Journal of Chemistry of Goods and Traditional Medicine

J Chem Good Trad Med, Volume 4, Issue 2, 2025 Traditional Medicine

ANTIRADICAL ACTIVITY OF FOOD ADDITIVES «ASFAM» AND «ASFALAYM»

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DOI: https://doi.org/10.55475/jcgtm/vol4.iss2.2025.490

Keywords:
Asfam, Asfalaym, food supplement, alcoholic extract, antiradical activity, 2,2-diphenyl-1-picrylhydrazyl

Annotation
The evaluation of the antiradical activity of alcoholic extracts from the food supplements "Asfam" and "Asfalaym," developed from the peels of kiwi, mandarin, lemon, and lime—considered as waste materials—demonstrated their high ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. It was found that the minimum volume of 96% ethanol extract required to neutralize 50% of DPPH free radicals under standard conditions was 162.6 µL for "Asfam" and 134.4 µL for "Asfalaym" respectively. These results suggest the promising potential of these products for medical use as therapeutic agents against oxidative stress.

How to Cite
Askarov, I. R., Alieva, F. A., Abdulloev, S. K., & Sattarova, B. N. ANTIRADICAL ACTIVITY OF FOOD ADDITIVES «ASFAM» AND «ASFALAYM». Journal of Chemistry of Goods and Traditional Medicine, 4(2). https://doi.org/10.55475/jcgtm/vol4.iss2.2025.490

Introduction. Radical-based reactions play a crucial role in both normal and pathological metabolic processes across all cellular forms of life. It is well established that oxidative stress is a key factor in the development of various chronic diseases, as many pathological processes are accompanied by the activation of antioxidant enzymes in the mitochondrial electron transport chain, which react with oxygen via a single-electron transfer mechanism [1]. The formation of superoxide as a result of the one-electron reduction of oxygen is considered the primary initial step in the initiation of free radical oxidation processes in living organisms [2]. Free radicals are responsible for the degradation of biologically important organic molecules, lipid peroxidation in food, and are implicated in the development of various diseases such as cardiovascular diseases, diabetes, and cancer. Furthermore, free radicals can adversely affect lipids, proteins, and DNA, which is a major cause of aging and numerous diseases [3]. An increase in the concentration of free radicals in the

body is considered a leading cause of general aging and age-related degenerative diseases. One of the most effective strategies for reducing oxidative stress is the consumption of natural exogenous antioxidants, which are typically of plant origin and rich in beneficial components [4, 5].

Subtropical fruits such as lemon (*Citrus limon*), lime (*Citrus aurantiifolia*), mandarin (*Citrus reticulata*), and kiwi (*Actinidia deliciosa*) are among the fruits highly valued worldwide for their nutritional content, exotic flavor, medicinal properties, and appeal. Their industrial processing results in the accumulation of significant amounts of waste, particularly peels. However, due to the richness of these peels in carbohydrates, flavonoids, carotenoids, folic acid, vitamins, pectin, and essential oils, they are now regarded as a promising source of biologically active compounds [6, 7, 8].

The biologically active food supplements "Asfam" and "Asfalaym" developed by us [9, 10], based on kiwi and mandarin peels as well as lemon and lime peels, with an optimized content of bioactive substances, can be used in both modern and traditional medicine as promising agents with antioxidant and antiradical properties.

This article presents the results of a study conducted to evaluate the antiradical activity of the food supplements "Asfam" and "Asfalaym"

Materials and methods.

Evaluation of Antiradical Activity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical, which is stable in ethanol solution and has a violet color, is widely used in the spectrophotometric assessment of antiradical activity due to its ability to become decolorized as a result of electron pairing with various antiradical (antioxidant) compounds [11, 12, 13]. In this study, the antiradical activity of the extracts from the food supplements "Asfam" and "Asfalaym" was evaluated using a modified version of the DPPH• free radical scavenging method proposed by Blois [12, 13].

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

Preparation of "Asfam" and "Asfalaym" Extracts. A 0.75 g sample of each food supplement was extracted in 25 mL of 96% ethanol for 20 minutes at 60 °C using an ultrasonic bath. The resulting extract [14] was filtered through a 0.45 µm syringe filter.

Evaluation of the Antiradical Properties of the Extracts. A quartz cuvette with a volume of 4 mL and a path length of 10 mm was filled with 3 mL of DPPH solution in ethanol and 100 µL of ethanol. The cuvette was placed in a spectrophotometer, and the optical density (D1) was measured every 5 minutes for 30 minutes at a wavelength of 517 nm using a YOKE K7000 spectrophotometer [15]. The measured D1 value remained unchanged over time.

To determine the antiradical activity of the "Asfam" and "Asfalaym" extracts, four separate 10 mm quartz cuvettes, as described above, were each filled with 25, 50, 75, and 100 µL of the filtered plant extract. To each cuvette, 3 mL of DPPH solution was added and mixed, and the total volume was then adjusted to 4.1 mL using ethanol. The resulting solutions were immediately mixed, and the optical density (D_2) was measured at a wavelength of 517 nm, following the procedure described earlier.

The measured optical density values and the corresponding antiradical activity (ARA, %) of the "Asfam" and "Asfalaym" extracts were calculated using the following formula:

$$ARA, \% = \frac{D_1 - D_2}{D_1} \cdot 100,$$

the obtained values are presented in Tables 1 and 2.

Table 1. Optical Density Values of the Alcoholic Extract of the Food Supplement "Asfam" Added to the DPPH Solution in Various Volumes, Measured at a Wavelength of 517 nm, and the Calculated Antiradical Activity (ARA), %

Extract volume, µl	Time, minutes.	Optic density, D	ARF, %	Extract volume, µl	Time, minutes.	Optic density, D	ARF, %
25	0	1,004	0.00	75	0	1,004	0.0
	5	0.927	7.67		5	0.842	16.1
	10	0.91	9.36		10	0.808	19.5
	15	0.899	10.46		15	0.785	21.8
	20	0.892	11.16		20	0.768	23.5
	25	0.886	11.75		25	0.755	24.8
	30	0.881	12.25		**30**	0.744	25.9
50	0	1,004	0.00	100	0	1,004	0.00
	5	0.888	11.55		5	0.827	17.63
	10	0.863	14.04		10	0.787	21.61
	15	0.848	15.54		15	0.758	24.50
	20	0.836	16.73		20	0.737	26.59
	25	0.827	17.63		25	0.721	28.19
	30	0.82	18.33		**30**	0.707	29.58

Table 2. Optical Density Values of the Alcoholic Extract of the Food Supplement " Asfalaym " Added to the DPPH Solution in Various Volumes, Measured at a Wavelength of 517 nm, and the Calculated Antiradical Activity (ARA), %

Extract volume, µl	Time, minutes.	Optic density, D	ARF, %	Extract volume, µl	Time, minutes.	Optic density, D	ARF, %
25	0	1,009	0.00	75	0	1,009	0.0
	5	0.974	3.47		5	0.854	15.4
	10	0.939	6.94		10	0.783	22.4
	15	0.923	8.52		15	0.76	24.7
	20	0.913	9.51		20	0.743	26.4
	25	0.906	10.21		25	0.729	27.8
	30	0.901	10.70		**30**	0.717	28.9
50	0	1,009	0.00	100	0	1,009	0.00
	5	0.911	9.71		5	0.803	20.42
	10	0.855	15.26		10	0.71	29.63
	15	0.841	16.65		15	0.677	32.90
	20	0.824	18.33		20	0.656	34.99
	25	0.808	19.92		25	0.638	36.77
	30	0.799	20.81		**30**	0.624	38.16

Results and Discussion.

An analysis of the results presented in the tables above shows that both food supplements exhibit high antiradical activity. At a constant extract dose, the antiradical activity (ARA) initially increases sharply for all extract volumes, and then, after approximately 25–30 minutes, it practically stops changing. Therefore, using the ARA values obtained at the 30th minute, it is possible to determine the minimum volume of the food supplement extract required to neutralize 50% of DPPH free radicals—i.e., the IC50 value [13].To do this, a graph is plotted showing the dependence of antiradical activity (ARA, %) at the 30th minute on the volume of the food supplement extract used in the experiment. Then, using the method of least squares [16], a mathematical formula for the linear function that best fits the experimental data points is determined (see Figure 1).

Using this method, the R^2 parameter values were also calculated, characterizing the degree of fit between the experimental data points and the theoretical straight line. As can be seen from the figures, the R^2 values for both food supplements are close to one. This confirms that the derived linear equations can be used to calculate IC_{50} values with high accuracy.

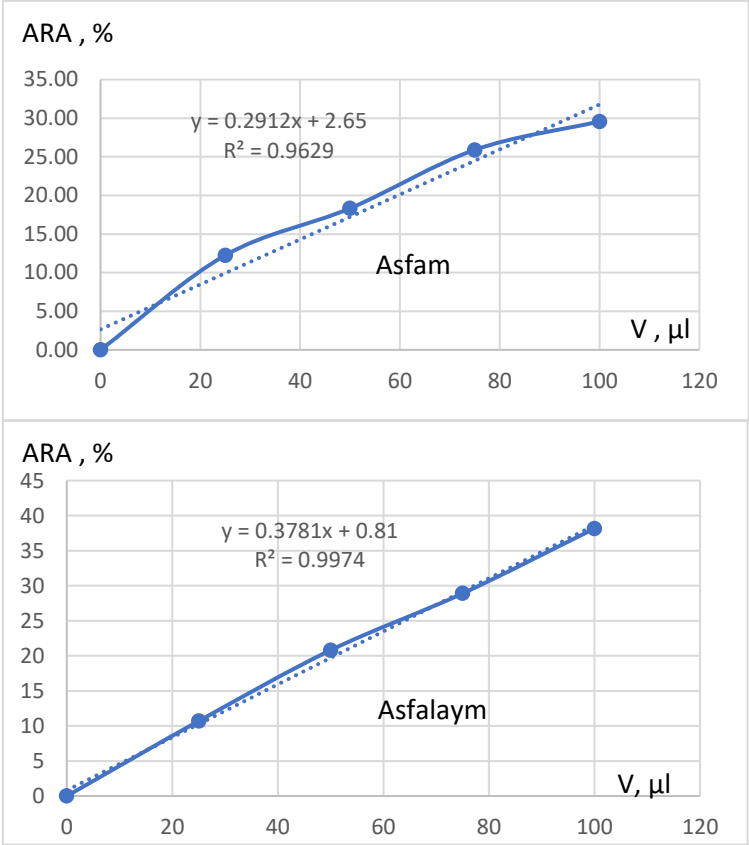


Figure 1. Graphs of the Dependence of Antiradical Activity (ARA, %) at the 30th Minute on the Volume of Extracts of "Asfam" and " Asfalaym"**,** along with the corresponding linear equations and R^2 values, which indicate the degree of fit between the linear trend and the experimental data points.

Thus, based on the theoretical formula of the straight-line $y=mx+b$, which best fits the experimental data points, the volume corresponding to 50% antiradical activity (IC_{50}) was calculated using the formula: $x=(y-b)/m$.

The IC_{50} value for "Asfam" is calculated as:

$$IC_{50} = \frac{(50 - 2.65)}{0{,}2912} = 162{,}6\ mkl$$

And for " Asfalaym":

$$IC_{50} = \frac{(50 - 0.81)}{0{,}3781} = 134{,}4\ mkl$$

The obtained values confirm that the food supplements "Asfam" and " Asfalaym" possess high antiradical activity, are rich in biologically active compounds that counteract oxidative stress, and can be considered promising therapeutic agents for use in medicine.

Conclusion. Experiments testing the antiradical activity of the alcoholic extracts of the food supplements "Asfam" and "Asfalaym", developed by us based on compositions from kiwi and

mandarin peels, as well as lemon and lime peels—considered waste products—showed that they possess high antiradical activity. The minimum volume of the alcoholic extracts of the food supplements "Asfam" and " Asfalaym" required to suppress 50% of DPPH free radicals under standard conditions was 162.6 µL and 134.4 µL, respectively. Therefore, these natural waste materials could potentially be used in medicine as therapeutic agents in the future.

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