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EVALUATION OF THE ANTIRADICAL ACTIVITY OF COMPOSITIONAL MIXTURES OF ST. JOHN'S WORT (HYPERICUM PERFORATUM L.) AND CHAMOMILE (MATRICARIA CHAMOMILLA L.)

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Annotation

In this study, the antiradical activity of alcoholic and aqueous extracts of mixtures of St. John's wort (Hypericum perforatum L.) and chamomile (Matricaria chamomilla L.) in ratios of 1:1, 3:1, and 1:3 was evaluated using the DPPH• (2,2-diphenyl-1-picrylhydrazyl) method. Extraction was carried out in 96% ethanol and distilled water in an ultrasonic bath for 20 minutes. Antiradical activity (ARA, %) was determined spectrophotometrically at 517 nm, and IC_{50} values were calculated graphically. The highest activity was observed for the aqueous extract of the mixture at a ratio of 3:1 (IC_{50} = 12.08 µL). The results demonstrated the presence of a synergistic effect in the plant composition. The obtained data may serve as a scientific basis for the development of functional teas and phytopreparations based on medicinal plants.

How to Cite

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Introduction. Free radicals intensify oxidative stress in the body and contribute to the development of many chronic diseases [1]. Phenolic compounds, flavonoids, and terpenoids present in plants possess strong antioxidant properties [2].

Hypericum perforatum contains phenolic compounds such as hyperoside, rutin, quercetin, and hypericin, which exhibit antioxidant and anti-inflammatory properties [3]. *Matricaria chamomilla*, in turn, is rich in biologically active compounds such as apigenin, luteolin, and chamazulene [4].

The DPPH assay is widely used for the evaluation of antioxidant activity as a rapid and reliable method [5, 6]. In the present work, the antiradical properties of mixtures of these two plants in different ratios were comparatively assessed.

Materials and Methods.

In this study, an improved method for evaluating the scavenging of DPPH• free radicals was applied [7].

Plant material. Dried aerial parts of St. John's wort (*H. perforatum*) and chamomile (*M. chamomilla*) were used. These plant materials were ground in a mill, and their compositional mixtures were prepared in weight ratios of 1:1, 1:3, and 3:1.

Preparation of the DPPH• working solution. A 7.92 mM DPPH• working solution was prepared in ethanolic medium in a 100 mL volumetric flask. To reduce photo-oxidation and degradation under the influence of light, the solution was wrapped in aluminum foil and kept in the dark at room temperature for 30 minutes.

Preparation of sample extracts. A 1 g portion of each dried plant composition was taken, 25 mL of 96% ethanol (or distilled water) was added, and extraction was performed in an ultrasonic bath at 60 °C for 20 minutes. The resulting extract was filtered through a syringe filter with a pore size of 0.45 μm and used for analysis. Since preliminary tests showed that the ethanolic and aqueous extracts of St. John's wort and chamomile possessed high antiradical activity, the filtered extracts were diluted tenfold with 96% ethanol (or water) prior to quantitative assessment of antiradical activity.

Determination of antiradical activity. In a 4 mL quartz cuvette, 3 mL of DPPH solution was placed and mixed with 100 μL of ethanol (blank sample). The cuvette was then placed in the spectrophotometer, and the optical density (D1) was measured every 5 minutes over a period of 30 minutes at a wavelength of 517 nm using a YOKE K7000 spectrophotometer.

To evaluate the antiradical activity of the plant extracts, 25, 50, 75, and 100 μL portions of each extract were mixed with 3 mL of DPPH solution, the total volume was adjusted to 3.1 mL with 96% ethanol, and the optical density (D2) was measured at 517 nm following the same procedure.

The antiradical activity of the samples was calculated using the following formula:

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

Results and Discussion

The dynamics of the optical density values of DPPH and the blank sample over time after the addition of alcoholic and aqueous extracts of the studied compositional mixtures are shown in Figure 1.

Dynamics of DPPH radical inhibition by the extracts

As can be seen from Figure 1, upon the addition of various volumes of tenfold diluted ethanolic and aqueous extracts of St. John's wort and chamomile compositions to the DPPH solution, a gradual decrease in optical density values at 517 nm over time was observed. This indicates that the DPPH radical reacts with antioxidant components and becomes decolorized.

On the one hand, these results indicate the presence of antioxidant components in the extracts of the compositional mixtures that are capable of scavenging radicals; on the other hand, they show that the reaction is time-dependent, and the decrease in optical density continues throughout the process lasting up to 30 minutes (Figure 1). The presence of such dynamics indicates that the compounds contained in the extracts react with DPPH via a certain kinetic pathway. Usually, phenolic substances and some vitamins react rapidly, whereas others exhibit slower kinetics; as a result, the overall ARA value may increase with time [9].

Evaluation of IC50 values of the compositional mixture extracts

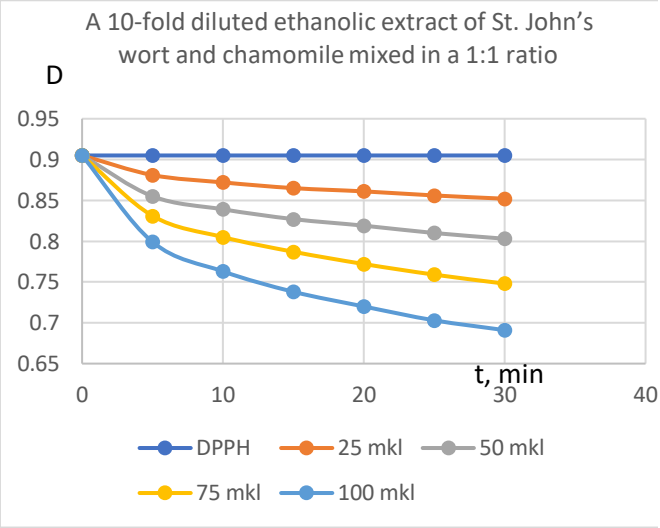
The IC50 value was defined as the extract volume corresponding to 50% inhibition of the DPPH solution. For each sample, a graph was plotted based on the ARA (%) values at 30 minutes (Table 1) and the extract volumes, and the value of x at y = 50 was determined from the trendline equation (y = mx + b) fitted to the experimental points using the formula x = (y - b)/m. Then, taking into account the tenfold dilution, the IC50 value for the actual extract was recalculated by dividing by 10.

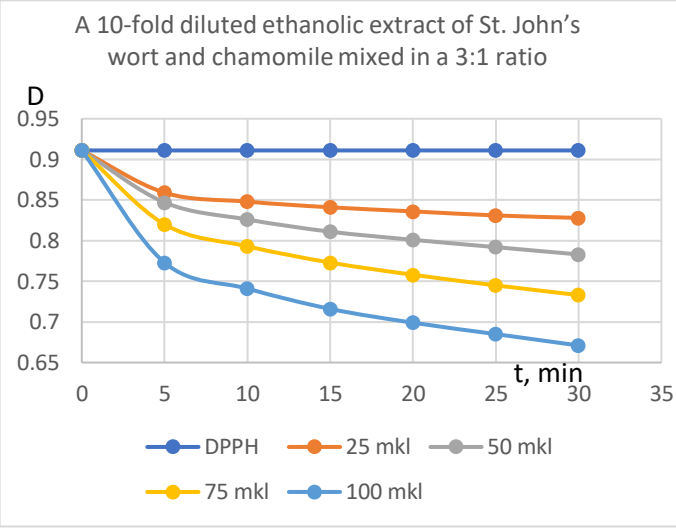
Table 1. Relationship between the extract volume used in the experiment and the antiradical activity (ARA, %) of St. John's wort and chamomile compositions in different weight ratios measured at 30 minutes

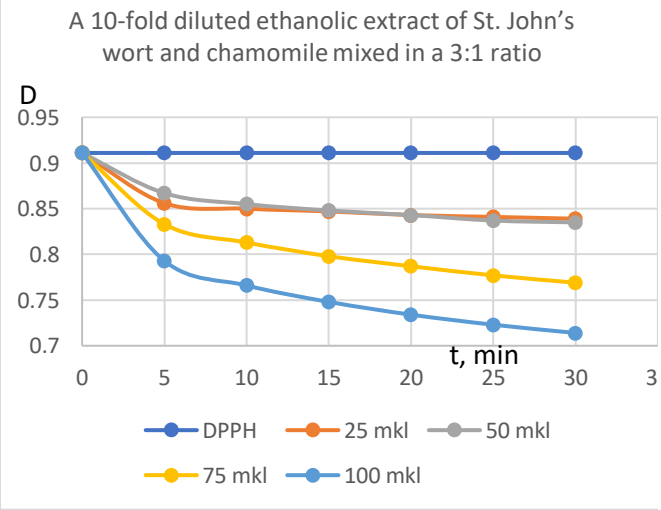
St. John's wort : chamomile ratio	Extract volume, μL	Ethanolic extract, ARA, %	Aqueous extract, ARA, %
1:1	25	5.86	10.10
1:1	50	11.27	14.09
1:1	75	17.35	20.32

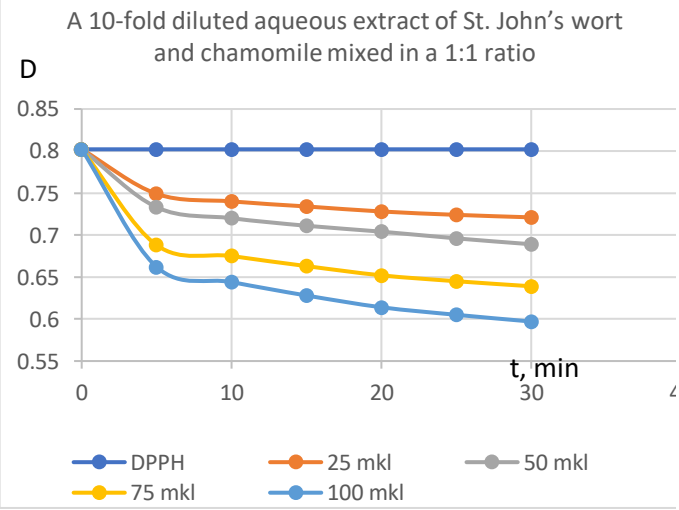
1:1	100	23.65	25.56
1:3	25	9.11	12.61
1:3	50	14.05	21.31
1:3	75	19.54	32.66
1:3	100	26.34	40.48
3:1	25	7.90	9.95
3:1	50	8.34	12.47
3:1	75	15.59	15.62
3:1	100	21.62	20.53

Using the values presented in the table above, graphs of the dependence of ARA (%) at 30 minutes on the extract volume were constructed for the ethanolic and aqueous extracts of each compositional mixture. By means of the least-squares method, the trendline equations, the coefficients of determination (R^2) reflecting the closeness of the experimental points to the calculated straight line, and the IC50 values for the samples were determined (Table 2).









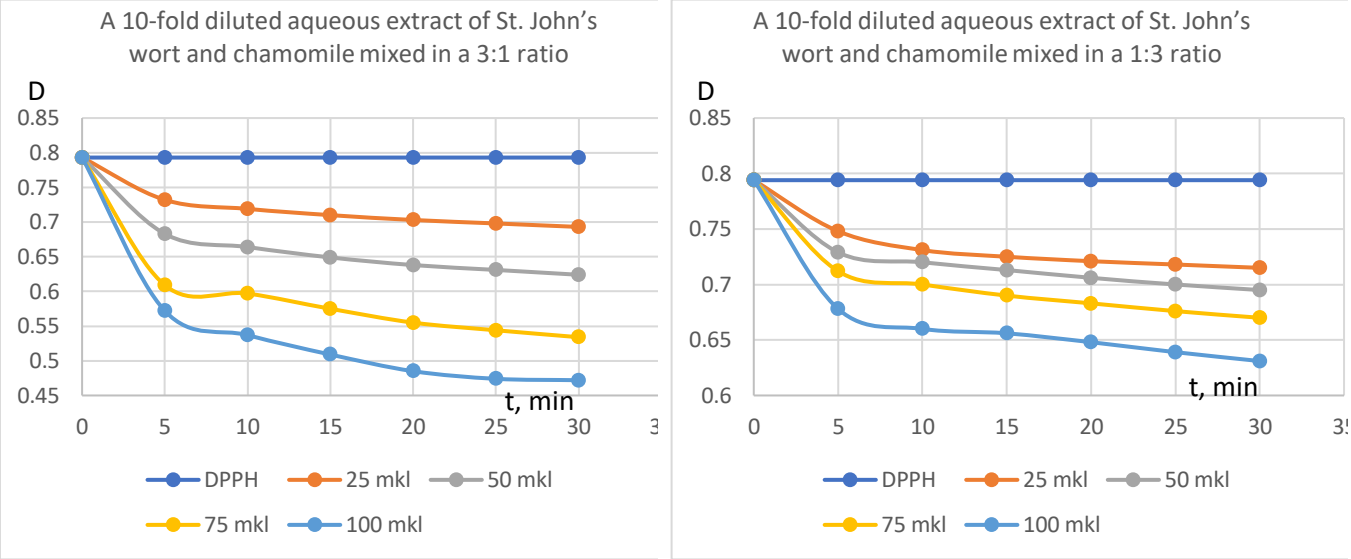


Figure 1. Dynamics of the optical density values of DPPH and the blank sample over time after the addition of alcoholic and aqueous extracts of the compositional mixtures of the aerial parts of St. John's wort and chamomile.

Table 2. Linear equations, coefficients of determination (R^2), and IC50 values calculated on the basis of the relationship between antiradical activity (ARA, %) and the extract volume of St. John's wort and chamomile compositions in different ratios

St. John's wort: chamomile weight ratio	Linear equation	R^2	IC_{50}
Ethanolic extract			
1:1	*y = 0.2352x - 0.132*	0.9993	21.20
1:3	*y = 0.2037x + 0.504*	0.9569	24.30
3:1	*y = 0.2524x + 1.186*	0.9890	19.34
Aqueous extract			
1:1	y = 0.2454x + 1.746	0.9778	19.66
1:3	y = 0.1869x + 2.368	0.9337	25.49
3:1	y = 0.404x + 1.210	0.9944	12.08

The IC_{50} parameter is an important criterion characterizing the antioxidant activity of an extract: the lower its value, the higher the antiradical activity of the sample. According to the results presented in Table 2, among the ethanolic extracts, the lowest IC_{50} value was observed for the St. John's wort and chamomile composition in the 3:1 ratio, amounting to 19.34. This result indicates that in this ratio the biologically active compounds extracted by ethanol possess a high inhibitory capacity toward the DPPH radical. In the ethanolic extract with the 1:1 ratio, the IC_{50} value was 21.20, whereas in the 1:3 sample this parameter was 24.30. Thus, in ethanolic extracts, a certain decrease in antiradical activity was observed with an increasing proportion of chamomile. This may be explained by the more efficient extraction of certain phenolic compounds and flavonoids of St. John's wort in ethanol.

Among the aqueous extracts, the highest antiradical activity was also recorded for the 3:1 composition, with an IC_{50} value of 12.08. This value was the best result not only among the aqueous extracts but also among all studied samples. This indicates that in the composition with a higher proportion of St. John's wort, the content and activity of water-soluble antioxidant compounds are higher. The IC50 value for the aqueous extract with the 1:1 ratio was 19.66, whereas that of the 1:3 aqueous extract was 25.49. Therefore, in aqueous extracts as well, the efficiency of DPPH radical inhibition decreased as the proportion of chamomile increased.

Scientific literature also reports that these plants possess high radical-scavenging capacity in the DPPH assay [8]. The occurrence of a synergistic effect in compositional mixtures may be explained by the mutual regeneration mechanisms of antioxidants.

The obtained results also indicate a significant difference between ethanolic and aqueous extracts. In particular, for the 3:1 composition, the IC_{50} value of the aqueous extract (12.08) was considerably lower than that of the ethanolic extract (19.34), indicating that water is a more effective solvent for extracting the active compounds of this composition. In the 1:1 ratio as well, the aqueous extract exhibited slightly higher activity than the ethanolic extract (19.66 and 21.20, respectively). Only in the 1:3 sample was the IC_{50} value of the ethanolic extract slightly lower than that of the aqueous extract (24.30 and 25.49, respectively). This indicates that extraction efficiency depends not only on the type of solvent but also directly on the ratio of plant components in the composition.

The R^2 values calculated for the trendlines ranged from 0.9337 to 0.9993, indicating a high degree of agreement between the experimental results and the theoretical regression lines. In particular, the values of 0.9993 for the 1:1 ethanolic extract and 0.9944 for the 3:1 aqueous extract confirm the reliability of the calculated IC50 values. Although the R^2 value for the 1:3 aqueous extract was relatively lower (0.9337), this value still indicates that the regression model adequately describes the experimental results.

Conclusion. In general, it was established that the antioxidant activity of compositional mixtures of St. John's wort and chamomile significantly depends both on their mutual ratio and on the nature of the solvent used for extraction. The most favorable result was observed for the aqueous extract with a higher proportion of St. John's wort in the 3:1 ratio. This may be explained by the high activity of water-soluble antioxidant compounds contained in St. John's wort. Thus, in the development of phytocompositions with pronounced antioxidant properties, mixtures with a predominant proportion of St. John's wort, especially in the form of aqueous extracts, may be considered a promising option.

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