Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.yu • www.shd.org.rs/JSCS
*J. Serb. Chem. Soc.* 73 (6) 597–607 (2008) UDC **Sempervivum marmoreum* L.+66.061:615.28
*JSCS–3742* *Original scientific paper*

# Comparative screening of the anti-oxidant and antimicrobial activities of *Sempervivum marmoreum* L. extracts obtained by various extraction techniques

SAŠA S. STOJIČEVIĆ[1], IVANA T. STANISAVLJEVIĆ[1#], DRAGAN T. VELIČKOVIĆ[2], VLADA B. VELJKOVIĆ[1#] and MIODRAG L. LAZIĆ[1*#]

[1]*Faculty of Technology, Bulevar oslobodjenja 124 and* [2]*Zdravlje-Actavis, Vlajkova 199, 16000 Leskovac, Serbia*

(Received 10 September, revised 31 December 2007)

*Abstract*: This paper presents a comparative study of the anti-oxidant and anti-microbial activities, total phenolic compounds and total flavonoids in extracts obtained from houseleek (*Sempervivum marmoreum* L.) leaves by the classical (maceration), ultrasonic and Soxhlet extraction (CE, UE and SE, respectively). The extract obtained by the CE contained higher amounts of phenolic and flavonoid compounds and showed a better antioxidant activity than those obtained using other two techniques. All the extracts, independent of the extraction technique applied, showed antimicrobial activities against *Aspergillus niger* and *Candida albicans* only but not against the tested bacteria.

*Keywords*: antimicrobial activity; anti-oxidant activity; extraction; houseleek; *Sempervivum marmoreum* L.; ultrasound.

## INTRODUCTION

Houseleek (*Sempervivum marmoreum* L., syn. *S. erytreum, S. italicum*) is an evergreen endemic plant on the Balkan Peninsula.[1] The genus *Sempervivum* contains about 50 species which occur predominantly in rocky places and at higher altitudes.[2] Therapy with preparations of *Sempervivum* species can be traced back to the origins of antique herbal medicine but the extracts of their leaves have not yet been introduced into medical practice. The earliest publications concerning the therapeutic use of this drug deal with its influence on pharingitis, tracheitis, thrush, combustion and otitis.[3] Strewn over wounds, sores, burns, abscesses or painful areas, the fresh juice from squeezed leaves of *S. tectorum* is used as a refrigerant and astringent, a sure remedy for insect bites, as well as to ease ear pains and inflammations, while the tea prepared from its leaves is recommended for ulcer treatment.[4]

# Serbian Chemical Society member.
* Corresponding author. E-mail: lmiodrag@yahoo.com
doi: 10.2298/JSC0806597S

The benefits of juice and tea prepared from the leaves of *S. tectorum* can be attributed to the presence of many bioactive compounds, such as oligomeric and polymeric polyphenols, phenol carboxylic acids, ascorbic, citric malic acids, flavones- and flavonol-mono- and diglycosides with kaempferol and quercetin as aglycones, tannins, coumarines, oligo- and polysaccharides.[3,5,6] Polyphenols are present in 4.2 %, flavonoids in 0.70 % and polysaccharides in 11.2 %.[7] Glycosides of kaempferol, quercetin, isorhamnetin, scutellarein, myricetin, herbacetin, proanthocyanidins (prodelphinidin and procyanidin) and delphinidol were isolated from *Sempervivum* sp. extracts.[2,4] Rhamnose, arabinose, xylose, mannose and galactose are characteristic monosaccharides.[3] The extract of *S. tectorum* exhibited a genuine superoxide scavenger activity in a cell-free system, which suggested that this extract might also act as a direct scavenger of $O_2^-$ in biological samples.[8] The antioxidant activity and antinociceptive effect of *S. tectorum* extracts were shown both *in vivo* and *in vitro*.[3,9] It was also found that *S. tectorum* extracts had a lipid-lowering effect in rats,[7] free radical scavenger, membrane stabilizing, and antimicrobial activity against *Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis* and *Geotrichum* sp., while the growths of *Escherichia coli, Proteus morgani* and *Saccharomyces cerevisiae* were slightly inhibited.[4] The dominant cations are Ca (76.2 mg/g), K (40.47 mg/g) and Mg (817.85 mg/g),[3] while no toxic elements were detected in *S. tectorum* extracts.[10]

Recently, it was shown that ultrasound-assisted extraction (UE) is an efficient alternative to conventional extraction techniques.[11] The main benefits of the use of ultrasound are an increased extraction yield, a faster process and even an improved quality of the extracts. Ultrasound can also reduce the operating temperature, allowing the extraction of thermolabile compounds. UE has already been employed in the extraction of bioactive compounds of interest to the pharmaceutical and food industries, such as a phenolic component of coconut shell,[12] flavonoids from *Hypericum perforatum* L.[13] and anti-oxidants from *Rosmarinus officinalis*.[14]

To the best of our knowledge, there have been no reports on the extraction efficiency and kinetics of extractive substances (ES) from *S. marmoreum* leaves. Furthermore, no comparative study of the anti-oxidant and antimicrobial activities of its extracts obtained by different extraction techniques has hitherto been reported. To date, the extracts of *Sempervivum* sp. leaves have been prepared only by a two-step stirring-assisted maceration at room temperature, using methanol as the extracting solvent.[4]

In the present study, the extraction of ES from fresh leaves of *S. marmoreum* L. by UE, classical solvent extraction (CE) and Soxhlet extraction (SE) using methanol as the extracting solvent was investigated. The main goal was to compare the anti-oxidant and antimicrobial activities of the extracts obtained by the different extraction techniques. In addition, the yields of ES, total phenolic com-

pounds and flavonoids in the extracts obtained by the different methods were compared. The potential of methanolic extracts of *S. marmoreum* leaves for practical applications has not previously been studied in detail, thus this study provides important information concerning the natural anti-oxidants and antimicrobials present in the leaves of *S. marmoreum*.

## EXPERIMENTAL

### *Materials*

The plant material was harvested by hand in the morning, before the blooming period (South Serbia, Mt. Suva Planina, 1500 m, in the second half of May, 2006). The leaves were removed from the rosette and roots, washed, dried in the shadow, chopped and used immediately. The moisture content, determined by drying at 105 °C to constant weight, was 88.1 %.

Methanol was from Zorka-Pharma (Šabac, Serbia). Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazil (DPPH), gallic acid and rutin were obtained from Sigma (St. Louis, MO). Sodium carbonate, potassium acetate and aluminum chloride were purchased from Merck-Alkaloid (Skopje, FYR Macedonia).

Seven microorganisms were selected to test the antimicrobial activity: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763 and *Aspergillus niger* ATCC 16404 (Oxoid, England). The tested bacteria were grown on Trypton soya agar (TSA, Merck), while Sabouraud dextrose agar (SDA, Merck) was used to grow the yeast and the mould. Plate count agar (Merck) was used for determining the total number of microorganisms (CFU/ml).

### *Soxhlet extraction*

Chopped fresh leaves (10 g) and methanol (100 ml) were put into a Soxhlet apparatus. The solvent was boiled and refluxed for a period of 150 min (eleven extraction cycles). The liquid extract was evaporated under vacuum at 40 °C to constant weight. This yield of ES (g/100 g of fresh plant material) was taken to represent the content of ES in the plant material.

### *Classical extraction*

Chopped fresh leaves (5.0 g) and methanol (50 ml) were put into Erlenmeyer flasks and placed in a thermostated water bath. The extraction was performed at 25±0.1 °C for 2.5, 5, 10, 20, 40 and 60 min. At the end of the extraction cycle, the liquid extract was separated from the solid residue by vacuum filtration. The solid residue was washed twice with fresh solvent (20 ml). The filtrates were collected and the solvent was evaporated in a rotary vacuum evaporator at 40 °C.

### *Ultrasonic assisted extraction*

Chopped fresh leaves (5.0 g) and methanol (50 ml) were put into Erlenmeyer flasks (100 ml), which were then placed into an ultrasonic cleaning bath (Sonic, Niš, Serbia; total nominal power: 3×50 W and internal dimensions: 30 cm×15 cm×20 cm) operating at 40 kHz frequency. The sonication was performed for 2.5, 5, 10, 20, 40 and 60 min. The bath was filled with distilled water up to 1/3 of its volume (about 2.5 l). The extraction was carried out at 25 °C. The temperature was controlled and maintained at the desired level (±0.1 °C) by water circulating by means of a pump from a thermostated bath. The separation and further treatment of the filtrates were the same as described for the classical extraction.

*Hydroxyl radical assay*

Hydroxyl radicals were obtained by the Fenton reaction in the following system: 0.20 ml 10 mM $H_2O_2$, 0.20 ml 10 mM $FeCl_2$ and 0.20 ml 0.30 M DMPO as the spin trap and 0.20 ml DMF ("blank"). The influence of the extracts on the formation and transformation of hydroxyl radicals was investigated by adding a DMF solution of the extracts to the Fenton reaction system in the concentration range between 0.25 to 2.5 mg $ml^{-1}$. The ESR spectra were recorded after 5 min on a Bruker 300E ESR spectrometer (Rheinstetten, Germany) under the following conditions: field modulation, 100 kHz; modulation amplitude, 0.512 G; receiver gain, $5\times10^5$; time constant, 81.92 ms; conversion time, 163.84 ms; center field, 3440.00 G; sweep width, 100.00 G; *x*-band frequency, 9.64 GHz; power, 20 mW; temperature, 23 °C. The magnetic field scanning was calibrated using Fremy's salt (peroxylamine disulfonate).[15] Splitting constants were calculated from computer-generated second derivatives of the spectra, after optimizing the signal-to-noise ratios, and were verified by computer simulations. The scavenging effect, %, of the extract was defined as:

$$\text{Scavenging effect} = 100\frac{h_0 - h_x}{h_0}$$

where $h_0$ and $h_x$ are the height of the second peak in the ESR spectrum of the DMPO–OH spin adduct of the blank and the sample, respectively. Butylated hydroxyanisole (BHA) was used as the reference compound ($EC_{50}$ = 0.115 mg $ml^{-1}$).

*DPPH photometric assay*

The free radical-scavenging activity of the plant extracts was evaluated using the stable radical DPPH.[16] A series of extracts with different concentrations (1.00–0.01 mg $ml^{-1}$, seven different concentrations) were prepared in methanol. Then, 2.5 ml of the extract and 1.0 ml of a $3.0\times10^{-4}$ M DPPH solution in methanol were mixed and placed in the dark at the room temperature for 30 min. The absorbance of each sample of the plant extract containing DPPH ($A_s$) was measured at 517 nm using a Varian Cary-100 spectrophotometer. Methanol (1.0 ml) plus the plant extract solution (2.5 ml) was used as the blank, while the DPPH solution plus methanol was used as the control. All determinations were performed in triplicate. The DPPH anti-radical-scavenging activity, *DPPH* (%), of each plant extract was determined using the following equation:

$$DPPH = 100\left(1 - \frac{A_s - A_b}{A_c}\right)$$

where $A_s$ is the absorbance in the presence of the plant extract in the DPPH solution, $A_c$ is the absorbance of the control solution (containing only DPPH) and $A_b$ is the absorbance of the sample extract solution without DPPH. Ascorbic acid was used for comparison ($EC_{50}$ = = 0.0039±0.0007 mg $ml^{-1}$).

*Determination of total phenolic content*

The total phenolic content in the *S. marmoreum* extracts was determined according to the Folin–Ciocalteu method,[17] using gallic acid as the standard. Samples (200 µl, 20 µg $ml^{-1}$) were introduced into test tubes and the Folin–Ciocalteu reagent was added and allowed to stand for 8 min at room temperature. Then, 0.80 ml of sodium carbonate (7.5 %) was added, mixed and allowed to stand for 30 min. The absorbance was measured at 765 nm. The total phenolic content was expressed as gallic acid equivalents (*GAE*), in mg/g of dry extract, as the mean ±*SD* of three replicate measurements:

$$A(765\ \text{nm}) = 12.722 c_{\text{gallic acid}}\ (\mu\text{g ml}^{-1}) + 0.0034,\ R^2 = 0.9994$$

*Determination of total flavonoid content*

The total flavonoid content was determined according to the aluminum chloride colorimetric method.[18] Each plant extract (2.0 ml, 5.0 μg ml$^{-1}$) in methanol was mixed with 0.10 ml of 10 % aluminum chloride, 0.10 ml of 1.0 M potassium acetate and 2.8 ml of deionized water. After 40 min incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm against a deionized water blank. Rutin was chosen as the standard and the total flavonoid content was expressed as milligram rutin equivalents (*RE*) per gram of dry extracts, as the mean±*SD* of three replicate measurements:

$$A(415\ \text{nm}) = 7.2328 c_{\text{rutin}}\ (\mu\text{g ml}^{-1}) - 0.2286,\ R^2 = 0.9919$$

*Determination of antimicrobial activity*

An agar well-diffusion method was employed for the determination of the antimicrobial activities of the extracts.[19] 0.10 ml of micro-organism suspension, formed during 24 h culture on agar with 10 ml sterile 0.90 % NaCl was suspended in 10 ml of the nutrition medium (*ca*. $10^6$ CFU/ml). A Petri dish (86 mm internal diameter) was filled with this system. Wells (10 mm in diameter) were cut from the agar and 30 μl of extract solution (concentration 10 mg ml$^{-1}$ in methanol) was delivered into them. Erythromycin (997 μg/mg; [114-07-8]; approx. 98 %; $H_2O$ content 4 %; Sigma) and tylosin tartrate (950 μg/mg; [74610-55-2]; Sigma) were used as the positive controls (concentration in methanol 0.05 mg ml$^{-1}$). All extracts were filtrated using a 0.45 μm membrane filter (Sartorius, Germany) and tested three times. After incubation at 37 °C for 24 h, the agar plates were examined for any zones of inhibition. The diameters of the inhibition zones, mm, were measured using a Fisher Lilly Antibiotic Zone Reader (USA).

*Statistical analysis*

Each of the measurements described above was performed in three replicate experiments, except the extract yields which were in duplicate. Comparison of means was analyzed by the Student's *t*-test and differences were considered significant when $p < 0.05$.

## RESULTS AND DISCUSSION

### *Kinetics of the extractions*

The change of the ES yield from *S. marmoreum* during the CE and UE, using methanol at a leaves-to-solvent ratio (1 g:10 ml) and a temperature of 25 °C, are shown in Fig. 1. The obtained curves have the same shape as those for the extraction of ES,[20] flavonoids and active compounds[13] from St. John's wort (*Hypericum perforatum)*, pyrethrines from pyrethrum (*Chrysanthemum cineraria*) flowers[21] and ES from sage (*Salvia officinalis*).[22] Independent of the recovery technique, the extraction occurred in two main stages: first, dissolution of the material near the surface, characterized by a rapid increase in the ES yield at the beginning of the process (washing or fast extraction), and second, diffusion of the solute from the porous plant residue into the solution (slow extraction). The optimum time for both CE and UE was approximately 40 min, ensuring a near maximum ES yield.

The efficiency of UE exceeded that of CE. Fig. 1 shows that ultrasound improved both the ES yield and the rate extraction. The total ES yield obtained

by UE was 4.3 % higher than the one obtained by CE but it was only 82.8 % of the yield achieved by SE. This was explained by the higher extraction temperature and much longer extraction time in the case of the SE.

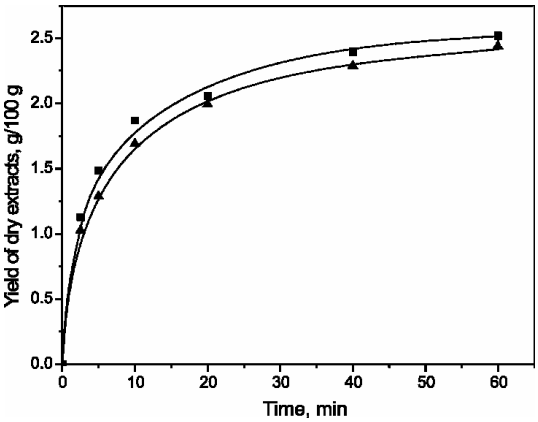


Fig. 1. Time course variation of the ES yield from *S. marmoreum* (CE: triangles; UE: squares).

*Anti-oxidant capacity*

The anti-oxidative effect of the houseleek extracts obtained by CE and UE was estimated by the ability of the extracts to scavenge hydroxyl radicals. This is very important because hydroxyl radicals are the major active oxygen species causing lipid oxidation.[15] Employing a spin trap, such as DMPO, it is possible to convert reactive hydroxyl radicals to stable nitroxide radicals (DMPO–OH adducts) with spectral hyperfine splitting that reflects the nature and the structure of these radicals. The relative intensity of free radical formation was determined by ESR spectroscopy, the height of the peaks being in proportion to the number of radical adduct molecules in the accumulating system.

As shown in Fig. 2, the reaction of $Fe^{2+}$ with $H_2O_2$ in the presence of the spin trapping agent DMPO generated a 1:2:2:1 quartet of lines with hyperfine coupling parameters ($a$N = $a$H = 14.9 G). The intensity of the ESR signal, corresponding to the concentration of the formed free radicals, was decreased in the presence of the extracts of *S. marmoreum.*

The anti-oxidative activity of the *S. marmoreum* extracts obtained by different extraction techniques was also estimated by the ability of the extracts to scavenge the stable DPPH radicals. The DPPH method was chosen to evaluate the anti-oxidant activity because it is one of the most effective methods for evaluating the concentration of radical-scavenging materials effective as a chain-breaking mechanism.[23]

The percentage of DPPH and the hydroxyl radicals-scavenging activity are plotted against the plant extract concentration in Fig. 3. To calculate the concentration of the extract necessary to decrease DPPH radical concentration by 50 % (called $EC_{50}$), the relationships of *DPPH* and the hydroxyl radicals-scavenging

activities on the concentration of the extract solution were determined by non-linear (sigmoid) and linear regression methods, respectively. The $EC_{50}$ value was used to measure the antiradical activity of the extracts: the lower the $EC_{50}$, the higher is the value of the antiradical activity.

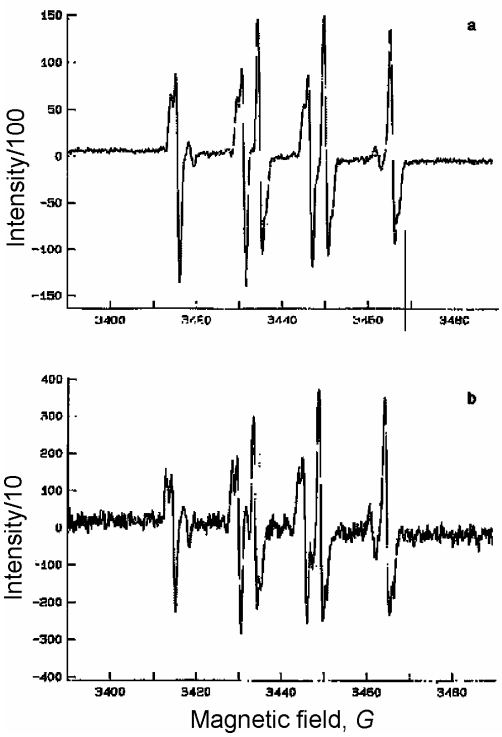


Fig. 2. ESR spectra of the DMPO–OH spin adduct recorded 5 min after mixing of a) 0.20 ml 0.30 M DMPO, 0.20 ml 10 mM $H_2O_2$ and 0.20 ml 10 mM $Fe^{2+}$ (blank) and b) the same as the blank but with a DMF solution of *S. marmoreum* extract, 0.50 mg $ml^{-1}$ (sample).

As it can be seen in Table I, the extract obtained by CE showed the highest anti-oxidant activity. This extract also contained the highest amount of total phenolic compounds and flavonoids. The differences observed between the anti-oxidant activity and the amounts of total phenolic compounds and flavonoids for all the extracts were statistically significant with a 95 % confidence level. The total amount of these compounds was accepted as an indication of the anti-oxidant potential because they acted as anti-oxidants, antimicrobials and photoreceptors in plants.[24]

It is believed that the observed reduction of phenolic and flavonoid compounds in the extract obtained by UE was the result of their degradation by interaction with the highly reactive hydroxyl radicals formed during sonication. The extractions were performed in methanol, a solvent which does not give rise to such a large proportion of radicals under cavitation,[25] but the water present in the fresh leaves of houseleek could.

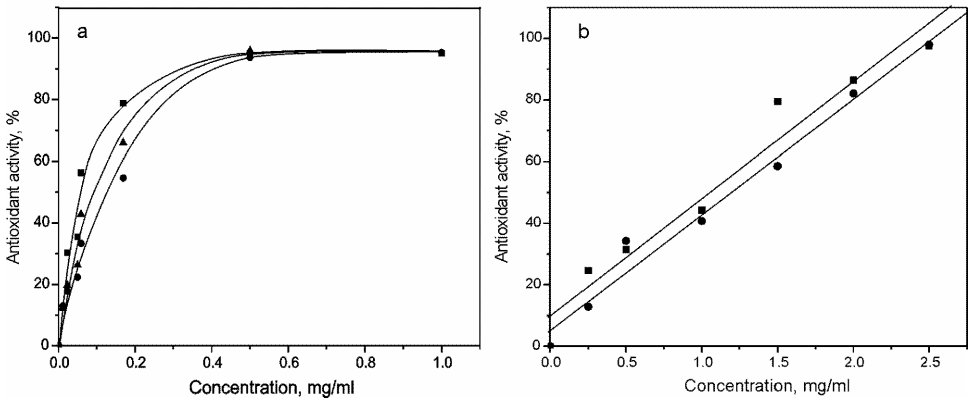


Fig. 3. Antioxidant activity against a) DPPH radical and b) hydroxyl radical for methanolic extracts of *S. marmoreum* obtained by different extraction techniques (CE: circles; UE: squares; Soxhlet extraction: triangles).

TABLE I. Yield of ES (g/100 g fresh plant material) obtained by the different extraction techniques, anti-oxidant capacity, total amount of phenolic compounds (mg gallic acid/g fresh plant material[a]) and flavonoids (mg rutin/g fresh plant material[a]) of *S. marmoreum* methanolic extracts

| Extraction technique | Yield of ES | $EC_{50}$ / mg ml$^{-1}$ | | Total phenolic content | Total flavonoids |
|---|---|---|---|---|---|
| | | DPPH assay[a] | Hydroxyl radical assay | | |
| CE[b] | 2.3 | 0.06±0.01 | 1.08 | 0.73±0.005 | 0.62±0.003 |
| UE[b] | 2.4 | 0.09±0.01 | 1.26 | 0.59±0.005 | 0.58±0.011 |
| SE[c] | 2.9 | 0.12±0.04 | ND[d] | 0.56±0.004 | 0.51±0.006 |

[a]Data are expressed as the mean of three replicates ± standard deviation; [b]40 min, 25 °C; [c]150 min, $T_b$; [d]not determined

The total phenolic content was correlated to the anti-oxidant activity of the extracts ($R^2$ = 0.884), as can be seen in Fig. 4. Thus, the anti-oxidant activity of an extract could be predicted from its total phenolic content. Statistically significant relationships were also observed between the total phenolics and the anti-oxidant activity in the case of virgin olive oils ($R^2$ = 0.991),[26] flaxseed ($R^2$ = 0.963) and cereal products ($R^2$ = 0.905).[27]

*Antimicrobial activity*

The antimicrobial activities of the methanolic extracts of *S. marmoreum* are given in Table II. As expected, the control treatment (methanol) had no inhibitory effect on any of the tested micro-organisms. All extracts showed a lower antimicrobial activity than the control antibiotics. Independent of the extraction technique, the methanolic extracts from *S. marmoreum* showed antimicrobial activity against only two of the seven tested microorganisms, the mould *Aspergillus niger* ATCC 16404 and the yeast *Candida albicans* ATCC 10231, with the diameters of the zone of inhibition ranging between 15.9 and 16.9 mm.

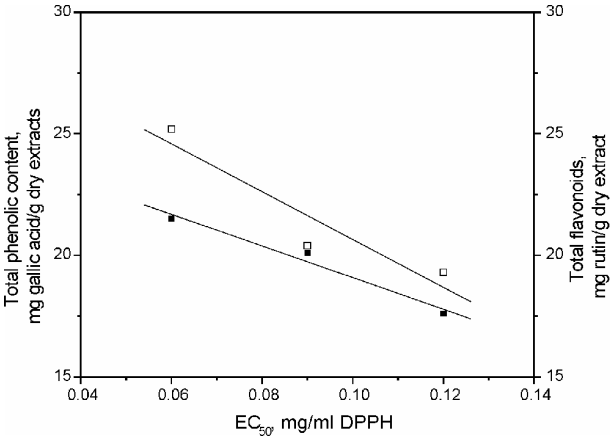


Fig. 4. Correlation between $EC_{50}$ (determined by the DPPH method), total phenolic content (open squares) and total flavonoids (black squares) of methanolic extracts from *S. marmoreum*.

TABLE II. Antimicrobial activity of methanol extracts of *S. marmoreum* and antibiotic sensitivity of selected microorganisms to the extracts (zone size, mm)

| Test microorganisms | Extracts (10 mg ml$^{-1}$) | | | Antibiotics (0.05 mg ml$^{-1}$) | |
|---|---|---|---|---|---|
| | UE[a] | CE[a] | SE[b] | Erythromycin | Tylosin tartrate |
| *Escherichia coli* ATCC 25922 | –[c] | – | – | 21.2±0.1 | 18.4±0.0 |
| *Pseudomonas aeruginosa* ATCC 9027 | – | – | – | 24.1±0.8 | 17.6±0.1 |
| *Bacillus subtilis* ATCC 6633 | – | – | – | 19.1±0.1 | 17.3±0.5 |
| *Staphylococcus aureus* ATCC 6538 | – | – | – | 23.4±0.1 | 18.4±0.4 |
| *Candida albicans* ATCC 10231 | 16.3±0.1 | 16.2±0.2 | 16.4±0.0 | 23.0±0.0 | 16.2±0.2 |
| *Saccharomyces cerevisiae* ATCC 9763 | – | – | – | – | – |
| *Aspergillus niger* ATCC 16404 | 16.9±0.3 | 16.4±0.4 | 15.9±0.3 | 20.5±0.5 | 18.1±0.1 |

Data are expressed as the mean of three replicates ± standard deviation; [a]10 mg ml$^{-1}$, 40 min, 25ºC; [b]150 min; [c]no antimicrobial activity

Independent of the tested micro-organism, there was no statistically significant difference (95 % confidence interval) in antimicrobial activities of the extracts obtained by UE and CE. The extract obtained by SE exhibited a slightly higher antimicrobial activity against *A. niger* and lower antimicrobial activity in the cases of *C. albicans*, than two other extracts.

The *S. marmoreum* extracts, similarly to *S. tectorum* extract,[4] had no antimicrobial activity against *Escherichia coli* and *Saccharomyces cerevisiae.*

## CONCLUSIONS

The present study is a comparative study of the anti-oxidant and antimicrobial activities, total phenolic compounds and total flavonoids in the methanolic extracts obtained from houseleek (*Sempervivum marmoreum* L.) leaves by traditional (CE and SE) and novel (UE) extraction techniques. The extracts of *S. marmoreum* showed antimicrobial activity against the mould *Aspergillus niger* and the yeast *Candida albicans*, but not against the tested bacteria. The methanolic extract obtained by CE showed stronger anti-oxidant, but similar antimicrobial activities, compared to the extracts obtained by UE and SE. Thus, the study suggests that methanolic extracts of *S. marmoreum* are both potential sources of natural anti-oxidants and natural antimicrobial preparations.

*Acknowledgements*. This work was supported by the Ministry of Science of the Republic of Serbia, project OI 142073B.

## И З В О Д

## ПОРЕЂЕЊЕ АНТИОКСИДАТИВНОГ И АНТИМИКРОБНОГ ДЕЈСТВА ЕКСТРАКАТА *Sempervivum marmoreum* L. ДОБИЈЕНИХ РАЗЛИЧИТИМ ЕКСТРАКЦИОНИМ ТЕХИКАМА

САША С. СТОЈИЧЕВИЋ[1], ИВАНА Т. СТАНИСАВЉЕВИЋ[1], ДРАГАН Т. ВЕЛИЧКОВИЋ[2], ВЛАДА Б. ВЕЉКОВИЋ[1] и МИОДРАГ Л. ЛАЗИЋ[1]

[1]*Технолошки факултет, Булевар ослобођења 124 и* [2]*Здравље-Actavis, Влајкова 199, 16000 Лесковац*

Рад представља компаративну студију антиоксидативног, антимикробног дејства и садржаја укупних фенолних и флавоноидних једињења у екстрактима добијеним из листа чуваркуће (*Sempervivum marmoreum* L.) мацерацијом, ултразвучном екстракцијом и екстракцијом по Сокслету. Екстракт добијен мацерацијом садржи више фенолних и флавоноидних једињења и показује веће антиоксидативно дејство него екстракти добијени осталим техникама. Сви екстракти независно од примењене технике показују сличну антимикробну активност према *Aspergillus niger* и *Candida albicans* али не и према тестираним бактеријама.

(Примљено 10. септембра, ревидирано 31. децембра 2007)

## REFERENCES

1. *Sempervivum marmoreum*, http://www.semper-vivum.de/kategorie/20/76/ (12 January, 2007)
2. J. F. Stevens, H. Hart, E. T. Elema, A. Bolck, *Phytochemistry* **41** (1996) 503
3. A. Blázovics, A. Lugasib, T. Keményc, K. Hagymásia, Á. Kéry, *J. Ethnopharmacol.* **73** (2000) 479
4. V. Abram, M. Donko, *J. Agric. Food Chem.* **47** (1999) 485
5. M. Šentjurc, M. Nemec, H. D. Connor, *J. Agric. Food Chem.* **51** (2003) 2766
6. A. Blázovics, R. Gonzáles-Cabello, I. Barta, P. Gergely, E. Fehér, Á. Kéry, G. Petri, *Phytother. Res.* **8** (1994) 33
7. Á. Kéry, A. Blázovics, N. Rozlosnik, J. Fehér, G. Petri, *Planta. Med.* **58** (1992) A661
8. A. Blázovics, L. Prónai, E. Fehér, Á. Kéry, G. Petri, *Phytother. Res.* **7** (1993) 95
9. 9. G. Kekesi, I. Dobos, G. Benedek, G. Horvath, *Phytother. Res.* **17** (2003) 1032
10. K. Szentmihályi, E. Fehér, P. Vinkler, Á. Kéry, A. Blázovics, *Toxicol. Pathol.* **32** (2004) 50

11. M. Vinatoru, *Ultrason. Sonochem.* **8** (2001) 313
12. S. Rodrigues, G. A. S. Pinto, *J. Food Eng.* **80** (2007) 869
13. A. Smelcerovic, M. Spiteller, S. Zuehlke, *J. Agric. Food Chem.* **54** (2006) 2750
14. S. Albu, E. Joyce, L. Paniwnyk, J. P. Lorimer, T. J. Mason, *Ultrason. Sonochem.* **11** (2004) 261
15. J. M. Canadanovic-Brunet, S. M. Djilas, G. S. Cetkovic, V. T. Tumbas, *J. Sci. Food Agric.* **85** (2005) 265
16. C. W. Choi, S. C. Kim, S. S. Hwang, B. K. Choi, H. J. Ahn, M. Y. Lee, S. H. Park, S. K. Kim, *Plant Sci.* **163** (2002) 1161
17. V. L. Singleton, J. A. Rossi, *Am. J. Enol. Vitic.* **16** (1965) 144
18. C. Chang, M. Yang, H. Wen, J. Chern, *J. Food. Drug Anal.* **10** (2002) 178
19. NCCLS (National Committee for Clinical Laboratory Standards), *Performance Standards for antimicrobial susceptibility testing*. 9th International Supplement, Wayne, PA, (1999), M100-S9.
20. V. Veljković, D. Milenović, *Hem. Ind.* **56** (2002) 60 (in Serbian)
21. M. Romdhane, C. Gourdon, *Chem. Eng. J.* **87** (2002) 11
22. D. T. Veličković, D. M. Milenović, M. S. Ristić, V. B. Veljković *Ultrason. Sonochem.* **13** (2006) 150
23. P. Maisuthisakul, M. Suttajit, R. Pongsawatmanit, *Food. Chem.* **100** (2007) 1409
24. P. Pietta, *J. Nat. Prod.* **63** (2000) 1035
25. L. Paniwnyk, E. Beaufoy, J. P. Lorimer, T. J. Mason, *Ultrason. Sonochem.* **8** (2001) 299
26. C. S. Sánchez, A. M. T. González, M. C. García-Parrilla, J. J. Q. Granados, H. L. García de la Serrana, M. C. L. Martínez, *Anal. Chim. Acta.* **593** (2007) 103
27. Y. S. Velioglu, G. Mazza, L. Gao, B. D. Oomah, *J. Agric. Food Chem.* **46** (1998) 4113.