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Research Article

Antioxidant and Antimicrobial Efficacy of Lemon (*Citrus limonum* L.) Peel

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Received: 08-07-2017; **Revised:** 24-08-2017; **Accepted:** 12-09-2017.

ABSTRACT

Waste materials such as peels and seeds obtained from post-harvest management are rich source of biologically active phytocomponents possessing various physiological functions. Usually these plant based products are often discarded as waste or utilized as feed or fertilizer. Nowadays the recent area of research interest includes exploiting the use of peels for the production of effectual, safe, inexpensive and novel nutraceuticals as they contain high amount of natural compounds with medicinal properties. The present study was carried out to determine the antioxidant and antimicrobial potential of lemon peel as it is a rich source of molasses, pectin, limonene and other secondary metabolites. The antioxidant activity of lemon peel was determined using DPPH assay, FRAP and phosphomolybdenum assay while the antibacterial activity was tested against four bacterial strains. The result indicated that acetone extract of lemon peel exhibited greater potential to scavenge free radicals and reducing power that increased with increase in concentration. Methanol extract of lemon peel showed 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 in value added food supplements is promising.

Keywords: lemon peel, phenolic compounds, nutraceutical property, antioxidant, antimicrobial.

INTRODUCTION

Vegetables and fruits yield about 25% to 30% of non-edible products such as peels and seeds.[1] In most cases these waste by-products contain high contents of antioxidant and antimicrobial compounds that can be successfully utilized as a source of phytochemicals and antioxidant agents.[2]

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.[3] During the manufacture of citrus juice, very large amounts of byproduct wastes, such as peels are formed every year.[4] Citrus peels exhibit a broad spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities.[5,6]

There are several *Citrus (C.)* species such as *C. linonum*(lemon), *C. aurantium* (bitter orange), *C. limetta*(sweet lemon), *C. jambhiri* (rough lemon) and *C. paradise* (grape fruit).[7] Lemon peel consists of two layers the outermost layer called zest, contains essential oils (6%) that are composed mostly of limonene (90%) and citral (5%) and a small amount of cintronelene, alphaterpincol, linayl and gernanyl acetate along with B complex vitamins. It is being used for dissolving gall stones and has anticancer properties too.[8]

Utilization of peel in several possible ways helps reducing solid-waste handling along with adding value to these peel waste.[9,10] Currently zest, a food ingredient obtained by scraping or cutting the outer skin of citrus fruits such as lemon and orange is commonly used as a flavoring agent in biscuits, puddings, candy, chocolates, pies, cakes and in sour condiments.

Due to rapid increase of antibiotic resistance in our country and use of synthetic anti-oxidant agents an attempt was made in the present investigation to determine the anti-oxidant and anti-microbial activity of lemon peel and to document the use of lemon peel as a natural food ingredient in value added food supplements.

MATERIALS AND METHODS

Collection of plant material

Fresh lemons were purchased from a local market in Chennai and washed thoroughly under tap water. The peels were removed using a sterile knife and shade dried for 4-5 days at room temperature. The dried peels were pulverized using an electric blender and stored in airtight containers for further use.

Preparation of peel extracts

Two different solvents namely methanol and acetone were used for extraction. 5grams of lemon peel powder was soaked in 100 mL of the respective solvents for 72 hours by maceration technique. The supernatant was filtered using Whatmann filter paper 1 and Buchner funnel and concentrated using rotary evaporator and dry residue was preserved at 5°C until further use.

Figure 1: Lemon

Figure 2: Dried lemon peel

Figure 3: Lemon peel powder

Estimation of total phenol content

Total phenolic content was estimated by Folin- Ciocalteau reagent method with slight modifications.[11]The extract (100 µg/mL) was mixed with 1 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water) and after 5mins later 1mL of 20% Na_2CO_3 was added. The mixture was allowed to incubate for 30 minutes and the absorbance was measured at 760 nm using spectrophotometer. The total phenolic content was expressed in terms of Gallic acid equivalent (µg/mg of extract), which is a common reference standard.

Estimation of total flavonoid content

The total flavonoid content was determined by $AlCl_3$ reagent method.[12]The extract (500µg/mL) was mixed with 0.5 mL of 5% $NaNO_2$ solution and allowed to stand for 5 minutes. Then 0.3 mL of 10% $AlCl_3$ solution was added and the mixture was allowed to stand for further 5 minutes. Finally, 1 mL of 1 M NaOH solution was added, and the final volume of the mixture was brought to 5 mL with distilled water. The mixture was incubated for 15 minutes at room temperature and absorbance was measured at 510 nm. The total flavonoid content was expressed as quercetin equivalent (µg/mg of extract), which is a common reference standard.

DPPH assay

One mL of peel extract was taken in various concentrations (50-300 µg/mL) and was mixed with 1 mL of 0.1 mM of DPPH solution in methanol. The reaction mixture was kept at room temperature for 30 minutes. Absorbance was read at 517 nm in spectrophotometer.[13] The percentage of the radical scavenging activity was calculated as follows.

$$\frac{\text{Absorbance in control} - \text{Absorbance in sample}}{\text{Absorbance in control}} \times 100$$

Phosphomolybdenum reduction assay

The antioxidant activity was evaluated by reduction assay method by the formation of green phosphomolybdenum complex.[14] 1 mL of various concentrations of peel extract was combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. The samples were cooled to room temperature and the absorbance of the mixture was measured at 695 nm against blank.

FRAP Assay

Different concentrations of the peel extract was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide (1%).The mixture was incubated at 50ºC for 20 min. 1 mL of 10% trichloroacetic acid was added to the mixture. Then 1 mL of 0.1% of freshly prepared ferric chloride was added and the absorbance of the resultant solution was measured at 700 nm.[15]

Antibacterial activity

The antibacterial activity was analysed by well diffusion method. 25 mL of Muller Hinton agar was prepared according to the standard procedure and poured into the plates and was allowed to solidify. The standard inoculum suspension was streaked over the surface of the media using sterile cotton swab to ensure the confluent growth of the organism and the plates were allowed to dry for 5 minutes. After drying, the different concentrations (50, 75 and 100 µg/mL) of the extract were poured into the wells. Tetracycline was used as a standard (1 µg/mL). Finally the inoculated plates were incubated for 24 hours at 37ºC for bacteria. The zone of inhibition was measured and noted.[16]

RESULTS AND DISCUSSION

Table 1: Total phenol and flavonoid content

Phytochemicals	Methanol extract	Acetone extract
Phenol content	26.58µg/mg GAE	119.6µg/mg GAE
Flavonoid content	4.5µg/mg QE	56.28 µg/mg QE

From the above table it is clear that flavonoids and phenolic compounds are present in lemon peel. Studies have reported that higher amount of phenolic compounds and ascorbic acids are found in the peel than in pulp.[17]Phenolic compounds possess different antioxidant properties such as oxygen scavengers, peroxide decomposers, and metal chelating agents and free radical inhibitors. Wang et al., [18] reported that lemon peel contains polymethoxylated flavones that are responsible for anti-cancer, anti-viral, anti-inflammatory activities, and reduced capillary fragility.

Table 2: Antioxidant activity of lemon peel using DPPH assay

S. No	Concentration µg/mL	% of inhibition	
		Methanol Extract	Acetone extract
1	50	15.51	40.29
2	100	32.92	46.52
3	150	38.95	68.50
4	200	41.63	82.78
5	250	47.66	97.44
6	300	53.91	98.53

Table 2 indicates that both the extracts showed free radical scavenging activity that increased with increase in concentration. Acetone extract exhibited potent radical scavenging activity as the IC_{50} value was found to be 107.48 µg /mL. The IC_{50} value of methanolic extract of lemon peel was 278.24 µg /mL. Different studies have shown that reactive oxygen species or free radicals present in the human organs cause oxidative damage to several molecules such as lipids, proteins and nucleic acids thereby leading to several degenerative diseases. Phenolic compounds present in fruits and vegetables peels are capable of neutralizing free radicals and thereby prevent the onset of degenerative diseases. Components such as tetrazene and coumarins present in lemon peel are capable of scavenging free radicals either by electron- or hydrogen-donating mechanisms, breaking the chain reaction or removing the ROS and RNS initiator by quenching chain initiator catalyst. [19]

Table 3: Antioxidant activity of lemon peel using FRAP assay

S. No	Concentration µg/mL	Absorbance at 700 nm	
		Methanol extract	Acetone extract
1	20	0.16	0.16
2	40	0.18	0.26
3	60	0.21	0.30
4	80	0.28	0.42
5	100	0.36	0.46
6	120	0.40	0.64

Reducing capacity of a compound also serves as a notable indicator of potential antioxidant activity. The reducing power of lemon peel was determined using FRAP assay and Phosphomolybdenum assay. Antioxidant assays such as FRAP and phosphomolybdenum assay are based on redox reactions. Electrons from oxidized antioxidant are transferred to the substrate by inhibiting oxidation of oxidant in this reaction.

Table 4: Antioxidant activity of lemon peel using Phosphomolybdenum assay

S. No	Concentration µg/mL	Absorbance at 695 nm	
		Methanol extract	Acetone extract
1	20	0.04	0.05
2	40	0.10	0.08
3	60	0.22	0.09
4	80	0.28	0.18
5	100	0.38	0.23
6	120	0.51	0.43

FRAP assay or Ferric Reducing Ability of Plasma is based on the reduction ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by the reductant while Phosphomolybdemun assay is based on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the sample with subsequent formation of a bluish green colored phosphate/Mo (V) complex. Greater absorbance indicates higher reducing potency. From the results obtained it is evident that both methanol and acetone extract exhibited good reducing power capacity which increased with increase with concentration. Majority of fruit peels exhibits 2 to 27 fold higher antioxidant activity than the fruit pulp.[20] Antioxidants donate electrons to free radicals thereby neutralizing the action of free radicals and making them more stable and unreactive. [21]

As the prevalence of antibiotic resistance still continues it is therefore essential to develop plant based products with antibacterial activity.Abdullah[22] reported that lemon juice showed inhibition against *S. aureus* and *K. pneumoniae* with inhibition zones 17.4 and13.3 mm respectively. The result of the present study indicated that lemon peel too showed antibacterial activity against all the tested bacterial strains due to the presence of components such as ascorbic acid, flavonoids, polyphenols and phenolic compounds. Methanolic extract of lemon peel exhibited highest activity against *S. flexneri*followed by *S. aureus* and *E.coli*.Acetone extract also inhibited the growth of *S. aureus* and *E.coli* which is on par with methanolic extract. Limonoids present in citrus fruit peels contributes to antibacterial and antifungal activity. [23]Alcoholic extract of lemon peel also exhibited antimicrobial activity against *P. aeruginosa* and *S. Typhimurium*. [24]

CONCLUSION

The hazardous effects of synthetic antioxidants along with the emergence of antibiotic resistant strains have revived the search for novel antioxidant and antimicrobial agents from natural sources. The present study paves a way to researchers and scientists to exploit the potential utilization of lemon peel in various domains such as food and nutraceutical industries as a natural, novel and economic source as lemon peel exhibited potent

antioxidant and antimicrobial activity. Utilization of fruit and vegetable peels in several ways not only improves the nutritional status of well-being but at the same time provides opportunity for income generation.

Table 5: Antimicrobial efficacy of lemon peel

S. No	Extracts	Pathogens	Standard Tetracycline	Zone of inhibition (mm)		
				50µL	75µL	100µL
1.	Methanol	*E.coli*	24	-	12	16
		Staphylococcus aureus	26	12	13	14
		Shigella flexneri	22	13	16	21
		Klebsiella pneumoniae	29	-	11	14
2.	Acetone	*E.coli*	20	11	15	16
		Staphylococcus aureus	25	-	15	17
		Shigella flexneri	24	-	-	-
		Klebsiella pneumoniae	23	-	-	12

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Source of Support: Nil, **Conflict of Interest:** None.