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OPTIMIZATION OF THE DPPH METHOD FOR THE EVALUATION OF ANTIRADICAL ACTIVITY OF PLANTS

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Annotation

This study is devoted to the optimization and validation of the DPPH method for the evaluation of antiradical activity of plant compositions. The optimal analytical parameters, including DPPH concentration, measurement wavelength, incubation time, and extract volume range, were determined. The main validation characteristics of the method — linearity, sensitivity, precision, and specificity — were assessed. The results confirm that the DPPH assay is a reliable and reproducible tool for determining the antiradical activity of plant extracts. The proposed approach can be applied for the standardization of phytocompositions and screening of natural antioxidants.

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Oxidative stress represents a state of imbalance between the production of reactive oxygen species (ROS) and the ability of biological systems to neutralize them and repair the resulting damage. Under physiological conditions, ROS perform important signaling functions and participate in protective mechanisms of the organism. However, excessive activation of free radical processes or insufficiency of antioxidant defense leads to the development of oxidative stress, which is considered a universal pathogenetic mechanism of many diseases [Halliwell, 2007]. Free radicals, including the superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), and others, are capable of damaging membrane lipids, proteins, nucleic acids, and other macromolecules. This results in disruption of the structure and function of cells, tissues, and organs. Oxidative stress plays a key role in the pathogenesis of diseases such as atherosclerosis, ischemic heart disease, arterial hypertension, diabetes mellitus, neurodegenerative disorders (Alzheimer's disease, Parkinson's disease), oncological diseases, as well as in processes of premature aging [Lobo et al., 2010].

In this regard, the search for effective and safe natural antioxidants is one of the priority tasks of modern pharmacy, medicine, and the food industry. Plants represent a unique source of biologically active compounds with antioxidant properties. During evolution, plants have developed complex defense mechanisms against oxidative stress by synthesizing a wide spectrum of secondary metabolites capable of neutralizing free radicals. Among these compounds, phenolic acids, flavonoids, tannins, carotenoids, vitamins, and other components are of particular importance, as they may be used in the development of medicinal products, dietary supplements, and functional foods [Pisoschi et al., 2016].

Common tansy (*Tanacetum vulgare L.*) is a perennial herbaceous plant of the Asteraceae family, widely distributed in the temperate climatic zone of Eurasia. In Uzbekistan, it grows in foothill and mountainous areas, along rivers and canals, in meadows, and as a roadside weed. The plant reaches a height of 50–150 cm and is characterized by bright yellow corymbose inflorescences and pinnately dissected leaves. It flowers in July–August, and the fruits ripen in August–September [Khodzhimatov, 1989].

The chemical composition of common tansy is rich and diverse. The main groups of biologically active compounds include: Flavonoids — luteolin, apigenin, quercetin, kaempferol, and their glycosides. These compounds exhibit pronounced antioxidant activity, the ability to chelate transition metal ions, and to inhibit enzymes involved in free radical processes [Williams et al., 2004]. Phenolic acids — chlorogenic, caffeic, ferulic, and protocatechuic acids. These compounds are effective free radical scavengers and demonstrate synergistic effects with flavonoids [Rice-Evans et al., 1996]. Essential oil (1.5–2%) — containing thujone (up to 70%), camphor, borneol, pinene, and limonene. Components of the essential oil also possess antioxidant properties, although their main biological activity is associated with antimicrobial and anti-inflammatory effects [Bail et al., 2008]. Sesquiterpene lactones — tanacetin, artemabsin, and tavulin. These compounds are responsible for the bitterness of the plant and exhibit anti-inflammatory activity. Tannins (up to 8%) — possess astringent and antioxidant properties. Carotenoids and ascorbic acid — enhance the overall antioxidant potential of the plant. Common tansy has long been used in folk and official medicine as a choleretic, anti-inflammatory, antispasmodic, anthelmintic, and wound-healing agent. Tansy preparations are used in diseases of the liver and biliary tract, in gastritis with low acidity, in enterocolitis, and externally for the treatment of purulent wounds and ulcers [State Pharmacopoeia of the USSR, 1990].

The antioxidant activity of tansy has been investigated in several studies. Ivănescu et al. (2018) demonstrated that tansy extracts possess a pronounced ability to inhibit the DPPH radical, which correlates with a high content of phenolic compounds. Studies by Bubenchikova et al. (2019) confirmed the presence of significant amounts of flavonoids and phenolic acids in the aerial parts of tansy, determining its antiradical properties. Field horsetail (*Equisetum arvense L.*) is a perennial spore-bearing herbaceous plant of the Equisetaceae family with cosmopolitan distribution. In Uzbekistan, it is found in river floodplains, moist meadows, along irrigation canals, in fields, and as a weed. A distinctive feature of horsetail is the presence of two types of shoots: spring fertile (spore-bearing) shoots and summer sterile (vegetative) shoots; for medicinal purposes, the summer shoots are used [Khodzhimatov, 1989].

The chemical composition of field horsetail is characterized by a unique combination of biologically active compounds: Silicon compounds (up to 10% calculated on dry weight basis) — mainly represented by water-soluble forms of silicic acid and organosilicon compounds. Silicon plays an important role in connective tissue metabolism, stimulates collagen and elastin synthesis, and participates in mineral metabolism [Holzapfel et al., 2009]. Flavonoids — isoquercitrin, kaempferol-3-sophoroside, luteolin-7-glycoside, apigenin-5-glycoside, and equisetrin. Horsetail flavonoids exhibit pronounced antioxidant, anti-inflammatory, and capillary-protective activities [Mimica-Dukić et al., 2008]. Phenolic acids — caffeic, ferulic, chlorogenic, and vanillic acids. These compounds enhance the antioxidant potential of the plant and demonstrate synergistic effects with flavonoids. Saponins (up to 5%) — equisetonin and other triterpene saponins with diuretic and anti-inflammatory effects. Alkaloids (in small amounts) — nicotine, palustrine, and equisetin. Organic acids — malic, citric, oxalic, and aconitic acids. Mineral substances — in addition to silicon, the plant contains potassium, calcium, magnesium, iron, manganese, zinc, and copper.

Field horsetail (*Equisetum arvense L.*) is widely used in official and traditional medicine as a diuretic, hemostatic, anti-inflammatory, and general tonic agent. Horsetail preparations are used in edema of cardiac and renal origin, in cystitis and urethritis, in pulmonary tuberculosis, and externally for the treatment of wounds and ulcers [State Pharmacopoeia of the USSR, 1990].

The antioxidant activity of field horsetail has been confirmed by numerous studies. Stajner et al. (2009) demonstrated that horsetail extracts effectively inhibit the DPPH radical and possess metal ion chelating ability. Nagai et al. (2005) attributed the antioxidant properties of horsetail to its high content of

flavonoids and phenolic acids. In studies by Kanarskaya et al. (2017), a correlation was established between the total phenolic content and the antiradical activity of field horsetail extracts.

The selection of common tansy (*Tanacetum vulgare L.*) and field horsetail (*Equisetum arvense L.*) as research objects was determined by the following factors:

Presence of antioxidant compounds — both plants contain significant amounts of flavonoids and phenolic acids, which are effective free radical scavengers. The total phenolic content in common tansy reaches 8–10%, while in horsetail it ranges from 5–7% [Ivănescu et al., 2018; Stajner et al., 2009].

Complementary chemical composition — common tansy is rich in flavonoids (luteolin, apigenin) and essential oils, whereas horsetail contains silicon compounds and specific flavonoids (isoquercitrin, equisetrin). The combination of these plants may provide synergistic enhancement of antioxidant effects due to the complementary action of different groups of biologically active compounds.

Availability of raw materials — both plants are widely distributed in the territory of Uzbekistan, ensuring accessibility of raw materials for practical use and the possibility of industrial harvesting.

Insufficient prior study — the antioxidant properties of tansy and horsetail compositions have not been systematically investigated previously. The literature lacks data on the influence of component ratios on antiradical activity and on the kinetics of interaction between extracts and free radicals, which determines the scientific novelty of the present work.

Traditional use — both plants have long been used in folk medicine, indirectly indicating their safety and effectiveness. Investigation of their antioxidant properties may provide a scientific basis for expanding their application in official medicine.

The method for evaluating antioxidant activity using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was first proposed by Marshall Blois in 1958 [Blois, 1958]. In his seminal work, Blois demonstrated that DPPH• is a stable free radical that can be used to study the antioxidant properties of various compounds, including amino acids, vitamins, and phenolic compounds. Since then, the method has undergone numerous modifications but has retained its popularity due to its simplicity, reliability, and informative value.

The principle of the method is based on the ability of antioxidants to reduce the stable radical DPPH•, which in its oxidized form exhibits an intense violet color with a maximum absorption at 517 nm. Upon interaction with compounds capable of donating a hydrogen atom or an electron (antioxidants), DPPH• is reduced to diphenylpicrylhydrazine, which has a yellow color. The degree of discoloration of the solution, recorded spectrophotometrically, is proportional to the amount and activity of antioxidants in the tested sample [Molyneux, 2004].

The reaction between DPPH• and an antioxidant (AH) can be represented by the following equation:

DPPH• (violet) + AH → DPPH-H (yellow) + A•

where AH is an antioxidant molecule capable of donating a hydrogen atom. The resulting radical A• is stabilized by resonance structures and does not initiate further chain reactions. In the case of phenolic compounds, which are the main antioxidants in plant extracts, the reaction mechanism involves the transfer of a hydrogen atom from the phenolic hydroxyl group to DPPH•, forming a semiquinone radical that subsequently dimerizes or interacts with other radicals [Prior et al., 2005].

The main advantages of the DPPH assay include: simplicity of performance — the method does not require complex equipment or lengthy sample preparation; rapid analysis — a single measurement takes 30–60 minutes; high reproducibility — when conditions are standardized, results are well reproduced across laboratories; reagent stability — DPPH is stable in solution under proper storage conditions; versatility — the method is applicable to both hydrophilic and lipophilic antioxidants; cost-effectiveness — it does not require expensive reagents or consumables; and the possibility of automation — the method can be easily adapted for high-throughput screening [Sharma & Bhat, 2009].

When interpreting the results of the DPPH assay, its limitations should be considered: the method measures only the ability of compounds to donate a hydrogen atom or an electron, which does not fully characterize their antioxidant potential in complex biological systems; colored components of plant extracts

may contribute to absorption at 517 nm, requiring appropriate control measurements; results may depend on analysis conditions (DPPH concentration, incubation time, solvent type, medium pH), emphasizing the need for method standardization; the method does not allow identification of specific compounds responsible for antioxidant activity; and some antioxidants may react slowly with DPPH•, requiring extended incubation time [Prior et al., 2005].

The aim of the present study was to optimize the conditions of the DPPH assay for evaluating the antiradical activity of alcoholic and aqueous extracts of mixtures of *Tanacetum vulgare L.* (common tansy) and *Equisetum arvense L.* (field horsetail) at different component ratios, and to validate the developed methodology.

To achieve this aim, the following objectives were addressed:

To select optimal parameters for plant material extraction (type of extractant, extraction method, and extraction time) ensuring maximum recovery of antioxidant-active compounds.

To determine optimal conditions for performing the DPPH assay (working solution concentration, wavelength, incubation time, measurement intervals, and range of extract volumes).

To evaluate the validation characteristics of the developed method: linearity, sensitivity, reproducibility (precision), and specificity.

To perform a comparative analysis of the antioxidant activity of alcoholic and aqueous extracts of the studied plants in order to identify the most effective extractant type.

To develop a standardized DPPH assay protocol suitable for further systematic investigation of the antioxidant properties of compositions of common tansy and field horsetail.

The objects of the study were the aerial parts of common tansy (*Tanacetum vulgare L.*) and field horsetail (*Equisetum arvense L.*). The plant material was collected during the flowering period (July–August 2024) in the Bostanlyk district of the Tashkent region of the Republic of Uzbekistan (coordinates: 41°45′ N, 70°05′ E; altitude 1100–1200 m above sea level). The collection area is characterized by foothill terrain with a moderately continental climate.

For common tansy, flower heads were collected at the beginning of the flowering phase by cutting them at a distance of 20–25 cm from the top. For field horsetail, green summer vegetative shoots were collected. Harvesting was carried out in dry weather, in the morning hours after the dew had evaporated.

The collected raw material was cleaned of foreign impurities, damaged parts, and other plant materials. Drying was performed by the air-shade method in a well-ventilated room protected from direct sunlight at a temperature of 25–30°C. The plant material was spread in a thin layer on paper and periodically mixed to ensure uniform drying. The drying process lasted 7–10 days until an air-dry state was achieved (residual moisture not exceeding 10–12%). The dried material was ground using a laboratory mill to obtain particles of 2–3 mm in size. The powdered material was placed in paper bags and stored in a dry, light-protected place at room temperature until analysis. The storage period did not exceed 3 months.

For the study, mixtures of common tansy and field horsetail were prepared in the following mass ratios: 1:1 (equal parts of tansy and horsetail); 1:3 (one part tansy and three parts horsetail); 3:1 (three parts tansy and one part horsetail). The mixtures were thoroughly homogenized to ensure uniform distribution of components. The following reagents were used: 2,2-diphenyl-1-picrylhydrazyl (DPPH), purity ≥95%, manufacturer “Sigma-Aldrich” (USA); ethanol 96% (analytical grade), manufacturer “Tashkent Chemical Reagents Plant” (Uzbekistan); distilled water obtained using a DE-4 apparatus (Russia). All reagents were used without additional purification.

The following equipment was employed: YOKE K7000 spectrophotometer (China) with a wavelength range of 190–1100 nm; “Sapphire” ultrasonic bath (China) with an operating frequency of 35 kHz and power of 100 W; “Shimadzu AUX-220” analytical balance (Japan) with an accuracy of 0.0001 g; syringe filters with a pore diameter of 0.45 µm (China); quartz cuvettes with an optical path length of 10 mm and a volume of 4 ml; Class A volumetric flasks (100 ml); variable volume pipettes (10–100 µl and 100–1000 µl); “IKA A11 basic” laboratory mill (Germany); membrane vacuum pump. For extract preparation, an accurately weighed portion (1.0000 ± 0.0005 g) of the plant mixture was placed into a 100

ml conical flask. The following extractants were used: 96% ethanol (for alcoholic extracts) and distilled water (for aqueous extracts). The choice of extractants was determined by the need for a comparative analysis of the efficiency of antioxidant compound extraction using polar (water) and less polar (ethanol) solvents.

Twenty-five milliliters of the corresponding extractant were added to the flask containing the weighed sample. The raw material to extractant ratio was 1:25 (w/v). Extraction was performed in an ultrasonic bath at room temperature. Ultrasonic treatment enhances the extraction process due to cavitation effects that disrupt cell walls and promote more complete release of biologically active compounds [Vinatoru, 2001]. The duration of ultrasonic treatment was 20 minutes. Preliminary experiments demonstrated that extending the extraction time beyond 20 minutes did not result in a significant increase in the yield of extractive substances. After completion of extraction, the obtained extracts were filtered through a syringe filter with a pore diameter of 0.45 μm to remove suspended particles and ensure sterility. The filtrate was collected in clean glass test tubes with airtight caps. Preliminary experiments showed that the alcoholic extracts of the studied plants exhibited high antioxidant activity and, when added in a volume of 100 μl, caused almost complete discoloration of the DPPH solution, exceeding the linear range of the method. Therefore, before analysis, alcoholic extracts were diluted 10-fold with 96% ethanol. Aqueous extracts were analyzed without dilution.

The mass of DPPH was calculated using the formula:

$$m = C \times M \times V$$

where m is the mass (g), C is the required concentration (mol/L), M is the molar mass of DPPH (394.32 g/mol), and V is the volume of solution (L).

To prepare 100 ml of a 7.92 mM DPPH solution:

$m = 0.00792$ mol/L × 394.32 g/mol × 0.1 L = 0.3123 g

The concentration of the working DPPH solution was 7.92 mM in ethanol. This concentration was selected based on literature data [Gulcin et al., 2005; Askarov et al., 2024] and provides optimal absorbance values (0.8–1.2 units) at 517 nm. An accurately weighed amount of DPPH (approximately 0.3123 g) was quantitatively transferred into a 100 ml volumetric flask, dissolved in a small amount of ethanol, diluted to volume with ethanol, and thoroughly mixed until complete dissolution.

The prepared DPPH solution was protected from light with aluminum foil and kept at room temperature in the dark for 30 minutes to allow stabilization and establishment of equilibrium radical concentration. To control the stability of the working solution, the absorbance of the control sample (3 ml DPPH + 100 μl ethanol) was measured at 517 nm at the beginning and end of each measurement series. The solution was considered stable if the change in absorbance did not exceed 5% during the analysis period. In a 4 ml quartz cuvette, 3 ml of the working DPPH solution and 100 μl of the corresponding extractant (96% ethanol for alcoholic extracts or distilled water for aqueous extracts) were added. The contents were thoroughly mixed and placed in the spectrophotometer. Absorbance at 517 nm was measured dynamically every 5 minutes over 30 minutes. The control sample was used to determine the initial absorbance D_1 applied in AOF% calculations.

For test samples, 3 ml of the working DPPH solution and 25, 50, 75, or 100 μl of the investigated extract (alcoholic or aqueous, diluted or undiluted depending on type) were added to the quartz cuvette. The total reaction mixture volume was adjusted to 3.1 ml by adding the corresponding extractant. The contents were thoroughly mixed and measurement was started immediately. The reaction mixture was incubated at room temperature (20–22°C) in the dark throughout the measurement period. Protection from light was necessary to prevent DPPH photolysis and secondary photochemical reactions. Absorbance was measured using the YOKE K7000 spectrophotometer at 517 nm immediately after extract addition (time 0 min) and then every 5 minutes for 30 minutes (5, 10, 15, 20, 25, and 30 min). Each measurement was performed in triplicate (n = 3).

Antioxidant activity (AOF%) was calculated using the formula:

$$AOF\% = \frac{D_1 - D_2}{D_1} \times 100\%$$

where: D_1 is the absorbance of the control sample (3 ml DPPH + 100 µl extractant) at the corresponding time point; D_2 is the absorbance of the test sample (3 ml DPPH + extract) at the corresponding time point. Calculations were performed separately for each time point.

Validation of the developed method was carried out in accordance with the ICH (International Conference on Harmonisation) guidelines for validation of analytical procedures [ICH Q2(R1), 2005]. Linearity was assessed by constructing calibration curves of AOF% versus the volume of added extract for each time point. Linearity was evaluated using the coefficient of determination (R^2), which was required to be not less than 0.98. The linear regression equation was calculated using the least squares method. The sensitivity of the method was evaluated based on the minimum extract volume at which a statistically significant difference in AOF% from zero was observed. The acceptance criterion was a signal-to-noise ratio > 3. Reproducibility was assessed from the results of three parallel measurements for each sample at each volume. The mean AOF%, standard deviation (SD), and relative standard deviation (RSD) were calculated.

The specificity of the method was evaluated by measuring the intrinsic absorbance of the extracts at 517 nm without the addition of DPPH. For this purpose, 3 ml of the corresponding extractant and 25–100 µl of extract were added to the cuvette, and absorbance was measured. The contribution of intrinsic absorbance was considered insignificant if it did not exceed 5% of the absorbance of the control sample D_1. All experiments were performed in triplicate (n = 3). Results are presented as mean ± standard deviation (mean ± SD). Statistical data processing was performed using Microsoft Excel 2019 and OriginPro 2021 software. Graphs and calibration curves were constructed using OriginPro 2021. The dried and ground plant material of common tansy (*Tanacetum vulgare L.*) consisted of yellow-green particles with a characteristic camphor-like odor. The yield of extractive substances was 18.3 ± 1.2% with ethanol extraction and 15.7 ± 1.1% with water extraction.

The raw material of field horsetail (*Equisetum arvense L.*) consisted of gray-green particles without odor. The yield of extractive substances was 12.5 ± 0.9% with ethanol extraction and 16.8 ± 1.3% with water extraction. Alcoholic extracts of all studied samples exhibited a more intense greenish-yellow color compared to aqueous extracts, which were pale yellow. This indirectly indicates more efficient extraction of pigments and phenolic compounds by ethanol. A comparative analysis of alcoholic and aqueous extracts showed that alcoholic extracts possess significantly higher antioxidant activity. For example, upon addition of 100 µl of the undiluted alcoholic extract of the 3:1 mixture, AOF% after 30 minutes reached 92.4 ± 3.8%, resulting in almost complete discoloration of the DPPH solution and exceeding the linear range of the method. Therefore, for accurate measurement, all alcoholic extracts were diluted 10-fold.

Aqueous extracts at the same volume (100 µl, undiluted) produced AOF% values in the range of 11–15%, which fit well within the linear range. Preliminary experiments established that increasing the duration of ultrasonic treatment from 10 to 20 minutes led to a 12–15% increase in AOF% for all samples. Further extension of extraction time to 30 minutes did not produce a statistically significant increase in activity ($p > 0.05$). Thus, an extraction time of 20 minutes was selected as optimal, ensuring maximum recovery of antioxidant compounds.

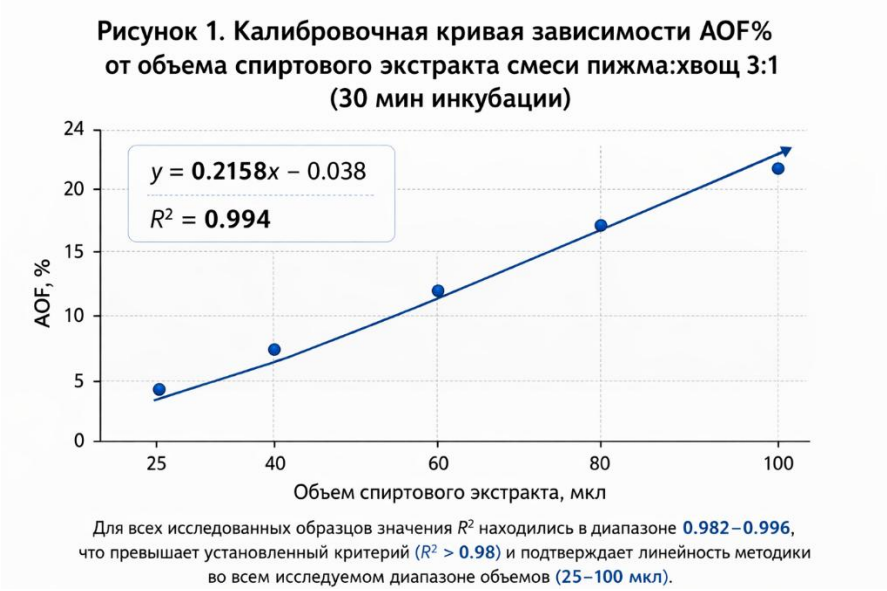
Table 1. Absorbance of the control sample D_1 at 517 nm over time (mean ± SD, n = 3)

Time, min	(D)	Deviation, %
0	1.055 ± 0.008	0.00
5	1.052 ± 0.009	-0.28
10	1.048 ± 0.007	-0.66
15	1.045 ± 0.008	-0.95
20	1.041 ± 0.009	-1.33
25	1.037 ± 0.010	-1.71
30	1.032 ± 0.009	-2.18

As shown by the presented data, the absorbance of the control sample slightly decreased during the 30-minute incubation period. The total decrease by the 30th minute was 2.18%, which does not exceed 5% and indicates sufficient stability of the DPPH working solution under the experimental conditions. The slight decrease in absorbance may be associated with partial degradation of DPPH under light exposure (despite protection with foil) or with interaction with traces of water in the solvent. The linearity of the method was evaluated by constructing calibration curves of AOF% versus the volume of added extract for each extract type and each component ratio.

Table 2. AOF% values for different volumes of the alcoholic extract of the common tansy:field horsetail mixture (3:1) (30 min incubation, mean ± SD, n = 3)

Volume, mkl	AOF%, %
25	5.31 ± 0.21
50	10.52 ± 0.35
75	16.59 ± 0.48
100	21.33 ± 0.62



The minimum detectable extract volume at which a statistically significant difference in AOF% from zero was observed (signal/noise > 3) was 25 µl. At this volume, AOF% values for all samples exceeded 2%, which is substantially above the noise level.

Table 3. AOF% values at the minimum volume (25 µl) for various extracts (30 min incubation)

Extract	AOF% in 25 mkl, %
1:1 alcohol	3.79 ± 0.18
1:3 alcohol	3.51 ± 0.15
3:1 alcohol	5.31 ± 0.21
1:1 water	3.13 ± 0.14
1:3 water	2.46 ± 0.12
3:1 water	3.41 ± 0.16

Reproducibility was evaluated based on three parallel measurements for each sample. As an example, data for the alcoholic extract of the 3:1 mixture are presented.

Table 4. Results of three parallel AOF% measurements for the alcoholic extract of the common tansy:field horsetail mixture (3:1) (30 min incubation)

Объем, мкл	Измерение 1	Измерение 2	Измерение 3	Среднее	SD	RSD, %
25	май.31	май.48	май.15	май.31	0.17	март.20
50	окт.52	окт.81	окт.23	окт.52	0.29	февр.76
75	16.59	16.98	16.20	16.59	0.39	февр.35
100	21.33	21.78	20.89	21.33	0.45	02.нояб

As seen from the table, RSD values for all volumes did not exceed 5%, indicating good reproducibility of the developed method. Similar results were obtained for all investigated samples.

CONCLUSION. As a result of the conducted research, the conditions of the DPPH assay were optimized for evaluating the antiradical activity of alcoholic and aqueous extracts of common tansy (*Tanacetum vulgare L.*) and field horsetail (*Equisetum arvense L.*). The following optimal parameters were established: DPPH concentration 7.92 mM in ethanol; wavelength 517 nm; incubation time 30 minutes with 5-minute measurement intervals; extract volume range 25–100 µl. The necessity of 10-fold preliminary dilution of alcoholic extracts to ensure linearity within the studied range was demonstrated. Optimal conditions for plant material extraction were determined: ultrasonic extraction for 20 minutes at room temperature using two types of extractants — 96% ethanol and distilled water. Alcoholic extracts were shown to exhibit higher antioxidant activity compared to aqueous extracts, due to more efficient extraction of phenolic compounds by ethanol. Validation of the developed method confirmed its reliability in terms of linearity, sensitivity, reproducibility, and specificity. Linearity was confirmed by high coefficients of determination ($R^2 > 0.98$) for all studied samples. Sensitivity was sufficient even for weakly active extracts (minimum detectable volume 25 µl, AOF% > 2%). Reproducibility was characterized by low relative standard deviation values (RSD < 5%), confirming the robustness of the protocol. Specificity was confirmed by the insignificant contribution of intrinsic extract absorbance (less than 2.5%) to the total signal. High stability of the DPPH working solution under experimental conditions was established (absorbance decrease over 30 minutes did not exceed 2.18%), ensuring the reliability of the obtained results. The developed and validated DPPH assay methodology provides a methodological basis for further systematic investigation of the antioxidant properties of compositions of common tansy and field horsetail, including analysis of activity dependence on component ratio, comparative study of alcoholic and aqueous extracts, kinetic analysis, IC_{50} determination, and development of practical recommendations. The proposed protocol may be recommended for screening evaluation of the antiradical activity of other medicinal plants of the flora of Uzbekistan, as well as for standardization and quality control of phytopreparations and dietary supplements.

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