ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF A NEW GENERATION PHYTO-GEL

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Antioxidant and antimicrobial activities of a new phyto-gel were studied in this work. The formulated gel is composed of propilene glycol extracts of St. John's worth (*H. perforatum* L.), comfrey (*S. officinale* L.), yarrow (*A. millefolium* L.), horsetail (*E. arvense* L.) and Na-usniate. The antioxidant activity was determined for the gel and each investigated extract of the gel separately by the DPPH test. The total phenols and flavonoids content was determined spectrophotometrically, for the gel and each investigated extracts. The antimicrobial activity of the gel and each compound separately was tested in vitro on the following microorganisms: *Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. The antioxidant activity of the investigated samples decreases in the series: *H. perforatum* (EC_{50} = 1.04 mg/mL) > Na-usniate (EC_{50} = 4.16 mg/mL) > *A. millefolium* (EC_{50} = 7.60 mg/mL) > gel (EC_{50} = 23.47 mg/mL) > *S. officinale* (EC_{50} = 30.10 mg/mL) > *E. arvense* (EC_{50} = 44.70 mg/mL). The formulated phyto-gel showed the antimicrobial activity. The results suggest that the obtained phyto-gel is a good source of natural antioxidants and antimicrobial agents, and it has potential as an alternative to synthetic products in pharmaceutical and cosmetic industries.

Keywords: antioxidant activity, antimicrobial activity, phyto-gel, plant extracts, Na-usniate

Introduction

The plants with the antioxidant activity have drawn attention in recent years due to the development of a large number of different diseases [1,2]. It has been hypothesized that these diseases are caused by action of free radicals in most cases [3]. Antioxidants have the ability to inhibit the free radicals generation or to remove the already formed free radicals by their direct action [4]. There are a number of natural antioxidants, e.g. α-tocopherol [5]; ascorbic acid [6,7]; retinol, thiamin and riboflavin, flavonoids [8] and phenolic acids [5,9], as well as a number of synthetic antioxidants [10,11]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have a potentially harmful effect on human health, leading to various dysfunctions [10]. One of the most important trends in the food and pharmaceutical industry today is searching for natural antioxidants from the plant material.

Medicinal plants have been used for centuries as remedies for various diseases because they contain bioactive-components of a therapeutic value [11-14]. There is the increasing use of a traditional medicine as an alternative form for treating various diseases due to the resistance of microorganisms to the existing synthetic antibiotics [13,15]. A large number of studies investigate the antimicrobial activity of natural phenolic compounds from plants [1,16-19] in order to find new, natural antimicrobial agents.

Flavonoids are synthesized in plants in response to microbial infection. It is therefore not surprising that these compounds have *in vitro* antimicrobial activity against a wide range of microorganisms [20,21]. Some phenolic compounds, such as resveratrol, hydroxytyrosol, quercetin and many phenolic acids may inhibit many pathogenic microorganisms [22-24].

There is considerable interest in preventive and traditional medicine for the development of natural antioxidants and antimicrobial agents obtained from plants [12].

The antimicrobial and antioxidant activities of these plant extracts were tested before [1,25-28], as well as the antimicrobial and antioxidant activities of usnic acid and related compounds from lichens [29,30].

The aim of our study was to determine antioxidative and antimicrobial properties of propylene glycol extracts of St. John's worth (*Hypericum perforatum* L.), comfrey (*Symphytum officinale* L.), yarrow (*Achillea millefolium* L.), horsetail (*Equisetum arvense* L.) and sodium-usniate in formulated phyto-gel. The investigated extracts contain polar substances - flavonoids and phenolcarboxylic acids (*H. perforatum, A. millefolium, E. arvense*), naphthodianthrones and phloroglucinols (*H. perforatum*), alantoin (*S. officinale*),

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sesquiterpene lactones (*A. millefolium*). The antioxidative activity was tested by DPPH test and the antimicrobial activity using agar-diffusion and a microdilution method.

Materials and methods

Plant material

The aerial parts of commercial samples of *H. perforatum, A. millefolium* (Adonis, Soko Banja, Serbia), *E. arvense* and *S. officinale* (Bilje Borča, Belgrade, Serbia), were used for the investigation. Sodium usniate was from Zdravlje-Actavis, Serbia.

Reagents and Chemicals

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazil (DPPH), gallic acid were obtained from Sigma (St. Louis, MO), and rutin from Merck. Sodium carbonate, potasium acetate and aluminium chloride were purchased from Merck-Alkaloid (Skopje, FYR Macedonia). Methanol was from Zorka-Pharma (Šabac, Serbia). HPLC grade acetonitrile (Merck, Darmstadt, Germany) and filtered bidistilled water were used for HPLC analysis. All standard components for HPLC analysis of the extracts were of HPLC reagent grade. All other chemicals were of analytical reagent grade.

Extraction method

The grained aerial parts of *H. perforatum, A. millefolium, E. arvense* and *S. officinale* were extracted with propylene glycol (1:10 m/v) at room temperature.

Preparation of new phyto-gel

Phyto-gel was prepared by gelation 50 g of carboxymethilcellulose with propylene glycol extracts (60%, v/v) of *S. officinale* (2 g), *H. perforatum* (4 g), *A. millefolium* (4 g), *E. arvense* (4 g) and 0.5 g of Na-usniat.

HPLC analysis

HPLC separation was performed using the Agilent 1200 Series HPLC system, equipped with a G-1312A binary pump, a G-1328B injector (loop of 20 µl) and G1315B DAD detector. The column used was a ZORBAX Eclipse XDB-C18 (4.6 × 250 mm, 5 µm) and operated at the temperature of 25 °C. A gradient elution was performed with the solvent A (H_2O and H_3PO_4, pH = 2.8) and B (acetonitrile) as follows: 20% B (5 min), 40% B (5 min), 50% B (5 min), 70% B (5 min) at a flow rate of 0.8 ml/min. The injection volume was 20 µl. The present compounds were determined on the basis of their retention times and UV spectra, and by direct comparison with available standards. The detection wavelength was 254 nm.

DPPH assay

The capacity of a compound to scavenge free DPPH radicals was determined by DPPH test [8,31-35]. The investigated extracts, sodium usniate, and gel were dissolved in methanol and various concentrations were prepared (the concentration range: 0.032-2.0 mg/ml for *H. perforatum*; 0.625-20.0 mg/ml for A. *millefolium*; 2.5-80.0 mg/ml for *E. arvense*; 0.82-80.0 mg/ml for *S. officinale*; 0.31-10 mg/ml for sodium usnate and 0.51-50.0 mg/ml for gel). A 1.0 ml of methanolic solution of DPPH (3×10-4 mol/l) was added to 2.5 ml of the sample and measured after 20 minutes of incubation at room temperature. The absorbance of the samples was measured on VARIAN UV–Vis Cary-100 Conc. spectrophotometer. The capacity of free radicals scavenging was calculated as follows:

$$DPPH\ radicals\ scavenging\ capacity\ (\%) = \left[1 - \frac{(A_S - A_B)}{A_C}\right] \cdot 100$$

where AS is absorbance of the sample at 517 nm (methanolic solution of the samples treated with DPPH radical solution), AB is absorbance of blank at 517 nm (the methanol solution of the samples not treated with DPPH radical solution), and AC is absorbance of control at 517 nm (methanolic solution of DPPH radical). A decrease of 50% of the initial DPPH concentration was defined as EC_{50}. The EC_{50} value (mg/ml) was determined for all samples. BHT was used as a reference compound (EC_{50} = 0.021 mg/ml).

Determination of total phenolic content

The total phenolic content in the investigated samples was determined spectrophotometrically according to the Folin-Ciocalteu method [36] using gallic acid (GAE) as a standard with small modifications [35]. The reaction mixture was prepared by mixing 1 ml of the methanolic solution of the samples (concentration 100.0; 1.0; 12.6; 32.0; 100.0 and 95.0 mg/ml for gel; sodium usniate; extracts of *H. perforatum, A. millefolium, E. arvense* and *S. officinale*, respectively); 9 ml of distilled water, 1 ml of Folin-Ciocalteu's reagent and 10 ml of 7% sodium carbonate. After the 90 minutes incubation at room temperature, the absorbance was determined spectrophotometrically at 765 nm. The total phenolic content was expressed as GAE in milligram per gram of the dry extract [35].

Determination of total flavonoids content

The total flavonoids content was determined according to the aluminium chloride colorimetric method [37] with small modification [35]. Each sample (2 ml, concentration of 100.0; 1.0; 12.6; 32.0; 100.0 and 95.0 mg/ml for gel; sodium usnate; propylene glycol extracts of *H. perforatum, A. millefolium, E. arvense* and *S. officinale*, respectively) in methanol were mixed with 0.1 ml of 10% aluminium chloride hexahydrate, 0.1 ml of 1 M potassium acetate and 2.8 ml of deionized water. After 40 minutes of incubation at room temperature, the absorbance of the reaction mixture was determined spectrophotometrically at 415 nm. Rutin was chosen as a standard and the total flavonoids content was expressed as milligram RE per gram of dry extracts [35].

Antimicrobial activity

Microorganisms and substrates. Eight microorganisms were selected to determine the antimicrobial activity: *Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (ATCC 10240), *Bacillus subtilis* (ATCC 6633BB), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCIMB

9111), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10259) and *Aspergillus niger* (ATCC 16404). Substrates used for the microorganisms growth: antibiotic agar no. 1 for microbiology for bacteria and tryptic soy agar for fungi (Merck, Darmstadt, Germany).

Disc-diffusion method

The disc-diffusion method [38] was used for testing the antimicrobial activity of propylene glycol extracts of *E. arvense*, *S. officinale*, Na-usniate, as well as the mixture of extracts and Na-usniate and formulated gel. The samples were dissolved in dimethyl sulfoxide (DMSO; BDH, Milan, Italy). The substrates were sterilized for 15 minutes in an autoclave at 121 ºC. To 50 ml of agar, 0.5 ml of microorganism was added and the samples of 10 ml each were poured to each Petri dish. Filter paper discs (12.7 mm dia., Schleicher & Schuell) were placed on inoculated substrates and impregnated with 50 µl (100 mg/ml for extracts and gel, 1 mg/ml for Na-usniate) of the sample. Plates were incubated for 24 hours at 37 ºC for bacteria, and 48 hours at 30 ºC for fungi. The antibacterial activity is expressed as the value of the inhibition zones (mm) obtained by the investigated samples.

Standardized discs of ampicillin (10 µg/disc), amikacin (10 µg/disc), and nystatin (100 U/disc) served as positive controls. Disc impregnated with 50 µl DMSO was negative control. All determinations were performed in duplicate and two positive growth controls were included.

Microdilution method

In order to investigate the antimicrobial activity (minimal inhibitory concentration - MIC) of *H. perforatum* and *A. millefolium* extracts, as well as Na-usniate, the modified microdilution technique was used [39]. Bacterial species were cultured overnight at 37 °C in Luria-broth (LB) medium. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The fungal and bacterial cell suspension was adjusted with sterile saline to the concentration of approximately 1.0×10^5 CFU/ml in a final volume of 100 µl per well. The inocula were stored at 4 °C for further use. Dilutions of the inocula were cultured on solid MH for bacteria and solid MA for fungi to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentrations (MIC) determination was performed by a serial dilution technique using 96-well microtitre plates. The investigated samples were dissolved in 5% DMSO (1mg/ml) and added in broth medium with inoculum. The microplates were incubated for 48 h at 37 °C for bacteria and 72 h at 28 °C for fungi. The lowest concentration without the visible growth was defined as MIC. Doxycycline and nystatin were used as positive controls [40].

Results and discussion

HPLC analysis

HPLC chromatograms of the investigated propylen glycol extracts from *H. perforatum, A. millefolium, E. Arvense* and *S. officinale* are shown in Figures 1 to 4, respectively.

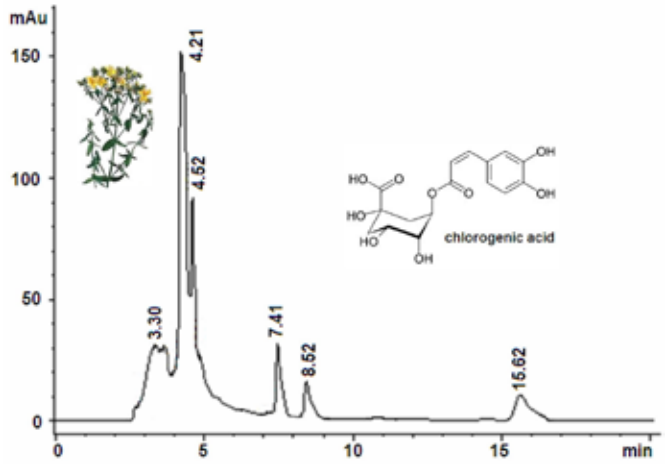

Figure 1. HPLC chromatogram of propylene glycol extract of *H. perforatum* (4.52 min – phenolcarboxylic acid derivatives; 7.41 min – quercetin glycoside; 15.61 min – apigenin glycoside)

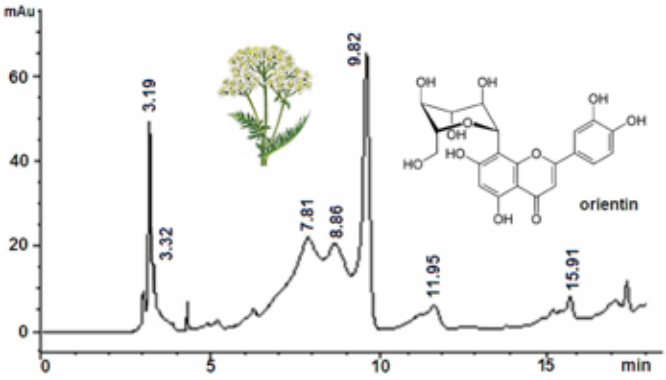

Figure 2. HPLC chromatogram of propylene glycol extract of *A. millefolium* (3.32 min – caffeic acid derivative; 7.81 min – orientin; 9.82 min – luteolin-7-O-glycoside; 11.95 min – apigenin-7-O-glycoside; 15.91 min – luteolin-glycoside)

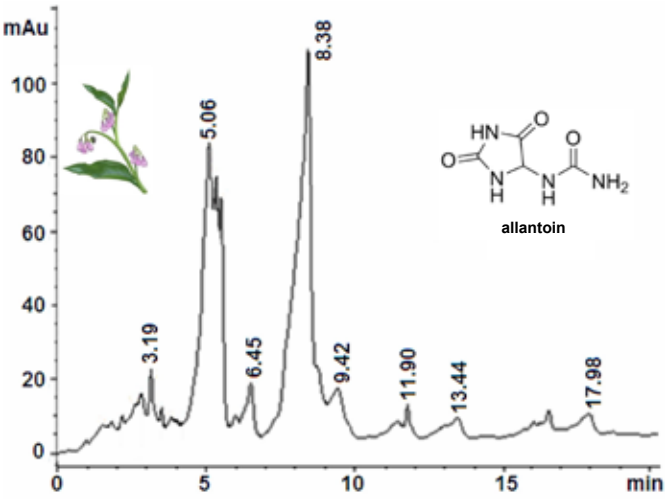

Figure 3. HPLC chromatogram of propylene glycol extract of *S. officinale* (5.06 min – caffeic acid; 8.38 min – chlorogenic acid; 9.42 min – allantoin; 13.44 min – luteolin-glycoside)

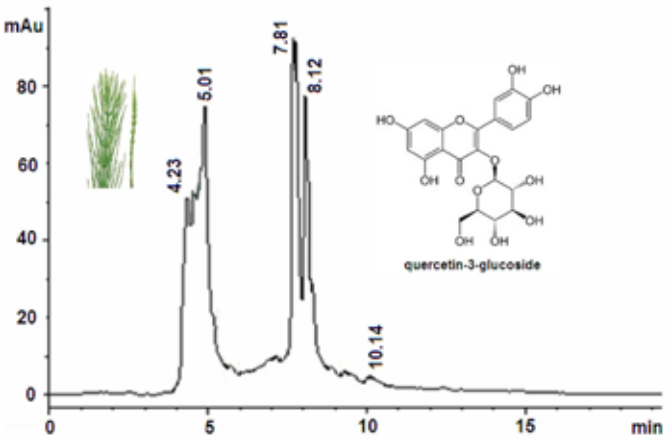

Figure 4. HPLC chromatogram of propylene glycol extract of *E. arvense* (4.23 min – phenolcarboxylic acid derivatives; 7.81 min – quercetin-3-O-glycoside; 8.12 min – quercetin-3-O-(6'-O-malonil)-glycoside; 10.14 min – pentacyclic triterpenoid saponin)

HPLC analysis of propylene glycol extracts showed the presence of the following compounds: a) phenolcarboxylic acid derivatives, as well as derivatives of quercetin and apigenin in *H. perforatum* extract; caffeic acid, quercetin glycosides and pentacyclic triterpenoid saponines in *E. arvense* extract; caffeic and chlorogenic acid, allantoin, luteolin glycoside in *S. officinale*; caffeic acid derivative, orientin, luteolin glycoside and apigenin glycoside in *A. millefolium* extract.

Based on presented results, it can be concluded that phenolic compounds (phenolic acids and flavonoids) are present in all extracts. Phenolic compounds are well known antioxidant and antimicrobial agents [5,23,24]. Allantoin, one of the components of *S. officinale* extract, has a strong pharmacological activity. Because of that, *S. officinale* extract has a wetting and keratolytic effect, increases smoothness of the skin leading to cell proliferation and wound healing, also has soothing, anti-irritating and protective effects on the skin [41].

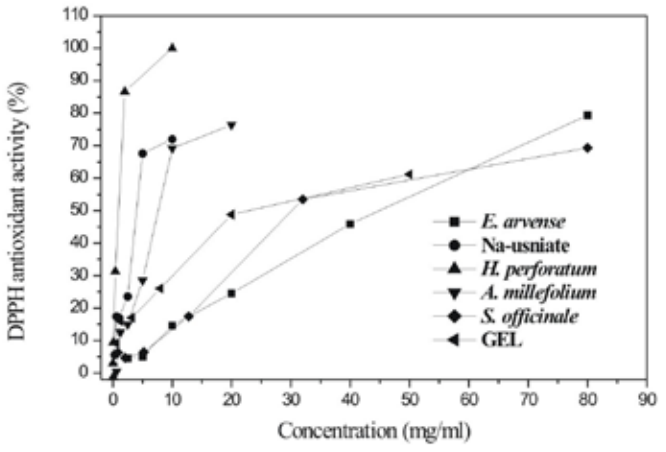

Figure 5. Antioxidant activity of *H. perforatum* (St. John's worth), *A. millefolium* (yarrow), *S. officinale* (comfrey), *E. arvense* (horstail), Na-usniate and gel estimated by DPPH test

Antioxidant activity

The DPPH test is based on the exchange of hydrogen atoms between antioxidants and stable DPPH free radical. Practically, the reaction is based on the reduction of DPPH radicals to the corresponding hydrazine, which is manifested by the colour change from violet to yellow, which is monitored spectrophotometrically [31,32]. The antioxidant activity of the extracts from *E. arvense, H. perforatum, A. millefolium, S. officinalis*, Na-usniate and formulated gel is shown in Figure 5.

The propylene glycol extracts of *H. perforatum, A. millefolium, E. arvense* and *S. officinale*, as well as tested Na-usniate and formulated gel, show a dose-dependent antioxidant activity in DPPH test. The EC_{50} value is a widely used parameter to measure the free radical scavenging activity. A lower EC_{50} indicates a higher antioxidant activity [42]. The antioxidant activity of the investigated samples decreases in the series: *H. perforatum* (EC_{50} = 1.04 mg/ml) > Na-usnate (EC_{50} = 4.16 mg/mL) > *A. millefolium* (EC_{50} = 7.60 mg/ml) > gel (EC_{50} = 23.47 mg/ml) > *S. officinale* (EC_{50} = 30.10 mg/ml) > *E. arvense* (EC_{50} = 44.70 mg/ml). Each value is a mean value of three measurements. The EC_{50} values, the amount of total phenolic and flavonoids for all extracts are given in Table 1.

In all investigated extracts (Figures 1-4) the presence of phenolic components (mainly phenolic acids and flavonoids) was determined, which is attributed a major role in the antioxidant activity of the plant extracts [43]. Since the antioxidant activity of the investigated extracts does not originate from phenolic compounds only, it is probably due to synergic action of these compounds with other biomolecules isolated from the plant material.

The extract of *H. perforatum* showed the highest while *S. officinale* extract showed the lowest antioxidant activity (Table 1 and Figure 1). The gel, as a formulation which contains all investigated extracts and Na-usniate, showed the antioxidant activity between these opposite values, which was expected.

In general, the samples with a high antioxidant activity showed a high phenolic content [42].

BHT concentration necessary for reaching EC_{50} in DPPH test after 20 minutes of incubation was 0.021 mg/ml (Table 1). The obtained data showed that all investigated extracts had or possessed lower antioxidant activities than BHT standard.

The results in Table 1 show that the extract of *H. perforatum* has a high phenolic content. This extract showed the best antioxidant activity, which probably comes from the high content of phenolic compounds in the extract.

The presence of hydroxyl group in phenolic compounds is probably the main factor for their antioxidant activity. Namely, phenolic compounds give stable aroxy radical by releasing H-atoms from OH-groups. The stable aroxy radicals react with other radicals (with DPPH, among others) and produce new stable compounds. So this is probably a mechanism through which phenolic compounds from the extracts exhibit their antioxidant activity [44].

Table 1. EC_{50} values, total phenolic and flavonoids for all investigated samples

Sample	Total phenolic content mgGAE/1g of extract	Total flavonoids, mg RE/1 g of extract	EC_{50} (mg/ml)
S. officinale extract	0.36	0.83	30.10
E. arvense extract	1.43	0.27	44.70
A. millefolium extract	4.43	0.44	7.60
H. perforatum extract	16.45	0.13	1.04
Na-usniate			4.16
Gel	1.35	0.66	23.47
BHT			0.021

The results suggest that formulated phyto-gel may be used in topical formulations in order to protect the skin from damage caused by free radicals and reactive oxygen species.

Antimicrobial activity

The results of antimicrobial activity determinations of the investigated extracts are shown in Tables 2 and 3. Microdilution assay was used for the determination of the minimal inhibitory concentration (MIC) for *H. perforatum* and *A. millefolium* extracts, as well as for Na-usniate (Table 2), and the agar diffusion method was used for antimicrobial properties of *S. officinale* and *E. arvense* extracts determination (Table 3).

Table 2. Minimal inhibitory concentration of Na-usniate, *H. perforatum* extract and *A. millefolium* extract

Samples	*S. a.* 1	*M.l.* 2	*B.s.* 3	*E.c.* 4	*K.p.* 5	*P.a.* 6	*C.a1.* 7	*A.n.* 8
Na-usniate (mg/ml)	0.78	1.18	0.58	1.22	1.36	4.68	37.51	18.78
H. perforatum extract (mg/ml)	7.23	7.81	7.25	7.55	15.6	7.53	115	98.63
A. millefolium extract (mg/ml)	7.85	7.85	7.81	7.81	15.6	15.6	450	230
Doxycycline (μg/ml)	0.81	nt.	1.56	0.84	nt.	11.45	nt.	nt.
Nystatin (μg/ml)	nt.	nt.	nt.	nt.	nt.	nt.	6.55	6.55

1 - *Staphylococcus aureus*; 2 - *Micrococcus luteus*; 3 - *Bacillus subtilis*; 4 - *Escherichia coli*; 5 - *Klebsiella pneumoniae*; 6 - *Pseudomonas aeruginosa*; 7 - *Candida albicans*; 8 - *Aspergillus niger*.

Table 3. Inhibition zone of *S. officinale* and *E. arvense* extracts, Na-usniate and gel

	The inhibition zone (mm)							
Microorganism	*S. officinale*	*E. arvense*	Na-usniate	Gel with Na-usniate	Gel without Na-usniate	Amikacin	Ampicillin	Nystatin
S. aureus	15.8	12.7	24.1	25.8	24.6	25.2	26.1	nt.
B. subtilis	13.2	11.5	27.4	27.1	26.3	32.1	15.2	nt.
M. luteus	-	26.3	22.4	25.0	21.5	31.2	32.4	nt.
E. coli	15.1	12.5	21.2	19.1	15.1	30.0	18.3	nt.
K. pneumoniae	17.2	20.1	20.0	15.7	12.3	25.5	23.4	nt.
P. aeruginosa	-	11.3	18.1	24.1	22.5	30.5	nt.	nt.
C. albicans	-	17.5	14.5	15.2	12.5	nt.	nt.	21.7
A. niger	-	17.1	15.1	15.2	13.1	nt.	nt.	22.2

According to the results shown in Table 2, it can be concluded that Na-usniate has the highest antimicrobial activity, while *H. perforatum* and *A. millefolium* extracts are weaker antimicrobial agents. Based on the inhibition zone diameter of *S. officinale* and *E. arvense* extracts (Table 3), it can be concluded that *E. arvense* extract has the effect on all tested microorganisms, but the highest effect was manifested on *M. luteus* microorganism. The antimicrobial activity of the formulated gel (with or without Na-usniate) and pure Na-usniate is shown in Table 3. Based on these results, it can be concluded that Na-usniate is mostly responsible for the gel antimicrobial activity, because Na-usniate itself shows a relatively strong antimicrobial activity. The activity of the formulated gel is also enhanced by the extracts which enter into its composition.

Our results are in accordance with the previously described. Candan and coworkers tested the antimicrobial and antioxidant activity of *A. millefolium* essential oil and the methanol extract [1]. The essential oil was more active than the methanol extract, with a very high antioxidant and weak antimicrobial activity. Conforti and coworkers [45] tested methanolic extracts of *H. perforatum*, and Ma & Xiao [46] showed a correlation between the antioxidant activity of *H. perforatum* and distribution of flavonoids in stems, leaves and flowers. The propylene glycol extract of *H. perforatum* had the highest antioxidant activity among the tested extracts with EC_{50} = 1.04 mg/ml. Bioguided fractionation led to flavonoids quercetin and biapigenin, responsible for a weak antimicrobial activity, while the recent research showed the antimicrobial activity of hypericin comparable to standard antibiotics which was not detected in our extract [28].

A recent publication about the antioxidant activity of *E. arvense* extracts showed a high DPPH (EC_{50} = 0.65 mg/ml) and hydroxyl radical scavenging (EC = 0.74 mg/ml) activity of *n*-butanol extract which was correlated with the total phenolic content. Ethylacetate, *n*-butanol and water extracts possessed a weak antibacterial activity against *P. aeruginosa, S. aureus* and *B. cereus* [27].

Formulated phyto-gel can find potential application as a natural antiseptic and antioxidant for topical use. Thanks to the presence of different extracts, this phyto-gel can find a potential application in a variety of skin diseases such as acne and eczema (due to the presence of the horsetail extract) [27], skin inflammation, wounds, purulent processes (due to the presence of the yarrow extract) [47], skin burns and wound infections (due to the presence of *H. perforatum* extract) [19], skin regeneration [48] and a antirheumatic agent (*S. officinale* extract) [49]. Usnic acid and its salts have well known antioxidant and antimicrobial properties [29,30], so Na-usniate is also an important component of the investigated phyto-gel in terms of the antioxidant and antimicrobial activity.

Conclusion

New generation phyto-gel was made of four plant extracts incorporated (*H. perforatum, A. millefolium, E. arvense* and *S. officinale* extract), as well as Na-usniate. The formulated herbal gel showed antioxidant and antimicrobial properties. *H. perforatum* extract is the most responsible component of phyto-gel in terms of the antioxidative activity, while the presence of Na-usniate is most responsible for the antimicrobial activity. The antioxidant and antimicrobial activity of the gel is probably a result of a synergistic effect of all components. New generation phyto-gel formulated from plant extracts by this method could potentially find application as a natural antioxidant and antiseptic agent for dermal use.

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Izvod

ANTIOKSIDATIVNA I ANTIMIKROBNA AKTIVOST FITOGELA NOVIJE GENERACIJE

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U radu je ispitivana antioksidativna i antimikrobna aktivnost fitogela – formulacija gela sa propilen-glikolnim ekstraktima *H. perforatum, S. officinale, A. millefolium, E. arvense* i Na-usninatom. Primenom DPPH testa određena je antioksidativna aktivnost svih ispitivanih ekstrakata, Na-usninata i gela. Primenom spektrofotometrijskih metoda određen je sadržaj ukupnih fenola i flavonoida u ekstraktima i formulisanom gelu. Antimikrobna aktivnost gela, kao i njegovih pojedinačnih komponenata, je ispitivana in vitro na sledećim mikroorganizmima: *Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans* i *Aspergillus niger*. Antioksidativna aktivnost ispitivanih uzoraka opada u nizu: *H. perforatum* (EC_{50}=1,04 mg/ml) > Na-usnate (EC_{50}=4,16 mg/ml) > *A. millefolium* (EC_{50}=7,60 mg/ml) > gel (EC_{50}=23,47 mg/ml) > *S. officinale* (EC_{50}=30,10 mg/ml) > *E. arvense* (EC_{50} 44,70 mg/ml). Formulisani fitogel je pokazao i antimikrobnu aktivnost. Dobijeni rezultati pokazuju da ispitivani fitogel predstavlja izvor prirodnih antioksidanasa i antimikrobnih agenasa, sa mogućom primenom u farmaceutsko-kozmetičkoj industriji kao alternativa sintetičkim proizvodima.

Ključne reči: antioksidantna aktivnost, antimikrobna aktivnost, fitogel, biljni ekstrakti, Na-usninat