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Potential application of waste fruit peels (orange, yellow lemon and banana) as wide range natural antimicrobial agent

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ABSTRACT

The antimicrobial potential of three abundantly available fruits peel waste, (orange, yellow lemon and banana) was evaluated on wide range of microorganisms. Three solvents methanol, ethyl acetate, ethanol and distilled water were used for extraction. The resultant extracts were used to test a six gram positive and six gram negative pathogenic bacteria in addition to two microscopic filamentous fungi (MFF) and two yeast species. Presence of trace metals were determined by Atomic Absorption Spectrophotometer and GC-MS Analysis was carried out to find out the total phenolic compounds, which may be responsible for the antimicrobial activity. The antibacterial activity was assessed by the well-bore method, reflected by the diameter of the zones of growth inhibition. Minimum Inhibitory Concentration (MIC) was also determined to confirm the antimicrobial potential of extracts and get quantitative results. Results shows that among the used solvents the extracts exhibited better performance in the order of Distilled water > Methanol > Ethanol > Ethyl acetate which reflects the suitability of solvent for fruit peel extraction. Effectiveness of fruit peel extracts was evaluated and found Yellow lemon > Orange > Banana peel. It was found that gram negative bacteria are more sensitive to the extracts and among them *Klebsiella pneumoniae* show the highest sensitivity against extract of yellow lemon peel and show the highest zone of inhibition (28 ± 1.4 mm to 3.5 ± 1.3 mm). Similarly, the MIC value was found to be 130 μg/mL which is the least value among other tested microorganisms. This may be attributed to the presence of high concentrations of zinc, magnesium and total phenolic content in the extract of yellow lemon peel. As multidrug resistant strains of microorganisms are emerging and treatment of their infection is becoming difficult with time, infectious diseases are a global cause of increase in death rate. Present study confirms the potential of studied fruit peel waste to be used for therapeutic purpose to combat the multidrug resistant microorganism infection. This will also result in reduction of waste material, reusing it for beneficial purpose in an economical and environmental friendly manner.

1. Introduction

Discovery of modern antibiotic medicines is linked to a Scottish biologist, Sir Alexander Fleming who discovered penicillin from the fungus *Penicillin notatum*, paving the road for the development of modern antibiotics (Aminov, 2010). Antibiotics are one of the most powerful tools to control infectious diseases; however, increase in the resistance to these drugs by targeted microorganisms is reported (Bandow et al., 2003; Dadashi et al., 2015). Microorganisms have the genetic ability to develop resistance to drugs which are used to treat infections. The effect of complex genotypic mutational patterns on virus drug susceptibility has been evaluated and phenotypic resistance was determined in various studies (Hertogs et al., 1998). This problem was augmented via the introduction of millions of tons of antibiotics annually to the environment. The environment is heavily flooded with these toxic compounds, which in turn has contributed significantly to the selection of resistant strains (Davies and Davies, 2010). At present, multidrug resistant strains of microorganisms are responsible for increase in untreatable bacterial infections and increasing death rates all over the world (Ventola, 2015).

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Herbal medicines are historically used for the treatment of many infectious diseases and found effective in many cases (Aminov, 2010). Most of the natural medicines are extracted from plant components such as leaves, flowers, fruits and stems. These extracts can be utilized to develop new antimicrobial compounds with different chemical structures and new mechanisms of action, to provide a barrier against multidrug resistant strains of microorganisms. There are an unlimited number of options to produce antimicrobial drugs from a variety of plants and their various components containing boundless chemical diversity (Martin and Ernst, 2003).

Fruit and vegetable peels are considered agro waste and are thrown into the environment instead of being used as a source of antimicrobial agents. Various studies conducted on peels revealed the presence of important constituents, which can be used for pharmacological or pharmaceutical purposes. Researchers extracted numerous components having antimicrobial, antioxidant and anti- inflammatory activities from different peels. This opportunity will provide an option to solve the problems in areas of antibiotic application in an economical and eco-friendly way, reducing the pollution from disposal of such agro waste. In the present study, the antimicrobial activity of peels of three fruits against a wide range of microorganisms was evaluated, particularly six gram positive, six gram negative, two MFF and two yeast strains were evaluated.

Oranges and lemons are important medicinal plants of the family *Rutaceae*. It is reported that their pulp as well as peel have antibacterial potential in addition to other properties (Parashar et al., 2014). Similarly, banana is an edible fruit and belongs to the berry family which is botanically known as genus *Musa*. Studies in the past reported that lemon has good antibacterial properties against clinically significant bacterial strains, such as *Pseudomonas aeruginosa* NCIM 2036, *Salmonella typhi* NCIM 5021, and *Micrococcus aureus* NCIM 5021 (Sultana et al., 2007; Dhanavade, et al., 2011). The antibacterial activity of orange peel extract derived from *Citrus medica L.* and *Citrus aurantium L.* was evaluated on bacterial strains and found effective against various bacterial strains like *E-coli* (MTCC No.118), *Staphylococcus aureus* (MTCC No.1349), and *Pseudomonas flourences* (MTCC No.103) (Anitha et al., 2016). Researchers also reported that the antimicrobial properties of banana peel against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella enteritidis, Escherichia coli,* were found to be effective (Mokbel and Hashinaga, 2005). This shows that fruit peel extracts could be used for the development of new antibiotics. However, there is still a need to investigate the effectiveness of these fruit peels against a wide range of microorganisms including bacteria, fungi s and yeast.

Global increase, in the present day, of antibiotic-resistant pathogenic microorganisms motivated researchers to explore the possibility of utilizing these fruit peels as antimicrobial agents. Present study investigates the antimicrobial activity of local orange (*Citrus sinensis L.*), yellow lemon (*Citrus limonia Osbeck*), and banana (*Musa acuminata*) peel extracts against selected microorganisms. Furthermore, this study also aimed to suggest a solution for agro waste minimization. The results obtained in this study helped to explore the possibility for future use of selected fruit peel wastes for therapeutic purposes to combat a wide range of multidrug resistant microorganisms.

2. Materials and methods

2.1. Collection of material

Local orange, yellow lemon and banana were obtained from the local market in Jubail city during month of June 2016. Fruits were cleaned and washed with tap water and then their peels were separated. Fruit peel was first dried in a drying oven at an average temperature of 35 °C. Samples were kept in the oven for three days till completely dried. After drying the samples were crushed and grinded with a mortar and pestle, than the samples were grinded to a powdered form using an electric grinder. The powder of the peel samples was stored in airtight glass bottles for further procedure.

2.2. Preparation of extracts

Methanol, ethyl acetate, ethanol and distilled water were used as solvents for the extraction of studied fruit peels. 10 g of each powdered sample was extracted with 100 ml of each solvent separately. During extraction samples were refluxed for two hours and later evaporated by distillation apparatus. Final concentrated extracts were obtained in round bottom flasks. The yield of each sample was calculated as gram (g) of extract per 10 g of dried peel. Each extract was stored in dark bottles and kept in refrigerator at 4 °C for further use. Stock solutions were prepared by dissolving each extract in appropriate amount of respective solvent to get concentration of 100 mg/mL. Dilutions were prepared accordingly when used in antimicrobial study.

2.3. In vitro antimicrobial activity study

In order to cover a wide range of microorganisms, six gram positive and six gram negative bacterial strains were tested. In addition, two microscopic filamentous fungi (MFF) and two yeast strains were also used for testing the antimicrobial activity of studied fruit peels.

2.3.1. Microorganisms and culture

Gram negative bacteria used are *Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens, Escherichia coli, Proteus vulgaris, Salmonella typhi* and gram positive bacteria studied are *Staphylococcus aureus, Enterococcus faecalis, Aeromonas hydrophila, Streptococcus pyogenes, Listeria monocytogenes,* and *Lactobacillus casei*. Studied MFF are, *Aspergillus niger* and *Penicillium citrinum*; two yeast are, *Candida albicans* and *Saccharomyces cerevisiae*. Bacterial cultures from solid media were sub-cultivated in Mueller Hinton broth, incubated for 24 h at 37 °C, and used as the inoculum for the determination of antibacterial activity. During study bacterial strains were maintained at 4 °C in Mueller Hinton agar slants.

2.3.2. Antimicrobial studies of extracts

In order to measure the antimicrobial activity of the studied fruit peels agar well diffusion method was adopted (Martin and Ernst, 2003). Molten Mueller Hinton agar medium at 40–45 °C was poured into sterile petri plate and seeded with different microbial cultures. For each bacterial test strain, 100 µl of a standardized bacterial stock suspension (10^8–10^9 CFU/ml) was used. The plates were kept for 15–20 min to solidify. Sterile borer was used to make three 8 mm wells in each petri plate. Next, 50 µl of each of the fruit peel solvent stock solution extracts containing a concentration of 5 mg were loaded into respective wells. As the positive control, 100 µl of broad spectrum antibiotic, Amoxicillin containing 50 µg was loaded. Negative controls were prepared by loading the respective solvents without extract. The bacterial culture plates were kept for 24 h at 37 °C and the yeast culture plates were kept at 30 °C for 48 h. The fungi plates were kept at 25 °C for 7 days in order to determine the zonal inhibition. After the incubation period the diameters of the zones of inhibition were measured (including well diameter) and tabulated for each fruit peel extract against each of the test microorganism. Solvent controls were run simultaneously.

2.4. Minimum Inhibitory Concentration (MIC) determination

Extracts demonstrating substantial inhibition zones based on agar well diffusion method were evaluated for their MIC. Bacterial strains were inoculated into 10 ml of aqueous SCD medium (soybean, casein, and digest) and cultured for 24 h at 35.5 °C. Dilutions of culture were prepared and adjusted to a concentration of 10^6–10^7 microorganisms per ml for further use as inoculum in the MIC test. For MFF culture, potato and dextrose agar slant medium which was cultivated for a week at 27 °C, was washed with salt-water having 0.05% Tween 80 (Rabbani et al., 2009). Concentration of spore suspension was adjusted to 10^6 microorganisms per ml for further use as inoculation in the MIC test. Yeasts were inoculated into 5 ml of glucose polypeptone (GP) medium and cultured for 48 h at 30 °C. The cultured fluid was diluted, adjusted to a concentration of 10^7–10^8 per ml, and used for inoculation in the MIC test. Peel extracts were suspended in water and the solution was then diluted with an SCD medium for bacteria and with a GP medium for MFF and yeast. Using them the twofold diluted solutions with concentrations of 1000 to 10 mg/mL were prepared. Each 1 ml of the culture medium containing various concentrations of the test material was inoculated with 0.1 ml of the microorganism suspension prepared above. Bacteria were cultured for one day at 35.5 °C, MFF for 7 days at 25 °C, and yeast for 2 days at 30 °C (Rabbani et al., 2009). Growth of the microorganisms was monitored during this period. When no growth of microorganism was observed in the medium containing the lowest concentration of the test materials, the MIC of the test material was defined at this point of dilution.

2.5. Elements determination

Elements that were determined included Ni, Cu, Fe, Zn, Mn, Cr, Cd, and Pb. The sample was prepared according to (Ryan et al., 1996). Elemental analysis was done using the Hitachi/Z-200 Zeemen flame/furnace Tandem Atomic Absorption Spectrophotometer following standard procedures (Clesceri et al., 1996).

2.6. GC-MS analysis

Total phenolic content was determined using GC-MS. 2 µl prepared samples were injected into an Elite-5MS column, with 5% Diphenyl/95% Dimethyl poly siloxane. The split ratio was 10: 1, temperature range was 500−3500 °C and Carrier gas flow was 1 ml/min. Gold Perkin Elmer Turbo mass detector was used. Spectral data from the library of the corresponding compounds and comparison of the retention times with authentic ones was used for identification of compound.

3. Results and discussion

Present study is an effort to test the potential of three common fruit peels as natural antimicrobial agents. Approach of the study linked with environmental sustainability using waste fruit peels for beneficial purposes and provided a means of solid waste management. Extraction was done using four different solvents, namely Distilled water, Methanol, Ethanol, and Ethyl acetate. Study shows that the yield of extracts varies with the selection of solvent in the order of Distilled water > Methanol > Ethanol > Ethyl acetate which, reflects the suitability of distilled water for fruit peel extraction (Table 1). This capability of solvent may be attributed to the inherent polar nature of distilled water. However, results reported by Arora and Kaur showed higher yield of distilled water after methanol. They reported similar results of antimicrobial study on *Staphylococcus aureus* (Arora and Kaur, 2013).

In the present study antimicrobial activity of studied fruit peel extracts was tested against a wide range of microorganisms (six gram positive, six gram negative, two MFF and two yeasts) as shown in Tables 2 and 3. Results of well-bore analysis measured by zone of growth inhibition show that studied fruit peel extracts show remarkable antimicrobial activity against the microorganisms. All the antimicrobial activity (well diffusion method) was done in triplicates and the results are presented in Table 2 as mean of three values It can be seen from the results presented in Table 2 that the effectiveness of fruit peel extracts for gram negative and gram positive bacteria is in the order of Yellow lemon > Orange > Banana peel. Results shown in Tables 1 and 2 reveal that there is a strong correlation between the yield of solvent extracts used in the study and the antimicrobial activity of peel extracts on studied microorganisms. It is also noted that the zone of inhibition is well comparable with the tested standard antibiotic (Amoxicillin) and shows very effective antimicrobial activity of yellow lemon extract against most of the gram negative and gram positive bacteria. It is also observed that these extracts are about 20–30% more effective on gram negative bacteria as compared to gram positive bacteria, which may be attributed to their inherent morphology such as structure and arrangement of their components and susceptibility of these microorganisms. Results reported by Dhanavade and coworkers shows that lemon is an effective antimicrobial agent against gram negative *Pseudomonas aeruginosa* which is due to the presence of coumarine and tetrazene compounds in the peel extract (Dhanavade et al., 2011). Banana peel extract shows least antimicrobial activity against tested bacteria. While comparing the present study results with the studies done in the past by Mandalari and coworkers, were shown comparable values with the reported results for both gram negative and gram positive bacteria (i.e. gram negative bacteria *Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris,* and gram positive bacteria *Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes*). However, slight difference in activities (10–15%) could be attributed to the different genera of citrus fruit, and ethanol as solvent used in their study (Mandalari et al., 2007). The antimicrobial activity study conducted by some researchers based on methanol solvent extract also reported similar results on *Pseudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram positive) bacteria (Khan and Kumar, 2011). Results presented in Table 2 show that the range of zone of inhibition is from 28 ± 1.1 to 35 ± 1.3 mm, with the largest diameter for *Klebsiella pneumoniae* tested with yellow lemon peel extracted with distilled water while banana peel shows minimum zone of inhibition ranging from 8 ± 0.2 to 16 ± 0.5 mm.

The Minimum Inhibitory Concentration (MIC) was also investigated to get quantitative results about the effectiveness of studied extracts and confirm the previous results. All the assays were performed in triplicate and results are presented in Table 3. In this

Table 1
Yield of extracts from studies fruit peels using various solvents.

Fruit peel	Solvent	Yield (g/100 g of peel)
Orange (*Citrus sinensis L.*)	Methanol	6.6
	Ethyl Acetate	0.61
	Ethanol	2.64
	Distilled Water	6.82
Yellow lemon (*Citrus limonia Osbeck*)	Methanol	2.82
	Ethyl Acetate	1.04
	Ethanol	2.59
	Distilled Water	5.86
Banana (*Musa acuminata*)	Methanol	3.84
	Ethyl Acetate	0.98
	Ethanol	2.15
	Distilled Water	4.34

Table 2
Antimicrobial activity of fruit peel extracts against wide range microorganisms measured by agar well diffusion method.

Peel Extract	Zone of Inhibition (mm)* Orange				Yellow Lemon				Banana				Antibiotic
Solvent	M	EA	E	DW	M	EA	E	DW	M	EA	E	DW	*Amoxicillin*
Microorganism (G−)													
Pseudomonas aeruginosa,	21 ± 1.4	10 ± 0.5	20 ± 1.7	25 ± 1.5	14 ± 0.8	30 ± 1.8	27 ± 1.9	32 ± 2.4	9 ± 0.4	12 ± 0.7	–	14 ± 0.9	35 ± 1.3
Klebsiella pneumoniae,	25 ± 1.2	12 ± 0.7	24 ± 1.1	29 ± 1.2	17 ± 0.8	28 ± 1.1	28 ± 1.4	35 ± 1.3	12 ± 0.4	14 ± 0.6	12 ± 0.5	16 ± 0.5	38 ± 1.1
Serratia marcescens,	22 ± 0.8	16 ± 0.6	20 ± 0.5	18 ± 0.7	20 ± 0.9	25 ± 1.2	17 ± 0.5	28 ± 1.0	–	12 ± 0.4	–	9 ± 0.2	32 ± 0.7
Escherichia coli,	18 ± 0.4	14 ± 0.3	17 ± 0.4	19 ± 0.5	15 ± 0.4	27 ± 0.9	24 ± 0.8	32 ± 1.1	9 ± 0.3	10 ± 0.3	–	12 ± 0.4	30 ± 1.1
Proteus vulgaris,	15 ± 0.5	10 ± 0.3	16 ± 0.3	18 ± 0.3	16 ± 0.2	27 ± 0.7	25 ± 0.8	32 ± 0.9	14 ± 0.3	15 ± 0.3	12 ± 0.2	16 ± 0.4	35 ± 1.2
Salmonella typhi	15 ± 0.5	12 ± 0.3	16 ± 0.4	16 ± 0.4	15 ± 0.3	25 ± 0.6	24 ± 0.9	30 ± 1.1	–	12 ± 0.4	10 ± 0.2	9 ± 0.2	34 ± 0.8
Microorganism (G+)													
Staphylococcus aureus	13 ± 0.4	12 ± 0.3	12 ± 0.3	14 ± 0.4	13 ± 0.3	21 ± 0.5	18 ± 0.4	25 ± 0.8	–	12 ± 0.4	10 ± 0.2	13 ± 0.3	38 ± 0.7
Enterococcus faecalis	17 ± 0.5	14 ± 0.3	16 ± 0.4	18 ± 0.4	18 ± 0.4	28 ± 0.6	20 ± 0.5	35 ± 0.8	–	11 ± 0.2	–	12 ± 0.3	45 ± 1.4
Aeromonas hydrophila	17 ± 0.4	18 ± 0.4	16 ± 0.3	17 ± 0.4	15 ± 0.3	19 ± 0.4	18 ± 0.4	28 ± 0.7	12 ± 0.3	13 ± 0.3	11 ± 0.2	14 ± 0.3	30 ± 1.1
Streptococcus pyogenes	18 ± 0.4	15 ± 0.5	18 ± 0.5	20 ± 0.6	16 ± 0.4	27 ± 0.6	21 ± 0.5	32 ± 1.0	–	10 ± 0.2	–	12 ± 0.3	38 ± 0.8
Listeria monocytogenes	20 ± 0.5	12 ± 0.2	20 ± 0.4	22 ± 0.4	18 ± 0.3	22 ± 0.5	25 ± 0.5	28 ± 0.6	9 ± 0.2	10 ± 0.2	10 ± 0.2	12 ± 0.2	32 ± 0.8
Lactobacillus casei	20 ± 0.4	14 ± 0.3	19 ± 0.4	21 ± 0.4	17 ± 0.3	20 ± 0.4	23 ± 0.4	25 ± 0.5	10 ± 0.2	10 ± 0.2	–	12 ± 0.2	34 ± 0.8
MFF													
Aspergillus niger	11 ± 0.2	10 ± 0.2	10 ± 0.2	15 ± 0.3	14 ± 0.3	12 ± 0.2	13 ± 0.2	16 ± 0.3	10 ± 0.2	9 ± 0.2	–	10 ± 0.2	12 ± 0.2
Penicillium citrinum	10 ± 0.2	9 ± 0.2	10 ± 0.2	15 ± 0.3	12 ± 0.2	10 ± 0.2	10 ± 0.2	15 ± 0.3	–	8 ± 0.2	–	9 ± 0.2	10 ± 0.2
Yeast													
Candida albicans	14 ± 0.3	10 ± 0.2	13 ± 0.2	14 ± 0.3	20 ± 0.4	16 ± 0.3	18 ± 0.4	27 ± 0.5	–	9 ± 0.2	–	14 ± 0.3	9 ± 0.2
Saccharomyces cerevisiae	15 ± 0.3	14 ± 0.3	16 ± 0.4	22 ± 0.6	18 ± 0.5	22 ± 0.6	19 ± 0.5	28 ± 0.8	12 ± 0.2	12 ± 0.2	–	20 ± 0.5	10 ± 0.2

'–' Means no zone of inhibition observed.
M = Methanol; EA = ethyl acetate; E = ethanol and DW = distilled water
* Mean value ± SD, n = 3 (the zone of inhibition is diameter also includes well of 8 mm).

Table 3
Antimicrobial Activities of fruit peel extracts evaluated by the minimum inhibitory concentration (MIC: μg/mL).

Peel Extract	Orange		Yellow Lemon		Banana		Antibiotic
Solvent	M	DW	M	DW	EA	DW	*Amoxicillin*
Antimicrobial Activity (in terms of (MIC: μg/mL)*							
Microorganism (G−)							
Pseudomonas aeruginosa,	350	210	210	160	410	380	90
Klebsiella pneumoniae,	310	200	140	130	370	350	100
Serratia marcescens,	280	250	200	150	500	290	70
Escherichia coli,	320	270	220	140	520	490	110
Proteus vulgaris,	290	210	220	130	300	280	90
Salmonella typhi	280	220	250	210	310	>1000	120
Microorganism (G+)							
Staphylococcus aureus	420	340	320	270	790	650	130
Enterococcus faecalis	400	260	260	140	>1000	>1000	100
Aeromonas hydrophila	410	400	280	280	670	630	110
Streptococcus pyogenes	370	340	210	250	720	590	90
Listeria monocytogenes	510	470	340	300	780	670	140
Lactobacillus casei	520	510	270	320	600	570	120
MFF							
Aspergillus niger	>1000	950	960	900	980	970	340
Penicillium citrinum	>1000	>1000	>1000	960	>1000	>1000	510
Yeast							
Candida albicans	>1000	>1000	>1000	920	540	510	510
Saccharomyces cerevisiae	>1000	930	660	960	670	530	530

M = Methanol; EA = ethyl acetate; E = ethanol and DW = distilled water
* >1000 means insignificant inhibition.

part of study only more effective extracts which were obtained from distilled water, ethyl acetate and methanol solvents, were used. In order to compare the antimicrobial results, experiments were done in parallel with standard antibiotic and fruit peel extracts. The antimicrobial activities of studied fruit peel extracts were evaluated by the MIC reported as μg/mL. Lower value of MIC means higher effectiveness of extract. MIC analysis results presented in Table 3 show that peel extracts have effectiveness in the order of Lemon > Orange > Banana peel. One of the reasons of effectiveness of Lemon as compared to orange and banana peels could be the presence of high concentrations of magnesium and zinc as shown in the results of trace metal analysis presented in Table 4. Rekha and colleagues reported that the surface area and surface defects of zinc and magnesium metal oxides particles play an important role in antimicrobial activities (Rekha et al., 2010). It is to be noted that the extracts are effective on almost all of the studied gram negative and gram positive bacteria. However, they are almost ineffective on *Penicillium citrinum* (MFF) and *Saccharomyces cerevisiae* (Yeast) and slightly effective in case of *Aspergillus niger* (MFF) and *Candida albicans* (Yeast). Therefore, results

Table 4
Results of trace metal analysis in fruit peels.

Constituent (μg/mL of extract)	*Orange*	*Yellow Lemon*	*Banana*
Cd	0.25	0.96	0.18
Cr	1.04	0.13	1.42
Cu	1.29	1.41	2.13
Fe	113.4	7.26	34.4
Pb	0.22	0.03	0.64
Ni	1.24	0.75	0.4
Mn	4.76	0.56	3.62
Mg	14.27	19.32	2.57
Zn	10.48	14.5	4.73

obtained by well-bore method, and confirmed by MIC analysis, show promising results about the potential of studied fruit peel extracts against microorganisms used in this study.

It is to be noted that the MIC value of standard antibiotic (*Amoxicillin*) is less than that of all studied extracts used in the study. This may be attributed to the fact that the compound of the antibiotic is pure and more refined in nature as compared to studied extracts. It can also be seen from Table 3 that the least value of MIC (130 μg/mL) was observed on bacteria *Klebsiella pneumoniae* and *Proteus vulgaris* while using yellow lemon peel extract. Results reported elsewhere shows similar findings when researchers used Turkish citrus peel oils which showed slightly lower efficiency with above microorganisms (Kirbaşlar et al., 2009).

Study done by Singh and coworkers revealed that the acetone demonstrated the highest yield and the extract exhibited strong antibacterial activity in *Citrus sinensis* (Singh et al., 2010). However, in this study extracts obtained from distilled water solvent exhibited the highest yields as well as antibacterial activity. Possibly, this difference could be attributed to the method of extraction, the variance in the phytochemical composition in different parts of the plant, and differences in the genotypes of the citrus plants used in their study. Differences in the antimicrobial activity of extracts, obtained from the same source utilizing different solvents showed that not all phytochemicals that are responsible for antibacterial activity are soluble in a single solvent. The total phenolic content obtained from used fruit peels and extracted by four solvents is presented in Table 5. Result shows that highest phenolic content was obtained from Yellow lemon while using distilled water as solvent followed by methanol. It means that the solvents providing higher yield and more effectiveness as antimicrobial agents are polar solvents, which are distilled water and methanol in this study.

As present study shows that gram negative bacteria were more sensitive than gram positive bacteria, this could be attributed to the differences in the cell wall structures of these bacteria. The gram positive bacteria have thick peptidoglycan cell wall in multilayer, which acts as an obstacle to various environmental materials, including natural as well as synthetic antibiotics. On the other hand, the gram negative bacterial cell has a single peptidoglycan outer layer, which is a less effective penetrability barrier, and also does not contain teichoic acid which is present in gram positive bacteria (Moreno et al., 2013). Furthermore, gram negative bacteria have low resistance to physical disruption due to a weak cell wall structure. In another study conducted by Al Zoreky and coworkers antimicrobial activity of pomegranate peels was investigated and presence of phenolic compounds and flavonoids in the extracts was found to be responsible for excellent antimicrobial activities (Al-Zoreky, 2009; Mehrotra et al., 2017).

Table 5
Results of total phenolic content in the fruit peel extracts using different solvents by GC-MS.

	Total phenolic content (mg/g extract) *Solvent Used*			
Fruit Peel	*M*	*EA*	*E*	*DW*
Orange	15.2 ± 0.4	12.4 ± 0.3	12.42 ± 0.3	25.21 ± 0.5
Lemon	18.7 ± 0.4	16.29 ± 0.4	17.37 ± 0.4	29.72 ± 0.6
Banana	8.6 ± 0.2	11.48 ± 0.2	11.48 ± 0.2	15.6 ± 0.4

M = Methanol; EA = ethyl acetate; E = ethanol and DW = distilled water.

4. Conclusions

The current study is reporting the quantitative results for the antimicrobial potential of three fruit peel extracts and focuses on the possibility of using these peel wastes that makes significant solid waste in the environment, as sources of novel, low cost natural antibiotics. The antimicrobial activity was found to be effective against gram negative, and gram positive bacteria, in addition to two microscopic filamentous fungi (MFF) and yeast, thereby demonstrating the application of extracts on a wide range of microbial populations. The results indicate that although all of the fruit peel solvent extracts demonstrated antimicrobial activity against the tested bacteria, MFF and yeasts, distilled water as a solvent was found to be the best choice of solvent, as compared to methanol, ethanol and ethyl acetate, to extract antimicrobial fractions from the waste peels. Hence, choosing an appropriate solvent is very crucial for the selective extraction of fractions with high antimicrobial activity from natural sources. Bacteria are more sensitive to the extract and among them gram negative have a higher sensitivity which may be attributed to their cell structure and morphology. Among studied microorganisms *Klebsiella pneumoniae* (gram negative) bacteria was found to be more sensitive against extracts of yellow lemon peel and showed the highest zone of inhibition (28 ± 1.4 mm to 3.5 ± 1.3 mm). Similarly, the MIC for the same bacteria was found to be 130 μg/mL, which is the least value among other tested microorganisms. The effectiveness of fruit peels are in the order of Yellow lemon > Orange > Banana peel. This may be due to the presence of high concentration of zinc, magnesium and total phenolic content in the extract of yellow lemon peel. Therefore, this study indicated that studied fruit peels having the remarkable potential to function as a precursor of newer, more effective and safer antimicrobial agents. Additionally, disposal of such drugs derived from natural fruits is safer than of conventional drugs.

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