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A Comparative Study on Antimicrobial Activity in Different Varieties of Bangladeshi Citrus Species

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Abstract: *Fruit peels are major byproduct of processing food and are not currently used commercially, but it can be a potential antimicrobial agent. As microorganism resistance to marketed antibiotics is prime concern now a day, the study concentrated on potential antibacterial activity of the following citrus species grown in Bangladesh. The purpose of the present study is to evaluate the peel of ten Bangladeshi citrus species namely C. maxima (local name, Batabilebu), C. reticulate (local name, Khasikomla), C. medica (local name, Jaralebu), C. sinensis (local name, Malta), C. lemon (local name, Lebu), C. hystrix (local name, Satkora), C. assamensis (local name, Adalebu), C. limittoids (local name, Sorbotilebu), C. aurantium (local name, Karuljamir), C. meyeri (local name, Chinalebu) against gram-positive and gram-negative bacteria such as Escherchia coli, Bacillus cereus, Streptococcus aureus, Shigella dysenteriae and Shigella sonnei. The disc diffusion method was used to evaluate the inhibition zones of bacterial growth and the results were compared with kanamycin, a commercial antibacterial agent. The C. limittoids peel of petroleum ether extract and C. aurantium peel of methanol extract exerted the highest antibacterial activity which exceeded the standard antibiotic kanamycin. Although higher antibacterial effects were observed in petroleum ether fraction however the effects were assumed to be much potent and predominant for methanol extract of peel. Our findings may explore new uses of peel and demonstrate that the C. aurantium peel of methanol extract is the best candidate among all because of its higher specificity to the microorganisms.*

Keywords*: Citrus peel, Antibacterial effects, Methanol extract, Antioxidants, Phytonutrients.*

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I. Introduction

Fruits and vegetables are considered as an important part of a good diet. Besides their delicious taste and flavor, they are known to reduce risk of several chronic diseases. Fruits and vegetables contain significant amounts of phytoconstituents which are negatively associated with the morbidity and mortality from cerebrovascular, cardiovascular and certain types of cancers [1,2]. Vegetables and fruits yield about 25% to 30% of non-edible products such as peels and seeds [3]. In most cases, these waste byproducts contain high contents of antioxidant and antimicrobial compounds that can be successfully utilized as a source of phytochemicals and antioxidant agents [4]. Lemon is an important medicinal plant that belongs to Rutaceae family. Citrus fruits such as orange, lemon, and lime, have been widely cultured and processed into juice. During the manufacture of citrus juice, very large amounts of byproduct wastes, such as peels are formed every year. Citrus peels exhibit a broad spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities [5,6].

Various types of citrus fruits such as lemons, oranges, limes and grapefruits are recommended to be responsible for the prevention of degenerative disease because of the availability of important nutrients. Vitamin C, folic acid, dietary fibers, carotenoids, potassium, selenium and a wide variety of phytonutrients are available in citrus fruit. Many chemical compounds such as alkaloids, flavonoids, glycosides, saponins, resins, oleoresins, sesquiterpene, phenolic compounds, fats and oils are reported to be present in citrus medicinal plants [7]. It has been revealed that citrus peel essential oils have natural antioxidant and antimicrobial properties [8, 9]. Citrus peel containing essential oils can be used as an alternate to the synthetic antibiotic due to their very prominent antimicrobial activity [10]. To increase morbidity and mortality of pathogens, the utilization of citrus peel as substitutive antibacterial agents of the high cost antibiotics can play a vital role.

Citrus byproducts, if utilized fully, could be major sources of phenolic compounds. The peels, in particular, are an abundant source of natural flavonoids and contain higher amount of phenolics compared to the edible portions. It has been reported that the contents of total phenolics in peels of lemons, oranges, and grapefruit were 15% higher than those in the peeled fruits [11]. Flavonoids in citrus are a major class of secondary metabolites. The peel contains the higher amount of flavonoids than other parts of the fruits and is involved in playing the vital biological and biochemical functions [11]. Flavonoids from citrus that have been extensively studied for anti-oxidative, anti-cancer, anti-viral, and anti-inflammatory activities, effects on capillary fragility and an observed inhibition of human platelet aggregation however, the extraction of these compounds are important aspects to get biological functions and their sensitivity to the different microorganisms. The strategy for extraction of compounds and their efficacy to the microorganisms are not clarified well.

It has been revealed that bioactive compounds present in citrus fruits cause the remedy of different complications affected by bacterial strains [2]. Phenolics are much diversified group of secondary plant metabolites, which includes simple phenolic, phenolic acids (benzoic and cinnamic acid derivatives), lignans, lignins, coumarins, flavonoids, stilbenes, flavonolignans and tannins. Many of phenolic compounds have shown strong antioxidant properties as oxygen scavengers, peroxide decomposers, metal chelating agents, and free radical inhibitors. Besides antioxidant activity, phenolic compounds have a wide range of action which includes antitumoral, antiviral, antibacterial, cardioprotective, and antimutagenic activities [12]. Flavonoids are polyphenolic antioxidants naturally present in vegetables, fruits, and beverages such as tea and wine. In vitro, flavonoids inhibit oxidation of low-density lipoprotein and reduce thrombotic tendency, but their effects on atherosclerotic complications in human beings are unknown. The peel of citrus fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants.

Based on several lines of evidences, it is assumed that lemon peel is a major phytonutrient essential for the prevention of different complications caused by microorganisms and shows clinical importance. Therefore, the present study involves the effectiveness of lemon peel of different varieties on antibacterial effects of different strains of bacteria. In this context, studies on the antimicrobial effect of lemon peel extract of different species were carried out against five bacterial strains (2 gram-positive and 3 gram-negative). The focal purpose of the current study is to explore the comparison between the antibacterial activity of petroleum ether extract and methanolic extract of ten Bangladeshi citrus fruits peel.

II. Materials and Methods

2. 1 Plant Materials

The fresh unriped ten citrus fruits, *C. maxima* (Ba), *C. reticulate* (Kh), *C. medica* (Ja), *C. sinensis* (Ma), *C. lemon* (Le), *C. hystrix* (Sat), *C. assamensis* (Ada), *C. limittoids* (So), *C. aurantium* (Ka) and *C. meyeri* (Chi) were collected from citrus research centre, Jointiapur, Sylhet and the garden of local farmer to get chemically treated free sample. All the samples were peeled off, dried, grinded into coarse powder and were extracted with petroleum ether for fat free and methanol under sonication bath for highest yield of extracts.
Local names of citrus species: Ba = Batabilebu; Kh = Khasikomla; Ja = Jaralebu; Ma = Malta; Le = Lebu; Sat = Satkora; Ada = Adalebu; So = Sorbotilebu; Ka = Karuljamir and Chi = Chinalebu.

2.2 Extraction Procedure

Dried peel powder of different citrus sample were taken in plastic cap containing glass bottle and extracted initially by petroleum ether under sonication bath (Trans sonicator, T-60). The objective was to remove the fatty constituents of peel. The sample was extracted by three times to get the maximum extract. The mixture (sample + solvent) was filtered through Whatman No.1 filter papers. The filtrate was then concentrated with a rotary evaporator under reduced pressure at 50°C to obtain brownish mass of peels. After getting the petroleum ether treated peel extract, residue peel powders were allowed to dry and these were dissolved in methanol for further extraction with the same process as mentioned above.

2.3 Test Organisms

For the assay of antibacterial activity of peel extracts, both gram-positive and gram-negative bacterial strains were used in this study. The tested bacteria were listed in the following table (Table 1). These organisms were collected from the Microbiological Research Laboratory, Department of Pharmacy, Rajshahi University, Bangladesh.

Table 1: Name of bacteria for the determination of antibacterial activity.

Gram-positive	Gram-negative
1. *Bacillus cereus*	1. *Escherichia coli*
2. *Streptococcus aureus*	2. *Shigella dysenteriae*
	3. *Shigella sonnei*

2.4 Preparation of Subculture

Nutrient agar media was used for the preparation of subculture of bacterial strains. The media was prepared according to manufacturer's instructions. The bacterial cultures were inoculated on nutrient agar and incubated at 37 °C for overnight as illustrated by researchers [13,14].

2.5 Determination of Antibacterial Activity

The peel extracts were tested for antibacterial activity using disk diffusion method on nutrient agar media as reported by Dash *et al.* (2005) [15]. All the extracts were individually dissolved in 1 mL of ethanol and the filter paper discs (6 mm diameter) were permeated with known amount of test substances and disc were prepared with different potencies. The discs were positioned on pre-seeded bacterial culture plates and kept at 4 °C temperature overnight for maximum diffusion of the components. The incubation of the plates was conducted at 37 °C for 12 h. After incubation, the diameter of the zones of inhibition (ZOI) of each extract were measured with a transparent scale for the assay of antibacterial activity and the values were compared with the standard disc Kanamycin (30 μg) (shown in Fig. 1). As negative control, blank disc were used.

Fig. 1. Measurement of zone of inhibition.

III. Results

3.1 Different Peel Extracts and Their Sensitivity to the Organisms

The antibacterial activity of peel extracts of different varieties of fruits was determined according to their zone of inhibition against some pathogenic bacteria and the result was compared with standard kanamycin disc. Table 2 shows the antibacterial activity of petroleum ether extract of citrus peel against gram-positive bacteria. For *Bacillus cereus* (gram-positive), the zone of inhibition (mm) of the test extracts So, Sat, Ja, Kh, Le, Ada, Ba, Chi, Ma and Ka were 20, 13, 16, 20, 10, 15, 10, 16, 11 and 11 mm respectively while for *Streptococcus aureus*, the values for the above mentioned extracts were 20, 15, 18, 20, 11, 16, 11, 17, 12 and 10 mm respectively. The zones of inhibition of standard kanamycin for the two organisms were noted as 22 and 17 mm respectively. The results indicated that the highest zones of inhibition were recorded as 20 and 20 mm for So (*C. limittoids*) and Kh (*C. reticulate*) extracts respectively against the organism *Bacillus cereus* when compared to the standard kanamycin (zone of inhibition, 22). Other varieties of extracts of traits also caused the enhanced zone of inhibition compared to the control disc. Similar effects (zone of inhibition) for So (*C. limittoids*) and Kh (*C. reticulate*) varieties of peel were found whenever the disc were inoculated with *Streptococcus aureus* (ZOI, 20, 20 mm). Other peel extracts also showed the considerable zone of inhibition (ZOI). The results argued that the different peel extracts were essential constituents extracted from petroleum ether and show antibacterial activity against the gram-positive bacteria however, *C. limittoids* and *C. reticulate* exhibited the potent effects regarding this phenomenon. The peels might be involved in prevention of pathogenic syndromes. The enhanced antibacterial activity in response to these two extracts might be due to the presence of some compounds.

In Table 3, the antibacterial activity of petroleum ether extract of citrus peel against gram-negative bacteria has been demonstrated. For *Escherichia coli* (gram-negative), the zone of inhibition (mm) of the test extracts So, Sat, Ja, Kh, Le, Ada, Ba, Chi, Ma and Ka were 18, 14, 15, 16, 10, 14, 12, 15, 13 and 12 mm respectively while for *Shigella dysenteriae*, the values for the above mentioned extracts were 22, 17, 19, 22, 12, 18, 14, 19, 12 and 12 mm respectively. The zones of inhibition of the standard kanamycin for these two organisms were 18 and 20 mm respectively. For *Shigella sonnei*, the zones of inhibition (mm) of the test extract were 25, 16, 20, 22, 12, 17, 15, 18, 15, 13 mm respectively while for the control, the value was recorded as 24 mm. The highest zone of inhibition (ZOI, 18 mm) for So (*C. limittoids*) extract against *Escherichia coli* was noted when compared to the standard kanamycin (zone of inhibition, 18). The other peels extract also showed the antibacterial effects however the values were lower than So (*C. limittoids*) variety. For *Shigella dysenteriae,* the highest antibacterial activities were recorded in response to So (*C. limittoids*) and Kh (*C. reticulate*) varieties of extracts (ZOI, 22, 22 mm) while other varieties also showed potent effects compared to standard kanamycin (zone of inhibition, 20). Similar increased antibacterial effects were demonstrated for different species of peel inoculated with *Shigella sonnei* however, So (*C. limittoids*) and Kh (*C. reticulate*) extracts of peel showed higher potency on antibacterial effects (ZOI, 25 and 22 mm) against this organism rather than other species. Other varieties of extracts of traits also caused the increased zone of inhibition when compared to standard kanamycin. The results indicated that these species of peel (*C. limittoids* and *C. reticulate*) have the higher antibacterial activity and might be involved in prevention of different pathogenic complications caused by gram-negative bacteria like *Escherichia coli*, *Shigella dysenteriae, Shigella sonnei.*

Table 2: *In vitro* antibacterial activity of petroleum ether extract of citrus peel against gram-positive bacteria.

Test organisms	Diameter of zone of inhibition (mm) of test extracts										Kanamycin
	So	Sat	Ja	Kh	Le	Ada	Ba	Chi	Ma	Ka	
Gram-positive											
Bacillus cereus	20	13	16	20	10	15	10	16	11	11	22
Streptococcus aureus	20	15	18	20	11	16	11	17	12	10	17

C. limittoids (So), *C. hystrix* (Sat), *C. medica* (Ja), *C. reticulate* (Kh), *C. lemon* (Le), *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma), *C. aurantium* (Ka) and Kanamycin (30 μg/disc).

Table 3: *In vitro* antibacterial activity of petroleum ether extract of citrus peel against gram-negative bacteria.
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Test organisms	Diameter of zone of inhibition (mm) of test extracts										Kanamycin
	So	Sat	Ja	Kh	Le	Ada	Ba	Chi	Ma	Ka	
Gram-negative											
Escherichia coli	18	14	15	16	10	14	12	15	13	12	18
Shigella dysenteriae	22	17	19	22	12	18	14	19	12	12	20
Shigella sonnei	25	16	20	22	12	17	15	18	15	13	24

C. limittoids (So), *C. hystrix* (Sat), *C. medica* (Ja), *C. reticulate* (Kh), *C. lemon* (Le), *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma), *C. aurantium* (Ka) and Kanamycin (30 μg/disc).

As illustrated in Table 4, the antibacterial activity of methanol extract of different varieties of citrus peel against gram-positive bacteria has been recorded. For *Bacillus cereus* (gram-positive), the zones of inhibition (mm) of the test extract So, Sat, Ja, Kh, Le, Ada, Ba, Chi, Ma and Ka were 12, 16, 14, 16, 14, 17, 12, 22, 11 and 24 mm respectively and for *Streptococcus aureus*, the ZOI values for the above mentioned extracts were 11, 18, 12, 15, 15, 18, 12, 20, 12 and 20 mm respectively obtained. Among the different extracts of peel, the maximum zones of inhibition for Ka (*C. aurantium*) (ZOI, 24 mm) and Chi (*C. meyeri*) (ZOI, 22 mm) extracts were noted for the organism *Bacillus cereus* when compared to the standard kanamycin (zone of inhibition, 22). Similar potent and increased antibacterial activities were demonstrated for the extract Ka and Chi (ZOI, 20, 20 mm) respectively when compared to the effect of kanamycin (ZOI, 17 mm) caused by gram-negative bacteria, *Streptococcus aureus* however, the effects were much higher for the extract Ka for *Bacillus cereus* showing that this peel extract may have the higher specificity to the organisms. Other varieties of extracts of traits also caused the enhanced zone of inhibition when compared to kanamycin. Therefore, it is evident that the methanol extract for different species causes higher antibacterial effects because of the presence of some compounds and might be involved in prevention of pathogenicity caused by these gram-positive bacteria. Although petroleum ether extract of the similar species of peel causes antibacterial effect against these organisms however the effects were assumed to be much potent for the extracts obtained from methanol (Table 2 and Table 4).

Table 5 shows the antibacterial activity of methanol extract of citrus peel against gram-negative bacteria. For *Escherichia coli* (gram-negative), the zone of inhibition of the test extract So, Sat, Ja, Kh, Le, Ada, Ba, Chi, Ma and Ka were 14, 18, 15, 17, 16, 18, 15, 18, 14 and 20 mm respectively and for *Shigella dysenteriae*, the values for the above mentioned extracts were 10, 15, 14, 12, 14, 17, 14, 16, 10 and 22 mm respectively obtained. For *Shigella sonnei*, the zones of inhibition of the test extract were recorded as 15, 20, 16, 20, 18, 20, 15, 22, 12 and 22 mm respectively. The highest zone of inhibition for Ka extract (*C. aurantium*, ZOI, 20 mm) for *Escherichia coli* was noted compared to the standard kanamycin (zone of inhibition, 18). Other varieties of extracts of traits inoculated with *Escherichia coli* also showed the potent zone of inhibition when compared to standard kanamycin. Similar increased values (zone of inhibition) for Ka (*C. aurantium*) extract from methanol treatment were recorded as 22 and 22 mm for the organisms *Shigella dysenteriae and Shigella sonnei* respectively when compared to kanamycin. The respective ZOI values for control kanamycin were 20 and 24 mm. The extract *C. meyeri* (Chi) inoculated with *Shigella sonnei* also caused the higher potency and antibacterial effects (ZOI, 22 mm) during methanol extraction. Other extracts of peel from methanol treatment also showed higher potency against these organisms. Therefore, all these different varieties of fruits have the potent antibacterial activities against the pathogenic bacteria. Although petroleum ether extract of this species of peel shows the potent antibacterial activity however methanol extract causes much higher results for this species (Table 5, Table 3). The peel extracts after methanol treatment produces higher specificity to the organisms thereby assumed to be effective extraction strategy for the separation of phytochemicals.

Table 4: *In vitro* antibacterial activity of methanol extract of citrus peel against gram-positive bacteria.

Test organisms	Diameter of zone of inhibition (mm) of test extracts										Kanamycin
	So	Sat	Ja	Kh	Le	Ada	Ba	Chi	Ma	Ka	
Gram-positive											
Bacillus cereus	12	16	14	16	14	17	12	22	11	24	22
Streptococcus aureus	11	18	12	15	15	18	12	20	12	20	17

C. limittoids (So), *C. hystrix* (Sat), *C. medica* (Ja), *C. reticulate* (Kh), *C. lemon* (Le), *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma), *C. aurantium* (Ka) and Kanamycin (30 µg/disc).

Table 5: *In vitro* antibacterial activity of methanol extract of citrus peel against gram-negative bacteria.
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Test organisms	Diameter of zone of inhibition (mm) of test extracts										Kanamycin
	So	Sat	Ja	Kh	Le	Ada	Ba	Chi	Ma	Ka	
Gram-negative											
Escherichia coli	14	18	15	17	16	18	15	18	14	20	18
Shigella dysenteriae	10	15	14	12	14	17	14	16	10	22	20
Shigella sonnei	15	20	16	20	18	20	15	22	12	22	24

C. limittoids (So), *C. hystrix* (Sat), *C. medica* (Ja), *C. reticulate* (Kh), *C. lemon* (Le), *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma), *C. aurantium* (Ka) and Kanamycin (30 µg/disc).

3.2 Screening and Comparative Analysis

In Fig. 2, the two fractions PE (petroleum ether) and Me (methanol) have been shown and their effects on ZOI were compared against different microorganisms. Among the petroleum ether fractions, *C. limittoids* (So) extract showed higher potency on antimicrobial effects when compared to the methanol fractions of the same species and the effects were almost similar to the standard kanamycin. The other two fractions for Sat (*C. hystrix)* variety (PE and Me) although produced effects however the values were lower than the standard kanamycin. The highest antibacterial activity was observed in the extract of *C. limittoids* (So) (PE fraction) for the *Shigella sonnei* strains. The results are appeared to indicate that some species of fruit peel exhibited higher potency to the microorganisms. Similar trends of antibacterial effects were demonstrated for Kh (*C. reticulate*) extract during preparations in petroleum ether extractions when compared to control while methanol fractions for this species of peel show lower potency on antibacterial effects (Fig. 3). Therefore, the results are good agreement that both these fractions have antibacterial effects on different microorganisms however the values of ZOI were higher than those of methanol fractions. Therefore, these citrus peels are essential phytoconstituents and sources causing antimicrobial effects and might be involved in prevention of infected diseases. It is reasonable that the antibacterial activity in some peel extract might be separated by different strategy and extraction procedures.

As illustrated in Fig. 4, the antibacterial activity to the organisms for the fractions *C. lemon* (Le) and *C. assamensis* (Ada) in methanol (Me) were much higher than petroleum ether (PE) fractions of the respective species. Although the zones of inhibitions (ZOI) values against standard kanamycin were lower, the extract showed much potency on antibacterial effects demonstrating that the methanol extract of the fruit peel may have some potential compounds responsible for the prevention of complications caused by the microorganisms. Among the PE fractions, Le showed much less ZOI values when compared to the effects of standard kanamycin. Similar results were noted for *C. meyeri* (Chi) species (methanol fractions) showing much increased antibacterial effects when compared to kanamycin and the effects were much lower for petroleum ether fractions (shown in Fig. 5). The ZOI values in *C. maxima* (Ba) were much lower than *C. meyeri* (Chi) and the values in PE fractions for this species were also lower than methanol fractions. Therefore, it is obvious that methanol extracts of the species may have some essential constituents demonstrating the higher effects to the organisms.

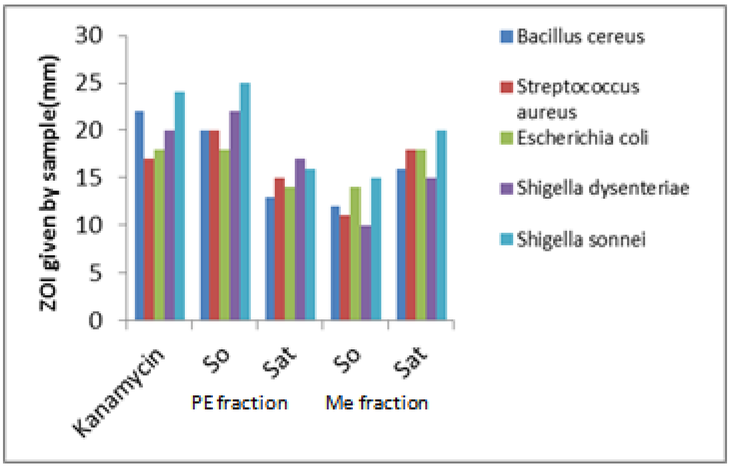

Fig. 2. Antimicrobial screening of petroleum ether and methanol extract of *C. limittoids* (So) and *C. hystrix* (Sat) varieties of citrus peel.

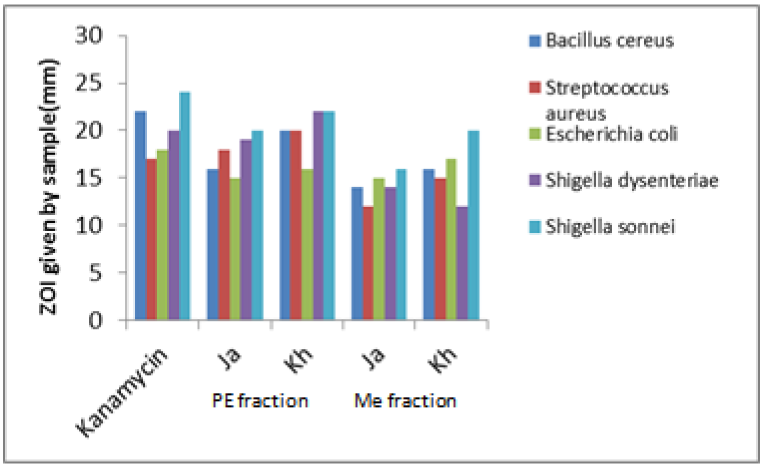

Fig. 3. Antimicrobial screening of petroleum ether and methanol extract of *C. medica* (Ja) and *C. reticulate* (Kh) varieties of citrus peel.

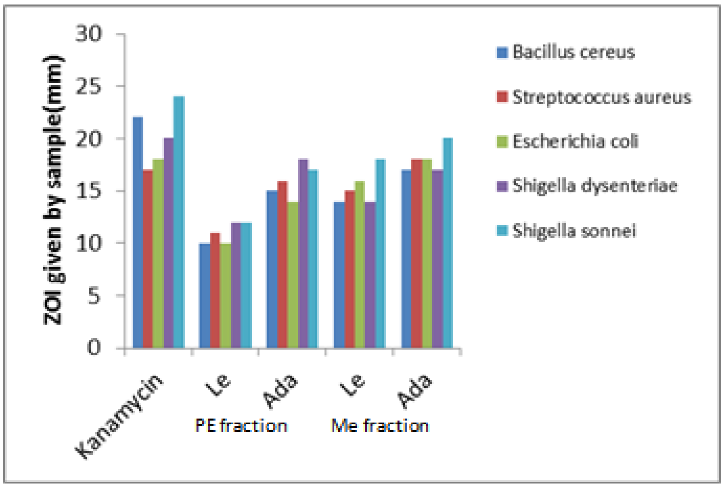

Fig. 4. Antimicrobial screening of petroleum ether and methanol extract of *C. lemon* (Le) and *C. assamensis* (Ada) varieties of citrus peel.

As demonstrated in Fig. 6, the antibacterial activities of another two species of fruit peel *C. sinensis* (Ma) and *C. aurantium* (Ka) for petroleum ether (PE) and methanol (Me) extractions have been shown. Compared to kanamycin treatment, the methanol extracts (Ka) (*C. aurantium*) showed higher potency to the different five microorganisms and the values were much lower for PE fractions. Another peel extract Ma (*C. sinensis*) produced a lower response on ZOI value when compared to the effects of *C. aurantium.* The increased ZOI values for this species, *C. aurantium* represent the potent antimicrobial effects on the bacterial species. Therefore, it is substantial to make strategy for the extraction of the compounds present in fruit peel and methanol extraction may produce much better results on the purification of the compounds having potent antibacterial effects.

It was observed that, among petroleum ether extract of citrus peel, the highest antibacterial activity was given by *C. limittoids* (So) peel against *Shegella sonnei* that exceeded the standard antibiotic kanamycin (Fig. 2, Table 3). On the other hand, the peels of *C. lemon (Le)*, *C. maxima* (Ba) and *C. aurantium* (Ka) showed lowest zone of inhibition against *Bacillus cereus, Escherichia coli and Streptococcus aureus* (Fig. 4, 5, 6). The trend of overall antibacterial activity of petroleum ether extract of citrus peel according to their zone of inhibition is given bellow:

So > Kh > Ja > Chi > Ada > Sat > Ma > Ka > Ba > Le

Among the methanol extract of citrus peel, the highest antibacterial activity was found by *C. aurantium* (Ka) peel against *Bacillus cereus* that exceeded the standard antibiotic kanamycin (Fig. 6, Table 4) and the lowest zone of inhibition was given by the peel of *C. limittoids* (So) and *C. sinensis* (Ma) against *Shigella dysenteriae* (Fig. 2, 6).

The trend of overall antibacterial activity of methanol extract of citrus peel according to their zone of inhibition is given bellow:

Ka > Chi > Ada > Sat > Kh > Le > Ja > Ba > So > Ma

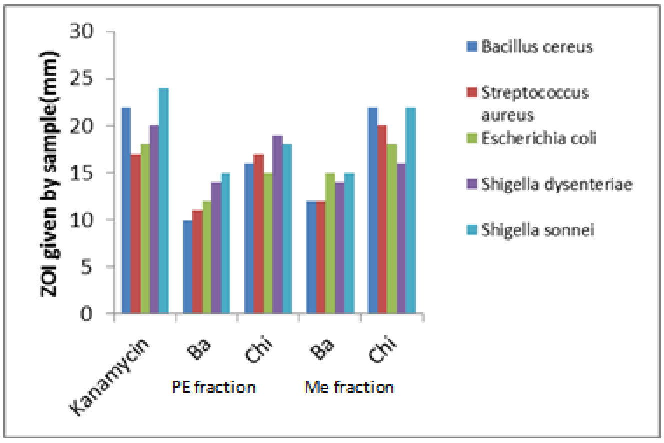

Fig. 5. Antimicrobial screening of petroleum ether and methanol extract of *C. maxima* (Ba) and *C. meyeri* (Chi) varieties of citrus peel.

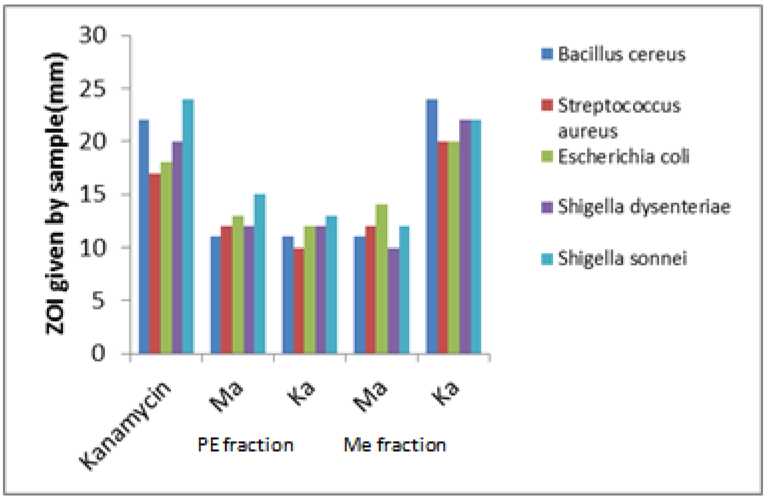

Fig. 6. Antimicrobial screening of petroleum ether and methanol extract of *C. sinensis* (Ma) and *C. aurantium* (Ka) varieties of citrus peel.

IV. Discussion

In the present study, we evaluated antibacterial efficacy of ten different Bangladeshi citrus peel extracts and designed to explore the comparative evaluation of antimicrobial activities of petroleum ether and methanol extracts of these fruit peels. Citrus fruits and crops contain active phytochemicals and protect health. Moreover, it provides an ample supply of vitamin C, folic acid, potassium and pectin. The contribution of citrus species in the prevention of life threatening diseases have been assessed and it has been reported that citrus fruits, citrus fruit extracts and citrus flavonoids exhibit a wide range of biological properties due to their presence of phenolic compounds and antioxidant properties [16,17]. Plants synthesize a diverse array of secondary metabolites (phytochemicals) known to be involved in plant defense against microbial and fungal pathogens, insect pests and several classes of phytochemicals have been shown to reduce the risk of various diseases. The epidemiological studies have pointed out that the consumption of fruits and vegetables enhances health benefits, e.g. reduced risk of coronary heart disease and stroke, as well as certain types of cancer. Apart from dietary fibre, these health benefits are mainly attributed to organic micronutrients such as carotenoids, polyphenols, tocopherols, vitamin C and others. Flavonoids from fruits and vegetables have been demonstrated to reduce risks of diseases associated with oxidative stress, including cancer [12].

Among the ten varieties of peel extracted with petroleum ether in our investigation, *C. limittoids* (So) extract showed the higher efficacy and microbial effects on different five microorganisms when compared to the control Kanamycin. However, the effects for this species were found to much lower in methanol extraction of the same species (Fig. 2). Among the five organisms, *Shigella sonnia* and *Shigella dycenteria* exhibited the highest specificity to the extract. Therefore, it is reasonable that the peel extract may have some essential constituents showing the preventive approach to the complications caused by these organisms. It has been revealed from the previous study that petroleum ether extract of fruit shows broad spectrum of antibacterial activity [18]. Therefore, the results are good agreement with their findings. The antibacterial effects of various citrus peels have been demonstrated in the previous studies [7,19]. Dubey *et al*. (2011) showed potent antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Shigella flexineri, Bacillus subtilis and Escherichia coli* in the extract from fruit of orange peels [20]. Cuishnie and Lamb (2005) concluded that the phenolic compounds in citrus peels had been responsible for antimicrobial activity [21]. The ethyl acetate extracts of the citrus peels exhibited more effect against food borne bacteria [22]. The other peel extracts also showed a higher antibacterial effect to the organisms demonstrating that the peel extracts were essential phytonutrients exerting their efficiency. The results argued that the activities had been widely distributed to the citrus species and might be due to presence of some compounds.

Citrus peels are rich in essential oil, flavonoids (flavone, flavonol and flavanone), terpenes, carotenes and coumarines that are medicinally important and are responsible for antimicrobial activity [23]. Citrus byproducts, if utilized fully, could be major sources of phenolic compounds. The peels, in particular, are rich source of vitamin C, fibre, and many nutrients including phenolics and flavonoids. Various potent antioxidants have been found in citrus peels and showed antioxidant effects including free radical scavenging and metal chelation activities. It is essential to explore the active phytochemicals in citrus peel as reactive oxygen species and play a major role in many diseases such as cancer, cardiovascular dysfunction, neurodegenerative diseases and process of ageing [24]. Flavonoids in citrus are a major class of secondary metabolites and the highest amount of flavonoids are present in the peel compared to the other parts of the fruits. Lemon (*Citrus sinensis*) peel is an agri-horticultural waste produced in large quantities from various fruit processing industries. It is normally discarded and dumped in the environment that can create environmental concerns. However, the peels are biologically important because of their essential phytonutrients or secondary metabolites and show the antibacterial efficacy as demonstrated by our investigation. Plants and microbes are well known to produce secondary metabolites, required for their survival and can be used as efficient drug candidates. Naturally occurring compounds like polyphenols in fruits, vegetables, beverages and cereals are plant secondary metabolites and generally involved in defense against aggression by pathogens and UV radiations [25]. Long term consumption of polyphenol rich diet offers protection against cancer, diabetes, cardiovascular diseases, neurodegenerative diseases and osteoporosis. It is well known that polyphenol rich food and beverages may increase plasma antioxidant capacity.

Among the methanol extracts of peel, the highest zones of inhibition (ZOI) values were observed and the extract shows the broad spectrum of effects. The results are much evident and predominant than the effects of petroleum ether extract. In this connection, *C. aurantium* (Ka) and *C. meyeri* (Chi) varieties of peel exhibited the enhanced antibacterial effects to the different organisms demonstrating that the peel may have some potent organic compounds showing the preventive approach to the organisms. Recent investigations reveal that similar results were observed for fruit peel extracted from methanol treatment. Therefore, the results are good agreement and are compatible to their findings [26]. They found that methanol extract of lemon peel had exhibited greater antimicrobial activity thereby indicating the effectiveness of lemon peel as a potent antimicrobial agent. Hence, lemon peel functions as a potent antioxidant and antimicrobial agent and that in future its use as an ingredient and food supplements might be promising. Moreover, some researchers investigated and found that the lemon peel extracts done by different solvents such as ethanol, methanol and acetone are subjected to antibacterial assay. Methanolic extract showed higher antimicrobial activity against tested microorganisms, *E. coli*, *S. aureus*, *Candida albicans* and *Trichophyton rubrum* [27]. Tumane *et al*., (2014) studied the antibacterial activity of *Citrus aurantium* (sour orange) and *Citrus medica* (lemon) fruit peel extracts after methanol treatment [28].

Antioxidants are naturally occurring molecules that combat oxidative damage in biological entities by free-radical scavenging. They are highly localized to citrus fruits and vegetables including plant flavonoids. An antioxidant achieves this by slowing or preventing the oxidation process that can damage cells in the body. Thus, antioxidants can also be termed as reducing agents. A free radical is any chemical species with one or more unpaired electrons and capable of independent existence. Once formed, radicals can react either with another molecule by different interactions or to another radical. Low levels of reactive oxygen species (ROS) are indispensable in many biochemical processes, involving apoptosis, cell differentiation, immunity and defense against pathogens. On the contrary, high doses of ROS result in oxidative stress causing severe metabolic malfunctions and damage to biological macromolecules [29]. The ROS can cause DNA modifications in several ways, which involve degradation of bases, pyrimidine or sugar bound modifications, deletion or translocations and cross linking with proteins [30]. Antioxidants may exert their effect by various mechanisms, like suppressing the formation of active species by reducing hydroperoxides (ROO•) and hydrogen peroxides and also by scavenging active free radicals, sequestering metal ions, repairing and clearing. Some antioxidants are also known to induce biosynthesis of other antioxidants or defense enzymes. Figure 7 represents an overview of antioxidant action on oxidative stress. Although not yet clarified, the citrus species mentioned in this study may exert their effects against different microorganisms accordingly.

The peel extracts of different species were effective against both groups of bacteria but its activity was assumed to be high in gram-negative bacteria as compared to gram-positive bacteria as demonstrated in the current study. These observations are in accordance with the earlier observations reported by Rakholiya *et al*. (2014) [31] and Hindi and Chabuck (2013) [32] who observed that gram-positive organisms were less susceptible to the extracts of fruit peel than gram-negative isolates. The reason for the different sensitivity of the gram-negative bacteria compared to that of gram-positive bacteria could be due to differences in their cell wall composition. Gram-positive bacteria contain an outer peptidoglycan layer, which is an effective permeability barrier, whereas gram-negative bacteria have an outer phospholipidic membrane [33].

Collectively, the antibacterial activities of methanol treated peel extracts were assumed to be higher because of the presence of flavonoid compounds as demonstrated by Ghasemi *et al*. (2009) [34] since the compounds have been recognized to be effective against bacteria. Among the petroleum ether and methanol extracts, the *C. limittoids* and *C. aurantium* peels showed much higher antimicrobial activity than other sample which indicated the presence of flavonoid compounds in them. Considering all the results, it is revealed that methanol extract of citrus peel has been shown to exert more potent antimicrobial effects than petroleum ether extract and can replace synthetic antimicrobials being natural agent.

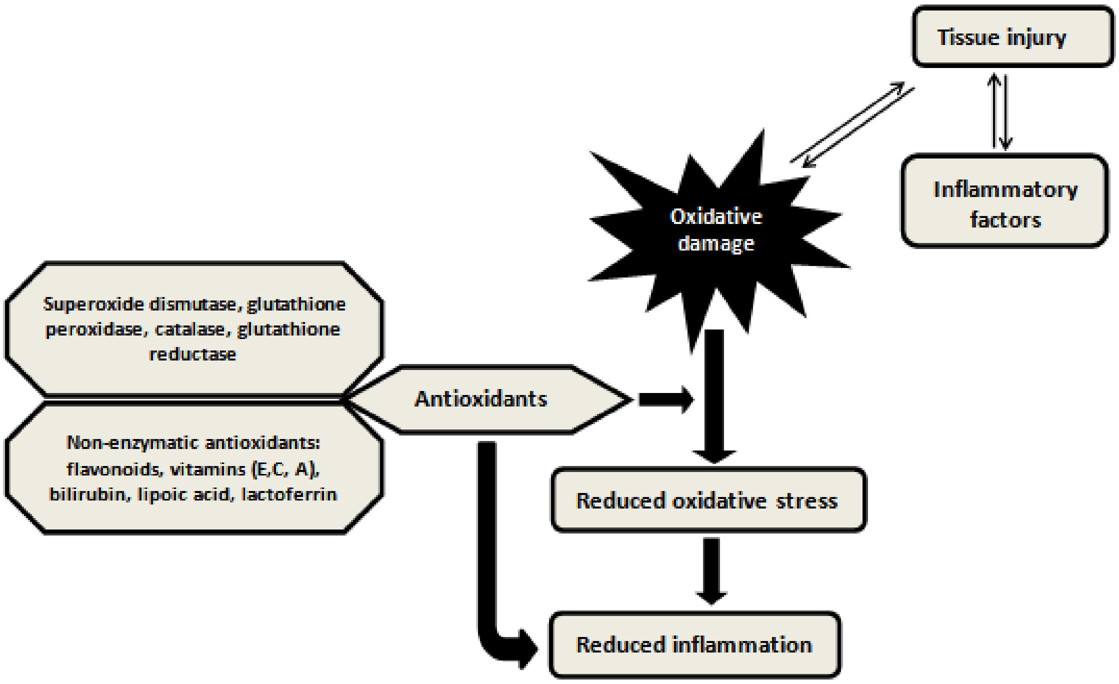

Fig. 7. Overview of antioxidant functions.

V. Conclusions

The citrus fruit peels usually cause waste and are thrown in our country. Therefore, it is essential to explore the use of citrus peel which might be a new window for advance research and helpful to mitigate our waste management. The current research involves the comparative antimicrobial activity of ten citrus fruits peel of Bangladesh. The potential of citrus fruits peel extract as natural antimicrobial agent might be exclusive resource of novel utilization of peel. To produce medicinally and commercially potential species, crossing or hybridization of these citrus peels might be employed. Although different varieties of citrus peel show the efficacy to the organisms in our study, however, the effects were separated on the basis of their purification pattern. Among the ten varieties of fruit peel, methanol treatment shows the higher specificity to the pathogenic organisms rather than petroleum ether extraction of the peels. The results are good insight to give a concept that these peel extracts might be beneficial and have clinical importance because of their essential phytoconstituents.

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