Comparative Study on Antimicrobial and Antioxidant Potential of Peelof *Citruslimon*(L.) and *Citrus aurantiifolia* (Christm.)

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Abstract

Citrus fruits are the main source of important phytochemical nutrients and for long have been valued for their wholesome nutritious and antioxidant properties. Many Citrus species are recognized for their medicinal, physiological, and pharmacologicalactivities including antimicrobial, antioxidant, anticancer, anti-inflammatory, and hypoglycemic activities. In this research, the commonly consumed citrus fruitsnamed*Citrus limon*(Lemon) and *Citrus aurantiifolia*(Lime) were selected to compare their some bioactivities (such as antioxidant and antimicrobial). The preliminary phytochemical investigation of the peel of selected citrus fruits was done by test tube method. The antimicrobial activity of two different citrus fruits peel against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas areuginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* were screened by agar well diffusion method. Antioxidant activity of crude extracts of two different citrus peel were determined by DPPH assay method. Total phenol content of the peel extracts was evaluated by Folin- Ciocalteu method. Total phenol content of watery extract of lime peel was found to be the highest (52.12 µg GAE/mg).

Keywords: *Citrus limon*(Lemon) and *Citrus aurantiifolia*(Lime), antimicrobial activity, antioxidant activity, total phenol

Introduction

The genus Citrus, which belongs to the family of Rutaceae, is rich in generalamong theedible fruits of various species, and they are one of the main fruit tree crops grown worldwide and are well – appreciated for their refreshing juice and health benefits. Numerous therapeutic properties have been attributed to citrus fruits, like anticancer, antiviral, anti-tumor, anti-inflammatory activities, cardiovascular diseases and age relatedmacula degeneration. These numerous health benefits of citrus fruits are linked to the high amounts of phytochemical and bioactive compounds such as flavonoids, carotenoids, vitamins and minerals available in citrus fruits. The citrus species have numerous applications in herbal medicine (Okwu, 2008). Citrus peels are also known to have high levels of phenolics which demonstrate strong antioxidant capability (Divya, 2019). The antioxidant properties of phenolic compounds, their abundance in diet and their probable role in the prevention of the various diseases associated with oxidative stress such as cancer, cardiovascular and degenerative diseases, are the main reasons for their popularity (Lau*et al.*, 2006). Citrus fruits are also known to contain bioactive compounds such as phenolics, flavonoids, vitamins, and essential oils which are believed to be responsible for a range of protective health benefits including antioxidative, anti-inflammatory, antitumor, and antimicrobial activities. In the present research, commonly consumed Citrus fruits namely, *Citrus limon* (Lemon) and also *Citrus aurantiifolia* (Lime) were selected to investigate their extent antioxidant potential and to assess their antimicrobial activity.

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Citrus Fruit (Lemon)

Scientific classification

Family	:	Rutaceae
Genus	:	*Citrus*
Species	:	*limon*
Scientific name	:	*Citrus limon* (L.)Osbeck
English name	:	Lemon
Myanmar name	:	Than-bayo

CitrusFruit (Lime)

Scientific classification

Family	:	Rutaceae
Genus	:	*Citrus*
Species	:	*aurantiifolia*
Scientific name	:	*Citrus aurantiifolia*(Christm.) Swingle
English name	:	Sour lime
Myanmar name	:	Than-baya

(Ancillo and Medina, 2015)

Figure 1.Photographs of (a) lemon (Than-bayo) and (b) lime (Than-bayar)

Materials and Methods

Collection of Sample

Two different common citrus fruits were purchased from a local market of Dagon Township, Yangon Region which was authenticated by the Taxonomist, Department of Botany, Dagon University. The fruits selected include lemon (*Citrus limon*) and lime (*Citrus aurantiifolia*) (Figure 2). After selection, fruits were washed under running tap water followed

by washing with distilled water to remove the surface debris. Then, the peel and pulp of the fruits were separated and the peels were air dried for one week and ground to a fine powder.

Qualitative Phytochemical Analysis

The phytochemical screening was performed by test tube method for testing the different chemical groups present in extracts of peel of all citrus fruits.

Figure 2.Selected citrus fruits

Screening of Antimicrobial Activity of Crude Extracts from Peel of Different Citrus Fruits by Agar Well Diffusion Method

For the examination of *in vitro* antimicrobial activity, solvent extracts such as Pet-ether, MeOH, EtOAc, EtOH and H_2O extracts from *Citrus limon*(Lemon) and *Citrus aurantiifolia*(Lime) were investigated by using agar well diffusion method.

Screening of Antioxidant Activity of Crude Extracts from Peel of Different Citrus Fruits

Free radical scavenging effects of ethanolicand watery extracts from the peel of two different citrus fruits were investigated by using DPPH assay method.

Determination of Total Phenol Content (TPC) of Crude Extracts of Peel of Different Citrus Fruits

One of the antioxidative factors, ethanolicand watery extracts from the peel of two different citrus fruits weremeasured spectrometrically according to the Folin-Ciocalteu Method (Santhosh*et al.,* 2013).

Results and Discussion

Phytochemical Investigation of Citrus Fruits Peel

The phytochemical screening was performed for testing the different chemical groups present in extracts of peel of all Citrus fruits. The phytochemical screening results of the peel extracts of all citrus fruits show that they contain alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, steroids and terpenoids. These secondary metabolites have been shown to have therapeutic activities in plants and function in a synergistic or antagonistic fashion for the treatment of diseases (Trease, 1996).

The results of the analysis are shown in Table 1.

Table 1. Results of Preliminary Phytochemical Investigation of Two Different Citrus Fruits Peel by Test Tube Method

No.	Test	Extracts	Test reagents	Observation	Result	
					L1	L2
1.	Alkaloids	1% HCl	Wagner's reagent	Brown ppt	+	+
			Mayer's reagent	White ppt	+	+
			Dragendroff's reagent	Orange ppt	+	+
2.	α - amino acids	H_2O	Ninhydrin reagent	Light purple spot	+	+
3.	carbohydrates	H_2O	10 % α-naphthol, conc: H_2SO_4	Red ring	+	+
4.	Cyanogenic glycoside	H_2O	conc: H_2SO_4, sodium picrate paper	No colour change	-	-
5.	Flavonoids	EtOH	Mg turning, a few drops of conc: HCl	Pink colour	+	+
6.	Glycosides	H_2O	10% lead acetate solution	White ppt	+	+
7.	Phenolic compounds	H_2O	5%$FeCl_3$ solution	Deep blue colour	+	+
8.	Reducing sugars	H_2O	Benedict's solution	Brick-red ppt	+	+
9.	Saponins	H_2O	Distilled water	No frothing	-	-
10.	Starch	H_2O	I_2 solution	No deep blue	-	-
11.	Steroids	PE	Acetic anhydride & H_2SO_4 (conc:)	Greencolour	+	+
12.	Tannins	H_2O	1 % Gelatin &10 % NaClsolution	Noppt	-	-
13.	Terpenoids	$CHCl_3$	Acetic anhydride, conc: H_2SO_4	Pink colour	+	+

L1 = Lemon L2 = Lime

(+) Presence (-) Absence (ppt) – Precipitate

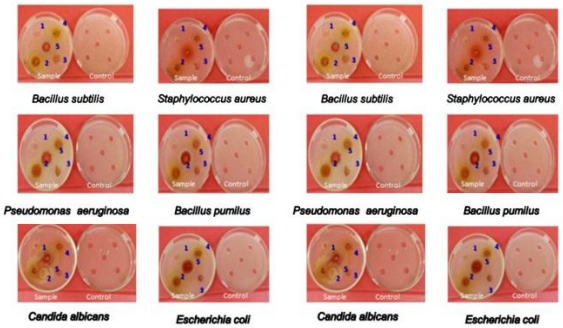
Antimicrobial Activity of Various Crude Extracts of Two Different Citrus Peel

In this research work, screening of antimicrobial activity of various crude extracts such as pet-ether, methanol, ethyl acetate, 95% ethanol and water were done by agar well diffusion method. The microorganisms used were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans*and*Escherichiacoli.* The measurable zone diameter, including the agar well diameter, shows the degree of antimicrobial activity. The larger the diameter of clear zone, the more potent the antimicrobial activity.

It was found that the aqueous extracts of two different citrus species peel possess antimicrobial activity. Inhibition zone ranges were 14 ~ 19 mm and 13 ~ 20 mm for lemon and

lime respectively. Watery extract of *C. limon (*lemon) peel had potent inhibition zone 19 mm against*E.coli*and *C. aurantiifolia*(lime) had 20 mm against *P.aeruginosa.* So, aqueous extracts have an important role as antimicrobial agents against microorganisms. They are natural, cheap, safe, and due to increase antibiotic resistance among bacteria.

The photographs showing the inhibition zone provided by crude extracts against six species of microorganisms are presented in Figures 3(a) and (b). The antimicrobial activities of two different citrus species peel were shown in Tables 2and 3.



(a) (b)

1 – PE extract 2 – MeOH extract 3 – EtOAc extract4 – EtOH extract5 – H_2O extract

Figure 3. Antimicrobial activity of various extracts (a)*C.limon*(Lemon)and (b) *C.aurantiifolia* (lime)peelson six microorganisms

Table 2. Inhibition Zone Diameters of Crude Extracts of *Citrus limon*(Lemon)Peelagainst Six Microorganisms by Agar Well Diffusion Method

Microorganisms	**Inhibition Zone Diameter (mm)**				
	PE	**MeOH**	**EtOAc**	**EtOH**	**H_2O**
Bacillus subtilis	15	17	16	17	14
Staphylococcus aureus	-	15	-	12	14
Pseudomonas aeruginosa	15	20	13	17	17
Bacillus pumilus	13	16	12	15	18
Candida albicans	-	13	15	12	18
Escherichia coli	12	15	14	15	19

Table 3. Inhibition Zone Diameters of Crude Extracts of the Peel of *Citrusaurantiifolia*(Lime)against Six Microorganisms by Agar Well Diffusion Method

Microorganisms	**Inhibition Zone Diameter (mm)**				
	PE	**MeOH**	**EtOAc**	**EtOH**	**H_2O**
Bacillus subtilis	16	19	13	15	14
Staphylococcus aureus	-	13	12	-	13
Pseudomonas aeruginosa	14	20	13	15	20
Bacillus pumilus	15	18	14	16	16
Candida albicans	-	-	-	12	17
Escherichia coli	12	18	12	16	14

Agar well – 10 mm

10 mm ~ 14 mm mild activity, 15 mm ~ 19 mm medium activity

20 mm above highest activity

Antioxidant Activity of Crude Extracts of Two Different Citrus Peel by DPPH Assay

Free radical scavenging effects of ethanolicand watery extracts from the peel of two different citrus fruits were investigated by using DPPH assay method.

This method is based on the capability of test sample to inhibit stable free radical that was followed by decrease in absorbance at λ_{max} 517 nm. Low absorbance value of test sample indicates high % inhibition against DPPH radical which in turn reveals high radical scavenging activity of that sample.

In this work, six different concentrations of (6.25, 12.5, 25, 50, 100 and 200 μg/mL) for each extract were prepared and determined for their radical scavenging activities. Ascorbic acid was used as a standard antioxidant for comparison purpose.

The absorbance and % inhibition of different concentrations of different extracts of the citrus were tabulated in Table 4. The data were compared to that of standard ascorbic acid. For each sample, it was found that the higher the concentration of extract, the lower the absorbance of DPPH solution and the greater the radical scavenging activity of the extract. The plot of % inhibition versus concentration of different extracts was shown in Figure 4.

Therefore, the lower value of IC_{50} indicates the higher antioxidant potential. Figure 5 shows the histogram of the comparison of IC_{50} values of crude extracts with standard ascorbic acid. According to the IC_{50} value, ethanolic extract of lime peel (IC_{50} = 81.59 μg/mL)is the highest antioxidant activity followed by watery extract of lime peel (IC_{50} = 118.89 μg/mL), watery extract of lemon peel (IC_{50} = 184.13 μg/mL) and ethanolic extract of lemonpeel(IC_{50} = 197.65 μg/mL) respectively.

Table 4. % Inhibition of Ethanolic and Watery Extracts from Two Different Citrus Fruits Peel and Standard Ascorbic Acid

Test sample		% inhibition in various concentrations (µg/mL) ± SD						IC_{50} (µg/mL)
		6.25	**12.5**	**25**	**50**	**100**	**200**	
Lemon	EtOH extract	31.12 ± 1.17	32.79 ± 0.17	34.58 ± 1.41	36.63 ± 1.17	42.51 ± 0.58	50.18 ± 1.41	197.65
	H_2O extract	26.65 ± 3.36	27.59 ± 3.08	29.45 ± 3.13	31.43 ± 3.43	40.46 ± 2.71	51.8 ± 3.61	184.13
Lime	EtOH extract	25.53 ± 2.68	34.82 ± 2.14	43.32 ± 2.33	47.41 ± 3.92	51.51 ± 2.66	54.70 ± 2.24	81.59
	H_2O extract	3.85 ± 1.93	7.08 ± 1.56	13.29 ± 2.86	26.98 ± 3.24	44.02 ± 2.68	76.9 ± 1.41	118.19

Standard ascorbic acid	% inhibition in various concentrations (µg/mL)					IC_{50} (µg/mL)
	2.5	**5**	**7.5**	**10**	**12.5**	
	26.08	46.05	69.51	90.77	91.42	5.3

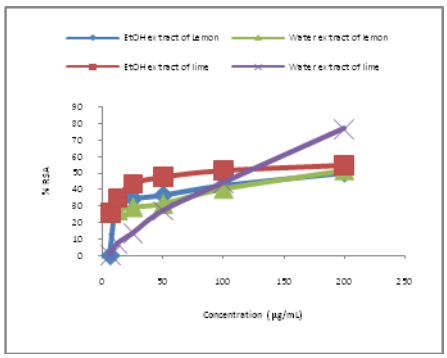


Figure 4. Percent radical scavenging activity (% RSA) vs concentration of ethanolic and watery extracts from two different citrus peel

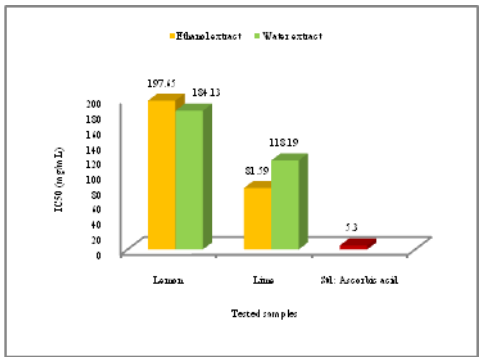


Figure 5. IC_{50} values of ethanolic and watery extracts of two different citrus peel

Total Phenol Contents of Crude Extracts of Two Different Citrus Fruits by Folin- Ciocalteu Method

Phenolic compounds are a diverse group of secondary metabolites and may contribute directly to antioxidant action. Folin-Ciocalteu (F.C) reagent was used to determine total polyphenol in sample extracts. The total phenol contents (TPC) of ethanol and water extracts were determined in terms of µg of gallic acid equivalents per mg (µg GAE / mg) and the results were displayed in Tables 5and Figure 6. Phenolic compounds have redox properties which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenol concentration could be used as a basis for rapid screening of antioxidant activity.

Watery extract of lime peel had the highest total phenolic content (52.12 µg GAE/mg) followed by ethanolic extract of lime peel (45.57 µg GAE/mg), watery extract of lemon peel(39.95 µgGAE/mg) and ethanolic extract of lemon peel (25.26 µg GAE/mg) were respectively. So, water is a good solvent for extraction of phenolic compounds than ethanol in citrus fruits peel and the presence of large amount of phenolic compounds are responsible for the most pronounced antioxidant activity of the extract.

Table 5. Total Phenol Content (TPC) of Ethanol and Water Extracts of Two Different Citrus Peel byFolin-Ciocalteu Method

Extracts		Mean absorbance ± SD	TPC (µg GAE/mg)
Lemon	EtOH	0.3299 ± 0.0071	25.26
	Water	0.540 ± 0.0029	39.95
Lime	EtOH	0.6204 ± 0.0206	45.57
	Water	0.714 ± 0.0038	52.12

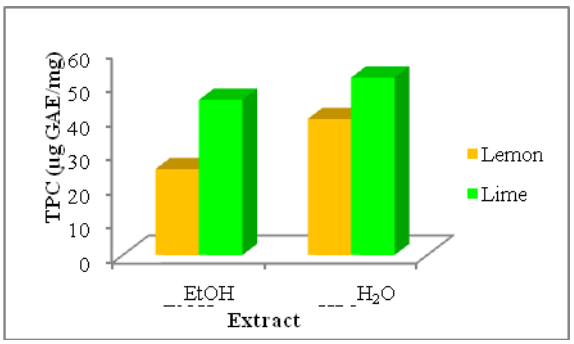


Figure 6. Histogram of total phenol contents of ethanol and water extracts of two different citrus peel

Conclusion

The preliminary qualitative phytochemical investigation documented that the peel extracts of two citrus fruits namely *Citrus limon*(Lemon) and also *Citrus aurantiifolia*(Lime) showed the presence of many bioactive compounds, namely, alkaloid, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars and terpenoids. Cyanogenic glycoside, saponins, starch and tannins are absent in all citrus fruits.

The antimicrobial activity of two different citrus fruits peel against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas areuginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* were screened by agar well diffusion method.The methanol, ethanol and aqueous extract ofcitrus peel possessed potent activity than that of other extracts. It was also found that aqueous extracts of lime peel is an important role as antimicrobial agents against microorganisms. They are natural, cheap, safe, and due to increase antibiotic resistance among bacteria.

The antioxidant activities of ethanolic and watery extracts of two citrus specieswere evaluated by DPPH free radical scavenging assay. In the present work, ethanolic extract of lime peel is the highest antioxidant activity with IC_{50}= 81.59μg/mL.

Total phenol contents of ethanolic and wateryextracts were measured by Folin- Ciocalteu method. Among peel extracts, watery extract of lime is highest TPC content. Total phenol contents of watery extract of lime was found to be the highest (52.12 μg GAE/mg).

In contrast, limepeelcontainshigh antioxidant activity compared to the peel of lemon citrus fruit used in this study. In general, the high content of antioxidant capacity and activity of citrus waste, particularly the lime peel, indicated that they may impart health and nutritional benefit when involving in the food industry as a natural antioxidant.

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