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ANTIRADICAL ACTIVITY OF ORANGE AND GRAPEFRUIT PEEL COMPOSITIONS

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Annotation.
The evaluation of alcoholic extracts of mixtures in various ratios prepared from orange and grapefruit peels, considered as waste materials, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method, demonstrated their high antiradical activity. It was found that, under standard conditions, the minimum volumes of extracts in 96% ethanol required to inhibit 50% of DPPH free radicals were 106.85, 109.05, and 105.72 μL for the mixtures with ratios of 1:1, 1:3, and 3:1, respectively. These results suggest that the mixtures of orange and grapefruit peels, especially the 3:1 composition, may be considered as promising therapeutic agents for the prevention of oxidative stress in medicine.

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Introduction. Orange (*Citrus sinensis* L. Osb.) and grapefruit (*Citrus paradisi*) belong to the Rutaceae family and are among the most widely cultivated and consumed citrus fruits in the world [1]. In the 2023–2024 period, the global production of oranges and grapefruits amounted to 48.82 and 6.9 million tons, respectively. Approximately 80% of the oranges grown worldwide are used for industrial purposes, primarily for juice production [2]. However, industrial citrus processing generates 15–25 thousand tons of waste annually, including peels, pulp, and seeds [3].

In recent years, researchers have found that citrus peels contain a significant amount of valuable biologically active compounds, including flavonoids, polyphenols, vitamins and essential oils, pectins, alkaloids, and minerals [4]. This discovery has led to their extensive use in the food, pharmaceutical, and cosmetic industries [5]. Specifically, these compounds are used to reduce cholesterol levels, improve intestinal function, neutralize inflammation and free radicals, slow aging, strengthen the immune system, and regulate fat metabolism in the human body [6].

In our previous studies, the antioxidant activity of the peels of various orange cultivars specifically those from Iran, Africa, China, and Turkey as well as yellow-green and red grapefruit varieties, was

investigated. According to the results obtained, the peels of Iranian oranges and red grapefruits exhibited high antioxidant activity. This article presents the findings of research aimed at determining the antiradical activity of mixtures made from Iranian orange and red grapefruit peels in the ratios of 1:1, 1:3, and 3:1, with the goal of developing a food additive with a standardized content of biologically active compounds.

Materials and Methods

Evaluation of Antiradical Activity. The stable violet-colored radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) in ethanol solution has the ability to become decolorized in the presence of compounds with antiradical (antioxidant) properties due to the pairing of its unpaired electron. For this reason, it is widely used in spectrophotometric assessments of antiradical activity [7 - 10].

In the present study, the antiradical activity of ethanol extracts from mixtures of Iranian orange and red grapefruit peels in the ratios of 1:1, 1:3, and 3:1 was evaluated using an improved method of DPPH• radical scavenging originally proposed by Blois [8] and further refined in [9].

Preparation of the DPPH• Working Solution. A DPPH• solution (7.92 mM) was prepared in 96% ethanol in a 100 ml volumetric flask. The flask was wrapped in aluminum foil to protect the solution from light and stored in a dark place at room temperature.

Extraction. To obtain the extracts, 0.75 g of a mixture of orange and red grapefruit peels—dried to constant weight at 35 °C in a drying oven and ground—was extracted in 25 ml of 96% ethanol at 60 °C for 20 minutes using an ultrasonic bath. The resulting extract was filtered through a syringe filter with a pore diameter of 0.45 μm.

Evaluation of the Antiradical Activity of the Extracts. A quartz cuvette with a volume of 4 ml and a thickness of 10 mm was filled with 3 ml of DPPH solution in ethanol and 100 μl of ethanol, then thoroughly mixed and placed in a YOKE K7000 spectrophotometer. The optical density (D_1) was measured at a wavelength of 517 nm every 5 minutes over a 30-minute period. The D_1 values remained stable (see Tables 1–3).

To assess the antiradical activity of the ethanol extracts of the Iranian orange and red grapefruit peel mixtures in ratios of 1:1, 1:3, and 3:1, 25, 50, 75, and 100 μl of each filtered plant extract were added to four separate 10 mm cuvettes. Then, 3 ml of DPPH solution was added to each cuvette, and the total volume was brought up to 4.1 ml with ethanol. The mixtures were immediately stirred, and their optical density (D_2) was measured at 517 nm.

Antiradical activity (ARA, %) was calculated using the formula: $ARA, \% = ((D_1 – D_2) / D_1) \times 100$.

The results of the antiradical activity measurements for the mixtures in the ratios 1:1, 1:3, and 3:1 are presented in Tables 1–3.

Table 1.

Optical density values measured at a wavelength of 517 nm and the calculated antiradical activity (ARA, %) of the ethanol extract of a 1:1 mixture of orange and red grapefruit peels, added in varying volumes to the DPPH solution.

Extract volume, μl	Time, minutes.	Optic density, *D*	ARF, %	Extract volume, μl	Time, minutes.	Optic density, *D*	ARF, %
25	0	1,03	0,00	75	0	1,03	0,0
	5	0,888	13,79		5	0,738	28,3
	10	0,879	14,66		10	0,717	30,4
	15	0,872	15,34		15	0,703	31,7
	20	0,867	15,83		20	0,691	32,9
	25	0,862	16,31		25	0,672	34,8
	30	0,858	16,70		**30**	0,656	36,3
50	0	1,03	0,00	100	0	1,03	0,00

	5	0,794	22,91		5	0,667	35,24
	10	0,775	24,76		10	0,639	37,96
	15	0,762	26,02		15	0,62	39,81
	20	0,753	26,89		20	0,6	41,75
	25	0,746	27,57		25	0,584	43,30
	30	0,74	28,16		**30**	0,569	44,76

Table 2.

Optical density values measured at a wavelength of 517 nm and the calculated antiradical activity (ARA, %) of the ethanol extract of a 1:3 mixture of orange and red grapefruit peels, added in varying volumes to the DPPH solution.

Extract volume, µl	Time, minutes.	Optic density, *D*	ARF, %	Extract volume, µl	Time, minutes.	Optic density, *D*	ARF, %
25	0	1,034	0,00	75	0	1,034	0,0
	5	0,904	12,57		5	0,734	29,0
	10	0,893	13,64		10	0,714	30,9
	15	0,886	14,31		15	0,699	32,4
	20	0,88	14,89		20	0,688	33,5
	25	0,876	15,28		25	0,679	34,3
	30	0,871	15,76		**30**	0,67	35,2
50	0	1,034	0,00	100	0	1,034	0,00
	5	0,817	20,99		5	0,651	37,04
	10	0,799	22,73		10	0,623	39,75
	15	0,786	23,98		15	0,607	41,30
	20	0,777	24,85		20	0,594	42,55
	25	0,77	25,53		25	0,583	43,62
	30	0,763	26,21		**30**	0,573	44,58

Table 3.

Optical density values measured at a wavelength of 517 nm and the calculated antiradical activity (ARA, %) of the ethanol extract of a 3:1 mixture of orange and red grapefruit peels, added in varying volumes to the DPPH solution.

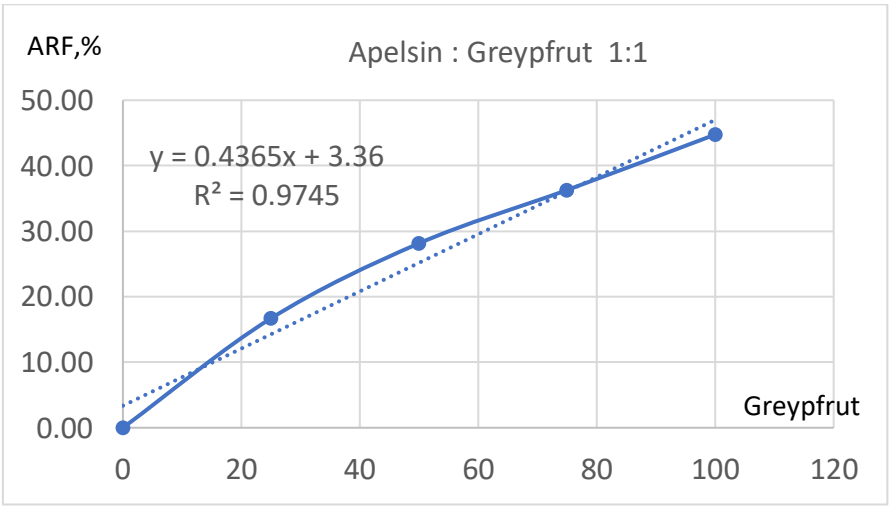
Extract volume, µl	Time, minutes.	Optic density, *D*	ARF, %	Extract volume, µl	Time, minutes.	Optic density, *D*	ARF, %
25	0	1,011	0,00	75	0	1,011	0,0
	5	0,883	12,66		5	0,696	31,2
	10	0,875	13,45		10	0,67	33,7
	15	0,87	13,95		15	0,661	34,6
	20	0,869	14,05		20	0,651	35,6
	25	0,864	14,54		25	0,644	36,3
	30	0,862	14,74		**30**	0,637	37,0
50	0	1,011	0,00	100	0	1,011	0,00
	5	0,796	21,27		5	0,609	39,76
	10	0,775	23,34		10	0,587	41,94
	15	0,76	24,83		15	0,576	43,03

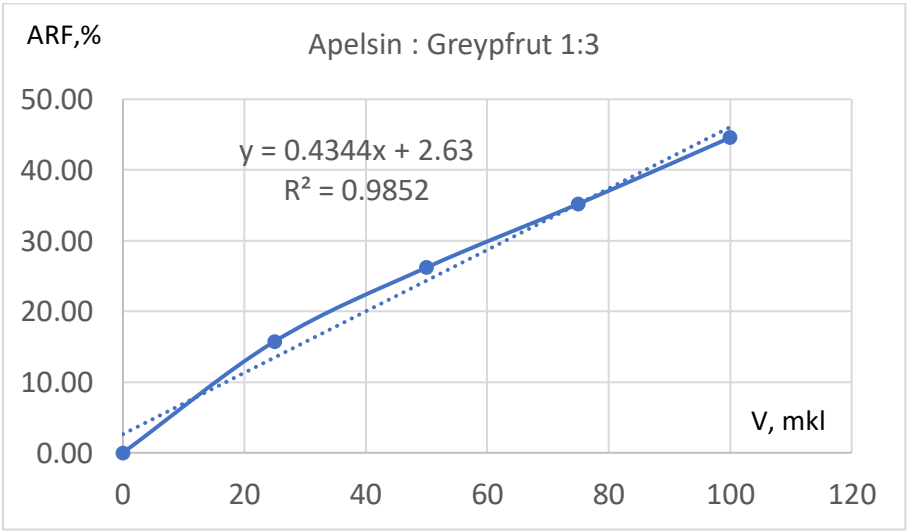
	20	0,755	25,32		20	0,566	44,02
	25	0,749	25,91		25	0,556	45,00
	30	0,744	26,41		**30**	0,55	45,60

Discussion of Results

Analysis of the results presented in the tables above shows that mixtures of orange and grapefruit peels in various ratios exhibit high antiradical activity. When the volume of the DPPH solution remains constant, the antiradical activity (ARA) of all extracts initially increases sharply and then stabilizes around the 25–30 minute mark. Therefore, ARA values measured at the 30-minute point were used for further calculations.

Based on these data, it is possible to determine the minimum volume of extract required to neutralize 50% of DPPH free radicals—this is known as the IC_{50} value. To determine this, a graph is plotted showing the dependence of antiradical activity (ARA, %) on the volume of extract (µl) used in the experiment. Then, using the least squares method [11], a mathematical equation for the straight line that best fits the experimental data is determined (see Figure 1). Thus, the method allows for a quantitative comparison of the antiradical properties of the tested mixtures and makes it possible to identify the most effective composition based on the IC_{50} value.





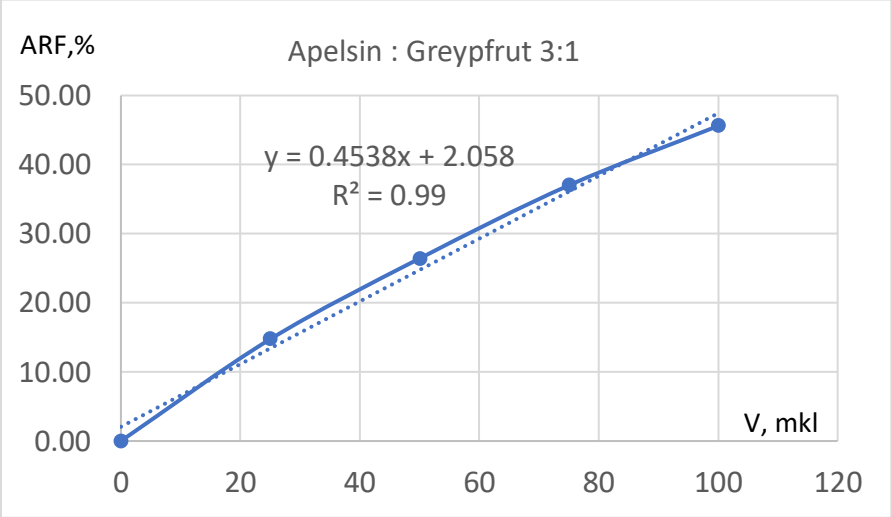


Figure 1. Graphs showing the dependence of antiradical activity values (ARA, %) at the 30th minute on the volume of ethanol extracts of orange and red grapefruit peel mixtures in different ratios (1:1, 1:3, and 3:1), along with the determination coefficients (R^2) indicating the degree of fit between the experimental data and the corresponding linear regression functions.

Using the method described above, the determination coefficients *(R^2)*, which indicate the degree of fit between the experimental data points and the theoretical straight-line regression, were also calculated. As can be seen from the graphs, the R^2 values for all mixtures are close to one. This indicates a high approximation accuracy and, therefore, justifies the use of linear equations to calculate the IC_{50} values.

Thus, based on the general linear equation $y = mx + b$, the volume of extract providing 50% antiradical activity (IC_{50} value) was calculated using the formula:

$x = (y - b) / m$

The calculations yielded the following results:

For the 1:1 mixture of Iranian orange and red grapefruit peel:

$IC_{50} = (50 - 0.4365) / 3.36 = 106.85\ \mu l$

For the 1:3 mixture:

$IC_{50} = (50 - 0.4344) / 2.63 = 109.05\ \mu l$

For the 3:1 mixture:

$IC_{50} = (50 - 0.4538) / 2.058 = 105.72\ \mu l$

These results show that all three extract variants possess comparable antiradical activity. However, the lowest IC_{50} value—indicating the highest efficiency—was observed in the mixture with a higher proportion of orange peel (3:1), making it particularly promising as an antioxidant food supplement.

These values confirm the high antiradical activity of the mixtures prepared from Iranian orange and red grapefruit peels, especially the 3:1 ratio, which showed the strongest effect. This suggests that these mixtures contain biologically active compounds capable of preventing oxidative stress in the human body. The results support the potential use of these compositions as health-promoting agents in medicine.

Based on these findings, we recommend the 3:1 (orange to grapefruit peel) mixture as a novel therapeutic and preventive food additive, which we provisionally name "Asshan."

Conclusion. Experiments aimed at determining the ability of ethanol extracts from mixtures of Iranian orange and red grapefruit peels—prepared in different weight ratios—to neutralize DPPH free radicals demonstrated their high antiradical activity. The minimum volumes of ethanol extracts required to suppress 50% of DPPH free radicals under standard conditions were 106.85 µl, 109.05 µl, and 105.72 µl for the 1:1, 1:3, and 3:1 mixture, respectively. Therefore, these natural waste products show promising potential for use as therapeutic and preventive agents in medicine.

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