

Investigation of the Correlation between the Antioxidant, Antimicrobial Activity and the Content of Phenolic Compounds of St. John's Wort (*Hypericum perforatum* L.) Liquid Extracts

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ABSTRACT

Infection diseases is a worldwide important problem for medicine and pharmacy. The purpose of work was study the total content of some biologically active substances (BAS), determine antimicrobial, anti-fungi and antioxidant activity of obtained St. John's Wort extracts, and study a correlation analysis between the content of natural compounds and antimicrobial/antifungal and antioxidant activity. Results demonstrate the highest amount of polyphenols, flavonoids, anthraquinone derivatives and organic acids were 1.37 ± 0.02 , 0.66 ± 0.01 , 0.05 ± 0.005 and $0.66 \pm 0.005\%$ in 60% EtOH extract, respectively. The hydroxycinnamic acids was dominated in 40% extract (0.72 ± 0.01). The most potent antioxidant property possessed 60% EtOH extract of John's Wort herb. The high correlation was found between the total polyphenols, flavonoids and antioxidant/antimicrobial/antifungal effects against all Gram-positive, Gram-negative bacterial strains and the fungus *C. albicans*. These findings have showed the great potential of St. John's wort in the development and creation of new medicines with antimicrobial, antioxidant and antifungal effects that are not inferior to, and even superior to, the effects of synthetic analogues.

1. Introduction

Nowadays, the problem of bacterial infection is still relevant. According to recent statistical studies, it has been found that every year 13.7 million people per year die from bacterial infections in the world. The mortality rate for all ages was 99.6 deaths per 100.000 population. Of the pathogens studied, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* accounted for 54.9% of the 7.7 million deaths, with *S. aureus* being associated with more than 1.1 million deaths. *S. aureus* was the leading bacterial cause of death in 135 countries and was associated with the largest number of deaths among people over 15 years of age (940.000)¹. In addition, this problem is compounded by the emergence of resistance in bacteria to widely used antibiotics, which makes treatment more complex, time-consuming and expensive². Thus, the search for new antimicrobial natural compounds is perspective for today.

Thousands of natural compounds were shown antimicrobial effects against Gram-negative, Gram-positive strains³. Natural compounds have a number of advantages over synthetic compounds: high efficiency, minimal side effects and low cost of the production method. Also, natural compounds, especially derivatives of phenolic compounds, have a high level of antioxidant effect, which is quite important in infectious diseases⁴.

The *Hypericum Taurin* ex L. is a genus with 508 species worldwide. The most widespread species is *Hypericum perforatum* L. that belong to *Hypericaceae* family. *H. perforatum* is an herbaceous perennial plants that origin to Europe, Asia and Africa⁵. *H. perforatum* contains derivatives of anthraquinone, flavonoids, prenylated phloroglucinols, hydroxycinnamic acids, volatile compounds and organic acids⁶. The main constituents of *H. perforatum* represented by 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 rich chemical composition the *H. perforatum* herb is applied in folk medicine for centuries. The *H. perforatum* herb has a wide range of application in medicine: inflammation of bronchs,

stomach ulcers, diabetes mellitus, wound healing, colds, obesity and depression⁸.

There are a lot of scientific researches about determination a level antioxidant activity of *H. perforatum* herb extracts^{9, 10}. However, there is no date about assessing antioxidant\antimicrobial\anti-fungi activity and its correlation with content of BAS by potentiometric method. So, the aim of the study was to determine the total content of polyphenols, flavonoids, hydroxycinnamic and organic acids, anthraquinone derivatives, moreover study antimicrobial and anti-fungi activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and fungi *Candida albicans*. In addition, it was aimed to study a correlation analysis between the content of BAS in *H. perforatum* herb extracts and antimicrobial/antifungal and antioxidant activity.

2. Materials and methods

2.1 Plant material

H. perforatum herb was the object of the study, which were collected in the places of its cultivation. The material was collected in 2022 during the flowering period in the vicinity of the village of Ternova, Kharkiv region.

2.2 Equipment

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

2.3 Extraction procedure

A six samples of 10.0 g (exact mass) of St. John's Wort herb had the size of particles 1-2 mm. The extraction was conducted with distilled water, 20%,

40%, 60%, 96% EtOH at 80° C within 1 hour with a condenser, ratio raw material/solvent – 1/20. The extraction technique was completed twice to provide totally extract all BAS, then the filtrates were joint and evaporated by vacuum rotary to ratio of extract to raw material 1:2. The six extracts of 96, 60, 40, 20% EtOH and aqueous were obtained. The green tea (*Camellia sinensis* L.) extract was obtained by the mentioned above method with 60% EtOH.

2.4 Quantitative analysis

The total content of phenolic compounds was measured by the Folin-Ciocaltau assay, the absorbance was measured at 760 nm. The calibration curve ($Y = 0.1055X + 0.1745$, $R^2 = 0.9951$). was plotted with interval concentrations 1.0 – 5.0 µg/ml, the calibration equation

The total phenolic compounds content was expressed as gallic acid according to equation 1.¹¹.

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{V} \quad (\text{Eq.1})$$

where, C_x – concentration of gallic acid according to the calibration curve, $C \times 10^{-6}$, g/ml; V – volume of extract, ml; K_{dil} – coefficient of dilution.

The sum of anthraquinone derivatives was determined by a molecular absorption analysis, the absorbance was measured at 591 nm. The total anthraquinone derivatives content was expressed as hypericin in extract according to equation 2.¹².

$$X(\%) = \frac{C_x \times K_{dil}}{718 \times V} \quad (2)$$

where, A – absorbance of analyzed solution; 718 – specific adsorption coefficient of hypericin; V – volume of extract, ml; K_{dil} – coefficient of dilution.

The total flavonoids were determined using assay of complex formation with AlCl_3 , the absorbance was measured at 417 nm. The total flavonoids content was expressed as rutin [13] calculated according to equation 3.

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{A_s \times V} \quad (\text{Eq.3})$$

where, A – absorbance of analyzed solution; A_{st} – absorbance of standard solution of rutin; V – volume of extract, ml; K_{dil} – coefficient of dilution.

The total hydroxycinnamic acids derivatives content was measured by assay of complex formation with NaNO_2 - Na_2MoO_4 , the absorbance was measured at 525 nm¹⁴. The total content of hydroxycinnamic acids derivatives was expressed as chlorogenic acid, was calculated according to equation (4)

$$X(\%) = \frac{C_x \times K_{dil}}{188 \times V} \quad (\text{Eq.4})$$

where, A – absorbance of analyzed solution; 188 – specific adsorption coefficient of chlorogenic acid; V – volume of extract, ml; K_{dil} – coefficient of dilution.

The total organic acids content was determined by acid-base titration with the fixation end-point by potentiometric method. The total content of organic acids was expressed as citric acid^{15, 16}, according to equation 5.

$$X(\%) = \frac{(V_{equiv} - V_x) \times 0.0032 \times K_{dil} \times K \times 100}{V} \quad (\text{Eq.5})$$

where, 0.0032 – the amount of citric acid, which is equivalent to 1 ml of sodium hydroxide solution (0.05 mol/L), g; V_{equiv} is the volume of sodium hydroxide solution (0.05 mol/L), which was used for titration, ml; V_x – the volume of sodium hydroxide solution (0.05 mol/L), which was spent for titration in a blank experiment, ml; V – volume of extract, ml; K_{dil} – coefficient of dilution; K is correction coefficient for 0.05 mol/L sodium hydroxide solution.

2.5 Antioxidant activity assay

Antioxidant activity of extract was evaluated by potentiometric method^{17,18}. It was calculated according to equation 6. and expressed as mmol-equiv./m_{dry res.}:

$$AOA = \frac{C_{\alpha} - \alpha \times C_{red}}{1 + \alpha} \times K_{dil} \times 10^{-3} \times \frac{m_1}{m_2} \quad (\text{Eq.6})$$

where, $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - E_{ethanol})nF/2.3RT}$, C_{ox} – con-

centration of $K_3[Fe(CN)_6]$, mol/L; C_{red} – concentration of $K_4[Fe(CN)_6]$, mol/L; $E_{ethanol} = 0.0546 \cdot C_{\%} - 0.0091$; $C_{\%}$ – concentration of ethanol; ΔE – change of potential; $F = 96485.33$ C/mol – Faraday constant; $n = 1$ – number of electrons in electrode reaction; $R = 8.314$ J/molK – universal gas constant; $T = 298$ K; K_{dil} – coefficient of dilution; m_1 – mass of dry residue; m_2 – mass of dry residue in 1.0 mL of extract.

The standardized green tea leaf 60% extract was used as the reference drug.

2.6 Test organisms

Museum strains of *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Proteus vulgaris* NTCS 4636, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885/653 were used in accordance with the recommendations for the assessment of antimicrobial activity of drugs.

2.7 Antimicrobial activity assay

In our study, we used 1% solution of extract, the solvent of which were 60% ethanol. The method of diffusion of the drug into agar carried out using the method of “wells”¹⁹. Gentamycin, and fluconazole were used as reference drugs for assessing antimicrobial and anti-fungal activity.

The standardized green tea leaf 60% EtOH extract was used as the reference drug.

2.6 Test organisms

Museum strains of *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Proteus vulgaris* NTCS 4636, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885/653 were used in accordance with the recommendations for the assessment of antimicrobial activity of drugs.

2.7 Antimicrobial activity assay

The method of diffusion of the drug into agar car-

ried out using the method of “wells”¹⁹. Preparation of microorganisms suspensions with determined concentrations of microorganisms (optical density) was carried out by the standard of turbidity (0.5 units according to scale of McFarland) with using of equipment of Densi-La-Meter (Czech, wavelength 540 nm). Suspensions were prepared according to equipment and information list. Colony forming unit was 107 microorganisms at 1 ml of growth medium and determined by standard of McFarland). On solidified agar, using a pipette under sterile conditions in Petri dishes made 1 ml of a suspension of microorganisms. After uniform distribution of microorganisms over the entire surface of the agar, the plates were incubated at room temperature for 15-20 minutes. Next, wells with a diameter of 6 mm were made in the cups, into which solutions of the test substances were introduced. The samples incubated at 37° C for 16-24 hours. After incubation, the plates were placed upside down on a dark matte surface so that light fell on them at an angle of 45° (accounting in reflected light). The diameter of the growth retardation zones measured using a caliper. Gentamycin, and fluconazole were used as reference drugs for assessing antimicrobial and anti-fungal activity.

Table 1. Interpretation criteria for microbial sensitivity

Microbial sensitivity	Diameter of the growth retention zone, mm
High sensitivity	>25
Sensitive	15-25
Low sensitivity	10-15
Not sensitivity	<10

2.7 Correlation analysis

Pearson's (r) correlation coefficient was used to analyze the correlation between antioxidant activity (AOA) and the amount of phenolic, catechin, flavonoid, hydroxycinnamic acids 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 correlation¹³

2.8 Statistical analysis

For all the experiments, two samples were analyzed and all the assays were carried out in 5 times. The results were expressed as mean values with confident interval. The MS EXCEL 7.0 and STATISTIKA 6.0 were used to provide statistical analysis.

3. Results

3.1 Qualitative analysis of BAS

According to obtained results shown in Table 1, the 60% EtOH extract ($1.37 \pm 0.02\%$) had the most significant amount of polyphenols, followed by 96% EtOH extract ($1.10 \pm 0.02\%$), whereas the lowest one – aqueous extract ($0.40 \pm 0.02\%$).

Table 2 demonstrates that the most significant content of flavonoids was found in 60% EtOH extract ($0.66 \pm 0.01\%$), whereas in the aqueous extract ($0.02 \pm 0.002\%$) was the lowest one. The percentage of flavonoids out of total of polyphenols was 54, 48, 17, 6 and 5% for 96%, 60%, 40%, 20% EtOH and aqueous extracts, respectively. The highest percentage of flavonoids was in 96% EtOH extract, whereas the lowest in aqueous extract.

The content of hydroxycinnamic acids increasing in the following order aqueous extract ($0.15 \pm 0.005\%$) > 20% EtOH extract ($0.26 \pm 0.005\%$) > 96% EtOH extract ($0.34 \pm 0.01\%$) > 60% EtOH extract ($0.54 \pm 0.01\%$) > 40% EtOH extract ($0.72 \pm 0.01\%$). The percentage of hydroxycinnamic acids out of total of polyphenols was 31, 39, 71, 49 and 38% for 96%, 60%, 40%, 20% EtOH and aqueous extracts, respectively. The highest percentage of hydroxycinnamic acids was in 40% extract, whereas the lowest in 96% EtOH extract. (Table 2)

The content of anthraquinone derivatives was found only in 96 and 60% extracts. The total content of anthraquinone derivatives of 96% extract was 40% lower than 60% extract. The percentage

of anthraquinone derivatives was 2 and 4% out of polyphenols for 96 and 60% extract, respectively. (Table 2)

The highest amount of organic acids was determined in 60% EtOH extract ($0.66 \pm 0.005\%$), followed by 96% EtOH extract ($0.39 \pm 0.005\%$), whereas the lowest one in 40% extract ($0.28 \pm 0.005\%$). The total content of organic acids was lower 65%, 52%, 71%, 34% than content polyphenols in 96%, 60%, 40%, 20% extracts, respectively. (Table 2)

3.2 Antioxidant activity

A potentiometric method for determining antioxidant activity was used to evaluate the effect of the obtained extracts of *H. perforatum* herb. Table 3 shows that the level of antioxidant activity increases in the following order: 20% EtOH extract (50.00 ± 1.00 mmol-equiv./m_{dry res.}) > aqueous extract (55.20 ± 1.10 mmol-equiv./m_{dry res.}) > 40% EtOH extract (59.59 ± 1.19 mmol-equiv./m_{dry res.}) > 96% EtOH extract (61.68 ± 1.23 mmol-equiv./m_{dry res.}) > 60% EtOH extract (70.71 ± 1.41 mmol-equiv./m_{dry res.}). In light of the data obtained, it can be established that the 60% EtOH extract has the highest level of antioxidant activity. In light of the data obtained, it can be established that the 60% extract has the highest level of antioxidant activity. According to the modern classification of antioxidant activity, which was previously developed by us²⁰, it was found that all extracts obtained have a high level of antioxidant activity. Moreover, a comparative analysis of the “strength” of antioxidant activity was carried out with the gold standard 60% EtOH extract of *C. sinensis* leaf. The *C. sinensis* leaf extract was obtained by the same technological method as *H. perforatum* herb extract. The obtained extracts were significantly inferior in antioxidant effect to *C. sinensis* leaf extract. Further, a 0.06 mol/L solutions (in terms of the amount of polyphenols expressed as gallic acid) of extracts of *H. perforatum* herb and *C. sinensis* leaf were prepared. As a result of the study, it was found that when compared at the same concentrations, the aqueous extract had the highest antioxidant effect, and the least - 60% EtOH extract. (Table 4)

Table 2. The sum of phenolic compounds, flavonoids, anthraquinone, hydroxycinnamic acids and organic acids in *H. perforatum* herb liquid extracts

Sample	Total phenolic compounds, % ± SD	Total anthraquinone, % ± SD	Total flavonoid, % ± SD	Total hydroxycinnamic acids, % ± SD	Total of organic acids, % ± SD
96% EtOH extract	1.10 ± 0.02	0.02 ± 0.005	0.59 ± 0.01	0.34 ± 0.01	0.39 ± 0.01
60% EtOH extract	1.37 ± 0.02	0.05 ± 0.005	0.66 ± 0.01	0.54 ± 0.01	0.66 ± 0.01
40% EtOH extract	0.98 ± 0.02	—	0.17 ± 0.001	0.72 ± 0.01	0.28 ± 0.01
20% EtOH extract	0.53 ± 0.02	—	0.03 ± 0.002	0.26 ± 0.01	0.35 ± 0.01
aqueous extract	0.40 ± 0.02	—	0.02 ± 0.002	0.15 ± 0.01	0.47 ± 0.01

Table 3. The level of antioxidant activity of *H. perforatum* herb liquid extracts

Sample	Antioxidant activity, mmol-eqv./m _{dry res.} ± SD	Conditional term of antioxidant level
96% EtOH extract	61.68±1.23	High level
60% EtOH extract	70.71±1.41	High level
40% EtOH extract	59.59±1.19	High level
20% EtOH extract	50.00±1.00	High level
aqueous extract	55.20±1.10	High level
<i>C. sinensis</i> leaf extract	548.79 ± 10.98	Very high level

*Note: SD – standard deviation, n=3, p<0.05

Table 4. Comparing the value of antioxidant activity of *H. perforatum* herb liquid extracts with *C. sinensis* leaf 60% extract at the concentration 0.06 mol/L expressed in the total phenolic compounds

Sample	Concentration, mol/L	Antioxidant activity, mmol-eqv./m _{dry res.} ± SD
96% EtOH extract	0.06 ^a	56.07±1.12
60% EtOH extract		51.61±1.03
40% EtOH extract		60.00±1.20
20% EtOH extract		100.00±1.02
aqueous extract		138.00±2.76
<i>C. sinensis</i> leaf extract		54.36±1.09
Epigallocatechin-3-O-gallate		61.20 ± 1.22

*Note: SD – standard deviation, n=3, p<0.05, a – molar concentration of *H. perforatum* herb liquid extracts and green tea leaf extract was calculated as total phenolic compounds expressed as gallic acid

3.3 Antimicrobial activity

In this research work, the antimicrobial activity of the obtained *H. perforatum* herb extracts was investigated against the following strains of *S. aureus*, *B. subtilis*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, as well as a strain of the fungus *C. albicans*. According to the obtained results, all extracts obtained from the *H. perforatum* herb had an effective antimicrobial effect. (Table 5)

S. aureus was most sensitive to the 60% EtOH extract (23.0 ± 0.3 mm) and least sensitive to the aqueous extract (15.0 ± 0.6 mm). When comparing the results of the gentamicin standard and the 60% EtOH extract, it was found that the 60% EtOH extract was 10% better at inhibiting the growth of the *S. aureus* strain of bacteria. According to the results presented in Table 4, it was found that *B. subtilis*, as well as *S. aureus*, was highly sensitive to the 60% EtOH extract (23.0 ± 0.3 mm), followed by 96% EtOH extract (22.0 ± 0.4 mm), and the aqueous extract inhibited the growth of the bacterial strain the least (17.0 ± 0.4 mm). The most resistant strains of bacteria to the action of *H. perforatum* herb extracts was *P. vulgaris*. A 60% EtOH extract of *H. perforatum* herb inhibited the growth of *P. vulgaris* by 20% better than the reference standard gentamicin. *E. coli* and *P. aeruginosa* were most sensitive to the action of 60% extract, in second place – 96% EtOH extract; the investigated bacterial strains were the most resistant to an aqueous extract. (Table 5)

When studying antifungal activity against *C. albicans*, the results showed that 96 and 60% EtOH extracts of *H. perforatum* herb most actively inhibited the growth of the fungus, and the fungus was the least sensitive to the action of 20% EtOH and an aqueous extract. When compared with the fluconazole standard, it was found that the 96 and 60% EtOH extracts inhibited fungal growth 10% better than fluconazole. (Table 5)

3.4 Correlation results

The dependence of antioxidant, antimicrobial and antifungal activity on the content of different groups

of BAS was studied using the method of linear regression. Table 6 shows that the correlation between the antioxidant effect and the content of polyphenols was very high, in the case of flavonoids it was high, in the case of hydroxycinnamic acids it was moderate, and the lowest correlation value was observed for organic acids.

According to the research results presented in Table 6 it was found that there is a high correlation between phenolic compounds and inhibition of the growth of *S. aureus*, in the case of flavonoids, hydroxycinnamic acids and antioxidant activity - moderate, and in the case of organic acids - there is no correlation.

The antibacterial effect against *B. subtilis* is very highly dependent on the content of polyphenols and flavonoids, in turn, the antioxidant effect and the content of hydroxycinnamic acids have a moderate effect, and in the case of organic acids there is no dependence.

The study showed that there is a significant correlation between phenolic compounds and inhibition of *E. coli* growth, while in the case of flavonoids, hydroxycinnamic acids and antioxidant activity there is a moderate correlation, and in turn, organic acids do not affect the growth inhibition of *E. coli*. (Table 6)

When studying the relationship between inhibition of growth of *P. vulgaris* and the content of different groups of BAS, it was found that there is a very high dependence of antimicrobial activity on the amount of polyphenols, in turn, with the amount of flavonoids and hydroxycinnamic acids there was a high correlation, and in the case of organic acids - a correlation was not found. The correlation between antimicrobial and antioxidant effects was also studied, and according to the results, it was found that this relationship is of a moderate level. (Table 6)

The correlation between the growth inhibition of *P. aeruginosa* and the sum of polyphenols and flavonoids is high, with the sum of hydroxycinnamic acids and antioxidant activity it is moderate, and in the case of organic acids it is absent. (Table 6)

A significant high correlation between inhibition of the growth of *C. albicans* and the content of polyphenols and flavonoids, in turn, with the sum

Table 5. The value of antimicrobial and anti-fungi activity of *H. perforatum* herb liquid extracts

Sample	Concentration, mM	Diameter of the growth retardation zone, mm ± SD					
		Gramm-positive		Gramm-negative			Fungi
		<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>P. vulgaris</i> ATCC 4636	<i>Paeruginosa</i> ATCC 27853	<i>C.albicans</i> ATCC 653/885
96% EtOH extract	0.018 ^a	22.0 ±0.3	22.0 ±0.4	19.0 ±0.4	16.0 ±0.5	19.0 ±0.4	18.0 ±0.4
60% EtOH extract	0.024 ^a	23.0 ±0.3	23.0 ±0.3	20.0 ±0.4	17.0 ±0.4	20.0 ±0.4	18.0 ±0.4
40% EtOH extract	0.018 ^a	21.0 ±0.3	21.0 ±0.3	19.0 ±0.3	16.0 ±0.5	18.0 ±0.4	17.0 ±0.4
20% EtOH extract	0.009 ^a	20.0 ±0.4	20.0 ±0.4	18.0 ±0.3	15.0 ±0.6	18.0 ±0.3	16.0 ±0.5
aqueous extract	0.006 ^a	15.0 ±0.6	17.0 ±0.4	14.0 ±0.6	12.0 ±0.6	15.0 ±0.5	15.0 ±0.5
Gentamycin	0.003	22.0 ± 0.3	24.0 ± 0.2	25.3 ± 0.3	25.0 ± 0.2	25.6 ± 0.6	12.0 ± 0.4
Fluconazole	0.003	18.0 ± 0.4	12.0 ± 0.6	14.3 ± 0.3	12.3 ± 0.3	10.0 ± 0.5	20.0 ± 0.4
96% EtOH		16.0 ± 0.4	16.0 ± 0.4	13.0 ± 0.5	13.0 ± 0.5	12.0 ± 0.5	12.0 ± 0.5

**Note: SD – standard deviation, n=3, a – molar concentration of extracts was calculated as total phenolic compounds expressed as gallic acid*

of hydroxycinnamic acids and antioxidant activity there is a moderate dependence, and in the case of organic compounds there was no correlation at all. (Table 6)

4. Discussion

4.1 Qualitative analysis

Tuhujac M. *et al.*²¹ reported about aqueous, 70% and 96% EtOH extracts of *H. perforatum* herb. According to their results, the content of total polyphenols and flavonoids in aqueous extract was 0.43 and 0.09%, in the 70% extract 0.63 and 0.13%, whereas in the 96% EtOH extract 0.50 and 0.11%, respectively. Compared with our research the highest content of flavonoids and polyphenols was found in 60%

EtOH extract. The extraction of BAS directly depends on the solvent polarity and chemical properties of compounds. In *H. perforatum* herb present flavonoid glycosides (rutin, hyperoside etc.), according to their chemical property they less soluble in polar solvent (water) and better in medium polar solvent. In arecent study Silva B.A. *et al.*²² investigated the content of hydroxycinnamic acids and anthraquinone derivatives in the methanolic extract of *H. perforatum* herb. The result of research showed that the total of hydroxycinnamic acids was 0.80%, whereas the total anthraquinone content was 0,04%, while we obtained 0.72% of hydroxycinnamic acids in the 40% extract, and 0.05% of anthraquinone in the 60% EtOH extract. The difference in the content of phenolic compounds, in our opinion, is associated with different brewing times, leaves/extractant ratio

Table 6. Correlation coefficients (R – Pearson’s coefficient) between antioxidant/antimicrobial activity and total phenolic compounds, flavonoids, hydroxycinnamic acids, organic acids content

	Total phenolic compounds content	Total flavonoids content	Total hydroxycinnamic acids content	Total organic acids content	Antioxidant activity
Antioxidant activity	0.9110	0.8816	0.5495	0.6799	—
Antibacterial activity against <i>S. aureus</i>	0.8780	0.7620	0.6580	0.1432	0.6192
Antibacterial activity against <i>B. subtilis</i>	0.9317	0.8395	0.6501	0.2534	0.7178
Antibacterial activity against <i>E. coli</i>	0.8506	0.6901	0.7205	0.0806	0.5775
Antibacterial activity against <i>P. vulgaris</i>	0.8838	0.7262	0.7347	0.1338	0.6329
Antibacterial activity against <i>P. aeruginosa</i>	0.8742	0.8002	0.5689	0.2750	0.6481
Antifungal activity against <i>C. albicans</i>	0.9580	0.9148	0.6075	0.7793	0.2500

used, species, climate, and geographical position.

4.2 Antioxidant activity

The potentiometric assay was chosen for evaluation antioxidant activity for several reasons, as it is highly sensitive, cheap and moreover accurate and precise. To compare the antioxidant effect, we used the obtained green tea leaf extract. The results showed that green tea extract inactivates free radicals significantly better than *H. perforatum* herb extracts. The 60% EtOH extract was found to have the highest level of antioxidant activity than other *H. perforatum* extracts. After this, we decided to compare the antioxidant effect of extracts at the same concentration of phenolic compounds, as a result, it was shown that green tea extract works at the same

level as 60% EtOH extract of *H. perforatum* herb. In addition, it was found that the order of levels of antioxidant activity of the extracts changed dramatically. At different concentrations of phenolic compounds, the 60% EtOH extract had the highest level of antioxidant activity, and when compared at the same concentration, the aqueous extract was the best.

Antimicrobial activity

The studied *H. perforatum* herb extract showed antimicrobial and antifungal activity against the strains of *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *B. subtilis* and *C. albicans*. According to the obtained data, at first glance it can be considered that the antimicrobial and antifungal activity of *H. perforatum* herb

extracts is significantly inferior to the action of gentamicin and fluconazole, because their concentration of solutions was significantly lower than the content of polyphenols in the extract. However, we would like to note that gentamicin has serious toxicity to the auditory nerve, kidneys and liver, which can lead to serious complications of the disease²³. Comparing the antifungal effects of fluconazole and *H. perforatum* herb extract, it was found that they inhibited the growth of the fungal strain at the same level, while the concentration of fluconazole was also lower, like gentamicin. We can declare that fluconazole is a leader as anti-fungi medicine, but at the same time it weakly inhibits the growth of gram-negative and gram-positive bacteria, but to *H. perforatum* herb extracts both strains of bacteria and fungus are sensitive. Thus, *H. perforatum* herb extracts is a combined pharmaceutical that affects different mechanisms of vital activity of bacteria and fungi, thereby having a wide spectrum of action against different strains of bacteria and fungi, and at the same time not possessing serious toxicity.

4.3 Correlation analysis

Audrone *et al.* reported about to study the correlation between antioxidant and antimicrobial activity and the content of polyphenols and flavonoids in extracts of raspberry shoots, leaves, seeds and fruits. It was found that there was a significant correlation between the content of BAS and the antioxidant effect, but in the case of antimicrobial activity there was no

correlation ($R = 0.3$). According to our data, it was shown that there is a high correlation between the sum of polyphenols, flavonoids and antioxidant/antimicrobial/antifungal effects against all Gram-positive, Gram-negative bacterial strains and the fungus *C. albicans*. The lowest correlations were observed in the case of organic acids. Therefore, polyphenols and flavonoids play a major role in antioxidant, antimicrobial and antifungal activities.

5. Conclusions

In the research, it has been determined the content of BAS, antioxidant, antimicrobial and antifungal activity of the obtained extracts of St. John's wort. The dominant content of the sum of polyphenols, flavonoids, anthraquinones and organic acids was observed. The 60% extract has a high level of antioxidant activity, and actively inhibits the growth of all studied Gram-positive, Gram-negative strains of bacteria and the fungus *C. albicans* in the range from 15 to 23 mm (diameter of growth inhibition). We have shown that there is a high correlation between the content of polyphenols, flavonoids and antioxidant\antimicrobial\antifungal action, in the case of hydroxycinnamic acids it is moderate, and in turn, for organic acids there is no correlation at all. These findings show the great potential of St. John's wort in the development and creation of new medicines with antimicrobial, antioxidant and antifungal effects that are not inferior to, and even superior to, the effects of synthetic analogues.

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