PHYTOCHEMICAL ACTIVITY OF BITTER ORANGE (Citrus aurantium L.) PEEL POWDER

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ABSTRACT
Citrus fruit peel apart from being physical and chemical barrier has several health benefiting constituents such as phytochemicals, fibre, pectin and essential oil that help to accomplish overall wellness. The present study was conducted to determine the presence of various phytochemicals present in Bitter orange (Citrus aurantium L.) peel powder using various solvents such as aqueous, acetone, ethanol, petroleum ether and chloroform. The ethanol extract of Citrus aurantium L. showed maximum number of phytochemicals present followed by acetone, aqueous, petroleum ether and chloroform extract. Estimation of total phenol, tannin and flavanoid content was carried out using three different extracts (aqueous, ethanol and acetone). The results indicated that total phenol content, total tannin content and total flavonoid content of Citrus aurantium L. peel powder was highest in ethanol extract i.e. 1.764 mg/Gallic acid g, 1.76 mg/TAE g and 0.32 mg Qe/g respectively.

KEYWORDS: phytochemicals, bitter orange, fruit peel powder.

1. INTRODUCTION
Fruits are an important source of compounds called phytonutrients such as ascorbic acid, flavonoids, carotenoids, phenolic acid, tocopherol and sulphur containing compounds, which possess antioxidant properties.[1] Citrus fruits belong to the family rutaceae, are one of the main trees grown throughout the world.[2] Citrus fruits are richer sources of bioactive compounds having beneficial effect on human health such as Vitamin C, caroteniods, flavonoids, limonoids, essential oils, acridone alkaloids, minerals and vitamin B complex.[3]
Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Most of the phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer.[4]

The Bitter orange (Citrus aurantium L.) is sometimes known as the sour, bigarde or Seville orange is a native of Southeastern Asia. The bitter orange (Citrus aurantium), although resembling the orange, differs by several characters. Its pulp is acidic and the albedo is more bitter.[5] Bitter orange peel contains a volatile oil with limonene (about 90%), flavonoids, coumarins, triterpenes, vitamin C, carotene, and pectin. In the oil of the peel of citrus fruits is a phytonutrient known as limonene, it reduces the activity of proteins that can trigger abnormal cell growth.[6] Bitter orange is used for inflammation of eyelids, conjunctivae, muscle pain, rheumatic pain and phlebitis.[7] Preparation from peel, flowers and leaves of bitter orange (Citrus aurantium L.) are popularly used in order to minimize central nervous system disorders.[8] The Bitter orange is grown in the United States for ornamental purposes and to be used as a stock in grafting as it is too acid to be used as a fresh fruit. It is grown in Spain extensively however, the fruits are used for marmalades, orangeade and candied orange peel. In India, it is used to prepare pickles.[9] In traditional Chinese medicine, the peel of the immature fruit is used for indigestion, abdominal pain, constipation, and dysenteric diarrhoea.[6]

![Figure 1: Bitter orange fruit](image)

2. MATERIALS AND METHODS

2.1 Collection, separation and drying of Bitter orange peel

The Bitter orange fruits were purchased. The peels were manually separated from the fruit. The peels were shade dried. The dried peels were collected and ground well to form a
powder. The powdered bitter orange peel was stored in an airtight container and used for various tests

2.2 Preparation of the peel extract
Preparation of the extracts was assessed by the following method as described by Janarthanam et al.\textsuperscript{[10]} One gram of dried bitter orange peel powder was extracted with 20 ml of aqueous, ethanol, acetone, chloroform and petroleum ether and soaked overnight at room temperature. The sample was then filtered through Whatman.No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extract was approximately 100%. The extracts were used for further tests.

2.3 Qualitative phytochemical analysis
The phytochemical tests were carried out using standard methods of analysis of tannins, saponins, quinones, flavanoids, glycosides, cardiac-glycosides, terpenoids, phenols, coumarins, steroids, alkaloids, anthocyanin and betacyanin.\textsuperscript{[11,12,13]}

2.4 Quantitative phytochemical analysis
2.4.1 Determination of total phenol content
The Folin-Ciocalteu’s reagent method has been used for estimation of total phenolic content, according to Lister and Wilson\textsuperscript{[14]} with slight modification. 100µl of crude extract of the bitter orange peel was mixed with 0.5 ml of Folin-Ciocalteu’s reagent (1/10) dilution and 1.5 ml Na2Co3 (2% w/v). The blend was incubated in a dark place at room temperature for 15 minutes. The absorbance of blue coloured solution of all samples was measured at 765 nm using a UV spectrophotometer.

The results were expressed in mg of gallic acid equivalent (GAE) per gram dry weight of plant.

2.4.2 Determination of total tannin content
Tannin content of bitter orange peel extract was estimated by the method described by Fagbeme et al.\textsuperscript{[15]} The peel extract (1 ml) was mixed with Folin-Ciocalteu’s reagent (0.5 ml) followed by the addition of saturated Na2Co3 solution (1ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 minutes at room temperature. The supernatant
was obtained by centrifugation and absorbance was recorded at 725nm using UV – visible spectrophotometer.

Tannin content was calculated as mg tannic acid equivalent obtained from a calibration curve.

Abs 725 nm = 7.061 x (TA) mg

Where, (TA) mg is the concentration of tannic acid taken as standard.

2.4.3 Determination of flavanoid content
The Aluminium Chloride calorimetric method was modified from the procedure reported by Woisky and Salantino.[16] Quercetin was used to make the calibration curve, 10 mg of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/ml. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95 % ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank.

3. RESULTS AND DISCUSSION
3.1 Collection, separation and drying of Bitter orange peel
The dried peels were collected, ground well to form a powder, stored in an airtight container and used for various tests.

![Figure 2: Drying and powdering of the Bitter orange peel](image)

3.2 Preparation of peel extract
The dried bitter orange peel powder was extracted using five different solvents (aqueous, etnanol, acetone, chloroform and petroleum ether).
3.3 Qualitative Phytochemical Analysis

The Qualitative phytochemical analysis of different extracts of Bitter orange peel powder is presented in Table 1.

Table 1: Qualitative phytochemical Analysis of *Citrus aurantium* L. peel powder

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Aqueous</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Betacyanin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

++ Present in moderate amount;  + Present in trace amount; - Absent

From table 1, it can be inferred that the bitter orange peel powder is a good source of phytochemicals such as tannins, saponins, flavonoids, cardiac glycosides, phenols, coumarins, alkaloids and betacyanin. Phytonutrients are vital in both; health promotion and disease prevention.**[17]** These phytochemicals scavenge the free radicals circulating in the body, thereby reducing the oxidative stress caused by the free radicals.**[18]**
Tannins have astringent properties, hasten healing of wounds, and inflamed mucous membrane.\(^{[17]}\) Saponins natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotics, helping the body to fight infections and microbial invasion.\(^{[19]}\) Flavonoids are another phytochemicals found in citrus fruits. They show anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activity.\(^{[20]}\) Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities.\(^{[21]}\) Alkaloids are known to have anticancer activities and antibacterial potential.\(^{[22]}\)

### 3.3 Quantitative phytochemical analysis

The results for total phenol content and total tannin content of *Citrus aurantium* L. peel powder (Table 2 and Table 3) shows that ethanol extract had greater concentration of total phenol and tannin. For total flavonoid content (Table 4) it was found that ethanol had greater concentration followed by aqueous and acetone.

#### Table 2: Estimation of total phenol content

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Extract</th>
<th>Absorbance (nm)</th>
<th>Total Phenol Content Concentration (mg Gallic acid equivalent/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>0.53</td>
<td>1.764</td>
</tr>
<tr>
<td>2</td>
<td>Acetone</td>
<td>0.44</td>
<td>1.464</td>
</tr>
<tr>
<td>3</td>
<td>Aqueous</td>
<td>0.36</td>
<td>1.44</td>
</tr>
</tbody>
</table>

#### Table 3: Estimation of total tannin content

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Extract</th>
<th>Absorbance (nm)</th>
<th>Total Tannin Content Concentration (mg tannic acid equivalent /g )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>0.22</td>
<td>1.76</td>
</tr>
<tr>
<td>2</td>
<td>Acetone</td>
<td>0.04</td>
<td>0.32</td>
</tr>
<tr>
<td>3</td>
<td>Aqueous</td>
<td>0.02</td>
<td>0.192</td>
</tr>
</tbody>
</table>

#### Table 4: Estimation of total Flavonoid content

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Extract</th>
<th>Absorbance (nm)</th>
<th>Total Flavonoid Content Concentration (mg Quercetin/gm )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>0.09</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>Acetone</td>
<td>0.01</td>
<td>0.036</td>
</tr>
<tr>
<td>3</td>
<td>Aqueous</td>
<td>0.02</td>
<td>0.086</td>
</tr>
</tbody>
</table>

A Study conducted by Silalahi\(^{[23]}\), indicate that flavonoids have been shown to be able to act as antioxidants by scavenging free radicals, an activity related to their phenol rings containing hydroxyl groups. Quercetin, myricitin, rutin, tangeritin, naringin and hesperidin are found amongst the common flavonoids in citrus fruits.\(^{[24]}\) Quercetin also shows remarkable anti-
tumor properties. Quercetin may have positive effects in combating or helping to prevent cancer, prostatitis, heart diseases, cataracts, allergies/inflammations and respiratory diseases such as bronchitis and asthma.\textsuperscript{[25]} Hesperidin is a flavonoid glycoside found abundantly in citrus fruits. Hesperidin reduces cholesterol and also has anti-inflammatory effects.\textsuperscript{[26]} The presence of tannin could be responsible for the bitter principle and sour taste of some citrus species.\textsuperscript{[17]}

4. CONCLUSION

The present study was conducted to determine the phytochemical activity of Bitter orange (\textit{Citrus aurantium} L.) peel powder. In conclusion it can be stated that the \textit{Citrus aurantium} L. peel powder has a high quantity of phytochemicals and antioxidants. The main idea of the study is to make use of fruit peels which are rich in phytochemicals and antioxidants usually discarded or wasted. As the fruit peels are high in phytochemicals it can be used in the treatment of diseases such as cancer, cardiovascular condition and diabetes mellitus. The \textit{Citrus aurantium} L. peel powder which has an excellent aroma can be incorporated into various recipes to enhance the flavour, taste and nutritive value of recipes.

5. REFERENCES


