

## Comparative Study for Citrus Fruits Peel Extract , Phytochemical Screening and In-vitro Antioxidant Activity

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### Abstract

Oxidative stress is one of the pharmacological & toxicology mechanisms and an important pathological mechanism proposed for many diseases including cancer . Antioxidants remove these free-radical intermediates and inhibit other oxidation thus stopping the harmful chain reactions for all living cells, There is an increasing interest in the antioxidants measurement of plant constituents which has antioxidant components replace synthetic ones , citrus genus which belongs to Rutaceae family includes some of the most widely cultivated crops in the world because of their many nutritional and health benefits. It is an important economically but the attention to leaves and peel had not given importance in comparison to fruits despite the presence of phenols quantity that varies among species , therefore, this comparative study aims involve ethanolic citrus peel extract preparation, phytochemical investigation and antioxidant determination of two different species *Citrus maxima* ( pomelo) and *Citrus sinensis* (sweet orange ) fruit peel.

The preliminary phytochemical analysis of the prepared peel extract showed the presence of several constituents of citrus peels as alkaloids, phenolics , flavonoids and rutin. Moreover , the result of in-vitro antioxidants activity of both citrus species showed that DPPH radical scavenging activity for peel extract exhibit a significant dose dependent inhibition with IC<sub>50</sub> was calculated (287.32 ug/ml and 341.89 µg/ml) for pomelo & orange respectively compared to IC<sub>50</sub> value of vitamin C which was 260.06 µg/ml as standard antioxidant . in conclusion , study results showed that pomelo peel show higher antioxidant activity compared to orange moreover, the citrus peel has significant antioxidant properties and it is potentially rich sources of natural antioxidants and PPE exhibited great efficiency in scavenging DPPH and reactive oxygen radicals .

**Keywords:** DPPH, Antioxidant capacity, Citrus maxima, Flavonoids, Vitamin C .

دراسة عقاقيرية- كيمونباتية ومقارنه تأثير مضادات الاكسدة لمركبات مستخلص قشور الحمضيات  
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### الخلاصة

الإجهاد التأكسدي هو أحد الآليات الدوائية ويعتبر آلية مرضية مهمة مقترحة للعديد من الأمراض بما في ذلك السرطان. مضادات الأكسدة تمنع الجذور الحرة الإشكالية التأكسدية الأخرى وبالتالي توقف التفاعلات المتسلسلة الضارة لجميع الخلايا الحية، وهناك اهتمام متزايد بقياس مضادات الأكسدة لمكونات النبات التي تحتوي على مكونات مضادة للأكسدة لتحل محل المكونات الاصطناعية فيحمية الأدوية والاعذية من التاكسد والتلف . يشتمل جنس الحمضيات الذي ينتمي إلى عائلة Rutaceae بعض المحاصيل المزروعة على نطاق واسع في العالم لما لها من فوائد غذائية وصحية عديدة. إنها مهمة من الناحية الاقتصادية ولكن الاهتمام بالأوراق والبذور لم يكن مهم مقارنة بالثمار على الرغم من وجود كمية من الفينولات التي تختلف بين الأنواع، لذلك تهدف هذه الدراسة المقارنة إلى تحضير مستخلص قشر الحمضيات الإيثانولي والفحص الكيميائي النباتي وتحديد مضادات الأكسدة من نوعين مختلفة من قشرفاكهة البوميلي *Citrus maxima* وقشر فاكهة البرتقال *Citrus sinensis* وقد أظهر التحليل الكيميائي النباتي الأولي للمستخلص المحضر وجود العديد من مكونات قشور الحمضيات مثل القلويدات، الفينولات، الفلافونويدات والرتين. بالإضافة إلى ذلك، أظهرت نتيجة نشاط مضادات الأكسدة في المختبر لنوعي الحمضيات أن نشاط الكبح للجهد التأكسدي DPPH بشكل تدريجي متوافق مع زيادة تركيز مستخلص القشر كما أظهر تثبيطاً قوياً من خلال قياس أقل تركيز من المستخلص الذي تسبب بتقليل التاكسد إلى ٥٠٪ (IC<sub>50</sub>). حيث أظهرت نتائج الدراسة ان كلا النوعين من الحمضيات لدية مضاد اكسدة عالي ولكن قشر فاكهة البوميلي اظهر تأثيراً اقوى بلغ (287.32) ميكرو غرام/مل=IC<sub>50</sub> ) بالمقارنة بمستخلص قشر البرتقال ( ٣٤١,٨٩ ميكرو غرام/مل=IC<sub>50</sub>) ومن خلال النتائج يتضح ان قشر فاكهة البوميلي هو الاقرب الى قيمة IC<sub>50</sub> (لفيتامين ج) النقي كمضاد أكسدة قياسي والتي بلغت

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Received: 13/7 /2022

Accepted: 4/ 10/2022

٢٦٠,٠٦ ميكروغرام/مل . في الختام، أظهرت نتائج الدراسة أن قشر البوميلو أظهر نشاطاً مضاداً للأكسدة أعلى مقارنة بالبرتقال وإن قشر الحمضيات له خصائص مضادة للأكسدة كبيرة ومن المحتمل أن تكون مصادر غنية بمضادات الأكسدة ويمكن الاستفادة منها في المحافظة على الاغذية والادوية المصنعة من التالف .

الكلمات المفتاحية : مضادات اكسدة ، حمضيات ، فلافينويدات ، فيتامين ج

## Introduction

Plants are valuable source of natural products for maintaining health in human beings. Phytochemicals present in plant extracts have potential antioxidant properties which may be useful in therapeutic applications<sup>(1)</sup>. Citrus fruit is rich in antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, beta-sitosterol, and polyphenols like flavonoids, flavones glycosides, and rutin<sup>(2,3)</sup>. This study aims to phytochemical investigation and determination the antioxidant properties in two varieties citrus fruit peel namely sweet orange (*Citrus sinensis*) and pomelo (*Citrus maxima*). The peels of *Citrus sinensis* and *Citrus maxima* were collected and the total phenolic content was calculated using Follin ciolcaletu's reagent<sup>(4)</sup>. The amount of terpenoids was found in greater amount in pomelo peel than in the orange peel<sup>(5)</sup>. It was also found that alkaloids were present in greater amount than the other phytochemicals in orange and pomelo peel<sup>(6)</sup>. Preliminary photochemical analysis and estimation of total phenolic content was done in Citrus fruit peel<sup>(7)</sup>. Thus, it is established that orange and pomelo peel can be used medicinally for their antibacterial and anti-oxidant activities due to the presence of significant amount of alkaloids in them<sup>(8)</sup>.

## Materials and Methods

### Preparation of citrus peel Extract (CPE)

An aqueous ethanolic citrus ( pomelo and sweet orange ) peel extract prepared according to the method reported by (Abeyasinghe et al. 2007)<sup>(9)</sup> with slight modifications, by soaking 10g of the dry powdered Citrus peel in 100ml of 80% ethanol. The mixture was poured into test tubes, covered with the aluminum foils, placed into water bath for extraction at 65°C for 3h and the whole solution was filtered and the filtrate allowed to evaporate into dryness in an oven at 60°C. The process was repeated several times and the yield was noted. The extract was refrigerated at 4°C for future use in experimental studies. Also, the required extract frequently prepared every 48 h and used fresh for each experiment<sup>(9)</sup>.

### Preliminary phytochemical screening

Qualitative phytochemical investigation of the prepared citrus (Pomelo or orange) peel ethanolic extract was carried out as follows via standard method involving :

#### 1-Alkaloids Determination Using\_Dragendroff Test

An aliquot of ethanolic extract was spotted on TLC plate , after development with

mobile phase (chloroform:methanol) (8:2) the TLC plate was dried and sprayed with dragendroff reagent.

#### 2-Terpenoids Determination test using (Salkowski test)

A 5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was added to form a layer. The formation of reddish-brown color indicates the presence of terpenoids.

#### 3- Total Phenolics Determination

##### Qualitative test (Ferric chloride test )

An aliquot of Plant extract added with 5ml distilled water and 5% FeCl<sub>3</sub>. Appearance of dark green color shows the presence of phenolics.

##### Quantitative test

The total phenolic compound (TPC) was determined by the Folin-Ciocalteu method to quantify the amounts of Phenolic contents of both types of citrus & pomelo peel extract . The total phenolic content in the methanol seeds extract of *C. fistula* was measured using Folin-Ciocalteu reagent based on procedures described by (Singleton *et al.* 1999)<sup>(10)</sup>, with some modifications . Briefly, 0.5 ml of methanolic seeds extract (1 mg/ml) was mixed with 1.5 ml (1:10 v/v diluted with distilled water) Folin -Ciocalteu's reagent and allowed to stand at 22°C for 5 min . Then 2 ml of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>, 7.5%, w/v) was added and the mixture were allowed stand for another 90 min and kept in the dark with intermittent shaking. Then the absorbance of the blue color that developed was measured at 725 nm using spectrophotometer (HITACHI U-1900 spectrophotometer 200V). The experiment was carried out in triplicates . Gallic acid was used for constructing the standard curve (25 to 150 µg/ml; Y= 0.0008X; R<sub>2</sub>= 0.9876) and the total phenolic compounds concentration in the seeds extract was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract<sup>(10)</sup>.

#### 4- Flavonoids Determination test

##### Qualitative tests

Ethanolic KOH 2% (2ml) was added to (1ml) of ethanolic extract of plant .The formation of yellow color indicate the presence of flavonoids.

##### Quantitative tests

Total flavonoid content was determined using Aluminium chloride colorimetric assay method used by (Chang *et al.* 2002)<sup>(11)</sup>, which involve the reaction mixture containing 0.1ml of 10% AlCl<sub>3</sub> in ethanol, 2.8 ml distilled water and 0.1 ml of 1 M potassium acetate (120 mM) was added to 0.5 ml of ethanol-water (80:20,v/v) extract of pomelo peel previously made, incubated at room

temperature for 30 min. Absorbance was read at 415 nm with (Shimadzu UV-visible spectrophotometer, Japan), the amount of 10% aluminium chloride was substituted by the same amount of D.W. in blank and the calibration curve was obtained using 80% alcoholic solution containing 10 grams of quercetin then diluted to (25, 50 and 100 µg/ml) used as standard solution to determine the flavonoid content of the sample extract <sup>(11)</sup>.

#### **Estimation of the antioxidant Citrus Peel Extract activity**

Citrus Peel Extract Antioxidant activity and minimum inhibitory concentration 50% (IC<sub>50</sub>) were determined by using 2,2-diphenyl-1-picrylhydrazyl DPPH assay, Antioxidant capacity of the citrus peel extract (CPE) for orang was measured using DPPH assay as described by (Tippani et al. 2010) <sup>(12)</sup> with minor modification. Briefly various concentrations of PPE starting from (500-25µg/ml) were made by serial dilutions from previously prepared ethanolic extract stock solution (4000 µg/ml). Equal volumes of each extract were pipetted into 0.2mM ethanolic solution of (1,1-diphenyl-2-picrylhydrazyl (DPPH)) to initiate the reaction for creating a calibration curve. After shaking, the mixture was incubated in dark for 30min. Ascorbic acid (vitamin C) widely studied antioxidant, was used for comparison or as a positive control.

The DPPH solution in the absence of CPE used as control and the 80% ethanol was used as blank discolorations were measured at 517 nm by using UV-spectrophotometer (HITACHI U-1900). Measurement was performed at least in triplicate (12). The percentage of the DPPH free radical was calculated using the following equation: DPPH scavenging effect (%) = ((A<sub>0</sub>-A<sub>1</sub>)/ A<sub>0</sub>) x 100

Where A<sub>0</sub> was the absorbance of the control and A<sub>1</sub> was the absorbance in the presence of the Citrus peel extract. The actual decrease in absorption induced by the test was compared with the positive controls.

The IC<sub>50</sub> (concentration providing 50% inhibition) values were calculated use the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding % of DPPH scavenging effect.

## **Results and Discussion**

### **The preliminary phytochemical analysis**

Screening tests of the prepared extract shown the presence of several constituents of citrus peels. The results are revealed in the table. The ethanolic extract showed different class of phytochemicals ( Table 1 ).

#### **Estimation of the antioxidant Citrus Peel Extract activity**

The result of in-vitro antioxidants activity of both citrus species, DPPH radical is commonly used as substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay. Citrus peel extract (CPE) prepared as stock and serial dilutions were screened for DPPH radical scavenging activity for peel extract exhibit a significant dose dependent inhibition of DPPH radical color according to the method previously described based on color change of DPPH solution from purple to yellow as the radical is quenched by the CPE antioxidant contents, measured quantitatively by spectrophotometer absorbance at 517 nm (Table 3, Figure 1.a). Moreover, concentration of the extract required to inhibit 50% of the initial DPPH free radicals (IC<sub>50</sub>) was calculated (287.32 ug/ml and 341.89 µg/ml) for pomelo & orange respectively compared to IC<sub>50</sub> value of vitamin C which was 260.06 µg/ml as standard antioxidant (Table 3, Figure 1.b). Basically, a higher DPPH radical-scavenging activity is associated with a lower IC<sub>50</sub> value. Previous studies showed that samples which had IC<sub>50</sub> lower than 50ug/ml was very strong antioxidant, 50-100 µg/ml was a strong antioxidant, 101-150 µg/ml was medium antioxidant whereas a weak antioxidant with IC<sub>50</sub> > 150µg/ml. <sup>(13)</sup> Therefore, citrus peel extract has good a free radical scavenger activity and both species activity was comparable to the previous study which represented that IC<sub>50</sub> of 13 different types of citrus peels were found to fall between 0.6-3.8 mg/ml. <sup>(14)</sup> Therefore, the current study results showed that pomelo peel had higher antioxidant activity compared to orange moreover, citrus peel possessed high antioxidant properties and it is potentially rich sources of natural antioxidants and PPE exhibited great efficiency in scavenging DPPH and reactive oxygen radicals.

**Table 1 . Qualitative tests for the citrus peel extract**

No.	Test	Orangepeel extract	Pomelo peel extract
1-	Alkaloids	+	+
2-	(Phenolic test) ferric chloride	+	+
3-	Terpenoids test	+	++
4-	Flavonoids test	+	+

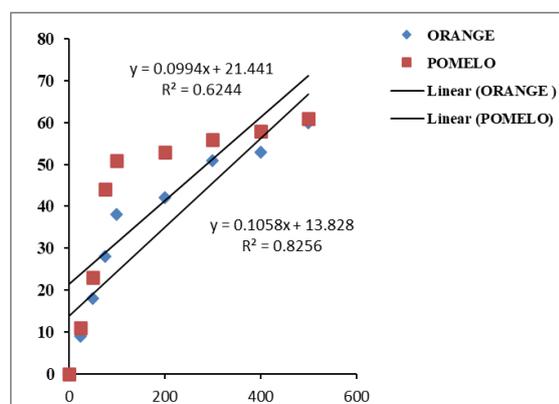
The (pomelo Peel Extracts) PPE exhibited higher total phenolic content (TPC)  $74.62 \pm 1.7$  mg/g GAE while the orange peel extracts (OPE) showed lower TPC of  $45.73 \pm 1.4$  mg/g GAE (Table 2) . Total flavonoids content (TFC) of PPE was  $21.12 \pm 4.1$  mg/g quercetin compared to  $23.2 \pm 2.3$  mg/g quercetin for OPE . (Table 2) .

**Table 2 . Quantitative determination of total phenolics & total flavonoids content of the citrus peel extract**

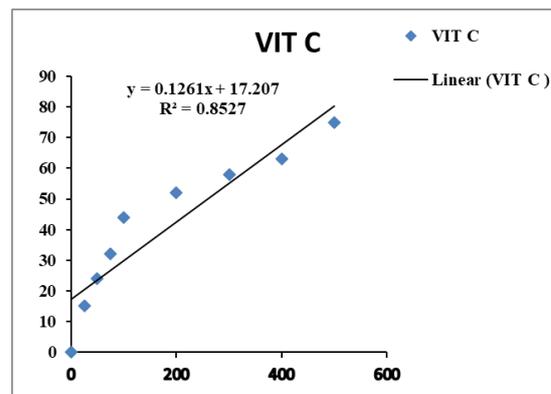
Citrus peel	Total phenolics(TPC) mg/gGAE	Total flavonoids (TFC)mg/g quercetin
Pomelo	$74.62 \pm 1.7$	$21.12 \pm 4.1$
Orange	$45.73 \pm 1.4$	$23.2 \pm 2.3$

**Table 3 . Determination of citrus extract Antioxidant activity compared to vitamin C standard**

% of DPPH INHIBITION			
Ext Conc.	Orange	Pomelo	Vit C.
0	0	0	0
25	9	11	15
50	18	23	24
75	28	44	32
100	38	51	44
200	42	53	52
300	51	56	58
400	53	58	63
500	60	61	75



**Figure 1. a – DPPH Antioxidant activity of pomelo peel compared to orange peel extract**



**Figure 1 b. DPPH Antioxidant activity of standard vitamin C**

**Acknowledgments**

The authors thank dean as well head manager of pharmacy department/Al-Rasheed University College for providing guidance and supply for all laboratory requirements to finished this work.

**Conflicts of Interest**

Authors declare that there was no conflicts of interest regarding the publication of this paper.

**Funding**

Authors declare that there was no funding received for conducting this study or assist with the preparation of this manuscript .

**Ethics Statements**

Authors declare that this study was in-vitro and phytochemical screening study need no ethical approval from an ethics committee .

**Author Contribution**

The authors confirm contribution to the paper as follows: study conception and design: Ihab I. Al-Khalifa, plant collection and extraction: Rand A. Aziz & Humam L.Qusay , data collection , analysis and interpretation of results : Mohammed K. Abbas & Mohammed M. Fadhil , draft manuscript preparation : Shaimaa M. Mohammed .

All authors reviewed the results and approved the final version of the manuscript.

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