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 (30-35°C). 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(CN)6] (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 phytochemical 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.

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

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Lemon peel</th>
<th>Orange peel</th>
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<tbody>
<tr>
<td></td>
<td>Aq</td>
<td>Et</td>
</tr>
<tr>
<td>Tannin</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Saponin</td>
<td>_</td>
<td>+</td>
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<tr>
<td>Steroids</td>
<td>+</td>
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<tr>
<td>Reducing sugar</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>++</td>
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Aq-Aqueous, Et-Ethanol, Ace-Acetone, Chl-Chloroform (+) Positive, (-) Negative

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. pneumonia*. 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. pneumonia*.
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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.

References


