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Study of the antioxidant activity of some active compounds in orange peels

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Abstract

The present study aimed to identify the active substances in orange peel powder (PO) and to extract beta-carotene (OR) from dried orange peel powder. Additionally, the study aims to examine the efficacy of these compounds as natural antioxidants. The levels of Vitamin C, phenolic compounds, flavonoids, and pectin were found to be significantly greater in OR compared to PO at (P≤0.01) level. Both PO and OR demonstrated a strong correlation between increasing concentrations with the removal of free radicals. The method of scavenging free radicals displayed a higher efficacy compared to the method of lowering ferric chloride (FeCl2). Additionally, it was observed that the elimination of free radicals increased with higher concentrations. The efficacy of both PO and OR as antioxidants was also assessed through implementing the method of introducing hydrogen peroxide (H2O2) by estimating the fragmentation factor of DNA)QB.(There were statistically significant differences at (P≤0.01) level, demonstrated by the reduction in QB with rising levels of PO and OR. The concentration of QB is 0 at 250 µg/ml for both PO and RO. This could be due to their efficacy as antioxidants, enabling them to eradicate free radicals that degrade DNA. The findings supported the hypothesis that orange peel powder (PO) and beta-carotene pigment (OR) function as potent natural antioxidants, effectively mitigating or eliminating oxidative processes induced by free radicals. These compounds are considered safe for human consumption and do not pose any health risks.

Keywords: Antioxidant, Ascorbic acid, Orange peel powder, Extract, β-carotene, DNA fragmentation factor.

1. Introduction

Antioxidants are molecules that limit or prevent cell damage caused by free radicals, a natural product of metabolism. The mechanism of antioxidants is through donating an electron to the free radicals, and interrupting oxidation chain formation in vivo and hence, becoming neutral. Antioxidants act as reducing factors against aging signs and in enhancing body immunity [1–3]. Antioxidants help prevent heart disease, because they prevent fat oxidation, and they reduce internal infections that increase the risk of heart disease and diabetes [4, 5]. Since the body produces free radicals during the body's various metabolic processes, antioxidants take control of these free radicals and neutralize them, which in turn increases the body's immunity and natural defenses [6, 7]. There are two types of antioxidants: those produced by the body known as endogenous antioxidants, and there are exogenous antioxidants that are obtained from natural, vegetable or industrial sources. Exogenous antioxidants can be obtained from the following sources: through food, especially fruits and vegetables, by vitamins such as vitamin E and vitamin C, which are considered effective antioxidants, and by antioxidant medicines, or antioxidant pills, which are nutritional supplements [3].

Antioxidants, such as polyphenols, flavonoids and β-carotene, can be found naturally in all parts of higher plants such as fruits, vegetables, seeds, and roots and it is suggested to act as a reducing agent, a metal chelator, a free radical scavenger, and a singlet oxygen quencher [8, 9]. Flavonoids in citrus are a major class of secondary metabolites that have significant impact on human life [10]. Due to its high phenolic content, β-carotene, which exists abundantly in orange peels, contributes to quality, color, antioxidant and flavor properties and therefore, to the treatment of many diseases [11, 12]. In addition, Carotenoids are fat-soluble phytonutrients that have anti-oxidant, anti-mutagenic and anti-cancer impact [13]. Fruits contain high nutritional value due to their high content of high-value nutrients such as dietary fiber, mineral salts, vitamins, polyphenolic compounds and carotenoids [14]. Blueberries and citrus, for example, contain high amounts of phenolic compounds, vitamin C, and carotenoids (amounting to. 30 mg/g dm) [15, 16]. Lemon and orange peels are by-products or waste products of food processing. In contrast, these by-products can be used in the production of pectins, flavonoids, essential oils and carotenoids [12, 17]. The study aimed to explore the contents of orange peels, as food wastes, and benefit from them as natural antioxidants due to their content of phenols and flavonoids in addition to extracting and estimating β-carotene, which is a source of the vitamin A. Vitamin C was also estimated, as oranges are one of the citrus fruits and it is a rich source of vitamins, especially vitamin C, in addition to the estimation of pectin found in peels, which is a source of fiber.

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2. Materials and methods

2.1. Sample preparation

The orange peels were washed and squeezed intensively to get rid of the pulp and fiber, then dried at room temperature for several days for the weight to be stabilized. Formerly, it was thoroughly ground by an electric grinder and kept in clean, sterile glass bottles until use. Subsequently, the β-carotene dye was extracted from the orange peel powder according to the Harborne method (1984) [18]. 60 g of orange peel powder was weighed and placed in a glass flask, then 160 ml of acetone was added to it and sealed tightly, then, placed in a vibrating incubator and in a dark place for 48 hours. The extract was then filtered using Whatman 0.1 filter paper to obtain the filtrate. The filtrate was placed in a separation funnel with ml volume and then a mixture of methanol and ether was added to it in a mixing ratio of 2:15 (h / h) with the addition of a small amount of sodium chloride salt to dispose of moisture, then was shaken vigorously until two layers were formed, then the separation funnel was left on the holder to stabilize the two layers separated clearly, then the lower layer (water) and the upper layer were separated in a 100 ml baker containing ether with carotene dye. The dye was then concentrated using a rotary evaporator at a temperature of 40 °C to eliminate the ether. The concentrated β-carotene dye was then collected and placed in a dark bottle at -18°C for the rest of the tests (Table 1).

2.2. Active compounds measurement

2.2.1 Measuring antioxidant activity

Antioxidant activity assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method:

The test material mixture underwent an oxidative antagonism analysis using the procedure described by [19]. The mixture was prepared by assaying different concentrations of β-carotene dye extract (OR) as well as orange-peel powder (PO) was dissolved in solvent (0.0,50,150, 200 and 250 мg\gm).

The amount of color change was measured using a spectrophotometer at the wavelength 515nm during 100 minutes of reaction at room temperature. As for the removal percentage, it was calculated according to the following equation:

$$\text{Removal percentage \%} = \frac{\text{model absorptivity} - \text{blank absorbance}}{\text{absorbance of standard solution}} \times 100 - 100$$

2.2.2. Examination of the antioxidant activity by ferrous reduction ($FeCl_2$) method

The reducing power of ferrous $FeCl_2$was measured for the samples prepared by following the method mentioned in [21], by adding 100 microliters of the test substance to 3 milliliters of methanol, and after good mixing of the mixture, 1 milliliter of 2 mmol of ferrous chloride $FeCl_2$ was added. The mixture was left at room temperature for 10 minutes. Then the color change is measured at the wavelength of 562 nm (Table 2).

2.2.3. Examination of the antioxidant activity by hydrogen peroxide H_2O_2 method

A sample of pure DNA isolated from the calf prepared by (BDH England) company was dissolved in a solution consisting of (0.0015) M NaCl and (0.00015) M Sodium citrate so that the final concentration of DNA is 5µg/ml at pH = 7. The DNA solution prepared above, which has an absorbance ratio of A260/A280 equivalent to 1.87, was treated with different test substance concentrations. β-carotene pigment extract (OR) as well as orange-peel powder was (PO) dissolved in solvent (0.0, 50, 150, 200 and 250 мg\gm) by mixing one volume of the test substance with a similar volume of the standard DNA solution with the presence of a concentration of 1×10^{-5}M of hydrogen peroxide solution (H_2O_2) prepared simultaneously (source of free ions). The mixture was incubated at 37°C for 10 minutes. Then, the effect of a test substance on the DNA molecule was followed up by measuring the absorbance at a wavelength of 260nm. The fragmentation coefficient (QB) of the nitrogenous base pairs (representing the breakdown of the DNA molecule) can also be calculated using the following equation and according to what was mentioned in [20].

2.2.4. Determination of total phenolic compounds

PO and OR total content of phenolic compounds was determined using standard Folin-Ciocalteu reagent according to the method described by Kaur and Kapoor [22] 100 µl of the extract, 500 µl of Folin-Ciocalteu reagent (Merck, Germany), and 1.5 ml of 20% sodium carbonate were mixed for the preparation of the reaction mixture using a vortex mixer and then addition of distilled water to make 10 ml of the final volume. Total phenolic content was calculated using the calibration curve created with gallic acid (Sigma Aldrich, Germany) after 2 hours of

Table 1. Substances were used to test the antioxidant activity according to DPPH method.

Additives	Blank (ml)	Standard	Test
DPPH	-	0.5	0.5
Ethanol 100%	3.3	-	3.3
Sample	0.5	0.5	0.5
DDW	0.5	3.3	-

DDW: Distilled deionized water

Table 2. Substances used in the antioxidant activity by the $FeCl_2$ reduction method.

Additives	Blank (ml)	Standard	Test
Fe Cl_2 solution	1	1	1
Solvent	3	-	3
Sample	-	100	100
DDW	100	3	-

DDW: Distilled deionized water

reaction. Per g of dry weight, the total phenolic content is represented as mg of gallic acid equivalent (mg of GA/g of extract).

2.2.5. Determination of total flavonoid compounds

Aluminum chloride colorimetric method, which was described by [23], was applied for the determination of total flavonoid content of crude extract. Briefly, methanol (up to 1 ml) was added to 50 µl of crude sample (PO and OR) (1 mg/ml solvent) plus 4 ml of distilled water with mixing. Afterwards, mixture was incubated for 5 min and 0.3 ml of 5% $NaNO_2$ and 0.3 mL of 10% $AICl_3$ solutions were added after incubation and were allowed to stand for 6 min. Subsequently, 2 ml of 1 mol/l NaOH solution and double-distilled water were added to make 10 ml of final mixture. After 15 minutes of standing time, the mixture was tested for absorbance at 510 nm. In order to calculate total flavonoid content, A calibration curve was applied, and results were expressed as ruin equivalents (mg RE)/g dry extracts.

2.2.6. Determination of pectin

Determination of pectin was executed according to the method described by Panchami and Gunasekaran [24]. In brief, 300 ml of 0.1N HCl was added to 50 gm of ground fruit and then boiled for 30 min. The mixture was filtered under pressure followed by the addition of boiled distilled water in order to collect filtrate. The same procedure was applied with 0.05N HCl and 0.3N HCl. The volume was made up to 500 ml with distilled water after all the filtrates were mixed. Afterward, 200 ml was relocated into a beaker followed by the addition of 250 ml of distilled water. Using the phenolphthalein indicator, 1N NaOH was added to the filtrate to neutralize the acid. The mixture was then left to stand for 24 hours. 50ml of 1N acetic acid was added and after 5 min, 25 ml of 1N CaCl was added with stirring and allowed to stand for 1 hour. Following a 2-minute boil, the mixture was filtered using Whatman No. 1 filter paper. Afterwards, boiled water was used to completely clear the precipitate until the filtrate was free of chloride. The total pectin content was calculated by transferring the filter paper at 55°C containing calcium pectate to a plate that had been pre-weighed and dried overnight. The following equation was used to calculate the weight after cooling and drying.

$$\% \text{ Calcium pectate} = \frac{\text{Weight of calcium pectate} \times 100 \times 500}{\text{ml of filtrate taken x Weight of sample taken for estimation}}$$

2.2.7. Sample preparation and spectrophotometer assessment of ascorbic acid

Determination of ascorbic acid was accomplished according to the method described by Majidi [25]. Ten grams of each sample was transferred into a 100 ml volumetric flask. Once colored solution formed, homogenization was accomplished by adding 50 ml of acetic acid solution together with 4–5 drops of bromine water. In order to remove the excess bromine and wait for a clear solution to be obtained, only a few drops of thiol urea solution were added to the samples. 2, 4-Dinitrophenyl hydrazine solution was added thoroughly to all standards and with the oxidized ascorbic acid, then, acetic acid was added to the solutions up to the mark. To determine the concentration of ascorbic acid, Shimadzu (1600-japan) UV-visible spectrophotometer was used to measure absorbance at 280nm.

2.2.8. Calibration curve

The ascorbic acid series of solutions was prepared from 500 ppm stock solution for the calibration curve.

2.3. Statistical analysis

The SAS (2018) software was applied to determine the impact of variance variables on the study parameters. In this investigation, the Least Significant Difference (LSD) was employed to compare between means using ANOVA/ One-way analysis.

3. Results

3.1. Comparison of active compounds between orange peel powder (PO) and β-carotene extract (OR)

First, orange peels (PO) were subjected to a drying process, after which the beta-carotene pigment (OR) was extracted from them. The content of active substances (vitamin C, alkylated phenols, and flavonoids) and pectin in (PO) and (OR) were quantified. It was observed that (OR) was significantly greater than (PO) at ($p \leq 0.01$) level. This difference may be attributed to the fact that beta-carotene is an abundant source of antioxidants, as illustrated in Table 3.

3.2. The effectiveness of (PO) and (OR) as antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Five solutions containing different concentrations of (PO) and (OR) were created in order to investigate their effectiveness as natural antioxidants. The activity of these solutions was evaluated using the DPHH method, as shown in Table (1). At a concentration of 250 µg/ml, PO exhibited significant efficacy in eliminating free radicals compared to other concentrations, with a statistical significance level of ($P \leq 0.01$). However, there were no significant differences in removal at concentrations of 50 and 100 µg/ml. (OR) also showed high activity in removing free radicals at a concentration of 250 µg/ml, which reached 54.07 ± 3.05 at the level ($P \leq 0.01$). While there were no significant differences in removal at concentrations of 200 and 150 µg/ml, it was higher in the removal of free radicals compared to 100 and 50 µg/ml at the level ($P \leq 0.01$). The results of the statistical analysis also showed the superiority of both (PO) and (OR) in the removal percentage at

Table 3. Active compounds of orange peel powder (PO) and β-carotene extract (OR).

Compounds	Peel of orange powder (PO)	β-carotene (OR)	T-teat (P-value)
Vitamin C (mg/100gm)	54.16 ±2.75 b	66.81 ±3.41 a	5.063 ** (0.0047)
Phenolic (mg/100gm)	21.85 ±1.08 b	27.34 ±1.52 a	2.902 * (0.0372)
Flavonoids (mg/100gm)	25.47 ±1.47 b	39.552.06 a	5.784 ** (0.0001)
Pectin %	18.21 ±0.89 b	38.74 ±2.25 a	5.169 ** (0.0001)

Means having the different letters in same row differed significantly. * ($P \leq 0.05$). ** ($P \leq 0.01$).

concentration 250 at the level (P≤0.01) compared to pure vitamin C, as well as compared to the $Fecl_2$ method (Table 4).

3.3. The effectiveness of (PO) and (OR) as antioxidant activity by reducing or removing ferric chloride ($FeCl_2$)

Five concentrations of (PO) and (OR) were prepared to study their activity as natural antioxidants using the FeCl2 method as shown in Table (2). The results of the statistical analysis showed that there were no significant differences between (PO) and (OR) at all concentrations at (P≤0.01) level in removing or reducing free radicals compared to pure vitamin C at a concentration of 50 µg/ml. The highest removal of (PO) and (OR) was at the concentration of 250 µg/ml, which reached 22.80 ± 1.31 and 27.19 ± 1.68, respectively, at the level of (P≤0.01), (Table 5).

3.4. The effectiveness of (PO) and (OR) against fragmentation factor of DNA by hydrogen peroxide (H_2O_2)

Five concentrations of both PO and OR were prepared and their effectiveness against DNA Fragmentation, was measured by estimating the (QB) fragmentation factor of DNA at each concentration using the H_2O_2 hydrogen peroxide method. The results showed high differences between the concentrations of PO, OR, and (QB), at the level of (P≤0.01). highest (QB) was observed for PO and OR at the concentration of 250 µg/ml compared to DNA + H_2O_2 (10-5×1), which had a (QB) of 1 ± 0.05. The results of the statistical analysis also showed that there is an inverse relationship between (QB) for PO and OR and the concentrations of OR and PO at the (P≤0.01) level (Table 6).

Table 4. Percentage orange peel powder (PO) and β-carotene (OR) of removing free radicals by DPPH.

Concentrations µg/ml	Abs. at 517nm of PO	PO % removal of free radicals	Abs. at 517nm of OR	OR % removal of free radicals	Abs. at 517nm of Ascorbic acid	Ascorbic acid % removal of free radicals
0	0.442	-	0.442	-	-	-
50	0.369	16.51 ±0.76 d	0.338	23.52 ±1.28 d	0.311	26.13
100	0.362	18.09 ±1.35 d	0.307	30.54 ±2.41 c	-	-
150	0.298	32.57 ±2.09 c	0.257	41.85 ±2.78 b	-	-
200	0.254	42.53 ±2.667 b	0.246	44.34 ±2.65 b	-	-
250	0.213	51.80 ±2.96 a	0.203	54.07 ±3.05 a	-	-
L.S.D.	-	7.494 **	-	7.001 **	-	-
P-value	-	0.0001	-	0.0001	-	-

Means having the different letters in same column differed significantly. ** (P≤0.01).

Table 5. percentage of orange peel powder (PO) and β-carotene(OR) free of removing radicals by $FeCl_2$.

Concentrations (µg/ml)	Abs. at 562nm of PO	$FeCl_2$ % reduction or removal by PO	Abs. at 562nm of OR	$FeCl_2$ % reduction or removal by OR	Abs. at 562nm of Ascorbic acid	Ascorbic acid
0	0.364	-	0.364	-	-	-
50	0.343	5.76 ±0.42 d	0.335	7.96 ±0.56 d	0.224	35.68
100	0.331	9.06 ±0.78 cd	0.321	11.81 ±0.81 cd	-	-
150	0.326	10.43 ±0.61 bc	0.312	14.28 ±0.82 bc	-	-
200	0.311	14.56 ±0.86 b	0.294	19.23 ±1.05 b	-	-
250	0.281	22.80 ±1.31 a	0.265	27.19 ±1.68 a	-	-
L.S.D.	--	4.371 **	--	5.026 **	-	-
P-value	--	0.0001	--	0.0001	-	-

PO= orange peel powder, OR = β-carotene extract of orange peel powder, (0) = standard solution. Means having the different letters in same column differed significantly. ** (P≤0.01).

Table 6. Antioxidant activity of (PO) and (OR) on fragmentation factor of DNA by Hydrogen peroxide (H_2O_2).

Concentration's µg/ml	Abs. at 260 nm of PO	QB of PO	Abs. at 260 nm of OR	QB of OR
50	0.318	0.836 ±0.12 ab	0.306	0.755 ±0.07 b
100	0.301	0.696 ±0.07 bc	0.276	0.526 ±0.07 cd
150	0.279	0.516 ±0.07 c	0.265	0.442 ±0.05 de
200	0.243	0.221 ±0.04 de	0.238	0.236 ±0.03 e
250	0.216	0 ±0 e	0.207	0 ±0 f
DNA +H_2O_2 (10^{-5}×1)	0.338	1 ±0.05 a	0.338	1 ±0.05 a
DNA	0268	--	0.268	--
L.S.D.	--	0.289 **	--	0.207 **
P-value	-	0.0084	--	0.0062

PO= Orange peel powder, OR= β-carotene extract of orange peel powder, QB= fragmentation factor of DNA. Means having with the different letters in same column differed significantly. ** (P≤0.01) .

4. Discussion

The results of the statistical analysis showed that OR was superior to PO in its content of vitamin C, phenolic and flavonoid compounds, as well as pectin at the level ($P \leq 0.01$) (Table 3). This may be due to beta-carotene being a rich source of antioxidants. [12] reported that the chemical properties of plant extracts affect their overall antioxidant capacity. As mentioned [26, 27], the composition and antioxidant content of vitamin C are involved in the correspondence between the total phenolic and flavonoid content and their antioxidant activity. In addition to the importance of vitamin C in contributing to supporting the body's immunity by supporting various cellular functions of both the main and adaptive immune systems, for example, in supporting the epithelial membrane barrier in resisting pathogens and also by enhancing oxidative scavenging activity [28, 29]. Pectin is an important source of fiber that improves the digestive process. Patil and Albishi stated that among the natural products present in citrus peels (such as vitamin C, sugars, folic acid, carotenoids, flavonoids, pectin and essential oils), phenolic compounds are one of the most important natural ingredients in orange peels, which have a function of great importance as useful antioxidants for human health and in the manufacture of functional foods [30, 31]. The main function of phenolic compounds as antioxidants is due to their ability to interact with free radicals by a specific mechanism, which is to donate electrons or hydrogen from hydroxyl groups to free radicals containing uncoupled electrons, thus, because of this process, free radicals can be neutralized [32, 33].

OR and PO had highly significant activity in removing free radicals by the DPPH method at the level of ($P \leq 0.01$) (Table 4). This activity can be attributed to the ability to donate a hydrogen molecule [34]. As a natural antioxidant in suppressing high free radicals. Likewise, the results showed that the removal of free radicals by the DPPH method, which relies on a single electron transfer (SET) reaction, was more effective in removing free radicals compared to the $FeCl_2$ method as shown in (Table 5). As a result, the oxidant receives one electron from the antidote molecule. for oxidation [35, 36]. Both PO and OR, with increasing their concentration, exhibited a highly significant activity at the ($P \leq 0.01$) level in reducing the value of QB (fragmentation factor of DNA) (Table 6), which resulted in preserving the DNA from being destroyed, as demonstrated in (Table 6). The relationship was inverse between the concentrations of PO, OR, and QB. This could be attributed to their high efficacy as potent antioxidants, as well as their composition of polyphenolic compounds that inhibit free radicals and thereby mitigate oxidative stress.

5. Conclusion

Phenolic compounds present in natural foods can potentially lower the likelihood of developing severe health conditions. The findings revealed that orange peel powder exhibited a high concentration of inherent antioxidants. Phenols, flavonoids, and related substances exhibit potent antioxidant properties, including the ability to scavenge free radicals and so alleviate oxidative stress. As a result, it eas concluded that they represent safer options compared to synthetic antioxidants and also help prevent the negative impact on human health caused by artificial food additives.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed consent

Not applicable.

Availability of data and material

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Authors' contributions

All authors contributed equally to this research study.

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