European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 03, 2020

Estimation Of Phenolic Compounds And Evaluation Of Their Antioxidant Activity Of Some Parts Of The Orange Plant (*Citrus Sinensis* L.)

Iman Mohsin Kadhom[1], Nagham Saddon Ibrahim[2], Najim Abdullah Al-zubaidy[3]

Ministry of Educat[1]
Diyala university/ Collage of Basic Education[2]
Diyala university/ Education Collage for Pure Science[3]

ABSTRACT:

Orange fruits were collected from one of the gardens of Baqubah / Diyala / Iraq in January of 2020, while samples of leaves and flowers were collected in March of the same year, for the purpose of completing the research procedures. The present study aims to estimation of phenolic compounds and evaluation of their antioxidant activity of the extract of some parts of the orange plant. The results show a variation in the parts of the orange plant in their content of phenolic compounds. Moreover, the statistical analysis pointed to the presence of significant differences at the probability level of P <0.05 among the parts of the orange plant, in Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). The leaves are superior in their (TPC) which record the highest concentration of 145.71 mg(GAE) .g^{-1} in comparison with the juice, which recorded the lowest (TPC) 57.18 mg(GAE) .g^{-1}. Though the highest (TFC) in the mesocarp of fruit is 11.14 mg Querecetin .g^{-1} compared to 5.81 mg.Querecetin. g^{-1} in the juice, which gave the lowest (TFC).

On the other hand, the result of statistical analysis showed significant difference among the parts of orange plant at the probability of P <0.05 in antioxidant activity. The result showed that the highest antioxidant activity, detected by using the DPPH scavenging test of the flowers is 2.64 mg. mL^{-1}. While the lowest value of the free radicals DPPH inhibition, which was 0.86 mg. mL^{-1} was obtained from exocarp. The statistical analysis results indicated a positive correlation among the (TPC), (TFC) and the antioxidant activity of DPPH free radical test with value of 0.385 and 0.484 respectively.

Key words: antioxidant activity, phenolic compounds ,Orange, DPPH, HPLC

1-INTRODUCTION:

Orange plant, which belongs to the *Rutaceae* family considered as one of the most important fruits. It is cultivated on a large scale around the world for the purpose of obtaining the pulp of the fruit, as well as its natural juice [1]. Due to the high content of secondary metabolites and natural antioxidants such as flavonoids, carotenoids and vitamins. [2], its natural juice works in strengthening the human body's immunity and increase its activity[3]. However, the waste from processing oranges accounts for about 50% of the fruits[4]. Antioxidants suppress the activity of free radicals, which are atoms, molecules, or ions with one or more (non-double) electrons, and they tend strongly to interact with other molecules to obtain the

required electron and reach a stable state [5]. Free radicals arise naturally in plant and animal cells as a result of the processes of air respiration and metabolism, and they are harmless when they are in low concentrations[6], but, When present in a high concentration, it causes oxidative stress which leads to cell damage and death [7]. Zhang et al.[8] conducted a study to evaluate the Total Phenols Content (TPC), Total Flavonoids Content (TFC) and the antioxidant activity of the different parts of citrus (peels, pulp residues, seeds and juices) of 19 different species. The results indicated the presence of a positive correlation coefficient between the TPC and the TFC with their antioxidant activity. The value of TPC ranged from 0.079 - 0.792, while the value of TFC ranged from 0.150 - 0.664. In another study conducted by Olyad et al.[9] to evaluate the flavonoid content and their antioxidant activity of the peels of four citrus species, cultivated in Ethiopia, C. *sinensis* and C. *reticulate*, C. *limon* and C. *aurantifolia*. The results showed a strong correlation between flavonoids content in citrus peel extracts and free radical removal activity, with a value of 0.975. Antioxidants are classified into natural and synthetic . Several synthetic antioxidants have been shown to have a toxic effect and stimulate the formation of cancer cells, therefore, researchers have resorted to finding safer natural alternatives [10] The purpose of this study is to estimate the phenolic compounds and evaluate their antioxidant activity of extracts of some parts of the orange plant (*Citrus sinensis* L.).

2-MATERIALS AND METHODS

: *2-1-Samples collection and preparation*

Orange fruits were collected from one of the gardens of Baqubah / Diyala / Iraq in January of 2020. After washing the fruits with distilled water for several times to remove impurities and soil, they were peeled, and the required parts were separated, which are the exocarp, mesocarp, seeds and juice. At the flowering stage, in March of the same year, samples of leaves and flowers were collect for the purpose of completing the research procedures.Some of the fruits were used to obtain fresh juice, while the remaining samples were dried in an electric oven and milled using a commercial coffee grinder. The dry powder was kept in a refrigerator at a temperature of (4C°) until the extraction operations were carried out.

2-2-Extraction of phenolic compounds:

Extraction of phenolic compounds was done based on a method described by [11]. One (1) gm of *Citrus* sample powder and 1 ml from juice were dissolved in 20 ml hexane to remove fat layer , then the organic layer dissolved in 100 ml of 80:20 (methanol: water), The extract was subjected to ultra-sonication (Branson sonifier, USA) at 60 % duty cycles for 25 min at 25°C followed by centrifugation at 7,500 rpm for 15 min. The clear supernatant of each sample was subjected to charcoal treatment to remove pigments prior to evaporation under vacuum (Buchi Rotavapor Re Type). Dried samples were re-suspended in 1.0 ml HPLC grade methanol by overtaxing , the mixture were passed through 2.5 μm disposable filter , and stored at 4°C for further analysis ,then 20 μL of the sample injected into HPLC system according the optimum condition.

2-3- Analysis and Diagnosis of phenolic Compounds

The separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A shimadzu, the eluted peaks were monitored by UV –Vis 10 A- SPD spectrophotometer. The main phenolic and flavonoids compound were

separated on FLC (Fast Liquid Chromatographic) column under the optimum condition. Column: phenomenex C-18 ,3µm particle size (50 x 2.0 mm I.D) column. Mobile phase: linear gradient of, solvent A 0.1% phosphoric acid : solvent B was (6:3:1, v/v) of acetonitrile : methanol: 0.1% phosphoric acid , linear gradient program from 0%B to 100%B for 10 minutes. Flow rate 1.0 ml/min. Detection: UV 280 nm.

The calculation of the phenolic and flavonoids concentrations ($mg.gm^{-1}$) in the samples was done by using the equation below:

$$\text{Concentration } (mg.gm^{-1}) = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{concentration of standard} \times \text{dilution Factor.}$$

2-4- Estimation of the Total Phenolic Content and the Total Flavonoid Content In Some Parts of the orange.

2-4-1- Estimation of the Total Phenolic Content

Estimated of Total phenolic contents was done based on a method that described in[12] with a slight modifications . In order to get 1mg/ml solution, 10 mg of each of the extract samples were dissolved in 10 ml of methanol. The extract of orang samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and kept on a water bath set at 45 °C. Then , 2.0 ml of a 7.5% w/v sodium carbonate (Na_2CO_3) solution were added. Mixture was then allowed to stand for 15 min. The phenols were determined by colorimetry at 765 nm. Standard curve was prepared by 50, 100, 150, 200, and 250 mg. ml^{-1} solutions of gallic acid in (methanol: water) (50: 50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg. g^{-1} of dry wight), which is a very common reference compound.

2-4-2- Estimation of Total Flavonoid Content:

Estimated of Total flavonoid contents was done based on the method described by [13] with a slight modification .Half (0.5) mL of extracts were mixed with 1.5 mL of methanol. 0.1 mL of 10% aluminium chloride was then added to this mixture, followed by 0.1 mL of 1M potassium acetate as well as 2.8 mL of distilled water. The mixture was then incubated at room temperature for 30 min. The absorbance of samples was measured by a spectrophotometer at 420 nm and the results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract).

2-4-3-DPPH Scavenging Activity:

The stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity assay was carried out following methods that described by[14] with slight modification. DPPH stock solution was prepared by adding 24 mg DPPH with 100 mL of methanol, whereas DPPH working solution was prepared by mixing 10 mL of the DPPH stock solution with 45 mL of methanol. Briefly, 500 µL of Citrus samples was added with 500 µL of DPPH working solution. The mixture was incubated for 2 h at room temperature in the dark. Absorbance of the mixture was determined at 515 nm against a blank. The results were expressed as EC50 value of DPPH assay in mg. mL^{-1}.

Statistical Analys

Statistical analysis was performed using (SPSS v.22 and Excel 2013). Quantitative data were described using (Mean ± Std. Error). The Least Significant Difference (LSD) test was used to compare between the averages. The correlation coefficient (r) was also used to know the type and strength of the relationship between the variables. The test was conducted at the probability level $P \leq 0.05$.

3-RESULTS AND DISCUSSION

3-1-Phytochemical analysis:

The results shown in Table 1 indicate a superiority of the flowers in their content of phenolic compounds as compared to other parts of orange. The flowers recorded the highest percentage of 23.86%, with an increment of 70.07% in comparison with 13.55% in leaves, which recorded the lowest percentage.

Table 1:
The concentrations of phenols in some parts of the orange plant

Compound	dry weight ($mg.g^{-1}$) Concentration						Total concentration $mg.g^{-1}$
	Flowers	Leaves	Exocarp	Mesocarp	Juice	Seeds	
Phenols	29.14	16.55	17.05	17.16	18.62	23.61	122.13

Figure 1 shows the presence of hesperidin with the highest percentage in all the parts of the orange compared to other phenolic compounds, as it reached 36.77%, with increment of 13.92%, however Diosmetin, recorded the lowest percentage of 2.46%. This confirms with the previous finding reported [15][16], which confirmed the presence of phenolic compounds in all the parts of the orange plant, especially the inedible parts such as leaves, seeds and peels. Moreover, the results are in a consistent with what was found by [17], [18] who confirmed that the most abundant phenolic compounds in the orange plant are hesperidin and naringin.

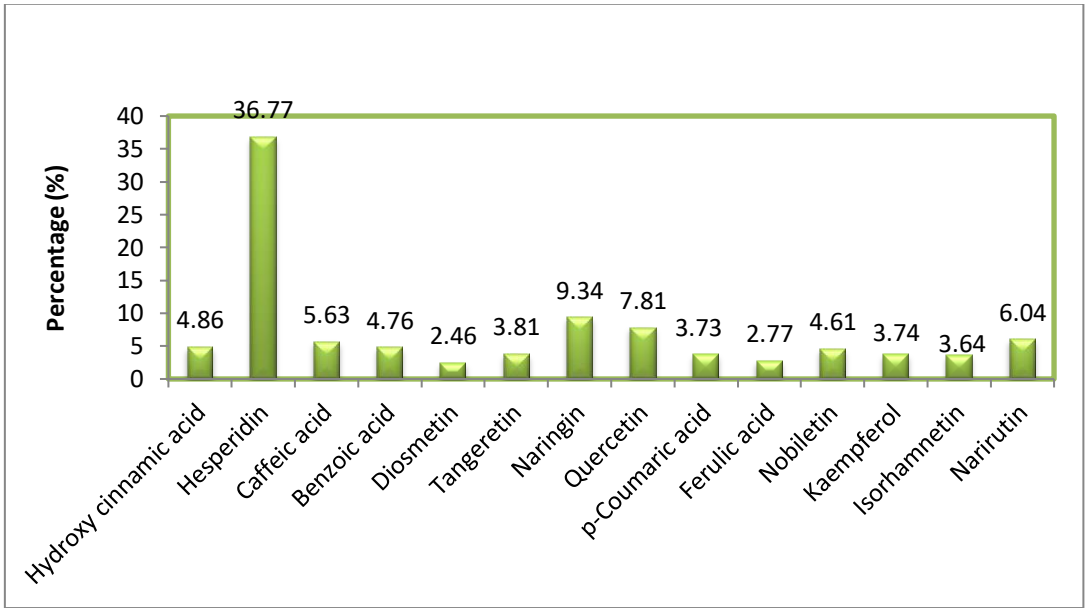

Figure 1: The percentages of phenolic compounds in some parts of the orange plant

3-2-Estimation of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The statistical analysis results shown in Figure 2, indicate significant differences at a probability level P <0.05 in the Total Phenolic Content (TPC). The results show that the highest value of TPC was 145.71 mg(GAE).g^{-1} dry weight in the leaves which was, followed by the flowers with a value of 135.71 mg(GAE).g^{-1} dry weight, while the juice recorded the lowest value of 57.18 mg(GAE).g^{-1} dry weight. These results are consistent with results of [19],[20],[21] who confirmed the presence of high concentration TPC in plant residues (peels, seeds and leaves) compared to the edible parts (pulp and juice).

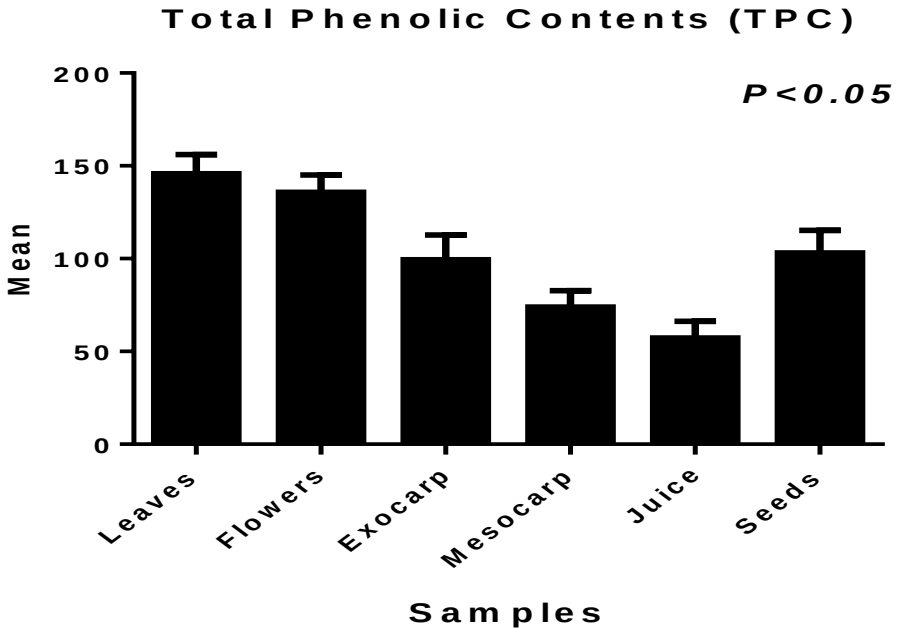

Figure 2: The Total Phenolic Content (TPC) in some parts of the orange plant

As for the Total Flavonoids Content (TFC), the statistical analysis results shown in Figure 3, pointed to the presence of significant differences at a probability level P <0.05 in TFC among the different parts of the orange plant. The highest TFC content was 11.14 mg Quercetin.g^{-1} dry weight , and it was obtained from the mesocarp. Seeds came in the second stage with a TFC value of 10.38 mg Quercetin .g^{-1} dry weight, though the lowest TFC (5.81 mg Quercetin.g^{-1}) was found in the juice. The results of the current study agree with the results of many previous studies [22],[23] which indicated to the presence of flavonoids in orange peels at a high concentration compared to other parts of the plant .

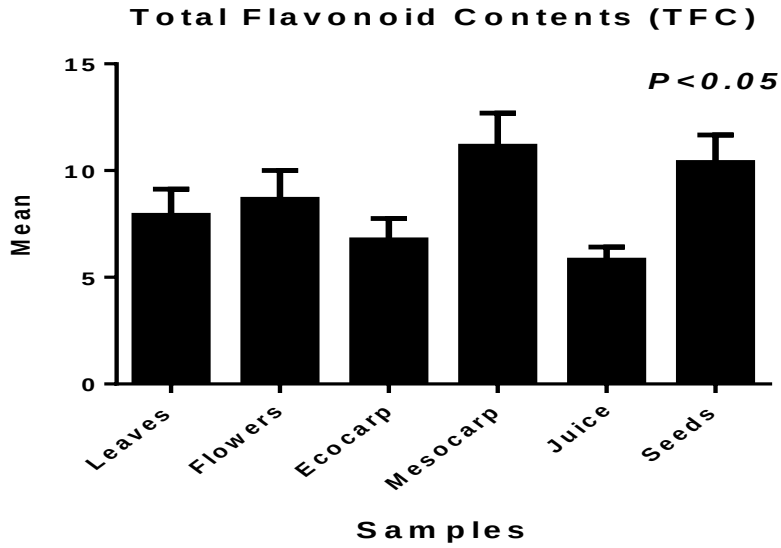

Figure 3: The Total Flavonoid Content (TFC) in some parts of the orange plant

3-3- Evaluation of Antioxidant Activity:

The results of the statistical analysis that have been shown in Table 2 indicate a significant differences at the level of P <0.05 among the different parts of the orange plant in their ability of free root DPPH inhibition. The flowers surpassed the other parts of the orange plant in their antioxidant activities, with a highest value of DPPH 2.64 mg.ml^{-1} followed by the seeds, with the DPPH value of 1.86 mg. ml^{-1}, while the lowest value of 0.86 mg.ml^{-1} was obtained from the exocarp.

Table 2:
The antioxidant activity of DPPH in some parts of the orange plant

DPPH mg .ml^{-1}	TFC mg . Querecetin. g-1	TPC mg.GAE.g-1	Samples
Mean± Std. Error	Mean± Std. Error	Mean± Std. Error	

1.46±0.29	7.91±1.22	145.71 ±10.33	Leaves
2.64±0.19	8.66±1.34	135.71 ±9.31	Flowers
0.86±0.08	6.76±0.99	99.41±13.27	Exocarp
1.79±0.12	11.14±1.55	73.85±8.98	Mesocarp
1.19±0.11	5.81±0.61	57.18±9.11	Juice
1.86±0.12	10.38±1.29	103.11±12.18	Seeds
1.02	3.42	3.55	LSD

On the other hand, the statistical analysis results presented in Table 3 indicate significant differences in the value of correlation coefficient at a probability level of (0.05) between the TPC and TFC of some parts of the orange plant with the antioxidant activity, using the manufactured free root scavenging method DPPH. The result shows that there is a positive correlation coefficient between TPC and TFC with the antioxidant activity and the values were 0.385 and 0.484, respectively.

Table 4:
Correlation coefficient between TPC, TFC, and DPPH

TFC	TPC	Pearson Correlation
0.484*	0.385	DPPH

Figure 4 shows the linear relationship between the TPC of some parts of the orange plant and the DPPH. As the value of the linear correlation coefficient reached 0.148. While Figure 5 shows the linear relationship between the TFC and the DPPH, the value of the linear correlation coefficient was 0.234.

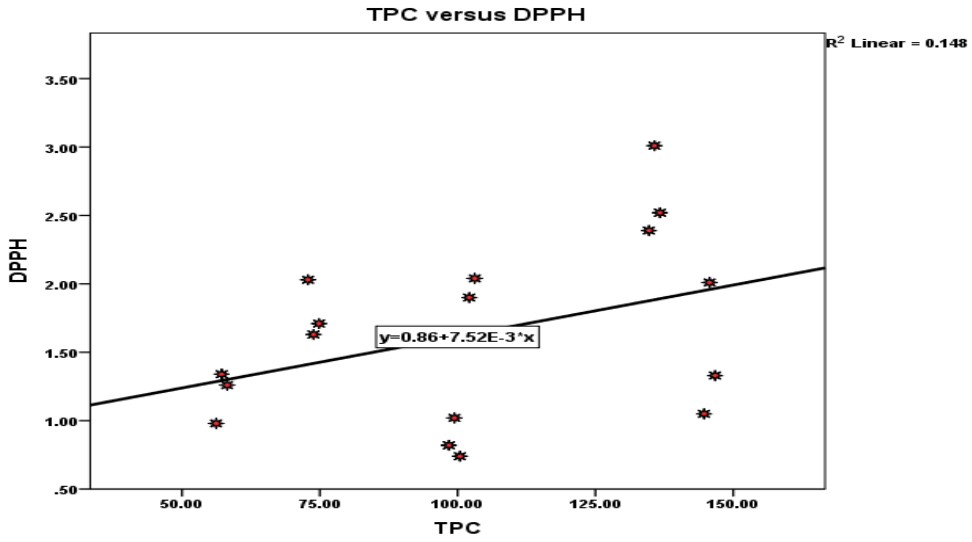

Figure 4: linear correlation between (TPC) and (DPPH)

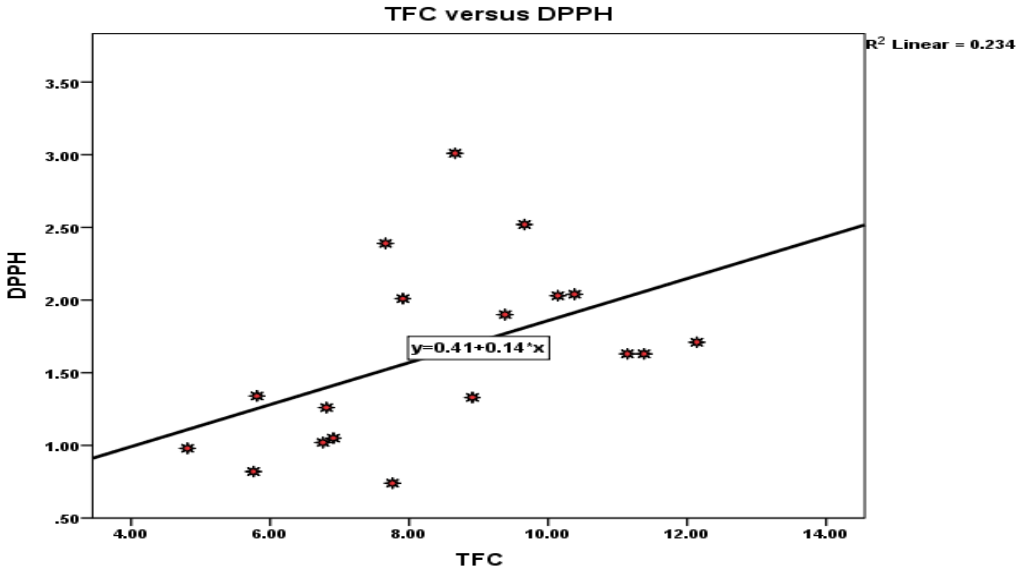

Figure 5: linear correlation between (TFC) and (DPPH)

This result is consistent with the findings reported by[24],[25] .The presence of statistically significant correlation coefficients between TPC and TFC with the antioxidant activity of the DPPH method confirms that the orange plant possesses many phenolic compounds that can contribute to the antioxidant activity, and this confirms what have been reported by[26],[27].

5. CONCLUSION

Through our findings we concluded the presence of significant differences between the different parts of orange plant in their content of active compounds, which act as natural antioxidants. The highest value of antioxidant activity was obtained from the seeds, followed by the flowers, which confirms that the orange plant residues are more effective than the edible parts. We also concluded from the results of the current research, the presence of a positive correlation between total phenolic content, total flavonoid content, and the antioxidant activity.

REFERENCES

Etebu, E., & Nwauzoma, A. B. (2014). A review on sweet orange (Citrus sinensis L Osbeck): health, diseases and management. American Journal of Research Communication, 2(2), 33-70. [1]

Al-Snafi, A. E. (2016). Nutritional value and pharmacological importance of citrus species grown in Iraq. IOSR Journal of Pharmacy, 6(8), 76-108. Citrus leaves and seeds ethanolic extracts. AABES London UK, 93-7. Pharmacognosy and Phytochemistry, 8(4), 157-161. [2]

Carr, A. C. (2020). A new clinical trial to test high-dose vitamin C in patients with COVID-19. Critical Care, 24(1), 1-2. [3]

Mahato, N., Sinha, M., Sharma, K., Koteswararao, R., & Cho, M. H. (2019). Modern Extraction and Purification Techniques for Obtaining High Purity Food-Grade Compounds and Value-Added Co-Products from Citrus Wastes. Foods, 8(11), 523-542. [4]

Carocho, M., Morales, P., & Ferreira, I. C. (2018). Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. Trends in Food Science & Technology, 71, 107-120 . [5]

Sies, H. (2020). Oxidative eustress and oxidative distress: Introductory remarks. In Oxidative Stress (pp. 3-12). Academic Press. [6]

Gupta, P., Jyoti, R. B., & Gupta, S. (2019). Free Radical Pharmacology and its role in various diseases. Journal of Drug Delivery and Therapeutics, 9(2), 690-694. [7]

ZHANG, H., YANG, Y. F., & ZHOU, Z. Q. (2018). Phenolic and flavonoid contents of mandarin (Citrus reticulata Blanco) fruit tissues and their antioxidant capacity as evaluated by DPPH and ABTS methods. Journal of Integrative Agriculture, 17(1), 256-263. [8]

Olyad, E. R. B. A., Atomsa, D., Chimdessa, M., & Gonfa, T. (2020). Determination of Flavonoid Contents and Evaluation of in vitro Antioxidant Activities of the Extract [9]

of Selected Citrus Fruit Peel. International Journal of Secondary Metabolite, 7(1), 8-18.

[10] Ismaeel, A., Holmes, M., Papoutsi, E., Panton, L., & Koutakis, P. (2019). Resistance Training, Antioxidant Status, and Antioxidant Supplementation. International journal of sport nutrition and exercise metabolism, 29(5), 539-547.

[11] Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. Journal of Agricultural and Food Chemistry, 50(13), 3660-3667.

[12] Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology, 299, 152-178.

[13] Ghasemi, K., Ghasemi, Y., & Ebrahimzadeh, M. A. (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak J Pharm Sci, 22(3), 277-281.

[14] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of food composition and analysis, 19(7), 669-675.

[15] Sir Elkhatim, K. A., Elagib, R. A., & Hassan, A. B. (2018). Content of phenolic compounds and vitamin C and antioxidant activity in wasted parts of Sudanese citrus fruits. Food science & nutrition, 6(5), 1214-1219.

[16] . Omoba, O. S., Obafaye, R. O., Salawu, S. O., Boligon, A. A., & Athayde, M. L. (2015). HPLC-DAD phenolic characterization and antioxidant activities of ripe and unripe sweet orange peels. Antioxidants, 4(3), 498-512.

Victor, M. M., David, J. M., Cortez, M. V., Leite, J. L., & da Silva, G. S. (2020). A High-Yield Process for Extraction of Hesperidin from Orange (Citrus sinensis L. osbeck) Peels Waste, and Its Transformation to Diosmetin, A Valuable and Bioactive Flavonoid. Waste and Biomass Valorization, 1-8. [17]

Oikeh, E. I., Oviasogie, F. E., & Omoregie, E. S. (2020). Quantitative phytochemical analysis and antimicrobial activities of fresh and dry ethanol extracts of Citrus sinensis L.Osbeck (sweet Orange) peels. Clinical Phytoscience, 6(1), 1-6 . [18]

Chen, Q., Wang, D., Tan, C., Hu, Y., Sundararajan, B., & Zhou, Z. (2020). Profiling of Flavonoid and Antioxidant Activity of Fruit Tissues from 27 Chinese Local Citrus Cultivars. Plants, 9(2), 196. [19]

. Farag, M. A., Abib, B., Ayad, L., & Khattab, A. R. (2020). Sweet and bitter oranges: An updated comparative review of their bioactives, nutrition, food quality, therapeutic merits and biowaste valorization practices. Food Chemistry, 127306. [20]

Molan, A. L., Ismail, M. H., & Nsaif, R. H. (2016). Phenolic contents and antioxidant activity of peels and seeds of orange (Citrus sinensis) cultivated in Iraq. World J Pharm Sci, 5(4), 473-482. [21]

Al-Anbari, A. K. H., & Hasan, M. A. (2015). Antioxidant activity in some Citrus leaves and seeds ethanolic extracts. AABES London UK, 93-7. [22]

Bentahar, A., Bouaziz, A., Djidel, S., & Khennouf, S. (2020). Phenolic content and antioxidant activity of ethanolic extracts from Citrus sinensis L. and Citrus reticulata L. fruits. Journal of Drug Delivery and Therapeutics, 10(5)308-313. [23]

Dzah, C. S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G., & Ma, H. (2020). The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. Food Bioscience, 100547 [24]

. [25]

Liew, S. S., Ho, W. Y., Yeap, S. K., & Sharifudin, S. A. B. (2018). Phytochemical composition and in vitro antioxidant activities of Citrus sinensis peel extracts. PeerJ, 6, e5331. [26]

Montero-Calderon, A., Cortes, C., Zulueta, A., Frigola, A., & Esteve, M. J. (2019). Green solvents and Ultrasound-Assisted Extraction of bioactive orange (Citrus sinensis) peel compounds. Scientific reports, 9(1), 1-8. [27]

Zacarías-García, J., Rey, F., Gil, J. V., Rodrigo, M. J., & Zacarías, L. (2020). Antioxidant capacity in fruit of Citrus cultivars with marked differences in pulp coloration: Contribution of carotenoids and vitamin C. Food Science and Technology International, 1082013220944018. [28]