ANTIMUTAGENIC AND ANTIOXIDANT POTENTIAL OF AQUEOUS AND ACIDIFIED METHANOL EXTRACTS FROM *CITRUS LIMONUM* FRUIT RESIDUES

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ABSTRACT

Aqueous and acidified methanol extracts from *C. limonum* fruit residues (CLFR) were evaluated for their total phenolic contents, antioxidant and antimutagenic activities. Total phenolic contents (TPC) of aqueous (30% and 70%) and acidified (0.5 N and 1.0 N) methanol extracts from CLFR were estimated by Folin-Ciocalteu reagent method whereas *in-vitro* antioxidant activity was assessed calorimetrically by measuring DPPH[•] scavenging capacity and inhibition of linoleic acid peroxidation. Anti-mutagenic potential of the extracts was appraised by Ames bacterial reverse mutation test. TPC, DPPH[•] scavenging capacity and inhibition of linoleic acid peroxidation were varied from 27.75-126.35 mg gallic acid equivalent (GAE)/g DW, 46-91%, and 34-83%, respectively. All the tested extracts of CLFR noticeably hunted mutagens (16.47-55.69 %) whereas none of these caused mutagenesis. Overall, acidified methanol extracts from CLFR exhibited higher extraction yields, TPC, inhibition of peroxidation and DPPH radical scavenging activity among others indicating a significant ($p < 0.05$) variation of these attributes in relation to residue samples and extraction media. The results support that CLFR (peel and pulp biomass) being a rich source of phenolic antimutagens, can be explored as a potential candidate for the development of natural chemo-preventive drugs and nutraceuticals.

Keywords: Citrus fruit; Hydrolysis; phenolic bioactives, antioxidants; antimutagenics

INTRODUCTION

Citrus belongs to *Rutaceae* family, which comprises more than 40 species distributed in Brazil, China, India, Mexico, United States, Spain, and Pakistan. Pakistan being sixth largest producer of citrus, harvests annually about 2.10×10^6 tons of citrus fruit comprising different varieties¹⁻². Citrus are one of the most important fruit crops having medicinal benefits to protect against coronary diseases such as chronic asthma, inflammatory, tumor and blood clotting³⁻⁴. *Citrus* species of various origins have been assessed for their antioxidant activity and phenolic constituents using different *in vitro* assays⁵⁻⁶. In addition to edible parts of citrus fruits, fruit residue of this species such as peel are also reported to be a promising source of natural antioxidants⁷. Multiple biological activities and medicinal health functions of citrus fruits have been accredited to the presence of bioactive compounds, such as ferulic acid, hydrocinnamic acid, cyanidinglucoside, hisperidine, vitamin C and flavonoids⁸⁻⁹.

Due to presence of pharmaceutically important compounds, especially phenolics, citrus can be a potential source of ingredients for the development of chemotherapeutic drugs and nutraceuticals but extraction of such bioactive compounds due to their structural complexity is a challenging task. In our recent study, we observed that aqueous methanol was a good choice for the extraction of citrus fruit phenolics but the recovery rates was not promising¹¹. The reason might be the presence of covalent linkage between phenolics (bound phenolics) and cell wall constituents. Conventionally, organic solvents such as methanol and ethanol are known to be efficient for the extraction of free phenolics (entrapped) but these solvents are often unable to extract and liberate citrus bound phenolics (more than 20 % phenolics are in bound form) from the well-organized cellulosic cell wall structure further fortified by lignin and in certain cases shielded by pectin¹⁰.

During the present study, therefore, a simple acid hydrolysis based process was applied to facilitate the liberation of bound phenolics from citrus fruit residues (peel and pulp biomass) during aqueous methanol extraction. Moreover, the extracts produced by different concentrations of acidified methanol were evaluated for their phenolic contents as well as antioxidant and antimutagenic activities using *in-vitro* assays.

EXPERIMENTAL

Materials, Chemicals, and Reagents

The healthy and mature fruit of *C. limonum* were collected from the botanical garden of University of Agriculture Faisalabad, during winter, 2012 and further authenticated by Dr. Mansoor Hameed (accession No. 149-13), Department of Botany, University of Agriculture, Faisalabad, Pakistan. The fruits were peeled off manually using a sharp knife to separate out peel whereas

pulp portion recovered was pressed by a domestic juice presser yielding pulp biomass. The fruit residue (peel and pulp biomass left after juice extraction) obtained thus were dried under ambient conditions ($32 \pm 5^\circ\text{C}$), pulverized into fine powder and stored under moisture free conditions for further analyses.

All the standards and reagents including 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,6-di-tert-butyl-4-methylphenol (BHT), Gallic acid, Folin-Ciocalteu reagent and linoleic acid were purchased from Sigma-Aldrich GmbH, Germany whereas other chemicals such as potassium dihydrogen phosphate, ferrous chloride, ammonium thiocyanate, dipotassium hydrogen phosphate and sodium bicarbonate were procured from Merck (Darmstadt, Germany). Two mutant strains *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100 and sterile chemical mutagens *i.e.* sodium azide and potassium dichromate were purchased from TRINOVA Biochem (GmbH Germany).

Extraction

Total phenolic components in *C. limonum* fruit residues (CLFR) were extracted using four solvents systems *i.e.* 30% aqueous methanol (30:70, v/v, methanol: water), 70% aqueous methanol (70:30, v/v methanol: water), 0.5N acidified methanol (4.5:95.5, HCl: methanol), and 1N acidified methanol (9:91, v/v, HCl: methanol). Briefly, an accurately weighed 5g of ground peel and pulp biomass of *C. limonum* were separately extracted with 100 mL of each of the extraction media at room temperature for 4 hours using orbital shaker (Gallenkamp, UK) at 120 rpm. The extracts were filtered, made free of solvent by evaporating at 40°C under vacuum in a rotary evaporator (EYELA, N-N series, Tokyo, Japan), weighed, and stored at 4°C , until used for further analyses.

Mutagenic and Anti-mutagenic Assay

The antimutagenic potential of the crude extracts from CLFR was evaluated by using the Ames bacterial reverse mutation test with some modification as described by Razaket *al*¹². Reagent mixture was prepared by mixing Davis Mingioli salt, D-biotin (0.01%w/v), D-glucose (40w/v), bromocresol purple (0.2% w/v), and L-histidine (0.01%w/v) aseptically, in a sterilized vial. Five sterilized vials labeled as Blank, Negative control, Positive control, Mutagen and, Antimutagen were totaled with 2.5mL of reaction mixture, 17.5mL deionized water and 5 μL of bacterial strain (except blank). The plant extract (5 μL of 1 mg/mL) in Dimethyl sulfoxide (DMSO) were added to mutagen and antimutagen assay vials whereas positive control vials were supplemented with 0.1 mL of 0.5% sodium azide and 30% potassium dichromate for *S. typhimurium* TA100 and *S. typhimurium* TA98, respectively. A 200 μL portion from each reaction mixture were transferred into 96-well ELISA plate, incubated at 37°C for 4 days and finally inhibition of mutagenesis was calculated as previously documented by Mosovska *et al*¹³.

$$\text{Antimutagenicity (\%)} = 1 - \frac{\text{No. of (+) wells in test sample} - \text{spontaneous mutation}}{\text{No. of (+) well in Positive control} - \text{spontaneous mutation}} \times 100$$

DPPH free radical scavenging capacity

The antiradical potential of CLFR extracts was assessed in terms of their capacity to bleach purple solution of DPPH in methanol using *in-vitro* spectroscopic assay¹⁴. Briefly, 3mL of each of the extract was mixed with 1mL of 0.1mM methanolic DPPH[•] solution and incubated at room temperature. After 30 min, 200 μ L were transferred into 96-well ELISA plate and absorbance was read at 517 nm to determine 50% inhibition (IC_{50}) against ascorbic acid and butylated hydroxyl toluene (BHT) as positive control following the equation below:

$$\text{Inhibition \%} = \frac{A_{\text{Control}} - A_{\text{sample}}}{A_{\text{Control}}} \times 100$$

Where A_{Control} and A_{sample} indicate the absorbance of DPPH[•] reagent and reaction mixture, respectively.

Inhibition to linoleic acid peroxidation

Antioxidant activity of methanolic extracts of peel and pulp of *C. limonum* towards lipid peroxidation was evaluated using thiocyanide assay¹⁵ with slight modification. Briefly, 0.5mg extract diluted with 0.5 mL of distilled water at pH 7 (0.05M phosphate buffer) was mixed with 0.5 mL of 2.51% linoleic acid in absolute ethanol. The resulting mixture was incubated at 40°C in dark for autooxidation and 0.1mL were taken sequentially at 12 h interval to check the degree of oxidation in terms of percent inhibition of lipid peroxidation relative to control. The oxidized lipids in reaction mixture were quantified by adding 9.7mL 75% ethanol, 0.1mL 0.02M in 3.5% HCl aqueous ferrous chloride, 0.1mL 30% aqueous ammonium thiocyanate and measuring absorbance at 500nm, until the absorbance of the control (containing all reagents except the test sample) reached maximum. Gallic acid was used as reference standard and inhibition potential (percent) of extract towards lipid peroxidation relative to control as shown by equation:

$$\text{Lipid peroxidation inhibition (\%)} = \left[1 - \frac{A_{\text{sample at 72 h}} - A_{\text{sample at 0 h}}}{A_{\text{Control at 72 h}} - A_{\text{Control at 0 h}}} \right] \times 100$$

Where A_{Control} and A_{sample} denote absorbance of sample and control, respectively.

Determination of total phenolic contents (TPC)

Folin-Ciocalteu reagent method¹⁶ was used to determine the total phenol contents in CLFR extracts using gallic acid as reference standard.

Statistical analysis

All the experiments were triplicated to get data as mean $\pm$ SD. The mean estimates for each for different solvents used and fruit parts investigated were compared by Duncan's multiple ranges test. The results were considered statistically significant at probability (p) < 0.05.

RESULTS AND DISCUSSION

Extract yield

The present research work was mainly planned to get comprehensive insight into anti-mutagenic and antioxidant potential of under-utilized and usually discarded fruit residues (peel and pulp biomass) of *C. limonum*. The bioactive components from *C. limonum* fruit residues were extracted using 30%, 70%, 0.5N and 1N acidified methanol, respectively.

The amount of extracts obtained from *C. limonum* fruit residues (CLFR) in aqueous and acidified methanol varied widely (3.87-54.52 g/100g DW) as given in Table 1. The maximum yield of active compounds from peel and pulp biomass of *C. limonum* was obtained with 1N acidified methanol whereas that of least was recovered by 30% methanol. The overall order of extract recovery by the solvents tested for CLFR was observed to be: 1N acidified methanol > 0.5N acidified methanol > 70% methanol > 30% methanol. Among CLFR examined, peel were found to have greater level of extractable bioactives for all the solvent systems used except in case of 30% methanol where pulp showed higher extraction yield.

The extraction of biologically active and potential plant metabolites including phenolics, flavonoids, phenolic acids, lignins, salicylates, stanols, sterols, glucosinolates is one of the most critical step towards the isolation and analytical characterization of such bioactives. Ideally, extraction should be quantitative, green, economic and quick¹⁴⁻¹⁶. Our previous study¹¹ and several other earlier reports revealed methanol and ethanol as relatively efficient

solvents for the extraction of phenolic antioxidants¹⁷⁻¹⁹ but still there exists constraints for accompanying yield, environmental and economic concerns²⁰. Keeping in view of the hydrolyze ability of acids, in the present work aqueous and acidified methanol extraction media were employed to elucidate their effect on the quantitative extract yields and recovery of phenolics and selected biological activities.

The results showed that the nature of extraction solvent as indicated by small alphabets superscripted in a column (Table 1) strongly affected these attributes. The results showed that the percent yield (g/100g) of extracts enhanced significantly with acidification of extraction media. The organized cell wall structure of citrus peel and pulp comprising cellulose, hemicellulose, pectin and lignin is a barrier towards the liberation of a diverse range of polyphenols entrapped in the form of glycosidic esters²¹. The acidified methanol not only swelled and macerated cell wall to improve the solvent distribution but also hydrolysed glycosidic bond into corresponding simpler phenolic acids and thus notably improved phenolic liberation rates. Furthermore, the mean range of available biologically active components from peel and pulp biomass of *C. limonum* (3.87-54.52 g/100 g DW) was found to be higher than our previous finding¹¹ and than the results of Zia-Ur-Rehman²² producing 24.20 g/100g and 19.87 g/100g citrus peel extracts, respectively, using aqueous methanol. Hence, acidification of methanol might be an effective tool to enhance the extraction yield for the isolation of plant bioactive from peel and pulp waste of *C. limonum*.

Total phenolics (TP)

The tabulated TP data of aqueous (30 and 70%) and acidified (1N and 0.5 N) methanol extracts of *C. limonum* residues ranged from 50.70-188.93 mg Gallic Acid Equivalents (GAE)/100g of DW (Table1). The results revealed that 1N acidified methanol extracted maximum amount of TP (188.93 $\pm$ 3.26 mg GAE)/100g of DW) among others with overall efficacy order: 1N acidified methanol > 0.5N acidified methanol > 70% methanol > 30% methanol. The higher amounts of total phenolic compounds in 1N acidified methanol extracts indicated that methanol acidification assisted the liberation of covalently bound phenolic compounds²¹. A comparison with previous reports supported that TPC extracted from *C. limonum* residues using 1N acidified methanol were higher than those investigated by some other researchers in different Citrus species^{9,22,23} and herbs²⁴ using different organic solvents and their aqueous fractions.

DPPH[•] scavenging activity

The data plotted indicates that overall peel extracts (Fig 1) exhibited better radical scavenging as compared to pulp biomass extract (Fig 2) of *C. limonum* and observed activities were found to be strongly correlated with the amount of total phenolics earlier discussed in their extracts ($R^2 = 0.79$). The antiradical potential of *C. limonum* residue extracted with aqueous and acidified methanol against DPPH[•] (per cent) were assessed at different concentrations of extract (1-5000 μ g) and found to be extract concentration dependent with maximum activity at 5mg/L as shown in Fig 1-2.

Fig 1: DPPH radical scavenging activity of extractable components from *C. limonum* fruit residues (CLFR) extracted using aqueous and acidified methanol.

The graphical plots also make it clear that extracts obtained with 1N acidified methanol scavenged DPPH[•] more efficiently as compared with other solvent systems used. The reason behind this trend might be enhanced recovery of phenolic compounds with acidified methanol. The results further disclosed that *C. limonum* pulp biomass metabolites when extracted with 0.5 N acidified methanol also showed comparable DPPH[•] scavenging activity (79.29-92.27%).

Table 1: Extract yield, total phenolic and inhibition of linoleic acid peroxidation of extracts from *C. limonum* fruit residues (CLFR).

Solvent used	Extract Yield ¹		TP ^M		Inhibition (%) ^N	
	Peel	Pulp biomass	Peel	Pulp biomass	Peel	Pulp biomass
1N acidified methanol	54.52±1.63.93 ^a	45.9±1.37 ^a	188.93±3.26 ^a	134.05±4.02 ^b	83.22±2.53 ^a	60.65±1.81 ^a
0.5 N acidified methanol	19.87±0.59 ^c	8.87±0.26 ^d	93.27±2.79 ^b	107.88±3.23 ^b	49.00±1.47 ^{bc}	54.87±1.64 ^{cd}
70% methanol	39.21±1.17 ^b	36.9±1.10 ^b	60.36±1.81 ^c	91.05±2.73 ^c	47.15±1.41 ^{cd}	51.21±1.53 ^d
30% methanol	3.87±0.11 ^d	9.5±0.28 ^d	50.70±1.52 ^d	72.02±2.16 ^d	27.23±0.81 ^d	31.30±0.93 ^d

¹ Values (g /100g) are mean±SD, ^M mg gallic acid equivalent (mg GAE)/g of dry matter, ^N The results were calculated against BHT (showing 84 % inhibition of linoleic acid peroxidation), Different small letters in superscript within the same column indicate significant differences of individuals at 95% confidence interval for mean based on pooled stdev ($p<0.05$) for extraction solvent used.

Fig 2: DPPH radical scavenging activity of aqueous and acidified methanol extracts from *C. limonum* fruit residues (CLFR)

Inhibition of peroxidation in linoleic acid system

The preservative and antioxidant characteristic of plant products has been evaluated by assessing their potential to inhibit peroxidation of linoleic acid.

The inhibition potential of aqueous and acidified methanol extracts obtained from CLFR varied significantly ($p<0.05$) in relation to extraction solvents as expressed by small alphabets in superscripts (Table 1). The results indicated that acidified methanol extract of *C. limonum* peel most effectively inhibited peroxidation in linoleic acid system among others thus reflecting a higher antioxidant activity of this extract (71.22±1.53). The higher antioxidant potential of acidified methanol extracts of *C. limonum* peel towards inhibition of lipid peroxidation can be ascribed to the greater amounts of liberated phenolics extracted with acidified methanol as compared to those with aqueous methanol. Interestingly, the extracts produced by 0.1 N acidified methanol in the present study offered relatively a better inhibition (83 %) of lipid peroxidation as compared to our previous study results (60 %) ¹¹.

Mutagenesis risks of extracts

Mutagens interrupt transcription and replication of the DNA protein and may cause aberrant, impaired or in severe cases, cell death and certain cancers. The tabulated data (Table 2) depicted that the isolated plant phenolic metabolites are safe with reference to mutagenesis risks and hence might be potentially explored for drug development, pharmaceutical applications and food flavoring purposes. Our results for mutagenic potential of *C. limonum* residues are in line with the earlier data reported by Chughtai et al. ²⁷ from Pakistan and Rahimifard et al. ²⁸ from Iran.

The results regarding mutagenic activities of extracts from CLFR in aqueous and acidified methanol are shown in Table 2.

Table 2: Mutagenic potential of CLFR extracts.

Test of <i>Salmonella</i> TA 98				Test on <i>Salmonella</i> TA 100		
Number of positive wells /96 wells				Number of positive wells /96 wells		
	peel	Pulp	Effect	Peel	pulp	Effect
¹ Background	8	8		14	14	
^M K ₂ Cr ₂ O ₇	93	93	+	NA	NA	NA
^N NaN ₃	NA	NA	NA	93	93	+
1N Acidified methanol	7	5	nm	20	8	nm
0.5 N acidified methanol	9	8	nm	8	11	nm
70% methanol	12	11	nm	27	17	nm
30% methanol	15	14	nm	5	23	nm
BHT	1	1	nm	1	1	nm

¹ Negative control, ^M positive control, ^N positive control, +mutagenic, nm non-mutagenic, NA Test not applied.

Anti-mutagenic activity

The results regarding percent antimutagenic potential ranged from 16.47-55.69 % (Table 3) and were found to be extraction solvent and extract dose dependent (R^2 0.987, 0.979). The strongest anti-mutagenic activity 55.69 %

was shown by 1N acidified methanol extract of *C. limonum* pulp biomass for *Salmonella* TA 100 against standard mutagen NaN₃ where as that of minimum by 30% methanol extract of *C. limonum* peel for *Salmonella* TA 98 against K₂Cr₂O₇.

Table 3: Antimutagenic potential of extracts from CLFR.

	<i>Salmonella</i> TA 98						<i>Salmonella</i> TA 100					
	Number of positive wells /96 wells						Number of positive wells /96 wells					
	Peel	%	Effect	Pulp	%	Effect	peel	%	Effect	Pulp	%	Effect
^L Background	8	NA	NA	8	NA	NA	14	NA	NA	14	NA	NA
^M K ₂ Cr ₂ O ₇	93	NA	--	93	NA	--	NA	NA	NA	NA	NA	NA
^N NaN ₃	NA	NA	NA	NA	NA	NA	93	NA	--	93	NA	--
1N acidified Methanol	56	43.52	++	48	52.94	++	51	53.16	++	49	55.69	++
0.5 N acidified methanol	67	30.58	+++	57	42.35	+++	55	48.10	+++	53	50.63	+++
70% Methanol	71	25.88	+++	64	34.11	+++	61	40.50	++	59	43.03	++
30% methanol	79	16.47	+++	69	28.23	+++	68	31.64	+	64	36.70	+++
BHT	37	65.88	++	36	67.05	++	40	67.08	++	37	70.88	++

^L Negative control, ^MPositive control, ^NPositive control, +++strong antimutagenic, ++ moderate antimutagenic, + weak antimutagenic - non-mutagenic and -- indicate mutagenic response and NA Test not applied.

Increased incidence of diet related cancers and degenerative diseases have built considerable interest in plant phenolic antioxidants²⁹⁻³⁰. The naturally occurring metabolites especially, polyphenols which are mainly present in herbs and medicinal plants have been reported to be fascinating candidates for development of chemo-preventive drugs.

Based upon considerable level of phenolic compounds and antioxidant activities observed in terms of DPPH[•] radical scavenging (83-91%) and inhibition of linoleic acid peroxidation (72-83%) of *C. limonum* peel and pulp biomass extracts were further tested for their antimutagenic potential towards direct mutagens against two bacterial strains of *Salmonella* (TA 100 and TA 98).

Table 4: Pearson's correlation between antioxidant, antimutagenic activities and total phenolic contents of peel of *C. limonum*

Pearson correlation <i>P</i> -Value	Solvent	Yield	TPC	DPPH	Inh	IC ₅₀
Yield	0.998*** 0.000					
TPC	0.979*** 0.000	0.986*** 0.000				
DPPH	0.847** 0.001	0.831** 0.001	0.798** 0.002			
Inh	0.877*** 0.000	0.867*** 0.000	0.749** 0.002	0.823*** 0.000		
IC ₅₀	-0.951*** 0.000	-0.932*** 0.000	-0.899*** 0.000	-0.818** 0.001	-0.801** 0.002	
Antimutagenic T98	-0.987*** 0.000	0.979*** 0.000	0.950*** 0.000	0.850*** 0.000	0.847** 0.001	-0.982*** 0.000
Antimutagenic T100	0.584* 0.046	0.591* 0.043	0.691* 0.013	0.601* 0.039	0.144 ^{ns} 0.654	-0.385 ^{ns} 0.216

TPC = total phenolic content, DPPH = DPPH[•] scavenging activity of extract, Inh = inhibition of linoleic acid peroxidation by the extract, antimutagenic = % antimutagenic activity of extract, ns = non-significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$ and *** = significant at $p < 0.00$

The overall order of antimutagenic potential of CLFR (peel and pulp biomass) among the tested solvents was noted to be 1 N acidified methanol > 0.5N acidified methanol > 70% methanol > 30% methanol. This data trends are in accordance with the amount of total phenolics and antiradical activity of CLFR. The observed trends for antimutagenic activity of peel extracts from

CLFR were found to be strongly correlated with TPC, DPPH and reducing power as indicated by their related Pearson's correlation values (Table 4). Furthermore, findings of the present study on antimutagenic activity of *C. limonum* peel extract were in good agreement with the reports by Kamran et al.²⁷ in Pakistan, and Maliheh et al.²⁸ in Iran.

Table 5: Pearson's correlation between antioxidant, antimutagenic activities and total phenolic contents of pulp biomass of *C. limonum*

Pearson correlation <i>P</i> -Value	Solvent	Yield	TPC	DPPH	Inh	IC ₅₀
Yield	0.933*** 0.000					
TPC	0.992*** 0.000	0.918*** 0.000				
DPPH	0.917*** 0.000	0.828** 0.001	0.878** 0.000			
Inh	0.926*** 0.000	0.753*** 0.005	0.908** 0.000	0.760** 0.004		
IC ₅₀	-0.975*** 0.014	-0.906*** 0.000	-0.947*** 0.000	-0.979*** 0.000	-0.949** 0.000	
Antimutagenic T98	0.687** 0.000	0.518 ^{ns} 0.084	0.702** 0.011	0.658* 0.020	0.760** 0.004	-0.676* 0.016
Antimutagenic T100	0.993*** 0.000	0.941*** 0.000	0.976*** 0.000	0.935*** 0.000	0.923*** 0.000	-0.985*** 0.000

TPC = total phenolic content, DPPH= DPPH° scavenging activity of extract, Inh= inhibition of linoleic acid peroxidation by the extract, antimutagenic= % antimutagenic activity of extract, ns=non-significant, *=significant at $p<0.05$, **=significant at $p<0.01$ and ***=significant at $p<0.00$

Data presented in table 4 and table 5 reveal the correlation (Pearson's correlation) between the extraction yield and phenolic constituents and total phenolic and the exhibited levels of *in-vitro* antioxidant and antimutagenic attributes. A highly positive correlation (r 0.933-0.998) was observed between crude extract yield and the polyphenol constituents (TPs). This established that the efficiency of selected extraction media is quite reasonable as well as selected solvent composition is optimum for high recovery rates. Similarly, correlation between radical scavenging (DPPH) potential and total polyphenol constituents (0.878-0.798) revealed that anti radical potential of CLFR (*C. limonum* peel and pulp biomass) extracts is mainly due to occurrence of these phenolic bioactives. Similarly, Pearson correlation values > 0.500 (Table 4 and table 5) supports that phenolic constituents in acidified methanol extracts of peel and pulp biomass of *C. limonum* are mainly attributable for the observed antimutagenic potential of this under-utilized fruit materials.

CONCLUSIONS

In the present investigation we observed that fruit residue (peel and pulp biomass) of *C. limonum* contained appreciable amount of total phenolics rendering these under-utilized fruit waste as a potential source of antioxidant as well as antimutagenic compounds. As far as the extraction efficacy of the solvent media is concerned, the data revealed that acidification of methanol enhanced the availability of phenolics as well as their antioxidant and antimutagenic activities *in-vitro*. The results further supported that the tested extracts from CLFR were mutagenically safe. Overall, it can be concluded from the findings of this study that CLFR (peel and pulp biomass) is a potential source of antioxidant and antimutagenic agents and can be explored for potential pharmaceutical and nutraceutical applications.

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