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Antioxidant Activity of a Multicomponent Remedy Based on Extracts of Purifying Pelargonium Root, Oregano, and IVY

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Abstract

Naturally-occurring antioxidants are of particular interest because of their ability to trap free radicals and prevent destructive processes caused by oxidative stress. The purpose of this study was to investigate the antioxidant activity of three herbal extracts from the root of pelargonium purifying (*Pelargonium sidoides*), ivy leaves (*Hedera helix L., Hedera caucasigena Pojark*) and oregano (*Origanum vulgare L.*) with different combinations of their ratios. These plants were chosen based on their wide use by traditional medicine healers to treat various diseases. Extraction conditions were selected for each plant separately, and ethanol was used as an extractant. Thick extracts of the studied plants were dried to obtain dry extracts with a moisture content not exceeding 1%. A definition of antioxidant activity (AA) of the studied multicomponent drug solutions was performed in vitro using DFPH (2,2-diphenyl-1-picrylhydrazyl) free-radical method. The results obtained show high AA of solution #7 in which the ratio of active pharmaceutical ingredients was almost the same (tannins: hederocoside: thymol and carvacrol = 1: 1: 1.25). Solutions #3 and #4, which contained 1.4 mg of tannins, 1 mg of hederocosids, and 3 mg of thymol and carvacrol in 100 ml of ethanol, also showed high AA. Multicomponent drugs #5 and 6 were the least effective. Thus, the antioxidant activity of multicomponent drugs based on extracts from purifying pelargonium root, common ivy leaf, and common oregano herb was investigated. The results of the research indicate the promising use of multicomponent drugs #7, 3, and 4 as antioxidant remedies.

Keywords: Tannins, Hederocoside, Thymol, Carvacrol, Medicinal plants, Antioxidant activity

Introduction

Antioxidants protect human cells in various ways (Abdulsahib *et al*., 2020; Akshita *et al*., 2020). This results from the conversion of reactive oxygen to safer forms, disrupting the oxidative chain reaction, which in turn leads to a decrease in localized oxygen concentrations (Oroian and Escriche, 2015). Synthetic antioxidants have a fairly wide application due to their high stability and low cost (Basavegowda and Baek, 2021). However, in the case of their long-term use, there is the appearance of certain side effects, which can subsequently lead to some diseases (Lourenço *et al*., 2019; Ousji and Sleno, 2020). Various plants are well-known for their antioxidant properties. The interest in plant raw materials as a source of antioxidants is due to their high activity and relatively easy availability.

For this reason, many researchers have focused on natural antioxidants, and in the plant world, purely natural compounds and numerous crude extracts have antioxidant properties (Anwar *et al*. 2018). Natural antioxidants in the form of crude extracts or their chemical components are very effective in preventing destructive processes that are caused by oxidative stress. Therefore, the use of drugs that have antioxidant properties in addition to their main therapeutic effect may have certain treatment benefits.

Thus, the main purpose of this study is to investigate the antioxidant activity of three selected plant extracts under different combinations of their ratios. These results may provide insight into whether the extracts in different combinations will cause stronger antioxidant effects than when used alone.

Literature Review

Reactive oxygen species (ROS)/free radicals cause over 100 diseases including atherosclerosis cirrhosis, diabetes, neurodegenerative disorders, cancer, inflammation, nephrotoxicity, etc. (Ray *et al*, 2000; Checa and Aran, 2020; García-Sánchez *et al*., 2020; Akhigbe and Ajayi, 2021). All aerobic organisms, including humans, have antioxidant defense mechanisms, which protect them from oxidative damage. However, natural antioxidant defense mechanisms may not be sufficient, so the intake of antioxidant components with food is important and recommended (Gülçin, 2012; Kurutas, 2015). Natural antioxidants can absorb free radicals, reducing agents, pro-oxidant metal complexes, etc.

Recently, there has been a significant increase in interest to find natural antioxidants for use in medical products and food to replace synthetic antioxidants whose use is limited by their side

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effects, such as carcinogenicity. Thus, plants are a major source of natural antioxidant molecules that can eliminate or neutralize harmful ROSs (Kasote *et al*., 2015). The medicinal plants' importance in preventing or controlling the disease is due to the antioxidant properties of their constituents, such as phenols and flavonoids (Anwar *et al*., 2018; Tungmunnithum *et al*., 2018). Polyphenol compounds are thought to have suppressive activity and chemo-preventive against cancer cells by inhibiting quite a few metabolic enzymes that are involved in the stopping the cell cycle or activation of potential carcinogens (Niedzwieck *et al*., 2016). Natural antioxidants can protect the human body from free radicals and slow many chronic diseases down from developing such as cancer, heart disease, stroke, etc., as well as increase plasma antioxidant capacity (Chanda and Dave, 2009; D'Antuono *et al*., 2015).

In many countries around the world, multicomponent herbal preparations are used to treat a wide range of diseases (Builders, 2018). The concept of multicomponent herbal therapy can be useful when individual plants or extracts of plant parts in preparation have different efficacies, providing additive or synergistic effects. It may also reduce the required doses of individual components compared to mono-component herbal therapies. This area of research is still little explored in the scientific world, so appropriate research into possible interactions between different herbal preparations, especially those frequently used.

The pelargonium purifying (*Pelargonium sidoides*) is one of the medicinal plants used to treat upper respiratory diseases (Šmejkal and Rjašková, 2016). The main active substances in pelargonium aqueous-alcoholic extract are coumarins, flavonoids, phenolic acids, pro-antioxyanids (tannins, etc.), Phenols, organic acids, etc. It is known (Panche *et al*., 2016) that tannins and some flavonoids have the potential ability to bind free radicals. Moreover, a comparison of these two classes of components has shown that tannins have greater anti-radical potential than flavonoids (Panche *et al*., 2016). The effectiveness of the use of the medical product based on Pelargonium sidoides extract has been demonstrated in several clinical studies (Careddu and Pettenazzo, 2018).

The common oregano herb (Origanum vulgare L.), of the Lamiaceae family, is listed in the State Pharmacopoeia of Ukraine and Europe (European Pharmacopoeia-8th ed., 2013). Different classes of biologically active substances, such as flavonoids, tannins, ascorbic acid, as well as an essential oil, which includes thymol, carvacrol, sesquiterpenes, geranyl acetate, free alcohols, represent the chemical composition of the oregano herb. Scientists have confirmed the promising use of oregano herb for the production of extracts and the creation of drugs based on them, which have anti-inflammatory and antioxidant properties (Sakkas and Papadopoulou, 2017).

Ivy (Hedera helix L., Hedera caucasigena Pojark.) is an evergreen climbing liana plant from the Araliaceae family. Biologically active compounds are responsible for the medical use of ivy - Triterpene saponins (2.5-6%): bidesmoside glycosides hederogenin: hederocoside C (1.7-4.8%), hederocoside D (0.4-0.8%), hederocoside B (0.1-0.2%), and monodesmoside α-hederin (0.1-0.3%). Ivy leaves are used for chronic catarrh of mucous membranes, tuberculosis, rickets, and other diseases, as an external remedy for burns, for dressing purulent wounds. Common ivy has antiseptic, anti-inflammatory, antioxidant, expectorant, diuretic, hypotensive, wound healing, and antispasmodic effects (Deng *et al*., 2015).

Thus, the main purpose of this study is to investigate the antioxidant activity of three selected plant extracts under different combinations of their ratios. These results may provide insight into whether the extracts in different combinations will cause stronger antioxidant effects than when used alone.

Materials and Methods

The Material

The objects of the study were the root of purifying pelargonium (Pelargonium sidoides), ivy leaves (Hedera helix L., Hedera caucasigena Pojark), and common oregano (Origanum vulgare L.). Plant raw materials were ground using a laboratory cam crusher and determined the fractional composition and humidity: the root of purifying pelargonium - less than 1 mm -46%, 1-5 mm-54% and 8.0% moisture; ivy leaves - less than 1 mm -16%, 1-5 mm-35%, 5-10 mm - 49% and 8.5% humidity; Oregano herb - less than 1 mm - 4%, 1-10 mm -96% and 10.5% humidity.

Extraction

The extraction was performed using ethanol as an extractant (15% for the roots of purifying pelargonium; 40% - ivy leaves; 90.6% - oregano). The extraction time was 8 hours for the roots of purifying pelargonium and 12 hours for 2 other medicinal plant raw materials. The obtained extracts were used to determine the dry residue content (State Pharmacopoeia of Ukraine, 2016) and the quantitative content using absorption spectrophotometry (State Pharmacopoeia of Ukraine, 2016) and gas chromatography (State Pharmacopoeia of Ukraine, 2016).

After that, thick extracts were obtained, with a dry matter content of not less than 75%, evaporating the obtained solutions on a laboratory rotary evaporator LABOROTA 4001. To reduce the boiling point, a deep vacuum pump VT6 (0.8-0.9 kg/cm^2) was used. The optimum temperatures for obtaining a thick extract of oregano, pelargonium, and ivy are 60-70 ºC, 80-90 ºC, and 65-70, respectively. The thick extracts were dried to obtain dry extracts with a moisture content not exceeding 1%.

The drying technology was tested **(Table 1)** on dryers of 4 types: contact dryers: vacuum-drying cabinet, roller-belt dryer; convective dryers: multi-belt dryer, spray dryer.

Table 1. Selection of technology for drying thick extracts of oregano, pelargonium, and ivy.

Dryer type:	Thick extracts		
	oregano	pelargonium	ivy

	Process duration, hours	Manufacturability of the dry extract	Process duration, hours	Manufacturability of the dry extract	Process duration, hours	Manufacturability of the dry extract
vacuum drying cabinet	Over 48	No	64	Yes	48	Yes
roller-belt dryer	Over 48	No	-	No	8	Yes
multi-belt dryer	Over 48	No	6	Yes	6	Yes
spray dryer	Over 48	No	4	Yes	-	No

Preparation of a multicomponent drug

Dry extracts of pelargonium (tannins), ivy (hederocoside), and thick extract of oregano (thymol and carvacrol) were dissolved in 40% ethanol at the rate of:

Table 2. The ratio of active pharmaceutical ingredients (API) in multicomponent drugs

API	The amount of API, in mg per 100 ml of 40% ethanol						
	solution number						
	1	2	3	4	5	6	7
Tannins	1	1	1,4	1,4	1,8	1,8	2,0
Hederocoside	3	2	1	1	3	2	2
Thymol and carvacrol	2	2	3	3	1	1	2,5

Antioxidant activity (AA)

The determination of AA of the investigated solutions was performed in vitro using DPPH - free radical method (Brand-Williams *et al*., 1995). The amount of absorption was determined on a spectrophotometer at a wavelength of 515 nm immediately after the addition of DPPH solution and after 30 min relative to distilled water for all test solutions and the solution comparison. AA was calculated as a percentage by the formula:

$$\text{Antioxidant activity } \% = [\text{DPPH}]_0 - [\text{DPPH}]_t \times 100/[DPPH]_0 \quad (1)$$

where $[DPPH]_0$ – the rate of absorption of the solution-comparison $DPPH$;
$[DPPH]_t$ – the rate of absorption of the solution $DPPH$ with the studied multicomponent drugs after 30 min.

Statistical Analysis

Statistical processing of experimental data was performed using Student's test (t). All results are presented as the average values ± standard error (SEM). Differences with a value of P <0.05 were considered statistically significant.

Results and Discussion

To date, there is ample evidence to suggest a key role for reactive oxygen species (ROS) and other oxidants in the development of many different disorders and diseases (Di Meo *et al*., 2016). As noted above, the available literature has drawn the attention of scientists to the recognition of antioxidants for the prevention and treatment of disease and the maintenance of human health (Di Meo *et al*., 2016; Salanță *et al*., 2020). Studies of herbs, vegetables, and fruits have shown the presence of antioxidants such as phenols, flavonoids, tannins, and pro-anthocyanids. Antioxidants that are present in medicinal plants can help protect the body from various diseases (D'Antuono *et al*., 2015). Naturally-occurring antioxidants are of particular interest because of their ability to trap free radicals (Kurutas, 2015). The use of medicinal plants with high levels of antioxidant components has been proposed as an effective therapeutic approach (Kirichenko *et al*., 2020).

By extraction were obtained **(Table 3)** and dried API (tannins, hederocoside, thymol, and carvacrol) from the root of purifying pelargonium, ivy leaves, and oregano. Ethanol was used as the extractant.

Table 3. Extraction conditions and quantitative content of active pharmaceutical ingredients in the extracts

Medicinal plant raw materials	Extractant - ethanol,%	The ratio of raw materials: extractant	Duration of extraction, h	The quantitative content of API, units	Dry residue,%
Purifying pelargonium root	15	1:3,625	8	0,21 tannins	1,53
Ivy leaves	40	1:3,475	12	0,12 glycosides	1,4
Oregano herb	96,5	1:3,525	12	0,28 thymol and carvacrol	2,1

Thick extracts contained 75% dry matter. Since the compounds thymol and carvacrol are sensitive to high temperatures, the optimal temperature for obtaining a thick extract was 60-70 ºC. Due to the low ethanol content (15%), the duration of evaporation at low temperatures and the lack of temperature influence on the quantitative content of tannins in the thick extract, the optimal conditions for obtaining a thick pelargonium extract are 80-90 ºC. While, the optimal evaporation temperature of ivy extract was 65-70 ºC, and the duration of evaporation was about 4 hours.

In the course of this work, thick extracts of oregano, ivy leaves, and pelargonium roots with a dry matter content of at least 75% were obtained, from which dry extracts were subsequently obtained and their antioxidant activity was determined. Based on experiments on the selection of technology for drying a thick extract of oregano we were unable to obtain technologically malleable raw materials, a lamellar mass was formed. Perhaps in the future, we should consider the option with freeze-drying, although in the literature it is not recommended to use freeze-dried in the case when the product remains ethanol, due to significant costs and inefficiency of drying on the shafts. The best option for drying a thick extract of pelargonium roots and ivy leaves is to use radar and a multi-belt dryer, respectively.

There are several methods commonly used to determine antioxidant activity in vitro for rapid screening of substances, as substances that have low antioxidant activity in vitro are also likely to show little activity in vivo (de Torre *et al*., 2019; Salanță *et al*., 2020). The DPPH-free radical method was used to evaluate and compare the antioxidant activity of the studied multicomponent plant extracts.

It is known that the presence of three aromatic rings in DPPH makes its molecule very stable (Ichikawa *et al*., 2019). This radical has a maximum absorption at 517 nm. Any substance that can capture DPPH reduces absorption at this wavelength, so DPPH analysis can be considered as an appropriate method to assess the ability of samples to capture free radicals (Ichikawa *et al*., 2019).

The dependence of the antiradical efficacy of the studied multicomponent drugs can be depicted as follows **(Figure 1)**:

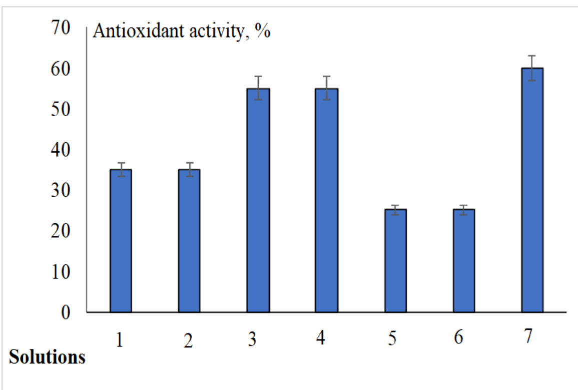

Figure 1. Antioxidant activity of the studied multicomponent drugs (solution 1-7).

According to the data shown in the figure, the antioxidant efficacy of the obtained multicomponent drugs based on extracts of purifying pelargonium root (Pelargonium sidoides), common ivy leaf (Hedera helix L., Hedera caucasigena Pojark), and common oregano herb (Origanum vulgare L.) decreases in the following series:

7 > 3 > 4 > 1 > 2 > 5 > 6

The greatest antioxidant activity was shown by solution # 7, which contained 100 ml of 40% ethanol 2 mg of tannins and hederocoside, as well as 2.5 mg of thymol and carvacrol. Also, high AA showed solutions # 3 and 4, which contained 1.4 mg of tannins, 1 mg of hederocoside, and 3 mg of thymols and carvacrols in 100 ml of ethanol.

The least effective were multi-component drugs # 5 and 6, which contained the same amounts of tannins (1.8 mg in 100 ml of 40% ethanol) and thymol and carvacrol (1 mg in 100 ml of 40% ethanol) but differed in the content of hederocoside (3 and 2 mg, respectively, in 100 ml of 40% ethanol).

It can be concluded that the multi-component drug, in which the ratio of active pharmaceutical ingredients was almost the same, had the highest antioxidant activity and may be promising as an antioxidant.

Conclusion

The antioxidant activity of multicomponent drugs based on extracts from roots of purifying pelargonium (Pelargonium sidoides), common ivy leaves (Hedera helix L., Hedera caucasigena Pojark), and common oregano herb (Origanum vulgare L.) was studied.

The highest antioxidant activity had multicomponent medicinal product #7, in which the ratio of the active pharmaceutical ingredients was almost the same (tannins: hederocoside: thymol and carvacrol = 1: 1: 1.25). Also, high antioxidant activity was shown by solutions #3 and #4, in which the composition was quantitatively dominated by thymol and carvacrol. The results of the research indicate the promising use of multicomponent drugs # 7, 3, and 4 as antioxidant agents.

Practical value of the study

Multicomponent drugs # 7, 3, and 4, which are based on extracts from the roots of pelargonium (Pelargonium sidoides), ivy leaves (Hedera helix L., Hedera caucasigena Pojark), and oregano (Origanum vulgare L.), can be used as the antioxidant for disease prevention and control. These drugs can protect the human body from free radicals and slow the development of many chronic diseases such as stroke, heart disease, and cancer, as well as increase the capacity of antioxidant for plasma. In addition, extracts of pelargonium root, ivy leaves, and oregano can be used in food production, or directly during eating.

Prospects for further research

Numerous studies should be carried out to determine the spectrum of biological activity of these extracts in vitro or in vivo. For example, to determine the activity concerning the trapping of nitrogen and hydroxyl radicals, and to establish the antimicrobial activity of the extracts.

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