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The study of the total antioxidant capacity of St. John's Wort (*Hypericum perforatum* L.) herb

Aim. To determine the total antioxidant capacity of St. John's Wort herb.

Materials and methods. The content of phenolic compounds, anthraquinone derivatives, flavonoids, and derivatives of hydroxycinnamic acids was determined by the spectrophotometric analysis, while organic acids were identified by the alkalimetric method; the antioxidant activity of extracts obtained was evaluated by the potentiometric method.

Results and discussion. The total antioxidant capacity of St. John's Wort herb was 91.35 mmol-equiv/m^{dry weight}, the total content of phenolic compounds was 22.70 mg of gallic acid/mL, anthraquinone derivatives – 0.46 mg of hypericin/mL, flavonoids – 8.10 mg of rutin/mL, derivatives of hydroxycinnamic acids – 7.90 mg of chlorogenic acid/mL and organic acids – 10.80 mg of citric acid/mL in St. John's Wort herb extracts obtained during the sequential exhaustive extraction. The correlation analysis showed a very high positive correlation between the antioxidant activity and the total content of phenolic compounds, flavonoids, hydroxycinnamic acid derivatives in St. John's Wort herb extracts.

Conclusions. The total antioxidant capacity of *H. perforatum* herb was 91.35 mmol-equiv./m^{dry weight}. The results of the analysis of the BAS content and the antioxidant activity of *H. perforatum* herb extracts revealed that the aqueous extract had a significant content of phenolic compounds, flavonoids, hydroxycinnamic acids, organic acids and the antioxidant activity, while anthraquinone derivatives dominated in 96 and 60 % EtOH extracts. The quantitative analysis showed that flavonoids and hydroxycinnamic acids dominated among phenolic compounds. The results can be applied in developing optimal technologies for obtaining drugs based on the extract of *H. perforatum* herb.

Keywords: *St. John's Wort herb; total antioxidant capacity; correlation; sequential*

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Дослідження сумарної антиоксидантної ємності трави звіробою (*Hypericum perforatum* L.)

Мета дослідження – визначити сумарну антиоксидантну ємність трави звіробою.

Матеріали та методи. Вміст фенольних сполук, антрахінонових похідних, флавоноїдів та похідних гідроксикоричних кислот визначали методом спектрофотометричного аналізу, тоді як органічні кислоти визначали алкаліметричним методом; антиоксидантну активність отриманих екстрактів оцінювали потенціометричним методом.

Результати та їх обговорення. Сумарна антиоксидантна ємність трави звіробою становила 91,35 ммоль-екв./г_{сух. зап.}, сумарний вміст фенольних сполук – 22,70 мг галлової кислоти/мл, антрахінонових похідних – 0,46 мг гіперіцину/мл, флавоноїдів – 8,10 мг рутину/мл, похідних гідроксикоричних кислот – 7,90 мг хлорогенової кислоти/мл, органічних кислот – 10,80 мг лимонної кислоти/мл в екстрактах трави звіробою, отриманих шляхом послідовного вичерпного екстрагування. Проведений кореляційний аналіз засвідчив дуже високу позитивну кореляцію між антиоксидантною активністю та сумарним вмістом фенольних сполук, флавоноїдів, похідних гідроксикоричних кислот в екстрактах трави звіробою.

Висновки. Сумарна антиоксидантна ємність трави *H. perforatum* становила 91,35 ммоль-екв./г_{сух. зап.}. Результати аналізу вмісту біологічно активних речовин та антиоксидантної активності екстрактів трави *H. perforatum* засвідчили, що водний екстракт мав значний вміст фенольних сполук, флавоноїдів, гідроксикоричних кислот, органічних кислот та антиоксидантної активності, тоді як антрахінонові похідні домінували в екстрактах з 96 % та 60 % етанолом. Кількісний аналіз засвідчив домінування флавоноїдів та гідроксикоричних кислот серед фенольних сполук. Результати можуть бути використані для розробки оптимальних технологій отримання лікарських засобів на основі екстракту трави *H. perforatum*.

Ключові слова: трава звіробою; сумарна антиоксидантна ємність; кореляція; послідовне вичерпне екстрагування

Introduction. Reactive oxygen species (ROS) are produced by exogenous and endogenous ways, for instance, ROS actively generated during tricarboxylic acid pathway and the respiratory chain that linked to the inner mitochondrial membrane [1]. The production of ROS is important metabolites for the proper protection and survival of cells with physiological limits [2]. But, the disruption neutralization process of ROS may lead to increasing free radicals in cells and may induce oxidative stress. Oxidative stress associated with various pathologies in

humans, such as cancer, diabetes, chronic renal diseases, mitochondrial dysfunction, and neurodegenerative diseases, aging and DNA damage [3]. Therefore, it is recommended to consume foods and beverages, medications, dietary supplements that are rich in antioxidants to neutralize the negative effects of ROS [4].

Hypericum Taurin ex L. is a genus with 508 species worldwide. The most widespread species is *Hypericum perforatum* L. belonging to *Hypericaceae* family. *H. perforatum* is an herbaceous perennial plant native to Europe,

Asia and Africa [5]. *H. perforatum* contains derivatives of anthraquinone, flavonoids, prenylated phloroglucinols, hydroxycinnamic acids, volatile compounds and organic acids [6]. The main constituents of *H. perforatum* are hyperforin (2-4,3 %), hypericin (0.1-0.15 %), hyperoside (0.4-0.8 %), rutin (0.8-1.6 %) and catechins (0.5-0.9 %) [7]. Due to the rich chemical composition, *H. perforatum* herb is used in folk medicine for centuries. *H. perforatum* herb has a wide range of application in medicine: inflammation of bronchi, stomach ulcers, diabetes mellitus, wound healing, colds, obesity and depression [8].

The antioxidant activity of *H. perforatum* herb was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [9], 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) [10] and ferric reducing antioxidant power (FRAP) [11]. However, no studies have been conducted to determine the total antioxidant capacity of *H. perforatum* herb. In our opinion, the total antioxidant capacity of the raw material is essential for the further development of medicines, dietary supplements and cosmetic products.

The aim of the study was to determine the total antioxidant capacity and the content of biologically active substances (BAS) of *H. perforatum* herb extracts obtained by the sequential exhaustive extraction.

Materials and methods. The study object was *Hypericum perforatum* herb collected in the places of its cultivation. The material was harvested in 2022 during the flowering period in the vicinity of the village of Ternova, Kharkiv region.

The pH meter HANNA 2550 (Germany) with a combined platinum electrode EZDO 50 PO (Taiwan) was applied for potentiometric measurements. The quantitative analysis of BAS was carried out on an UV – 1000 UV-spectrophotometer (China) with matched 1 cm quartz cells. Weighing was done using an AN100 digital analytical balance (AXIS, Poland) with $d = 0.0001$ g.

First 10.0 g of *H. perforatum* herb were ground to 1-2 mm in size. The extraction was carried out one by

one using distilled water, 20 % ethanol, 40 % ethanol, 60 % ethanol, 96 % ethanol and chloroform in the raw material/solvent ratio of 1/20 (*m/v*) on a water bath at 80°C with reflux for 1 hour. After cooling, the solutions were filtrated and concentrated to 20 mL by a rotary evaporator at 40 ° C under vacuum.

The total phenolic compounds were quantified using the Folin-Ciocaltau method, with absorbance readings taken at 760 nm [12]. The total content of flavonoids was found by $AlCl_3$ assay where the absorbance was measured at 415 nm [12]. The total content of hydroxycinnamic acids derivatives was measured by the complex formation assay with $NaNO_2$ - Na_2MoO_4 , the absorbance was measured at 505 nm [12]. The total content of organic acids was determined using the acid-base titration and the potentiometric method to determine the end-point [12]. The total content of anthraquinone derivatives was determined by the molecular absorption analysis [13], the absorbance was measured at 591 nm.

The antioxidant activity of extracts was evaluated by the potentiometric method [14].

The Pearson's (r) correlation coefficient was used to analyze the correlation between the antioxidant activity and the amount of phenols, catechins, flavonoids, hydroxycinnamic acid derivatives and organic acids. The correlation coefficient takes a value in the range of -1 to +1. Correlation is very high if it is within the range from 0.90 to 1.00; from 0.70 to 0.90 is a high correlation; from 0.50 to 0.70 is a moderate correlation; from 0.30 to 0.50 is a low correlation; from 0.00 to 0.30 negligible correlation [15].

Result and discussion. The aqueous extract had the most significant content of phenolic compounds (12.50 mg/mL), while other *H. perforatum* herb extracts showed much lower content of these compounds. The total content of phenolic compounds of *H. perforatum* herb extracts was 22.70 mg/mL (Table 1).

The total content of anthraquinone derivatives was 0.46 mg/mL. The highest amount of anthraquinone

Table 1

The quantitative content (mg/mL) of anthraquinone derivatives, flavonoids, derivatives of hydroxycinnamic acids, organic acids, the antioxidant activity, and the dry residue calculated in the extraction of *H. perforatum* herb (the raw material/solvent ratio – 1:20)

Extractant	Dry residue, %	Total phenolic content calculated with reference to gallic acid	Total content of anthraquinone derivatives calculated with reference to hypericin	Total flavonoid content calculated with reference to rutin	Total content of hydroxycinnamic acid derivatives calculated with reference to chlorogenic acid	Total content of organic acids calculated with reference to citric acid	Antioxidant activity, mmol-equiv./m _{dry weight}
Distilled water	6.69 ± 0.07	12.50 ± 0.25	Not observed	4.60 ± 0.09	4.00 ± 0.08	5.00 ± 0.10	42.67 ± 0.43
20 % EtOH	4.45 ± 0.04	3.30 ± 0.07	Not observed	1.30 ± 0.03	1.20 ± 0.03	3.50 ± 0.07	11.56 ± 0.12
40 % EtOH	0.23 ± 0.01	1.70 ± 0.03	0.06 ± 0.001	0.60 ± 0.01	0.70 ± 0.01	0.60 ± 0.01	6.00 ± 0.06
60 % EtOH	0.56 ± 0.01	2.30 ± 0.05	0.20 ± 0.01	0.80 ± 0.01	0.70 ± 0.01	0.60 ± 0.01	14.56 ± 0.15
96 % EtOH	0.68 ± 0.01	2.30 ± 0.05	0.20 ± 0.01	0.80 ± 0.01	0.70 ± 0.01	0.60 ± 0.01	14.76 ± 0.15
Chloroform	0.23 ± 0.01	0.60 ± 0.01	Not observed	Not observed	0.60 ± 0.01	0.50 ± 0.01	1.80 ± 0.02
The total content of BAS	12.84	22.70	0.46	8.10	7.90	10.80	91.35

Table 2

The Pearson's (r) correlation coefficient between the antioxidant activity and the BAS content in *H. perforatum* herb extracts

	Total phenolic content	Total content of anthraquinone derivatives	Total flavonoid content	Total content of hydroxycinnamic acid derivatives	Total content of organic acids
Antioxidant activity	0.9723	Not conducted	0.9722	0.9412	0.6220

derivatives was observed in 60 % and 96 % EtOH extracts (0.20 ± 0.01 mg/mL), followed by 40 % EtOH extract, while in other extracts anthraquinone derivatives were not found. According to the results, the part of the total anthraquinone derivatives out of phenolic compounds was 2.0 % (Table 1).

The total flavonoid content was 8.10 mg/mL. The highest amount of flavonoids was observed in the aqueous extract (4.60 ± 0.09 mg/mL), followed by other EtOH extracts (Table 1). According to the results, the part of the total flavonoids out of phenolic compounds was 35.7 % (Table 1).

The total content of hydroxycinnamic acid derivatives was 7.90 mg/mL. The highest amount of hydroxycinnamic acid derivatives was observed in the aqueous extract (4.00 ± 0.08 mg/mL), followed by other EtOH extracts (Table 1). The part of the total hydroxycinnamic acid derivatives out of phenolic compounds was 35.0 % (Table 1).

The aqueous extract had the highest content of organic acids (5.00 ± 0.10 mg/mL), while other *H. perforatum* herb extracts demonstrated much lower content of organic acids. The total content of organic acids was 10.80 mg/mL (Table 1).

The total antioxidant capacity of *H. perforatum* herb was 91.35 mmol-equiv./m_{dry weight}. The antioxidant

activity of extracts increases in the following order: 96 % EtOH < 60 % EtOH < 40 % EtOH < 20 % EtOH < aqueous extract.

The Pearson's (r) coefficients between the antioxidant activity and phenolic compounds, flavonoids, hydroxycinnamic acid derivatives, and organic acids were 0.9722, 0.9723, 0.9412, and 0.6220, respectively (Table 2).

In our previous study [16], we created and described an exhaustive sequential extraction approach. This type of extraction is based on the extraction of the same raw material with extractants of different polarity, for example, distilled water as a more polar extractant and ethanol solutions of different concentrations as a less polar extractant. The sequential exhaustive extraction allows extracting hydrophilic and lipophilic BAS completely. In this case, the raw material was not dried after the extraction; therefore, the volume of the extractant absorbed by the raw material was considered. Previously, the absorption coefficient for the raw material was found using the extraction with different solvents.

The antioxidant activity values of the extracts under study were estimated using the potentiometric method. This method was chosen due to its high sensitivity, rapid analysis procedure, and relatively low cost of equipment and reagents [17]. However, certain acceptance criteria are required to substantiate the choice of extraction

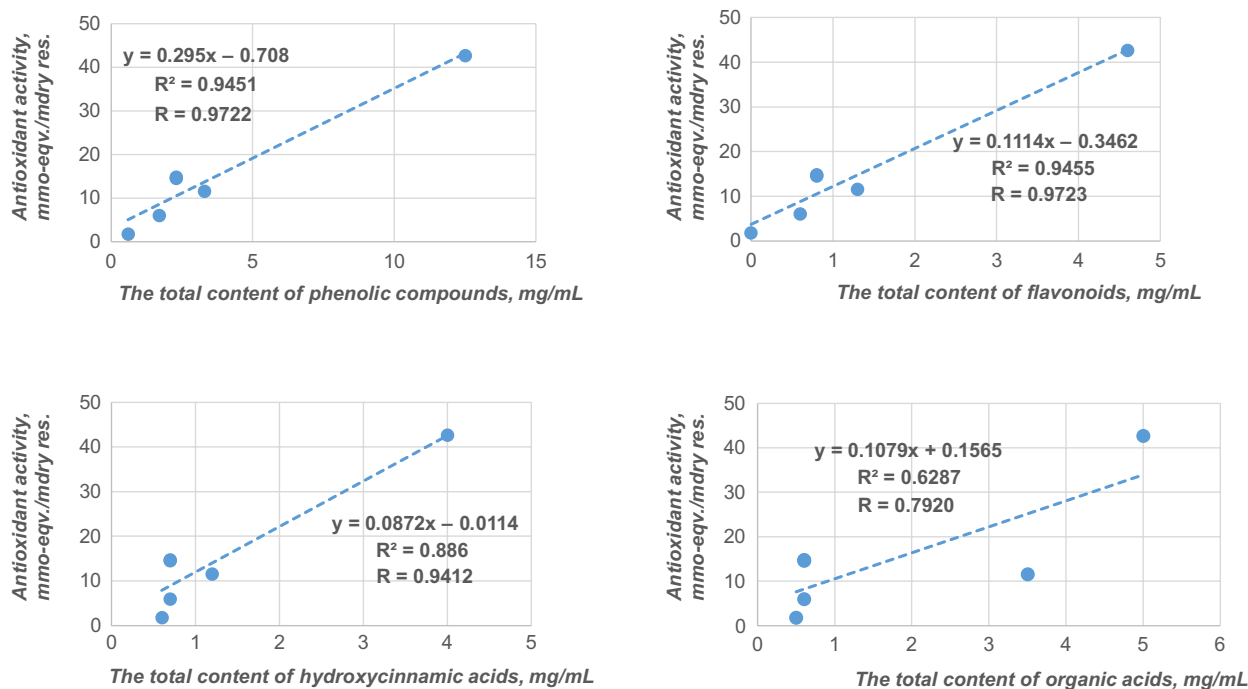


Fig. 1. The correlation relationship between the value of the antioxidant activity and the total content of phenolic compounds, flavonoids, hydroxycinnamic acids and organic acids

conditions. According to the available literature, the main criterion for acceptability is to obtain the maximum content of phenolic and extractive compounds. We propose to use the total antioxidant capacity of the raw material as an acceptance criterion for choosing the optimal extraction conditions for several of reasons, firstly, the antioxidant activity and the content of phenolic compounds have a high positive correlation, secondly, the determination of the antioxidant activity requires less time than the determination of the content of BAS and extractive compounds. The term “the total antioxidant capacity of the raw material” means the total content of the antioxidant activity of all hydrophilic and lipophilic BAS contained in the raw material analyzed.

In our study, the highest value of the correlation coefficient was between the antioxidant activity and the total content of phenolic compounds ($r = 0.9723$), the second place was taken by flavonoids ($r = 0.9723$) and the third one – by derivatives of hydroxycinnamic acids ($r = 0.9412$), while the correlation with the total content of organic acids was the worst (Fig. 1, Table 1). The correlation analysis was not conducted in the case of anthraquinone derivatives because of the limited numbers of values (only

three points). Therefore, phenolic compounds, flavonoids and hydroxycinnamic acid derivatives affected the antioxidant activity of *H. perforatum* herb extracts.

Conclusions. The total antioxidant capacity of *H. perforatum* herb was determined by the potentiometric method. The results of the analysis of the BAS content and the antioxidant activity of *H. perforatum* herb extracts revealed that the aqueous extract had a significant content of phenolic compounds, flavonoids, hydroxycinnamic acids, organic acids and the antioxidant activity, while anthraquinone derivatives dominated in 96 and 60 % EtOH extracts. The quantitative analysis showed that flavonoids and hydroxycinnamic acids dominated among phenolic compounds. The correlation analysis revealed a strong positive linear relationship between the antioxidant activity and phenolic compounds, flavonoids and hydroxycinnamic acids, while the worst correlation was in the case of organic acids. The results can be applied in developing optimal technologies for obtaining drugs based on the extract of *H. perforatum* herb.

Conflict of interests: authors have no conflict of interests to declare.

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