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Extracts from unripe and ripened peels of *Citrus limon* reveal variation in composition of bioactive compounds and exhibit antibacterial activity in relation to different extraction solvents

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ABSTRACT

The goal of present study was to appraise the bioactivity of compounds using TFC (total flavonoid content), DPPH scavenging activity, reducing power assay and ABTS (radical scavenging activity) of different solvent extracts, from *Citrus limon* (unripe and ripened) peels. In unripe peels the total flavonoid contents, DPPH radical scavenging activity, ABTS radical scavenging activity and reducing power ranged from 18.23-57.56 mg/g CE (catechin equivalent), 21.69-65.82%, 38.96-84.83% and 0.01-1.8 mg /mL, respectively. For ripened lemon peels all these indicators ranged from 10.53-47.88 mg/g CE, 37.99-81.40%, 42.96-95.93% and 0.07-1.47 mg /mL respectively. Lemon peel extracts exhibited good antimicrobial activity against *Escherichia coli, Bacillus subtilus*, *Staphylococcus aureus* and *Salmonella typhimurium*. Overall ripened lemon peel extracts showed higher antioxidant activity than unripe peels. The data presented in present study is an important factor to select ripened lemon peels as high potential values for nutraceutical, pharmaceutical and cosmetic industries.

Key Words: *Citrus limon*, Extraction solvent system, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Citrus limon is commonly available in Pakistan and generally known as *limo.* It has considerable economic value for its peels essential oil and is documented to be the source of many bioactive compounds such as minerals, vitamin C, flavonoids, phenols, limonoids, folic acid (Deyhim *et al.,* 2006). Lemon peels bioactive compounds have been used for their antioxidant, germicidal and anticarcinogenic activities (Guenther, 1948; Mukhopadhyay, 2000).

Presently, world over attention is focused to extract valued compounds from natural sources so as to explore their commercial uses in cosmetics, medicines and food protection. C. *lemon* fruit peels, are a possible source of numerous bioactive compounds such as flavonoids, tannins, and specifically limonoids which are infrequent to other plants. These components have significant biological activities including antioxidant, antimicrobial, anti-inflammatory and anti-cancer (Sikora *et al.,* 2008).

Extraction is the chief significant step in the recovery and purification of active constituents from plant materials (Delfanian *et al.,* 2015). Solvent extraction technique is a traditional method for extraction and is more regularly used for the separation of bioactive compounds. In this process, extraction yield of bioactive compounds is reliant on conditions of extraction and the solvent polarity etc.

Extract yield is dependent on method of extraction and nature of solvent used (Goli *et al.,* 2004). The extraction process must allow the complete extraction of required compounds and should avoid chemical modification of compound of interest (Zuo *et al.,* 2002). Various solvent systems are used for extraction of phenolic compounds from plant extracts (Chavan *et al.,* 2001).

Some recent studies have shown that *C. limon* is a rich source of flavonoids, phenolic compounds and essential oil, hence it would be interesting to evaluate different solvent extracts to maximally obtain the valuable bioactive compounds found in it. In this scenario, this particular project was initiated to obtain and analyze the different chemical constituents obtained from unripe and repined peels of lemon

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by applying various organic solvents and their dilution. To the best of our knowledge no such comparative study has yet been reported *on C. limon.* The commonly used solvent extraction technique is utilized to separate antioxidant compounds from peels. This report describes the antioxidant properties of different extracts from unripe and ripened lemon peel by using *in-vitro* antioxidant assays.

MATERIALS AND METHODS

Plant material

Unripe (110 days of fruiting) and ripened (150 days of fruiting) *C. limon* peels were harvested at two different time intervals from local farms of district Sargodha. Lemons were peeled manually and dried at ambient conditions.

Chemicals and reagents

Analytical grade chemicals of Sigma-Aldrich Chemical Corporation, Germany including Trichloroacetic acid (TCA), Ascorbic acid, Catechin, Methanol, Butylated hydroxyl tolune (BHT), Distilled water, Ferric chloride, Acetone Potassium ferricynide, Sodium carbonate, Sodium dihydrogen phosphate, Sodium nitrite, Sodium hydroxide, Trichloroacetic acid (TCA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS, MnO_2 Nitrium hydrogen phosphate, n-Hexane, Broth, nutrient agar, Folin-Ciocalteu reagent were used in this work.

Preparation of extracts and determination of antioxidant activity

Extraction

Dried sample of unripe and ripened lemon peels were ground into fine powder in a grinder. Pulverized peel sample of both unripe and ripened peels (20g) were individually mixed with 200mL of extraction solvents;100 % methanol (pure methanol), 100% ethyl acetate(pure ethyl acetate), 100% chloroform(pure chloroform), 70 % methanol(methanol:water,70:30 v/v) 50% methanol (methanol:water,50:50v/v),70% ethyl acetate (ethyl acetate:water,70:30 v/v), 50% ethyl acetate (ethyl acetate:water, 50:50 v/v),70 % chloroform (chloroform:water,70:30 v/v) 50% chloroform (chloroform:water,50:50 v/v) in conical flasks. Extraction was carried out in an orbital shaker (Optima OS-752) for 27 hours. Each extract was filtered and solvents were evaporated using rotary evaporator (HB Heidolph digital laborota 4001 efficient) under reduced pressure. The concentrated crude extracts of unripe and ripened lemon peels were stored under refrigeration for further analysis.

Determination of TFCs

The total flavonoid contents (TFCs) were calculated by a method stated by (Zhishen *et al.,* 1999). For TFC, 1mL crude extract aqueous solution (10mg/1mL) was taken and filled up to 5mL with distill water in 10 mL volumetric flask. Then (0.3 mL of $NaNO_2$ 1:20) was added in each mixture and incubated for 5 minutes at room temperature. After 5 minute incubation, 0.3 mL of $AlCl_3$ (1:10) was added in sample and again kept at room temperature for 6 minutes and then a 2mL of NaOH(1M) was added and filled up to 10 mL using distilled water. Absorbance was measured at 510 nm against blank (having all chemicals in equal amount without extract). The results were reported as CE (Catechin Equivalent) mg/g of DW (Dry Weight). Absorbance was measured three times for each sample and then average mean reading was obtained. Each test was performed in triplicates.

Assessment of reducing power of crude extracts

The reducing power of all extracts was determined by using method described by (Jayaprakasha *et al.,* 2008) with slight modifications. Different concentration of extracts (2-10) mg were made in a mixture (1:1) phosphoric acid buffer (0.2 M, pH 6.6) and Potassium ferricyanide (1%) ($K_3Fe(CN_6)$. These were mixed and placed for 20 min at 50^{o}C in water bath and then chilled rapidly with ice. Then added 1 mL 10% trichloroacetic acid to each concentration and allowed to react for 10 minutes in dark. Then 1 mL of distilled water and 0.8 mL of 0.1% ferric chloride (FeCl3) was added and incubated in dark for further 10 min. Absorbance of reaction mixtures was taken at 700 nm. Absorbance value relates positively with reduction power. The reduction ability tests were run in triplicate.

Estimation of ABTS radical scavenging activity

The ABTS radical scavenging activity was determined by using the procedure reported by (Proestos *et al.,* 2013). First ABTS was dissolved in distilled water and made 7mM solution. MnO_2 solution of 2.45 mM was prepared. Both solution were mixed in 1:1 ratio and kept it in the dark for 24 hours. 1ml of ABTS was added to 25ml of aqueous methanol. A volume of 20µL (diluted 1:10) of aqueous plant extract was added to two mL of ABTS•+ radical cation solution, and the mixture was kept at a standard temperature of 30 °C. The absorbance was taken at 734 nm directly. The results were measured as % inhibition of ABTS•+ radical cation by dried sample. The

following formula was used to calculate percentage radical scavenging activity.

$$I\% = \frac{AO - AS}{AS} \times 100$$

Where I = ABTS•+ inhibition (%), A0 = absorbance of control and AS = absorbance of a tested sample.

Determination of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity

Antioxidant potential taken as DPPH radical scavenging capacity assay was calculated using method of Clarke *et al.,* 2013. Briefly 20µL of plant extract solution (4mg/mL) was taken and then added to 180 µL of DPPH solution (40 µg/mL). The Mixture was incubated for 15 min in the dark and measured spectrophotometrically at 540 nm by UV- Vis spectrophotometer and the results were expressed as % inhibition of dried sample. The % inhibition was calculated as

$$I\% = \frac{AO - AS}{AS} \times 100$$

I = (% inhibition of DPPH), Ao = (absorbance of control sample) (t = 0 h) and AS = (absorbance of a tested sample at the end of the reaction) (t = 15 minutes). Results were calculated as % inhibition of DPPH radical scavenging activity; maximum values of DPPH scavenging are associated with stronger antioxidant activity.

Evaluation of antimicrobial activity

Antimicrobial activity of lemon peel extracts

Antimicrobial activity of all extracts was calculated using method described by Afzal *et al.,* 2014. Bacterial strains used were *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. Briefly, 100mL inoculum of each bacterial strain was poured into the nutrient agar. Then filter paper discs of 6mm were placed on medium and each disc was loaded with 100 µl of sample extract followed by incubation at 37 ˚C for 24 hours. Inhibition zones were developed by extracts showing antimicrobial activity.

Statistical analysis

Two way ANOVA was employed through the software SPSS 16.0 and evinced the whole data as mean ± standard deviation. Significant differences for mean values were determined and $p>0.05$ revealed non-significant difference.

RESULTS AND DISCUSSION

Yield of crude extracts

The extraction yield for antioxidant constituents from lemon with methanol (100, 70 and 50 %), ethyl acetate (100, 70 and 50 %) and chloroform (100, 70 and 50 %) has been presented in Table I. The extract yield of different unripe lemon peel extract varied over a range of 5.29 – 28.12 % (g / 100g). Maximum yield (28.12 %) was obtained with 70 % methanol whereas least yield (5.29%) with 100 percent chloroform. So, it can be considered that there is significant difference ($p < 0.05$) in relation to the different solvents used for extraction of antioxidant components from lemon. The present results showed 17.48 % extract yield of mature lemon peels with 100 % methanol which was in exact agreement with the percentage yield reported by Sekar *et al*., (2013) for methanolic extracts of mature lemon peels. Sultana *et al*., (2014) reported 16.62% extraction yield of clove with 70 % methanol but that percentage yield was lower than that observed in our present study of unripe and ripened lemon peels which was 28.12 and 20.54 % respectively. The present results also showed higher extraction yield with methanol as compared to chloroform which was comparable to percentage yield of methanol and chloroform extract of thyme reported by (Hossain *et al*., 2013). Sultana *et al*. (2007) reported 11.3% extract yield of corncob with ethyl acetate which was greater than present results of 100% ethyl acetate of unripe and ripened which were 8.13, 7.34 %, respectively.

Present results showed higher percentage yield of ripened lemon peels than unripe lemon peels which was in agreement with those reported by Gull *et al*., 2012 for unripe and fully ripe guava fruit. Kumar *et al.,* (2011) reported the lemon peels extract yield of 18% using ethyl acetate as solvent which was higher than our results of using 100% ethyl acetate but lower than 50 % ethyl acetate yield (20.20%).

Table I: Extract yields (g/100g) from different peel extracts of *Citrus limon.*

Solvent	Unripe peels			Ripened peels		
	100%	70%	50%	100%	70%	50%
MeOH	19.28±0.36***	28.12±0.13***	26.49±0.48***	17.48±0.34***	20.54±0.39***	17.57±0.50***
EtOAc	8.13±0.11***	7.11±0.12***	13.6±0.38***	7.34±0.48***	15.27±0.42***	20.20±0.177***
$CHCl_3$	5.29±0.27***	7.04±0.06***	5.14±0.17***	6.52±0.46***	13.29±0.27***	16.32±0.44***

- Values represent mean ± standard deviation (n=3) of three separate samples of extracts individually analyzed
- Stars show significance, p<0.001=***, p<0.01=**, p<0.5=*, ns=non-significant.

Total flavonoid contents

Total flavonoid contents from different unripe lemon peel extracts (Table II) obtained by using different solvents such as methanol, ethyl acetate and chloroform at different concentration (100% ,70% and 50%) ranged from 18.23 to 54.76 CE (mg/g) of dry weight (DW). Details are given in Table II. The lowest value of ripened lemon peel was obtained for 100% chloroform extract of ripened lemon peel (10.53 CE mg/g), while highest value was obtained for 100 % methanol extract of unripe lemon peel (47.88CE mg/g).

Eghdami *et al.* (2013) reported higher flavonoid contents of methanolic extracts of thyme which is in good agreement with our trend of methanolic extracts of unripe and ripened lemon peel extracts of present study. Present work also showed higher total flavonoid contents with methanolic extracts followed by chloroform and ethyl acetate which are also supported by the results of Hossain *et al.,* 2013 for thyme extracts using methanol, chloroform and ethyl acetate as solvents.

Results of present study showed higher flavonoid contents in unripe lemon peels than ripened one, which agree with results obtained by Gull *et al.,* (2012) for different stages of ripening of guava fruit but are not in agreement with results reported by Mahmood *et al.,* (2013) that cherry fruits contain higher flavonoid contents at ripening stage than at un ripened stage.

The higher concentrations of total flavonoid compounds of present study in younger un-ripe lemon fruit as compared to those in fully-ripe lemon fruits can be explained by the fact during ripening, at various stages different phenolic acid compounds might be condensed to form complex phenolic acids such as lignin and tannins etc (Ben-Ahmed *et al.,* 2009). So, due to conversion of phenolic acids to complex phenolic compounds during maturity, ripened fruits possess lower concentrations of flavonoids than un-ripe fruits. Differences in total flavonoid contents of lemon peels at two different stages of maturity could also be explained by a report that stated that phenolic presence was influenced by species genetic makeup, growing conditions, soil circumstances and nutrients availability at harvesting stages (Jaffery *et al.,* 2003).

Table II: Total flavonoid contents (CE mg / g (DW) of different extracts from *Citrus limon.*

Solvent	Unripe peels			Ripened peels		
	100%	70%	50%	100%	70%	50%
MeOH	54.76±0.68***	48.03±0.05***	41.77±0.69***	47.88±0.83***	33.22±0.38***	38.2±0.38***
EtOAc	23.16±0.28***	40.06±0.11***	36.37±0.25***	27.33±0.83***	31.33±0.57***	29.30±0.51***
$CHCl_3$	18.23±0.40***	34.77±0.69***	41.26±0.46***	10.53±0.50***	32.03±0.06***	37.23±0.40***

- Values represent mean ± standard deviation (n=3) of three separate samples of extracts individually analyzed
- Stars show significance (p<0.001=***, p<0.01=**, p<0.5=*, ns=non-significant)

DPPH radical scavenging activity

The % DPPH radical scavenging activity was carried out for unripe lemon peels extracts of different solvents: methanol, ethyl acetate and chloroform at varying concentrations (100,70 and 50 %) ranged from 21.69 to 65.82 % (Table III). The lowest value of unripe lemon peel was obtained for 50% chloroform (21.69 %) while highest value was obtained for 100% methanol extract of unripe lemon peel (65.82 %). Likewise % DPPH radical scavenging activity for ripe lemon peels was determined by using various solvents: methanol, ethyl acetate and chloroform in varying concentrations (100, 70 and 50 %) ranged from 37.99 to 81.40 %. The lowest value obtained for ripened lemon peels was for 100% methanol (37.99 %) and highest for 100 % ethyl acetate (81.40 %). Details are given in Table III. Substantial rise in reducing potential was observed in relation to the ripening/maturity of fruit. The present results showed significant rise in DPPH scavenging activity at progression of maturity which is also supported by the previous study on cherry fruits at varying stages of ripening (Mahmood *et al.,* 2013). Our trend of increasing DPPH radical scavenging activity was not in agreement to the results shown by other authors for various ripening stages of guava orange and lemon juice (Gull *et al.,* 2012; Omoba *et al.,* 2015; Kumari *et al.,* 2014).

Table III: DPPH radical scavenging activity of different extracts from *Citrus limon.*

Solvent	Unripe peels			Ripened peels		
	100%	70%	50%	100%	70%	50%
MeOH	65.82±0.75***	50.46±0.50***	51.59±0.52***	37.99±0.11**	55.89±0.84***	61.66±0.57***
EtOAc	44.97±0.95***	43.98±0.97***	62.18±1.04***	81.40±0.52***	77.67±0.58***	76.52±0.50***
$CHCl_3$	32.50±1.32***	60.49±0.50***	21.69±0.59***	47.68±0.59***	68.78±0.69***	77.97±0.95***

- Values represent mean ± standard deviation (n=3) of three separate samples of extracts individually analyzed
- Stars show significance (p<0.001=***, p<0.01=**, p<0.5=*, ns=non-significant)

ABTS radical scavenging activity

The ABTS radical scavenging action detected for unripe lemon peels using 100, 70 and 50 % of Methanol, ethyl acetate and chloroform (100, 70 and 50 %) ranged from 38.96 to 84.43 % (Table IV).The lowest value of unripe lemon peel was obtained for 100% ethyl acetate (38.96 %) whereas maximum value was obtained for 100 % methanol extract (84.43%). Likewise ABTS radical scavenging activity for ripened lemon peels using various solvents: methanol, ethyl acetate and

chloroform at varying concentrations (100, 70 and 50 %) ranged from 43.31 to 96.81 %. The lowest value obtained (Table IV) for ripened lemon peels were for 70% ethyl acetate (43.31 %) and highest for 50 % chloroform (88.53 %). Our present findings showed that ripened lemon peels possessed higher ABTS radical scavenging activity than unripe which was in good agreement to the work done by (Omoba *et al.,* 2015) for unripe and ripened orange peels**.**

Table IV: ABTS radical scavenging activity of different extracts from *Citrus limon*

Solvent	**Unripe peels**			**Ripened peels**		
	100%	70%	50%	100%	70%	50%
MeOH	84.43±0.38***	50.33±0.57***	72.96±0.94***	89.00±0.95***	68.64±0.72***	64.31±0.30***
EtOAc	38.96±0.95***	41.84±0.77***	58.31±0.30***	42.96±0.94***	43.36±0.38***	60.76±0.30***
$CHCl_3$	75.33±0.57***	60.38±0.56**	70.38±0.53***	75.58±0.52***	58.40±0.52***	95.93±0.90***

- Values represent mean ± standard deviation (n=3) of three separate extracts of extracts individually analyzed
- Stars in above table shows significance (p<0.001=***, p<0.01=**, p<0.5=*, ns=non-significant)

Reducing power assay

The lowest and highest reduction potential of unripe and ripened lemon peel extracts measured at concentration range of 2-10 mg/mL was in the range of 0.009-1.62 and 0.075-1.47, respectively (Fig., 1A and 1B). Methanolic, ethyl acetate and chloroform extracts of unripe lemon peels showed highest reductive potential of 0.853, 1.65 and 1.77 at 10 mg/mL, respectively. Similarly the ripened lemon peel extracts of methanol, ethyl acetate and chloroform showed highest reductive potential of 0.67, 1.21 and 1.47 at 10 mg/mL, respectively. Relation between the reduction potential and concentration was linearly increasing as shown in graph which was in good agreement to previous report (Manzoor *et al.,* 2013).

Antimicrobial activity by disc diffusion method

Lemon is considered an important medicinal plant cultivated for alkaloids which shows antibacterial and anticancer activities (Pandey *et al.,* 2011). Antimicrobial activity of different extracts of lemon peels was determined using disc diffusion method. Ciprofloxacin was used as a standard in present study.

Zones of inhibition of different unripe and ripened methanolic (100%, 70% and 50%) of lemon peel extracts against different bacterial strains were found to be in following ranges: 5.50 – 28.83mm against *Bacillus subtilus*, 3.63 – 21.70mm against *Staphylococcus aureus*, 1.00-18.60mm against *Salmonella typhimurium* and 3.00 – 22.00mm against *Escherichia coli* (Table V (A)).

Zones of inhibition of different unripe and ripened ethyl acetate (100%, 70% and 50%) lemon peel extracts against different bacterial strains were found to be in following ranges: 2.23 – 18.27 mm against *B. subtilus*, 5.43 – 11.33mm against *S. aureus*, 10.06-23.53mm against *Salmonella typhimurium* and 5.18 – 16.23mm against *E.coli* (Table V (B)) .

Zone of inhibition of different unripe and ripened chloroform (100%, 70% and 50%) lemon peel extracts against different bacterial strains were found to be in following ranges: 10.20 – 30.43 mm against *B. subtilus*, 10.30 – 31.33mm aginst *S. aureus*, 5.1-30.13mm against *S. typhimurium* and 0 – 10.11mm against *E. coli* (Table V (C).

Present study of methanolic extracts of unripe lemon peel extracts showed good antimicrobial activity against *E. coli* and *S. aureus* which is greater than that reported by Pandey *et al*., (2011) for methanolic and ethyl acetate extracts of lemon peels against various strains of bacteria. While ethyl acetate extracts activity of lemon peels against *Escherichia coli* and *Staphylococcus aureus* observed in present study was lower than that reported by Pandey *et al*., (2011) for lemon peel extracts. Kumar *et al*., (2011) reported the antimicrobial activity of ethyl acetate extracts of lemon peels against all four strains: *Bacillus subtilus, Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium* which was higher than our present research findings.

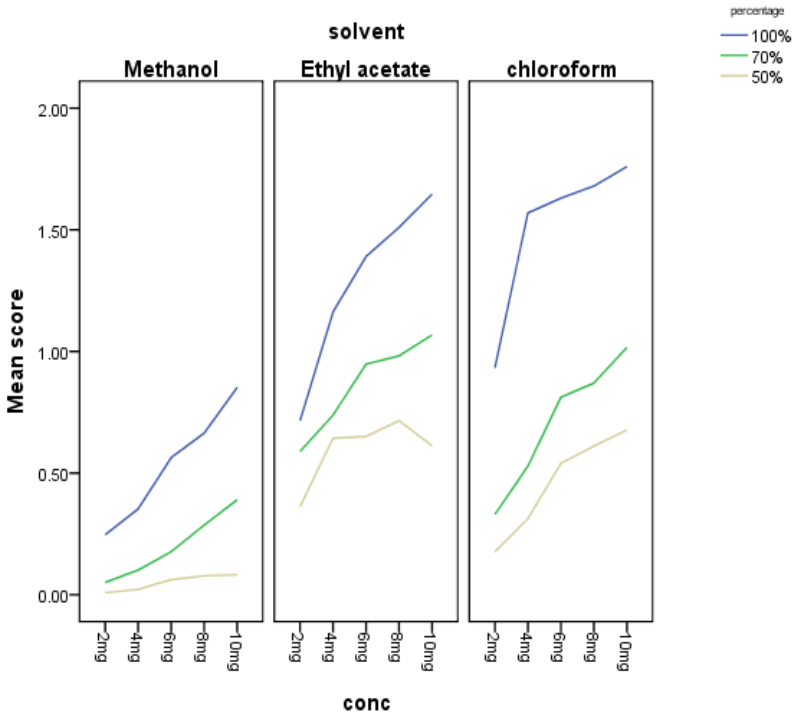
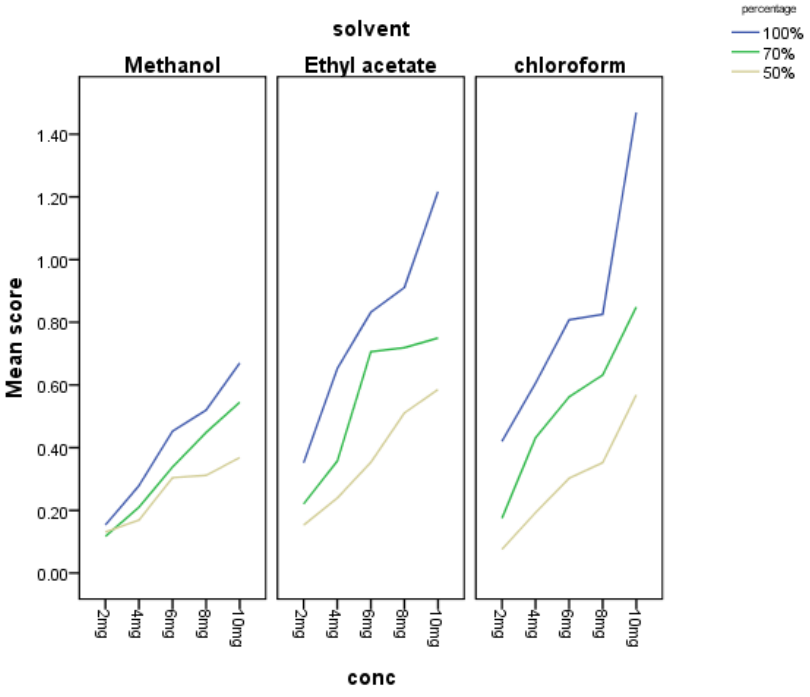


Fig., 1A: Reducing power assay of Unripe *Citrus limon*



peel

Fig., 1B: Reducing power assay of Ripened Citrus Limon peels

Table V (A): Antimicrobial activity of methanolic extracts of *Citrus limon*

Sample	Solvents	Bacterial strains			
		Bacillus subtilus ZOI(mm)	*Staphylococcus aureus* ZOI(mm)	*Salmonella typhimurium* ZOI(mm)	*Escherichia coli* ZOI(mm)
Unripe peels	100% MeOH	10.39±0.53***	10.33±0.57***	18.60±0.52***	10.66±0.57***
	70% MeOH	28.83±0.76***	20.26±0.30***	1.00±0.01***	22.00±1.00***
	50% MeOH	5.50±0.50***	21.70±0.60***	5.50±0.50***	4.46±0.50***
Ripened Peels	100% MeOH	15.63±0.55***	3.63±0.55***	18.03±0.05***	12.07±0.12***
	70% MeOH	10.33±0.57***	12.50±0.50***	16.23±0.25***	15.20±0.34***
	50% MeOH	20.33±0.57***	11.33±0.57***	3.1±0.17***	3.00±0.10***
Drug	Ciprofloxacin	40.00±0.01***	30.10±0.00***	31.00±0.00***	40.08±0.00***

- Values represent mean ± standard deviation (n=3) of three separate extracts of extracts individually analyzed
- Stars show significance (p<0.001=***, p<0.01=**, p<0.5=*, ns=non-significant)

Table V (B): Antimicrobial activity of ethyl acetate extracts of *Citrus limon*

Sample	Solvents	Bacterial stain			
		Bacillus subtilus ZOI(mm)	*Staphylococcus aureus* ZOI(mm)	*Salmonella typhimurium* ZOI(mm)	*Escherichia coli* ZOI(mm)
Unripe peels	100% EtOAc	22.3±0.20***	10.53±0.47***	10.31±0.30***	5.18±0.31***
	70% EtOAc	10.63±0.55***	5.43±0.40***	20.33±0.57***	7.55±0.48***
	50% EtOAc	5.07±0.06***	11.33±0.57***	10.06±0.11***	16.23±0.19***
Ripened Peels	100% EtOAc	12.33±0.57***	8.10±0.17***	10.20±0.34***	10.14±0.25***
	70% EtOAc	18.27±0.25***	8.14±0.25***	23.53±0.50***	12.23±0.40***
	50% EtOAc	23.3±0.32***	10.20±0.34***	22.30±0.36***	12.40±0.40***
Drug	Ciprofloxacin	48.00±0.00***	32.00±0.03***	40.10±0.00***	40.00±0.01***

- Values represent mean ± standard deviation (n=3) of three separate extracts of extracts individually analyzed
- Stars show significance (p<0.001=***, p<0.01=**, p<0.5=*, ns=non-significant)

Table V (C): Antimicrobial activity of chloroform extracts of *Citrus limon*

Sample	Solvents	Bacterial stain			
		Bacillus subtilus ZOI(mm)	*Staphylococcus aureus* ZOI(mm)	*Salmonella typhimurium* ZOI(mm)	*Escherichia coli* ZOI(mm)
Unripe peels	100% $CHCl_3$	20.10±0.10***	2.03±0.05***	21.13±0.15 ns	3.10±0.10***
	70% $CHCl_3$	22.00±1.00***	10.30±0.30***	20.44±0.50***	0.00±0.00***
	50% $CHCl_3$	30.43±0.15***	31.33±0.57***	5.1±0.17***	10.10±0.17***
Ripened Peels	100% $CHCl_3$	20.38±0.54***	15.23±0.40***	21.50±0.50***	2.00±0.10***
	70% $CHCl_3$	10.20±0.26***	20.33±0.57***	20.18±0.31***	5.18±0.15***
	50% $CHCl_3$	14.83±5.92***	15.13±0.15***	30.13±0.15***	10.11±0.10***
Drug	Ciprofloxacin	48.00±0.00***	40.08±0.10***	40.03±0.57***	40.01±0.56***

- Values represent mean ± standard deviation (n=3) of three separate extracts of extracts individually analyzed
- Stars show significance (p<0.001=***, p<0.01=**, p<0.5=*, ns=non-significant)

CONCLUSION

Current research project provides a comprehensive study on antioxidant potential of various extracts of unripe and ripened lemon peels cultivated in Pakistan. It has been concluded from results that antioxidant activity of *Citrus limon* (unripe and ripened) peels was greatly affected during ripening stages and nature of solvents that were employed to obtain bioactive compounds. Based on present findings it can be concluded that ripened lemon peels has high potential value for development and supply of highly valuable compounds. It can be said that assessment of antioxidants characteristics of *Citrus limon* peels needs selection of appropriate extraction solvent and multiple assays analysis. As such both the time of harvesting fruits and nature of extraction solvent has prominent effects on the antioxidant and phenolics compounds from citrus peels. Generally, the results showed that limon peel extracts were a potential source of natural antioxidants and exhibited potential for antibacterial activities.

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