids in the extract.

*Detection for glycosides*

The pink colour formed by mixing 2 mL of aqueous extract of peels with 3 mL of chloroform and 1 mL of 10% ammonium solution indicated the presence of glycosides in the extract.

*Detection of anthocyanosides*

The formation of pale pink colour upon mixing 1 mL of aqueous extract of peels and 5 mL of diluted HCl indicated the presence of anthocyanosides

in the extract.

*Detection of steroids*

The red-brown ring at the interface formed by adding 0.5 mL of aqueous extract of peels to 2 mL of chloroform and 1 mL of concentrated $H_2SO_4$ indicated the presence of steroids in the extract.

*Detection of phenols*

The formation of green or blue colour by adding 1 mL of aqueous extract of peels to 2 mL of distilled water and a few drops of 10% ferric chloride indicated the presence of phenols in the extract.

*Detection of proteins*

A total of 1 mL of aqueous extract of peels was boiled with 1 mL of 0.2% solution of ninhydrin. The formation of violet colour indicated the presence of amino acids and proteins in the extract.

*Detection of carbohydrates*

By boiling the aqueous extract of peels with 1 mL of Benedict's reagent, reddish-brown precipitation was formed which indicated the presence of carbohydrates in the extract.

*Antibacterial activity by agar well diffusion method*

The antimicrobial activity of aqueous extract of peels was assessed by the agar well diffusion method (Rios *et al.,* 1988). Nutrient agar (Oxoid, UK) and nutrient broth (Oxoid, UK) were used for bacterial cultivation. The bacteria were activated by inoculating a loopful in 25 mL of nutrient broth and incubated at 37°C on a rotary shaker for 24 h. The overnight culture was mixed with freshly prepared nutrient agar at 45°C and poured into sterile Petri dishes (pour-plate method). Inoculated Petri dishes were kept at room temperature in a laminar flow for solidification. Following this, in each plate, three wells of 5 mm diameter were made. Approximately 30 µL of each of the crude extracts of fruit peel powder and control solvent were placed in each prepared well, and plates were incubated at 37°C for 24 - 48 h.

All solvents were also used as negative control. Microbial growth was determined by measuring the diameter of the zone of inhibition after 24 h in millimetre (mm). The diameter of the clear zones (if greater than 5 mm) around each well was measured with the help of the scale.

*Antibiogram assay for antibiotic susceptibility test*

Antibiotic susceptibility of human pathogens was determined by Kirby Bauer disc diffusion test on Mueller-Hinton agar (Biemer, 1973) by using four different antibiotics (cepharadin, ceftriaxone, pipemidic acid, and ofloxacin). For this purpose, six human clinical bacterial pathogens were used including *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* These pathogenic strains were acquired from Laboratory of Microbial Biotechnology, Department of Zoology, Azad Jammu and Kashmir University, Muzaffarabad, Pakistan.

*Statistical analysis*

Statistical analysis was performed using Graph-Pad Prism for Windows (version 5.03). To analyse the antibacterial activity of standard antibiotics and fruit peelts, one-way analysis of variance and Dunnett's multiple comparison tests with a probability level of 5% as the minimal criterion of significance were used. Data are presented as means ± SEM.

## Results and discussion

*Preliminary screening of powdered peel of pomegranate, banana, and orange*

Preliminary phytochemical analysis showed that peel extracts of all three fruits were rich in phytochemicals in aqueous as well as in ethanol solutions. Different coloration in different reagents showed the presence of different phytocompounds in the plant extracts.

In the present work, phytochemical analysis has revealed that fruit waste was a significant source of organic compounds including alkaloids, flavonoids, and glycoside with antibacterial activity against different pathogenic and drug-resistant bacteria by reducing membrane fluidity. A phenolic compound in the peel of the fruit may interact with enzymes responsible for the formation of the bacterial cell membranes (Miklasińska-Majdanik *et al.,* 2018). The phlobatannins were absent in both aqueous and ethanol extracts of each of three fruit peels. It was revealed that pomegranate peel was rich in alkaloids, flavonoids, anthocyanosides, tannins, phenols, and carbohydrates; whereas banana peel contained alkaloids, flavonoids, tannins, phenols, proteins, saponins, anthocyanosides, glycosides, terpenoids, and carbohydrates. Orange peel was rich in alkaloids, flavonoids, tannins, phenols, proteins, saponins, glycosides, steroids, and carbohydrates. Many phytocompounds have been found in pomegranate peels such as gallagic acid, gallotannins, punicalins (García-Villalba *et al.,* 2015), punicalagin, punicalins (Sun *et al.,* 2017), esters of hexahydroxydiphenic acid, and aldohexose or quinic acid (Clifford, 2004), glycosides (Singh *et al.,* 2018), anthocyanidins (Akhtar *et al.,* 2015), and flavonoids (Kumar and

Neeraj, 2018). The potential applications of banana peels rely on its chemical composition since completely different fruit elements contain different inhibitors and antimicrobial elements. Unripen banana peels contain copper, zinc, sodium, potassium, calcium, phosphorus, and iron (Singh *et al.,* 2016). Additionally, banana peel contains several nutrients such as dietary fibres, essential amino acids, proteins, and unsaturated fatty acids (González-Montelongo *et al.,* 2010). Citrus fruits carry components of peculiar fragrance because of flavonoids and limonoids within the peel and these fruits are rich sources of antioxidants and flavonoids (Oikeh *et al.,* 2016).

The antioxidant / radical scavenging capability and reducing ability of various extracts of peels were investigated and results showed that ethanolic extract carried best values for yield i.e., total phenolic contents, total flavonoid contents, chelating, and inhibitor activities (% DPPH scavenging activity). It had been demonstrated that solvents play an important role in the extraction of the plant constituents. Specifically, methyl alcohol and ethyl alcohol were extremely polar among the solvents used in the present work (Hegazy and Ibrahium, 2012).

*Antibacterial activity of aqueous and ethanolic extracts of powdered peel of pomegranate, banana, and orange against six human clinical pathogenic bacteria*

Antibacterial activities of aqueous and ethanolic extracts against six human bacterial pathogens were performed using agar well diffusion method as shown in Table 1. Antibacterial activities of ethanolic extract of pomegranate peel showed maximum zone of inhibition against *M. luteus* (29.66 ± 1.63 mm), whereas aqueous extract exhibited maximum antibacterial activity against *E. coli* (24.33 ± 0.88 mm). Antibacterial activity of ethanolic extract of banana peels was highest against *M. luteus* (27.00 ± 0.33 mm), while aqueous extract exhibited maximum antibacterial activity against *B. subtilis* and *M. luteus* (23.00 ± 1.13 mm). Ethanolic extracts of orange peel had a maximum zone of inhibition against *K. pneumonia* (29.00 ± 0.57 mm) and aqueous extract exhibited highest antibacterial activity against *M. luteus* (26.33 ± 0.33 mm). The results showed that the waste material of fruits (pomegranate, orange, and banana) contained high potency against human pathogenic bacteria.

The results of the present work showed that bioactive compounds in fruit peels have a potent antibacterial effect. It has also been revealed from many previous studies that fruit peels carry highest antibacterial activity compared to other parts of fruit because of the presence of bioactive compounds. These activities were mainly attributed to flavonoids, phenolics, and tannins. Both aqueous and ethanolic extracts showed the antibacterial potential of peels of the fruits. However, ethanolic extract showed a significantly large inhibition zones as compared to aqueous solution because alcohol is a better solvent for active antimicrobial compounds (Sadeghian *et al.,* 2011). As reported by antimicrobial principles, either polar or non-polar organic solvent medium are more favourable for phytochemicals extraction (Ehiowemwenguan *et al.,* 2014).

*Antibiogram assay*

Antibiotic susceptibility of studied bacteria was determined by Kirby Bauer disc diffusion assay on Mueller-Hinton agar using antibiotics including cepharadin, pipemidic acid, ceftriaxone, and ofloxacin. The results showed that the *B. subtilis* showed

Table 1. Antibacterial activity of aqueous extract and ethanol extract of pomegranate, orange, and banana peel powder against human pathogenic bacteria.

| Pathogen | Zone of inhibition in mm (mean ± SEM) | | | | | |
|---|---|---|---|---|---|---|
| | Pomegranate (*Punica granatum*) peel | | Banana (*Musa acuminate*) peel | | Orange (*Citrus reticulata*) peel | |
| | Ethanolic extract | Aqueous extract | Ethanolic extract | Aqueous extract | Ethanolic extract | Aqueous extract |
| *Bacillus subtilis* | 28.33 ± 1.68** | 24.00 ± 0.60 | 23.33 ± 1.49 | 23.00 ± 0.67 | 27.66 ± 0.33 | 24.66 ± 0.33 |
| *Micrococcus luteus* | 29.66 ± 1.63** | 24.00 ± 0.60 | 27.00 ± 0.33 | 23.00 ± 1.13 | 27.66 ± 0.57 | 26.33 ± 0.33 |
| *Pseudomonas aeruginosa* | 23.00 ± 0.94 | 22.00 ± 0.51 | 20.33 ± 1.23 | 20.33 ± 0.94 | 24.0 ± 0.57** | 23.00 ± 0.57 |
| *Staphylococcus aureus* | 27.33 ± 1.98** | 23.33 ± 0.99 | 21.33 ± 1.12 | 21.00 ± 0.84 | 24.33 ± 0.33 | 23.33 ± 0.33 |
| *Klebsiella pneumonia* | 28.33 ± 0.33 | 23.66 ± 0.33 | 25.00 ± 0.57 | 22.66 ± 0.33 | 29.0 ± 0.57** | 25.33 ± 0.33 |
| *Escherichia coli* | 27.00 ± 0.57 | 24.33 ± 0.88 | 25.00 ± 0.57 | 22.00 ± 0.57 | 28.3 ± 0.66** | 23.66 ± 0.33 |

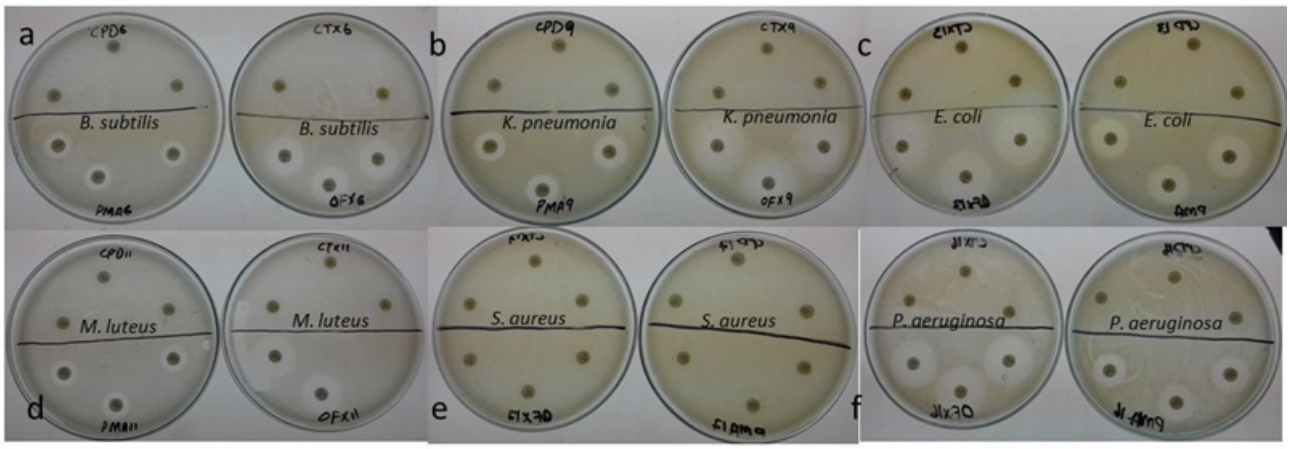


Figure 1. Antibiogram analysis against *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa,* and *Staphylococcus aureus.*
Note: a = *Bacillus subtilis*; b = *Klebsiella pneumonia*; c = *Escherichia coli*; d = *Micrococcus luteus*; e = *Staphylococcus aureus*; f = *Pseudomonas aeruginosa*; CPD = cepharadin; CTX = ceftriaxone; PMA = pipemidic acid; OFX = ofloxacin.

Table 2. Antibiogram analysis against *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa,* and *Staphylococcus aureus.*

| Pathogen | Zone of inhibition in mm (mean ± SEM) | | | |
|---|---|---|---|---|
| | **Cepharadin** | **Ceftriaxone** | **Pipemidic acid** | **Ofloxacin** |
| *Bacillus subtilis* | 1.66 ± 0.27 | 1.33 ± 0.27 | 7.60 ± 0.27 | 12.66 ± 0.27 |
| *Micrococcus luteus* | 1.33 ± 0.33 | 1.00 ± 0.00 | 7.66 ± 0.33 | 12.66 ± 0.33 |
| *Pseudomonas aeruginosa* | 1.66 ± 0.33 | 1.66 ± 0.33 | 14.33 ± 0.33 | 8.33 ± 3.17 |
| *Staphylococcus aureus* | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.33 ± 0.27 | 0.00 |
| *Klebsiella pneumonia* | 1.66 ± 0.33 | 1.00 ± 0.00 | 8.60 ± 0.33 | 14.00 ± 0.57 |
| *Escherichia coli* | 1.33 ± 0.33 | 1.66 ± 0.33 | 14.00 ± 0.57 | 13.00 ± 0.57 |

resistance against cepharadin and ceftriaxone; and susceptibility against ofloxacin and pipemidic acid. *K. pneumoniae* appeared resistant against cepharadin and ceftriaxone; and susceptible against ofloxacin and pipemidic acid. On the other hand, *M. luteus* showed resistance against cepharadin and ceftriaxone; and susceptibility against ofloxacin and pipemidic acid. *P. aeruginosa* showed resistance against cepharadin and ceftriaxone; and susceptibility against ofloxacin and pipemidic acid. *S. aureus* showed resistance against cepharadin and ceftriaxone; and susceptibility against ofloxacin and pipemidic acid. *E. coli* showed resistance against cepharadin and ceftriaxone; and susceptibility against ofloxacin and pipemidic acid as shown in Figure 1. Antibiogram analysis against the six pathogenic strains are shown in Figure 1, Figure 2, and Table 2.

The statistical analysis revealed that there was a significant difference in inhibition of the six tested human pathogens between peel extracts (ethanolic and aqueous) and standard available antibiotics ($p < 0.05$). This may be due to antibiotic resistance of pathogens against antibiotics whereas fruit peels had bioactive compounds that have potential against pathogenic bacteria, thus can be further utilised as alternative for antibiotics.

The phytochemical compounds play a vital role against different microorganisms (Singh *et al.,* 2018) and are vital microbial growth inhibitors (Orak *et al.,* 2011). Tannins in pomegranate had antibacterial activity against streptococci (Kunte *et al.,* 2018), *E. coli*, bacilli, and *S. aureus* (Pagliarulo *et al.,* 2016). The synthetic resin compounds in pomegranates possess inhibitory and atom scavenging activities (Dreher *et al.,* 2017). The banana peel has been found to carry highest inhibitors and antimicrobial activities (Vu *et al.,* 2018), and possessed antifungal activities (Velázquez-Nuñez *et al.,* 2013). These findings were further verified by the results of Okeke *et al.* (2015) where the antibacterial activity of citrus fruit were studied in great details.

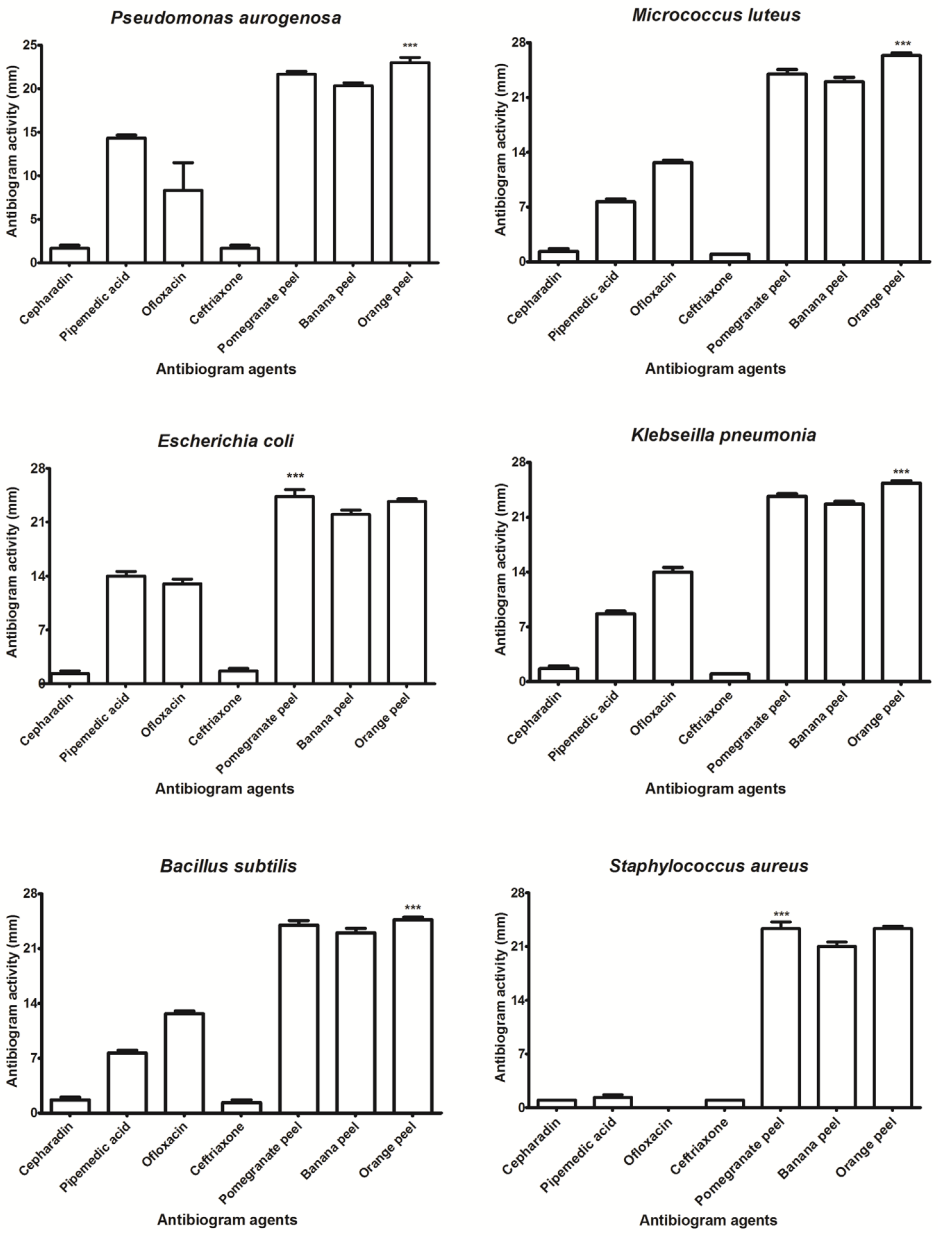


Figure 2. Antibiogram analysis of antibiotics and fruit peels against *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Micrococcus luteus, Klebsiella pneumoniae,* and *Staphylococcus aureus*.
Each bar represents the mean value of three replicates ($n$ = 3) ± SEM; * = the significant difference between antibiotics and fruit peels; *** = $p \leq 0.001$.

## Conclusion

The preliminary phytochemical analysis demonstrated in the present work highlights the potential of fruit peels in carrying multiple and crucial bioactive compounds. This potential may used for the development of novel therapeutics in the future. Assessment of the antibacterial activity of fruit peels revealed a significant zone of inhibition against important human pathogens. The results obtained also confirmed that the studied microorganisms were more susceptible to fruit peels than standard antibiotics, highlighting the capabilities of fruit peels to substitute synthetic drugs and leveraging the development of potent novel antibiotics.

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