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Phytochemical Screening, Antioxidant, Antibacterial Activities of *Citrus Limon* and *Citrus Sinensis* Peel Extracts

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Abstract

The study has demonstrated the occurrence of significant amount of secondary metabolites such as tannin, steroids, reducing sugar, proteins and high content of carbohydrates from the aqueous and chloroform extracts and other secondary metabolites from citrus fruit peel wastes of *Citrus limon* and *Citrus sinensis* where as the ethanolic extract indicated the unique presence of saponin too. The fruit peel ethanolic extract of *C.limon* and *C.sinensis* exhibited potent anti-oxidant activity and antibacterial activity against Gram positive and Gram negative organisms. The present study has shown the usefulness of the extraction methodologies adopted for efficient extraction, processing and utilization of these citrus fruit peel wastes and also to characterize the phytochemicals, antioxidant property and antibacterial activities of fruit peel wastes of lemon and oranges.

Keywords: Lemon (*Citrus limon*); Orange (*Citrus sinensis*); Phytochemical Screening; Antioxidant and Antibacterial activity

Introduction

Citrus fruits are highly nutritious, medicinal and are found to be commonly in cultivation throughout the tropics. It belongs to the genus Citrus, subgenus Papeda, and related genera. The endocarp is the palatable portion, partitioned into 10-14 sections segregated by thin septa, containing up to 8 seeds/septa, but it was appeared regularly with one. Each segment consists of juice vesicles ("pulp"), with long stalks attached to the juice containing outer wall, Citrus fruits include oranges, lemons, limes

and grapefruits, in addition to tangerines and pomelos. Citrus fruits constitute only 0.9% of total daily calories and 1.7% of daily carbohydrate intake while the Peel waste are highly perishable and seasonal which could be a wealth for the farmers if the processing industries and monitoring agencies evolve methodologies to use them and take attention in bringing useful products from citrus waste materials [1]. Though there were many studies on antioxidant and antibacterial effect of juice and edible parts, there are meager literature on the wastes of citrus fruits of lemon and oranges of different varieties. The citrus peel wastes are rich in nutrients and contain many phytochemicals. The peel of Citrus fruits is a rich wellspring of flavonoid, glycosides, coumarins, β and γ-sitosterol, glycosides and volatile oils and these can be efficiently used as drugs or as food supplements. Most of the phytochemicals are though non-nutritive plant chemicals, are known to have some disease preventive properties. And thus they offer protection against pathogens [2] and these peels and pomace are a source of sugars, minerals and organic acids, dietary fibers and phenolics which have a wide range of actions which includes antioxidants, antimutagenic, cardio preventive, antibacterials and antiviral activities [3]. Use of waste as a source of polyphenols and antioxidants may have considerable economic benefit to food processors. Moreover the wastes and by-products of fruits are an abundant source of antioxidant polyphenols fruits while the vegetable processing in India generates substantial quantities of waste, income and employment [4]. Therefore there is an urgent need for novel methodologies for efficient extraction, processing and utilization of these citrus fruit wastes. The present investigation is aimed to investigate and characterize the fruit peel wastes of lemon and oranges using phytochemical analysis, antioxidant property and antibacterial activity [5].

Materials and Methods

The plants used in this study were *Citrus limon L.* (Lemon) peel and *Citrus sinensis L.* Sweet orange) peel. The peels were collected from the local market and fruit juice shops. After collection, the peels were shade dried at room temperature (30-35ºC). 100 gm of peels of lemon and oranges were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was dried in an oven at 40°C for 24 h.

Preparation of different solvent Extracts

The *Citrus limon.L* (Lemon) and *Citrus sinensis* (Sweet orange) peel powder was extracted with different solvents such as ethanol, acetone, chloroform and water. 10 gm of peel powder of each fruit was suspended in 200 ml of solvents. Extraction was done using soxhlet apparatus for 5 hours at a specific temperature of each solvents but not exceeding the boiling point [6]. The attained extract was filtered through syringe filter and the solvent was removed by evaporation using Buchi rota vapor under reduced pressure at 45°C with 5 bar to get a constant mass and concentration of 1g. The resulting crude extract was then stored at 4°C until use [7].

Phytochemical Screening

Test for carbohydrates: Molisch's reagent with 2 ml of extract to a little amount of concentrated sulphuric acid was added to it and allowed to form a layer. The mixture was shaken well, and allowed to stand for few minutes then diluted by adding 5 ml of distilled water [7]. Purple precipitate ring indicated the presence of carbohydrates.

Test for saponins: 0.5 gm of extract was boiled and the mixture was filtered. To 2.5 ml of the filtrate, 10 ml of distilled water was added in a test tube. It was shaken well for few minutes and was allowed to stand. Frothing along with the formation of honey comb indicated the presence of saponins.

Test for tannins: 3 gm of extract was added to 6 ml of distilled water, which was filtered and few drops of 10% ferric chloride solution was added to it. A bluish green colour indicated the presence of tannins.

Test for reducing sugars: A little amount of Fehling's reagent was added to the extract, and the mixture was boiled for 2 minutes. A brick red colour indicated the presence of glycosides.

Test for proteins: 0.5 ml of extract was treated with equal volume of 1% sodium hydroxide, a few drops of copper sulphate solution was gently added. The solution turning to purple colour indicated the presence of proteins.

Test for steroids: 0.5 ml of the extract was dissolved in 3 ml of chloroform and was filtered. To the filtrate, concentrated sulphuric acid was added by the sides of the test tube, which formed a lower layer.

A reddish brown colour ring with a slight greenish fluorescence was taken as the indication for the presence of steroids [8].

Antioxidant Assay

Ferric reducing power activity: Various concentration (50, 100, 250, 500 µg /ml) of extracts were prepared in

1ml of distilled water was mixed with phosphate buffer (2.5ml, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe (CN6] (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material described by Perumal et al. [9].

Antibacterial Assay

Disk diffusion method: Prepare Petri plates of sterile molten Mueller Hinton Agar (20g) with 100ml of distilled water and 0.5g agar–agar at around 121ºC for 15 minutes and solidify the Petri plates. A bacterial culture was used to lawn Muller Hinton agar plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. After solidification 6 mm wells were prepared. In these wells solvent extracts of the *Citrus limon* and *Citrus sinensis* peel were added. The plate was incubated overnight at 37ºC. After incubation the zones of inhibition were measured and recorded [10,11].

Results

The phytochemical analysis of the different extracts of *Citrus limon* and *Citrus sinensis* are profiled for the secondary metabolites and are presented in the Table 1. The results of the phyto chemical analysis of *C. limon* aqueous and chloroform extract confirmed the presence of tannin, steroids, reducing sugar, proteins and high content of carbohydrates (Table 1). Besides the secondary metabolites, the presences of saponin were noticed in the ethanolic. By contrast, the acetone extract exhibited the absence of tannin and saponin but showed the presence of other common metabolites reported as in other extracts. Ethanolic extract of *C. sinensis* showed the presence of secondary metabolites such as tannin, saponin, steroids, reducing sugar, protein and carbohydrates.

Phytochemical constituents	Lemon peel				Orange peel			
	Aq	Et	Ace	Chl	Aq	Et	Ace	Chl
Tannin	++	++	_	+	++	++	_	_
Saponin	_	+	_	_	_	+	_	_
Steroids	+	++	+	++	+	++	+	++
Reducing sugar	++	++	+	_	++	++	+	_
Protein	+	+	+	+	+	+	+	+
Carbohydrate	++	++	+	++	++	++	+	++

Aq-Aqueous, Et-Ethanol, Ace-Acetone, Chl-Chloroform (+) Positive, (-) Negative

Table 1: Phytochemical Analysis of *Citrus limon L.* (Lemon) and *Citrus sinensis L.* (Sweet Orange) Peel extracts.

The total antioxidant potential was evaluated in the skin outer peel extracts of *C. limon* and *C. sinensis* and the results are set out in (Figure 1). The Ethanolic extract showed the highest reducing power of both the peels *C-limon* 30% and *C-sinensis*-46%. *C-limon* of Acetone extract showed 25% reduction, Aqueous extract and chloroform extract showed 24% of reducing activity. *C-sinensis* of aqueous extract demonstrated 43% of antioxidant activity while chloroform extract showed 40% of antioxidant activity, followed by Acetone extract showing 33% reducing activity. Figure 1 emphasized that the aqueous and ethanolic extract of orange peel showed the highest total antioxidant activity than that of the lemon peel extract. The orange peel ethanolic and aqueous extracts possess strong antioxidant potential when compared with ascorbic acid standard antioxidant.

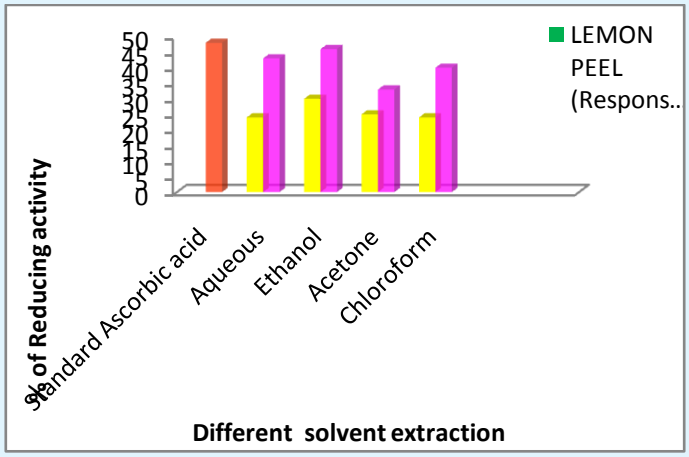
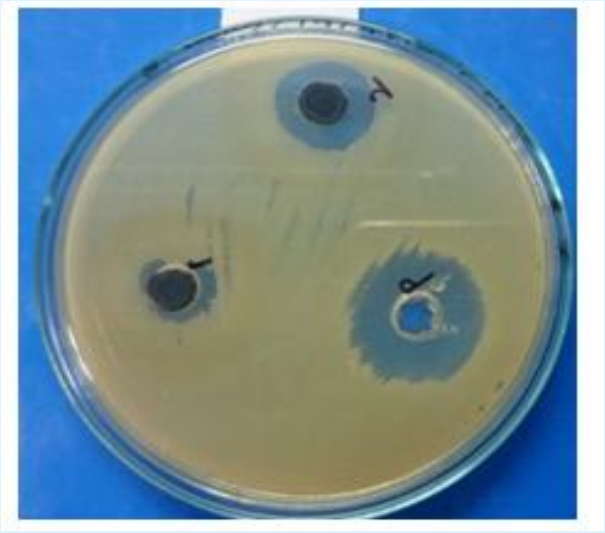


Figure 1: Antioxidant Analysis of Citrus limon L. (Lemon) and Citrus sinensis L. (Sweet Orange) Peel extracts.

The antimicrobial activity of *C. limon* and *C. sinensis* skin extracts against *K. pneumonia* and *E. coli* and the minimal inhibitory activity of the different extract are presented in the Figures 2 and 3. Ethanolic extract of *Citrus limon* and *Citrus sinensis* showed (Table 2) the highest zone of inhibition of *Klebsiella pneumonia* (10mm & 20mm) and *E.coli* (5mm&10mm).The antibacterial efficacy of the ethanolic extract of both the citrus fruits are presented in the (Figures 2 and 3) against *E. coli* and *K. pnuemonia*. Acetone extract showed the least zone of inhibition of *Klebsiella sp* (6mm & 5mm) whereas the *E. coli* exhibited 3mm zone of inhibition in *Citrus sinensis* when compared to other extracts. The aqueous and chloroform extracts did not show any inhibition on both the species viz. *E. coli* and *K. pnuemonia*.

P-Positive control ciproflacin (10µg), 1-Ethanolic extract of *C. limon* (50µg), 2- Ethanolic extract of *C. sinensis* (10µg).
Figure 2: Antibacterial activity of ethanolic extract of *Citrus limon* and *sinensis* on *Klebsiella pneumonia Sp.*



P-Positive control Ciproflacin (10µg), 1-Ethanolic extract of *C. limon* (50µg), 2- Ethanolic extract of *C.sinensis* (50µg).
Figure 3: Antibacterial activity of ethanolic extract of *Citrus limon* and *sinensis* on *E. coli.*

Pathogens	Lemon Peel Extract				Orange Peel Extract				Positive Control
	Aq	Et	Ace	Chl	Aq	Et	Ace	Chl	**Ciproflacin** (50µg)
E.coli	Nz	5mm	Nz	Nz	Nz	10mm	3mm	Nz	20
Klebsiella. pneumonia	Nz	10mm	6mm	Nz	Nz	20mm	5mm	Nz	20

Aq – Aqueous; Et – Ethanol; Ace – Acetone; Chl – Chloroform; Nz – No zone
Table 2: Antimicrobial activity of *Citrus limon L*. (Lemon) and *Citrus sinensis L.* (Sweet Orange) Peel extracts.

Discussion

The present study has shown the usefulness of the extraction methodologies adopted for efficient extraction, processing and utilization of these citrus fruit peel wastes and also to characterize the phytochemicals, antioxidant property and antibacterial activities of fruit peel wastes of lemon and oranges [5]. Our studies have demonstrated the occurrence of significant amount of secondary metabolites such as tannin, steroids, reducing sugar, proteins and high content of carbohydrates from the aqueous and chloroform extracts and other secondary metabolites from citrus fruit wastes *Citrus limon* and *Citrus sinensis* whereas the ethanolic extract indicated the unique presence of saponin too. These phytochemicals are well known to contain non-nutritive plant chemicals that possess varying degrees of disease- preventive antimicrobial and antioxidant molecules. The current study has shown valuable newer sources of raw materials for both traditional and orthodox medicine. Phytochemicals are known to display their health protective effects in diverse ways. They can act as antioxidants and protect cells against free radical damage, e.g. polyphenols, carotenoids etc [12], or in reducing risk for cancer by inhibiting tumor production [13] or antibacterial activity and hormonal stimulation [14]. The citrus peels are rich in nutrients and contain many phytochemicals with strong potential for use in drug production or as food supplements [15,16].

The increased reducing power in the citrus fruit waste extracts indicated that some of the phytochemical components in the extract were electron donors that could react with the free radicals to convert aqueous extract into more stable products and thus help to terminate radical chain reaction. Antioxidants are known to be strong reducing agents and this in principal is based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure [17].

The reducing power of the citrus fruit waste extracts was compared with a known reducing agent ASA and the reducing power of the tested extracts was markedly lower than of the reducing power of ASA. However, among the aqueous, ethanolic and chloroform extracts of the citrus fruit wastes, the ethanolic extract of orange peel has shown the highest reducing power. Also, the reducing power of the extracts exhibited increased reducing power with an increase in concentration of the extract.

The metal chelating capacity of the extracts is significant since it reduced the concentration of catalyzing transition metal in lipid per oxidation. Moreover, the chelating agents, which form ó-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion, therefore it is an important parameter. In all these fruit peels a maximum yield of antioxidants using ethanol as a solvent was extracted with orange peel. Ethanolic extract of Orange *C-sinensis* peel showed the maximum yield of 46% and *C-limon* peel showed the extraction yield of 30%.

The ethanolic extract showed inhibitory activity against all tested bacteria [18]. Comparing their results with our study with the same test organism and solvent shows that *Citrus limon* extract showed relatively similar results. On the other hand, the antibacterial activity of *Citrus sinensis L.* (Sweet Orange) showed excellent inhibitory action against the tested bacteria. The ethanolic extract showed maximum zone of inhibition against Klebsiella spp. The citrus peel extract exhibited higher antibacterial activity [19,20].

All bacteria tested in this study were multiple drug resistant bacteria. The difference in the antibacterial activity with the same source when extracted with different solvent has proved that not all phytochemicals that are responsible for antibacterial activity are soluble in a solvent. Hence solvents of different polarity should be employed as discussed in this study (polar: water, acetone, ethanol; non-polar: Chloroform). Sequential or successive solvent extraction is a good option for better solubility of many of the phytochemicals but it is always necessary to know the phytochemicals extracted by each

individual solvent so as to avoid the inclusion of unnecessary solvents for extraction process as well as to understand the role of each solvent in the extraction of a individual or class of phytochemicals [21].

Conclusion

Finally the present study has shown that ethanolic extract of citrus fruit peel wastes of *Citrus sinensis* and *C-limon* consisted of antioxidants and antibacterial compounds. It can be concluded from the study that peels due to its high antioxidant activity may prove to be a better substitute in place of synthetic antioxidants in extending the shelf life of food product by preventing the peroxide formation in the product containing fat and oil. In addition natural antioxidants are safe and impart health benefit to the consumer.

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