PHYSICAL CHEMISTRY 2014

12th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

The Conference is dedicated to the
25. Anniversary of the Society of Physical Chemists of Serbia

---

September 22-26, 2014
Belgrade, Serbia

PHYSICAL CHEMISTRY 2014

12th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Proceedings
Volume I

The Conference is dedicated to the
25. Anniversary of the Society of Physical Chemists of Serbia

---

September 22-26, 2014
Belgrade, Serbia

ISBN 978-86-82475-30-9

Title: PHYSICAL CHEMISTRY 2014 (Proceedings)
Editors: Ž. Čupić and S. Anić
Published by: Society of Physical Chemists of Serbia, Studenski trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan" Priting and Publishing Company; 200 Copies;
Number of pages: 6+ 441; **Format**: B5; Printing finished in September 2014.

Text an Layout: "Jovan"

200 - *Coppy priting*

CONTENTS

PHYSICAL CHEMISTRY 2014

12th International Conference on
Fundamental and Applied Aspects of
Physical Chemistry

Organized by
The Society of Physical Chemists of
Serbia

in co-operation
with

Institute of Catalysis Bulgarian Academy of Sciences

Boreskov Institute of Catalysis of Siberian Branch of the Russian Academy of Sciences

Faculty of Physical Chemistry, University of Belgrade, Serbia

Institute of Chemistry Technology and Metallurgy,
University of Belgrade, Serbia

Vinča Institute, University of Belgrade, Serbia

Institute of General and Physical Chemistry, Serbia

Faculty of Pharmacy, University of Belgrade, Serbia

ANTIOXIDANT POTENTIAL OF THE RESURRECTION PLANT RAMONDA SERBICA

N. Mandić-Kovačević[1*], S. Živković[2], T. Rakić[3] and A. Popović-Bijelić[1]

[1]*Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia, *nebojsa_m@msn.com*
[2]*Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia*
[3]*Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Beograd, Serbia*

ABSTRACT

The resurrection plant *Ramonda serbica* was completely dehydrated for 14 days, reaching 4.2 % of the relative water content, and then fully rehydrated for 24 h. During dehydration, the total phenolic and flavonoid contents of leaf extracts significantly increased, and then gradually decreased upon rehydration. These changes directly correspond to the observed antioxidant activity of extracts towards the hydrophobic free radical DPPH. The scavenging potential towards the hydrophilic radical Tempone was maximal after 3 h of rehydration, indicating a strong oxidative burst upon water uptake.

INTRODUCTION

An exceptionally small number of higher vascular plant species have the ability to dehydrate their vegetative tissues and overcome drought conditions in the state of physiological inactivity or anabiosis. With an increase in ambient humidity, they readily absorb water, and recover all their physiological activities within several hours or days. These plant species are able to survive repetitive cycles of dehydration and rehydration, without losing their viability, and therefore they are called desiccation-tolerant, poikilohydric or resurrection plants.

The poikilohydric, vascular flowering plant *Ramonda serbica* Panč. & Petrov. (Gesneriaceae) is an endemic and relict plant of the Balkan Peninsula [1,2]. Its highly effective antioxidant system protects cells from reactive oxygen species (ROS), which are one of the most harmful consequences of water deficit and/or metabolic perturbations in cells [3]. Previous studies have shown that during dehydration and rewatering, *R. serbica* plants undergo changes in antioxidant enzyme activities of ascorbate peroxidase, glutathione reductase, superoxide dismutase, and polyphenol oxidase [4-6]. In addition, changes of the total content of various compounds that participate in its antioxidative protection, such as ascorbate, dehydroascorbate, reduced glutathione, and phenolic acids, have been observed.

In this work, we investigated the antioxidant potential of *R. serbica* extracts during the dehydration-rehydration cycle using electron paramagnetic resonance (EPR) spectroscopy. Namely, the capacity of the extracts for the removal of two nitrogen-centered radicals, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 4-oxo-2,2,6,6-tetramethylpiperidine-N-oxyl (Tempone) were determined.

EXPERIMENTAL

Plants were collected from their natural habitat in the vicinity of the city of Niš (SE Serbia). They were allowed to acclimatize for two weeks during which they were regularly sprayed with water in order to maintain their optimal water status. After acclimatization, the plants were not irrigated for 14 days, and they consequently dehydrated. Desiccated plants were then rewatered for the next 24 hours. Mature leaves from the middle of rosettes were used for analyses. They were harvested from the well watered control plants (C), moderately and completely desiccated plants (D1 and D2, respectively), and after 1, 3, 6, and 24 hours after the beginning of rehydration (R1, R2, R3, R4, respectively). The relative water content (RWC) of all leaves was determined according to Barr and Weatherley [7].

Preparation of plant methanol extracts. Leaves were crushed into powder using liq.N_2 and extracted with 80 % methanol (1:10, w/v). After 20 min centrifugation at 10,000×*g*, the supernatants were filtered through 0.2 μm cellulose filters (Agilent Technologies, Santa Clara, USA) and stored at 4 °C until use.

Determination of total phenolic content. Total phenolic (TP) content was quantified using the modified Folin–Ciocalteau assay [8]. 50 μl of plant extract was mixed with 475 μl of the 5 % sodium carbonate solution. The mixture was left for 3–5 min and then 475 μl of the 50 % Folin–Ciocalteau reagent was added. The solution was mixed and allowed to stand at room temperature in dark for 1 h. Absorbance was measured at 724 nm using UV–visible spectrophotometer (Agilent 8453, Agilent Technologies, Germany). The TP content was calculated from a standard calibration curve based on gallic acid and the results were expressed as mg of gallic acid equivalents (GAE) per gram of leaf dry weight (mg GAE g^{-1} DW).

Determination of total flavonoid content. Total flavonoid (TF) content of the samples was determined according to [9], with some modifications. Briefly, 50 μl of plant methanolic extract was mixed with 600 μl ddH_2O, well shaken and mixed with 40 μl of 5 % KNO_2. The mixture was allowed to stand at room temperature for 6 min and subsequently 70 μl of 4.26 % $AlCl_3$ solution was added. After 5 min at room temperature, 240 μl of 1 M NaOH was added to the mixture and the solution was well mixed. The absorbance was measured at 510 nm. TF content in each extract was calculated from the standard curve based on rutin. The results are expressed as mg of rutin equivalents (RE) per gram of leaf dry weight (mg RE g^{-1} DW).

EPR spectroscopy. The samples used for EPR measurements contained 30 μl of 200× diluted leaf methanolic extracts and 0.02 mM DPPH or 0.1 mM Tempone. The X-band (9.8 GHz) EPR spectra were recorded on a Bruker Elexsys-II EPR

spectrometer at room temperature under the following conditions: microwave power 10 mW, modulation amplitude 2 G, modulation frequency 100 kHz, conversion time 120 ms, acquisition time 2 min. Spectra were analyzed using Bruker Xepr software. Antioxidant activities of methanolic leaf extracts were determined from the relative heights of the middle EPR peak (DPPH), and the low-field EPR peak (Tempone), as described in [10] using the following formula: $AA(\%) = (1 - I_{extract} / I_{cont}) \cdot 100$, where I_{cont} and $I_{extract}$ are the relative heights of the EPR peaks in the spectrum of the control, and the sample containing the leaf extract, respectively.

RESULTS AND DISCUSSION

The relative water content (RWC) of the well watered control plants (C) decreased from 78.7 % to 61.4 %, in moderately dehydrated plants (D1), and finally to 4.2 % in the completely dried ones (D2). Upon rehydration, the RWC restored rapidly to 19.71 % (R1), 25.08 % (R2), 40.95 % (R3), and 65.1 % (R4) after 24 hours.

The total content of phenolic and flavonoid compounds during the dehydration-rehydration cycle is shown in Figure 1a. It is observed that the dehydrated leaves have the highest content of phenolic compounds, and that this amount is significantly decreased during the first six hours of rehydration. This reduction may be correlated with their role in the antioxidant protection during the strong oxidative burst that occurs at the beginning of rehydration. As previously suggested, the phenolic compounds act against ROS in the initial phase of plant rewatering, being substrates for peroxidases [5,6].

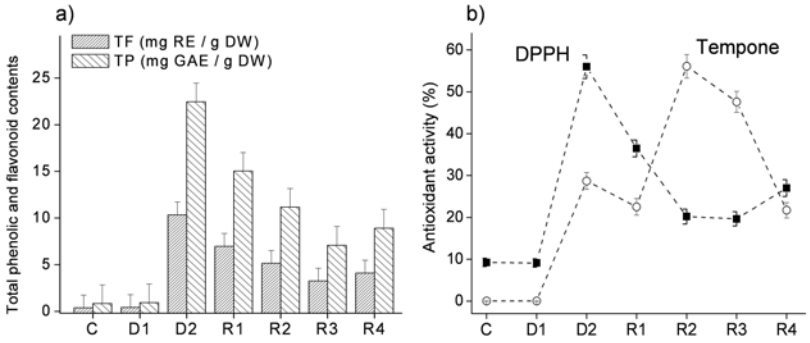

Figure 1. a) Total phenolic (TP) and flavonoid (TF) contents, and b) antioxidant activity towards free radicals, DPPH and Tempone, of *R. serbica* plants subjected to dehydration and rehydration. Error bars are standard deviation from two independent experiments.

The highest antioxidant activity of methanolic leaf extracts towards the free radical DPPH was detected in completely dehydrated and physiologically inactive leaves (D2), Figure 1b. The changes in the antioxidant potential towards DPPH correspond well to the changes in the concentrations of total phenolic and

flavonoid compounds, indicating their important role in protection against ROS, especially during dehydration.
The antioxidant activity of leaf extracts towards Tempone is also increased during dehydration (D2), however it reached its maximum after 3 h of rehydration (R2). This indicates that during the first few hours of rehydration, when the plants experience severe oxidative stress [6], they contain water-soluble compounds that can reduce the hydrophilic free radical Tempone. These compounds are likely to be part of the rehydration-induced antioxidant defense mechanism, and may protect the cells from elevated ROS production during the oxidative burst.
Taken together, the results show that the antioxidant defense system of *R. serbica* is well-coordinated during the dehydration and rehydration processes.

CONCLUSION

Ramonda serbica is a desiccation-tolerant plant that efficiently removes reactive oxygen species that form in water stressed plants, especially at the beginning of plant rewatering. The high capacity for the removal of the Tempone free radical in the initial phases of plant rehydration confirms the existence of the highly efficient ROS-detoxifying mechanisms during the strong oxidative burst. Our results also show that EPR spectroscopy is a valuable tool in the evaluation of the overall antioxidant capacity of plant extracts.

ACKNOWLEDGEMENT

This work was supported by the Ministry of education, science and technological development of Serbia (grants III41005, 173024, 173030).

REFERENCES

[1] P. Černjavsky, Journal de la Soc. Bot. de Russie 1928, T.13, IV, 1-2.
[2] S. Petrović, Glasnik Srpskog Učenog Društva 1885, Nova serija 62, 101-123.
[3] T. Rakić, M. Lazarević, Ž. Jovanović, S. Radović, S. Siljak-Yakovlev, B. Stevanović, V. Stevanović, Front. Plant. Sci. 2014, 4, 550, 1-9.
[4] A. Augusti, A. Scartazza, F. Navari-Izzo, C.L.M. Sgherri, B. Stevanovi, E. Brugnoli Photosynth. Res. 2001, 67, 79-88.
[5] C. Sgherri, B. Stevanović, F. Navari-Izzo, Physiol. Plant. 2004, 122, 478–485.
[6] S. Veljović-Jovanović, B. Kukavica, F. Navari-Izzo, Physiol. Plant. 2008, 132, 407-416.
[7] H.D. Barr, P.E. Weatherley, Austral. J. Biol. Sci. 1962, 15, 413–428.
[8] V.L. Singleton, J.A.Jr. Rossi, Amer. J. Enol. Viticult. 1965, 16, 144–158.
[9] F. Karadeniz, H.S. Burdulu, N. Koca, Y. Soyer, Turk. J. Agr. Forest. 2005, 29, 297–303.
[10] J.M. Dimitrić Marković, Z.S. Marković, I.A. Pašti, T.P. Brdarić, A. Popović-Bijelić, M. Mojović, Dalton Trans. 2012, 41, 7295-7303.