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Measurement of Some Citrus Species Content From Total Flavonoids and Antioxidant

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Abstracts: The aim of this study was to measure content of total flavonoids for most types of citrus cultivated in Iraq as well as the antioxidant activity. The antioxidant was highest in orange peels [5129] micromole Fe⁺² / gm dry matter. Citrus peels and seeds are a good source of natural antioxidants that can be used both in the medical and commercial fields.

Keywords: Flavonoids; Antioxidant; Citrus; Polyphenols.

I. Introduction

Citrus is cultivated in more than 80 countries around the world. It is one of the most important fruit trees in the world, as it occupies the first position in world production, which in 1999 reached 98,258 million tons [1]. The Iraqi production of the cultivated citrus varieties for the year 2019 and for the winter season, which are oranges, sour lemons, sweet lemons, and lankan citruses (133500, 5178, 1436, 4167 and 21498) tons, respectively. Salah al-Din, Wasit, Diyala and Baghdad occupied the first places in production [2]. Citrus fruits are native to East Asia, especially on the southern coast of China. They belong to the family Rutaceae under the scientific name Citrus sp. They are large evergreen trees, the leaves of which are simple green in color, veining, smooth and have a distinctive smell. Its flowers are white to small yellow and its green fruits turn into yellow or light orange to dark at ripeness and according to the type, and the size of the fruits also ranges from the small in the fruits of the tangerine [mandarin] to the large in the fruits [3]. Citrus fruits have a nutritional and preventive importance, as they work with vitamin C to reduce the possibility of cancer or heart attacks, strengthen bones and teeth, heal wounds, and make the skin supple [4].

Phenols are the second largest group of secondary metabolites in plants after alkaloids. Among the most important phenols in citrus fruits are flavonoids [or polyphenols], which are responsible for the color of fruits [5]. The peel and seeds of citrus fruits are very rich in phenols, such as phenolic acids and flavonoids. According to its molecular structure, it is divided into six groups, namely, flavones, flavanones, flavonols, isoflavones, anthocyanidins, flavanols or [catechins], as in Figure 1. [6] The peel of citrus fruits contains more of them than in the seeds [7]. 7-O-glycosylflavanones are the most abundant flavonoids of all citrus fruits. Whereas, neohesperidoside, naringin, neohesperidin, neoriocitrin are mainly found in bitter orange juice and grapefruit [8]. Flavonoids have an antiviral and antibacterial effect, and at the same time they increase immunomodulation and reduce symptoms associated with persistent bleeding and low calcium levels [9]. Flavonoids have antioxidant activity and this makes them such powerful essential stimulants that can be adopted as a good source of natural antioxidants for medical and commercial uses [10,11]. Therefore, the study dealt with measuring the flavonoids content of the peels and seeds of the types of citruses grown in Iraq and measuring the antioxidant activity to take advantage of them in several areas, especially as they are waste for food processing.

Fig. 1. The chemical composition of flavonoids [6].

II. Material and Methods

1. Raw Material

Five types of citrus fruits grown in Iraq and in Salah al-Din, Wasit, Baghdad and Diyala were used (orange, sour lemon, sweet lemon, Citrus aurantium, and mandarin) from the 2019 crop. Samples were kept in polyethylene bags by lyophilization.

2. Preparing of extracts

The method mentioned in [12] was used to prepare extracts of peels and seeds and their total flavonoids content were to be measured. 3 gm of sample powder was taken and placed in a 150 ml heat-tolerant beaker, 60 ml of 95% ethanol was added to it, and the beaker was placed in a shaking water bath for 90 minutes. Filter the extract with Whitman No.1 filter paper. Reduce the volume of ethanol using a rotary evaporator until there is residue. 5 ml of methanol was added to the sediment and the extract was placed in a marked test tube and kept in the refrigerator at $4 \,^{\circ}$ C.

3. Measuring of total flavonoids

The total flavonoids were measured according to the method mentioned [13]. 0.5 ml of the required sample extract was taken and placed in a test tube. To it, add 2.5 ml of ethanol and mix it well. 3 ml of aluminum chloride at a concentration of

0.01 mol was added to it. The tube was left for 10 minutes at room temperature. The optical absorption reading was taken by a Spectrophotometer at a wavelength of 400 nm, as well as for the blank tube that contains all the additives except for the sample extract.

4. Standard curve preparation

Standard curve was prepared using Rutin- $C_{27}H_{30}O_{16}$ at concentrations (5-50) mg / L. Its absorbance readings were taken and a straight-line equation was extracted for use in calibrating the readings and the results were attributed to mg / g dry weight. Fig. 2

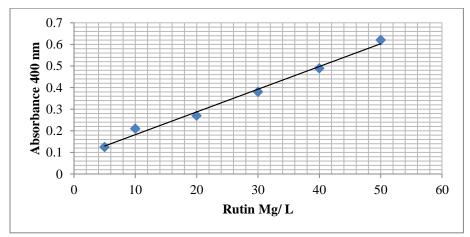


Fig. 2. Standard curve for rutin concentrations.

5. Measuring of antioxidant activity

Use the FRAP test. (Ferric Reducing Antioxidant Power) mentioned by [14] and [15]. In which the ferric Fe⁺³ ions are reduced to the Fe⁺² ferrous ions in the presence of antioxidants. The absorbance was measured at a wavelength of 590 nm (the effectiveness of antioxidants is expressed as the amount of iron they consume - micro mol Fe⁺² / g dry weight).

III. Results and Discussion

Figure 3. shows the content of the citrus species in this study (peels and seeds) of total flavonoids. The results showed that the peel content of flavonoids was higher than that of the seeds and for all types of citrus studied, and this is consistent with what was mentioned [7]. The highest value for lemon was (46.32) mg / g of dry matter. While it reached (40.89, 37.56, 28.34, 4.12) mg / gm in the peel and (11.72, 8.5, 12.8, 1.31) mg / gm in orange, sweet lemon, mandarin and aurantium seeds, respectively.

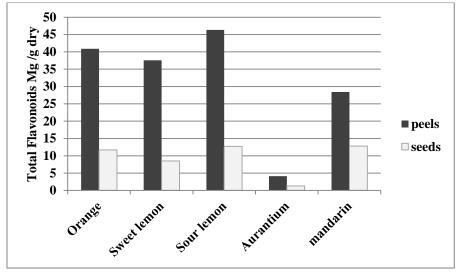


Fig.3. Total flavonoid content of the citrus species.

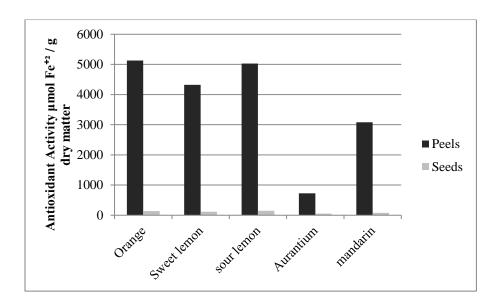


Fig. 4. Antioxidant activity measured in μ mol Fe⁺² / g dry matter of the citrus species.

The results show that the types of citrus under study have antioxidant activity. It showed the highest antioxidant activity in orange peels and reached 5129 micro mol Fe⁺² / g of dry matter. As for the seeds, it was highest in lemon seeds, reaching 147 micro mol Fe⁺² / g form 4. It is higher than what he mentioned [16]. The results of the research concluded that the secondary wastes of citrus species are a good source of naturally extracted antioxidants that can be used in different areas instead of their industrial counterparts.

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