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TOTAL ANTIOXIDANT CAPACITY IN THE MUSCLE TISSUE OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) UNDER *IN VITRO* INCUBATION WITH EXTRACTS FROM LEAVES OF VARIOUS SPECIES OF *SANSEVIERIA THUNB* (*ASPARAGACEAE*) (OVERVIEW)

Maryniuk M., Senior engineer

Kharchenko I., PhD

Buyun L., Doctor of biological science

M.M. Gryshko National Botanical Garden, NAS, Kyiv, Ukraine

Tkachenko H., PhD, assistant Professor

Witaszek M., student

Pazontka-Lipiński P., student

Osadowski Z., Professor, vice-rektor

Institute of Biology and Environmental Protection, Pomeranian University in Slupsk, Poland

The aim of this study was to evaluate in vitro the effect of buffer extracts obtained from leaves of various species from Sansevieria genus on the total antioxidant capacity (TAC) of the muscle tissue of the rainbow trout (Oncorhynchus mykiss Walbaum). The fully expanded leaves of Sansevieria plants, cultivated under glasshouse conditions, were sampled at M. M. Gryshko National Botanical Garden (NBG), National Academy of Science of Ukraine.

*The most potent antioxidant effect was demonstrated for the extracts of *S. caulescens*, *S. suffruticosa*, *S. hyacinthoides*, *S. canaliculata*, *S. aethiopica*, *S. gracilis*, and *S. parva* as compared to phosphate buffer control (46.6 %, 66.8 %, 77.3 %, 49.8 %, 71.1 %, 63.4 %, 39.4 %, respectively). However, there were no significant changes for other extracts screened. The results showed that extracts of *S. hyacinthoides* and *S. aethiopica* efficiently increased the TAC level in muscle tissue. The results of this study provide a new perspective for the use of various Sansevieria species as a medicinal plant to improve the antioxidant response of rainbow trout (*O. mykiss*). Further studies including the use of other medicinal plants as food additives in aquaculture, the assessment of its antioxidant effects on various tissues are in progress.*

Keywords: rainbow trout (*Oncorhynchus mykiss Walbaum*), total antioxidant capacity, aquaculture, sansevieria thunb. (*asparagaceae*), cultivars.

Nowadays, the growing interest of using plants in aquaculture has increased worldwide because they are easy to prepare, cheap, and contain natural organic compounds [3, 19]. The most important advantage of using plant-derived compounds as immunostimulant in aquaculture is that they contain natural organic materials that do not cause any threat to fish health or to the environment or to human health [1, 23]. Plant-derived products represent a promising source of bioactive molecules, being at the same time readily available, inexpensive and biocompatible. In addition, natural products are preferred because of their biodegradability in the environment. Many of herbal products as well as immunostimulant plants due to their secondary metabolites encompassing various phenolic, polyphenolic, alkaloid, quinine, terpenoid, lectine and poly-



peptide compounds, shown to be very effective alternative to antibiotics, chemicals or synthetic compounds and vaccines in aquaculture [1, 23]. There is evidence that the substances present in the plants have an important role in enhancing the fish immunity. Therefore, the development of new additives for aquaculture still attracts the attention of many researchers and fish farmers comprising the ecofriendly approach for the control of fish pathogens. This study was focused on various *Sansevieria* Thunb. species, a genus with diverse ethnobotanical uses in its geographical distribution range, which occupies an important place among plant genera applied for treatment of a broad spectrum of diseases and disorders [10, 21, 22].

These plants have been tested in the treatment of haemorrhoids, pain, smallpox, chicken-pox, and measles, venereal diseases, malnutrition, paralysis, epilepsy, convulsions, and spasm, pulmonary troubles, and as vermifuge, as well as remedy for parasitic infections. In studies carried out in Nakuru and Maragua districts of Kenya by Khalumba and co-workers [10], they identified five use categories of *Sansevieria* plants, namely medicine (33 % of the reports), fibers (24 %), soil conservation (22 %), fodder (18 %), and other uses (14 %) for four species, *Sansevieria ehrenbergii* Schweinf. ex Baker, *S. parva*, *S. raffillii* N.E. Br., and *S. suffruticosa* N.E. Br. On the other hand, Chhabra and colleagues [6] mentioned the use of *S. bagamoyensis* N.E.Br. for treatment of convulsive fever in Tanzania. Also, Watt and Breyer-Brandwijk [27] listed the use of *S. hacinthoides* in the treatment of toothache and earache and the use of the rhizome decoction of *S. kirkii* as a purgative both reported from East Africa. Yet, Kiringe [12] reported on the use of *S. volkensii* Gürke for the treatment of sexually transmitted diseases such as gonorrhoea. In Kenya, Owuor and Kisangau [16] included the use of *S. parva* leaf sap and *S. kirkii* extracts for treatment of snake bite wounds. Nevertheless, in spite of these data, Takawira-Nyenyanya with coauthors [22] reported that the documentation of ethnobotanical uses of genus *Sansevieria* is incomplete.

In our previous study [5, 25], we have evaluated the antibacterial capacity of ten species of *Sansevieria* genus against *Staphylococcus aureus* in order to validate scientifically the inhibitory activity for microbial growth attributed by their popular use and to propose new sources of antimicrobial agents. The leaves of *S. canaliculata*, *S. trifasciata*, *S. cylindrica*, *S. parva*, *S. fischeri*, *S. kirkii*, *S. aethiopica*, *S. metallica*, *S. caulescens*, *S. francisii* were used. Our results proved that the zones of inhibition ranged between 16 and 34 mm. Extracts from the leaves of *S. fischeri* and *S. francisii* were particularly active against tested organism (diameters of inhibition zones comprise up to 34 mm). This was followed by the activities of extracts from the *S. parva*, *S. kirkii*, *S. aethiopica*, *S. caulescens*, *S. metallica* leaves (diameters of inhibition zones were ranged between 25 and 31 mm). The ethanolic extracts of *S. canaliculata* and *S. trifasciata* showed less expressed antimicrobial activities (diameters of inhibition zones ranged between 16 and 16.5 mm). Additionally, the results proved that the ethanolic extracts from *S. fischeri*, *S. francisii*, *S. parva*, *S. kirkii*, *S. aethiopica*, *S. caulescens*, *S. metallica* exhibit a favorable antibacterial activity against *S. aureus* [5, 26].

Although antimicrobial activities of extracts obtained from leaves of various species of *Sansevieria* genus were investigated [4, 5, 24, 25], studies regarding their total antioxidant defences under *in vitro* incubation with the muscle tissue of the rainbow trout (*Oncorhynchus mykiss* Walbaum) have not been undertaken. The aim of this study was to evaluate *in vitro* the effect of buffer extracts obtained from leaves of various species from *Sansevieria* genus on the total antioxidant capacity (TAC) of the muscle tissue of the rainbow trout.

Total antioxidant capacity (TAC) is an indicator frequently used to assess the



antioxidant status of biological samples and can evaluate the antioxidant response against the free radicals produced in a given disease [18].

Materials and methods. *Collection of Plant Material.* The leaves of *Sansevieria* plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanical Garden (NBG), National Academy of Science of Ukraine. Specifically, the leaves of *Sansevieria francisii*, *S. caulescens*, *S. suffruticosa*, *S. roxburghiana*, *S. metallica*, *S. gracilis*, *S. hyacinthoides*, *S. cylindrica*, *S. canaliculata*, *S. aethiopica*, *S. kirkii*, *S. trifasciata*, *S. forskaliana*, *S. fischeri*, *S. dooneri*, *S. intermedia*, *S. parva* were sampled for study.

Preparation of Plant Extracts. Freshly collected leaves were washed, weighted, crushed, and homogenized in 0.1M sterile phosphate buffer saline solution (pH 7.4) (in proportion 1:19, w/w) at room temperature. The extracts were then filtered and investigated for their antioxidant capacity. All extracts were stored at -20°C until use.

Experimental fish. Clinically healthy rainbow trout with a mean body mass of 80-120 g were used in the experiments. The experiments were performed in water at $14.5 \pm 0.5^\circ\text{C}$ and pH 7.2-7.4. The dissolved oxygen level was about 9 ppm with additional oxygen supply, with a water flow of 25 L/min, and a photoperiod of 12 h per day. The same experimental conditions were used during the whole research. The water parameters were maintained under constant surveillance. The fish were held in square tanks (150 fish per tank) and fed commercial pelleted diet.

Collection of muscle tissue samples. The muscle tissue samples were homogenized in ice-cold buffer (100 mM Tris-HCl, pH 7.2) using a glass homogenizer immersed in ice water bath. Homogenates were centrifuged at 3000 g for 15 min at 4°C. After centrifugation, the supernatant was collected and frozen at -20°C until analyzed. All enzymatic assays were carried out at $22 \pm 0.5^\circ\text{C}$ using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) in duplicate. The enzymatic reactions were started by adding the tissue supernatant.

Experimental design. The supernatant of the muscle tissue was used to incubate with extracts of various species of *Sansevieria* (in a ratio 19:1) at room temperature. The control group (trout muscle tissue) was incubated with 100 mM Tris-HCl buffer (pH 7.2) (in a ratio 19:1). The incubation time was 2 hours. Total antioxidant capacity was studied in the incubated homogenate (control group and in samples with extracts of various species of *Sansevieria*).

Measurement of total antioxidant capacity (TAC). The TAC level in the sample was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm [8]. Sample inhibits the Fe^{2+} /ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. Briefly, 0.1 mL of sample was added to 2 mL of 1% Tween 80 reagent, 0.2 mL of 1 mM FeSO_4 , and 0.2 mL of 10 mM ascorbic acid. In the blank assay, 0.1 mL of distilled water was used instead of the sample. The mixture was heated in water bath for 48 hrs at 37 °C. After cooling, 1 mL of 20 % trichloroacetic acid was added. The mixture was centrifuged at 3000 g for 10 min. After centrifugation, 2 mL of supernatant and 2 mL of 0.25 % 2-thiobarbituric acid were mixed. The mixture was heated in a water bath at 95°C for 15 min. The absorbance of the obtained solution was measured at 532 nm. The absorbance of the blank was defined as 100 %. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

Statistical analysis. The mean \pm S.E.M. values was calculated for each group to determine the significance of intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). Significance of



differences between the total antioxidant capacity level (significance level, $p < 0.05$) was examined using Mann-Whitney U test (Zar 1999). All statistical calculation was performed on separate data from each individual with STATISTICA 8.0 software (StatSoft, Krakow, Poland).

Results and discussion. In present study, we investigated the influence of extracts of various species from *Sansevieria* genus on the total antioxidant capacity in the muscle tissue of rainbow trout after incubation with extracts under *in vitro* conditions. The most potent antioxidant effect was demonstrated for the extracts of *S. caulescens*, *S. suffruticosa*, *S. hyacinthoides*, *S. canaliculata*, *S. aethiopica*, *S. gracilis*, and *S. parva* compared to phosphate buffer control (46.6 %, 66.8 %, 77.3 %, 49.8 %, 71.1 %, 63.4 %, 39.4 %, respectively). However, there were no significant changes for other extracts studied. The results showed that extracts of *S. hyacinthoides* and *S. aethiopica* efficiently increased the TAC level in muscle tissue (Table).

Supplementation of herbal substances caused different antioxidant responses in muscle tissue of trout. Therefore, it would be reasonable to suggest that these different antioxidant effects are determined by their by-products (alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins, carbohydrates etc.). Indeed, the study on *S. roxburghiana* and *S. trifasciata* has revealed the presence of important compounds which were separated by thin layer chromatography [11]. Preliminary phytochemical screening of the extracts of *S. trifasciata* plant showed the presence of alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins and carbohydrates [2]. Additionally, the methanolic extract of the whole plant of *S. trifasciata* has yielded 12 steroidal saponins, 10 of which are new constituents [14].

The total antioxidant capacity (TAC) in the muscle tissue of rainbow trout after incubation with extracts from leaves of various *Sansevieria* species (M±m, n=6)

Species	Total antioxidant capacity, %	Significance, p
Control group	22.68±2.02	
<i>Sansevieria francisii</i>	23.22±2.64	0.874
<i>Sansevieria caulescens</i>	33.24±3.74*	0.015
<i>Sansevieria suffruticosa</i>	37.84±4.25*	0.002
<i>Sansevieria roxburghiana</i>	24.18±3.94	0.709
<i>Sansevieria metallica</i>	25.87±3.74	0.421
<i>Sansevieria hyacinthoides</i>	40.22±4.56*	0.001
<i>Sansevieria cylindrica</i>	22.12±2.35	0.869
<i>Sansevieria canaliculata</i>	33.97±4.60*	0.018
<i>Sansevieria aethiopica</i>	38.80±4.12*	0.001
<i>Sansevieria kirkii</i>	28.74±3.29	0.118
<i>Sansevieria trifasciata</i>	18.56±2.63	0.246
<i>Sansevieria forscaliiana</i>	27.46±5.67	0.338
<i>Sansevieria fisheri</i>	18.55±0.81	0.182
<i>Sansevieria dooneri</i>	23.54±1.76	0.786
<i>Sansevieria intermedia</i>	17.86±4.32	0.262
<i>Sansevieria gracilis</i>	37.06±3.86*	0.002
<i>Sansevieria parva</i>	31.61±1.70*	0.011

* – the changes are statistically significant ($p < 0.05$) compared to the control group.



Phytochemical analysis of the whole plant of *S. trifasciata* has resulted in the isolation of four new pregnane glycosides (1 β ,3 β -dihydroxypregna-5,16-dien-20-one glycosides) [14]. Gas chromatographic analysis of the leaves revealed the presence of alkaloids, allcins, glycosides and saponins [9]. Pettit and co-workers (2005) have isolated three new spirostanol saponins designated sansevierin A (1), sansevistatin 1 (2), and sansevistatin 2 (3) (10⁻⁵% yield) from the CH₃OH-CH₂Cl₂ extract of *S. ehrenbergii*, accompanied by three known steroidal saponins (4-6), using bioactivity-directed isolation procedures. Each of the saponins was evaluated against the P388 lymphocytic leukemia cell line and a panel of human cancer cell lines. Except for 1, all were found to cause inhibition of cancer cell growth. In addition, most of the saponins exhibited antimicrobial activity, particularly against the pathogenic fungi *Candida albicans* and *Cryptococcus neoformans* [17]. In addition, a new steroidal saponin from the leaves of *S. cylindrica* was isolated by Da Silva Antunes and co-workers [7]. Its structure was established as (3 β ,12 α ,15 α ,25S)-26-(β -D-glucopyranosyloxy)-22-hydroxyfurost-5-en-3-yl12-O-(6-deoxy- α -L-mannopyranosyl)-15-O-(6-deoxy- α -L-mannopyranosyl)- β -D-glucopyranoside. The steroidal saponin showed no haemolytic effects in the *in vitro* assays and demonstrated inhibition of the capillary permeability activity [7].

The presence of saponins, a group of naturally occurring plant glycosides with strong foam-forming properties in aqueous solution, has been reported in more than 100 families of plants out of which at least 150 kinds of natural saponins have been found to possess significant anti-cancer properties. There are more than 11 distinguished classes of saponins including dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes and steroids. Due to the great variability of their structures, saponins always display anti-tumorigenic effects through varieties of antitumor pathways [13]. A number of triterpenoids also have shown promise as antineoplastic agents. Members of the cycloartane, lupane, ursane, oleanane, friedelane (especially quinone methides), dammarane, cucurbitacin, and limonoid triterpenoids, have demonstrated anti-proliferative activity on various cancer cell lines [20].

According to the results obtained, we addressed the hypothesis that by-products in the extracts of various *Sansevieria* species, i.e. polyphenolic compounds, may be a major contributor to increase of antioxidant capacity of muscle tissue of rainbow trout after incubation *in vitro*. To prove this hypothesis, separation and characterization of secondary metabolites compound in plant extracts are required for further study.

Moreover, it should be noted that measurement of only total antioxidant capacity (TAC) can provide limited information about the antioxidant status, because TAC assays do not measure all antioxidant components [18]. Special attention should be given to the evaluation of the effects of plant extracts/products on fish growth, haematological profiles, immune responses and resistance to infectious diseases [3].

Conclusions. The present findings suggest that the extracts of various species from *Sansevieria* genus have shown remarkable antioxidant potential. According to the abovementioned antioxidant mechanisms, extracts of various species from *Sansevieria* genus may activate antioxidant enzymes and their synthesis *de novo*. **Taking into account existing experimental evidence**, it is reasonable to assumed that secondary plant metabolites, i.e. polyphenolic compounds in extracts of various species from *Sansevieria* genus extract may contribute to the antioxidant activity. In conclusion, the results of this study provide a new perspective for the use of various *Sansevieria* species as a medicinal plant to improve the antioxidant response of rainbow trout (*O. mykiss*). Further studies including the use of other medicinal plants as food additives in aquaculture, the assessment of its antioxidant effects on various tissues are in progress.



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ОБЩАЯ АНТИОКСИДАНТНАЯ ЕМКОСТЬ В МЫШЕЧНОЙ ТКАНИ РАДУЖНОЙ ФОРЕЛИ (*ONCORHYNCHUS MYKISS WALBAUM*) В ПРОБИРКЕ



ИНКУБАЦИИ С ЭКСТРАКТАМИ ИЗ ЛИСТЬЕВ РАЗЛИЧНЫХ ВИДОВ САНСЕВИЕРИЯ THUNB. (ASPARAGACEAE) (ОБЗОРНАЯ)

Маринюк М., Харченко И., Буюн Л., Национальный ботанический сад им. М.М. Гришко НАН Украины

Ткаченко Г., Виташек М., Пазонтка-Липински П., Осадовски З. Институт биологии и охраны среды Поморского университета (Слупск, Польша)

Целью исследования было оценить в лабораторных условиях влияние буферных экстрактов, полученных из листьев различных видов из рода Сансевиерия на общей антиоксидантной способности мышечной ткани радужной форели (*Oncorhynchus mykiss Walbaum*). Самый мощный антиоксидантный эффект был продемонстрирован для экстрактов *S. caulescens*, *S. suffruticosa*, *S. hyacinthoides*, *S. canaliculata*, *S. aethiopica*, *S. gracilis*, and *S. parva* по сравнению с фосфатным буфером управления (46.6 %, 66.8 %, 77.3 %, 49.8 %, 71.1 %, 63.4 %, 39.4 %, соответственно). Однако существенных изменений в отношении других скрининговых экстрактов не произошло. Результаты исследования показали, что экстракты *S. hyacinthoides* и *S. эфиопика* эффективно повышает уровень ТАС в мышечной ткани. Результаты данного исследования дают новую перспективу использования различных видов *Sansevieria* в качестве лекарственного растения для улучшения антиоксидантной реакции радужной форели (*O. mykiss*). Проводятся дальнейшие исследования, в том числе использование других лекарственных растений в качестве пищевых добавок в аквакультуре, оценка их антиоксидантного воздействия на различные ткани.

Ключевые слова: радужная форель (*Oncorhynchus mykiss Walbaum*), общая антиоксидантная емкость, аквакультура, сансевиерия thunb. (спаржа), сорта.

ЗАГАЛЬНА АНТИОКСИДАНТНА ЄМНІСТЬ В М'ЯЗОВІЙ ТКАНИНІ РАЙДУЖНОЇ ФОРЕЛІ (*ONCORHYNCHUS MYKISS WALBAUM*) ПРИ ІНКУБАЦІЇ З ЕКСТРАКТАМИ З ЛИСТЯ РІЗНИХ ВИДІВ САНСЕВІЄРІЯ THUNB. (ASPARAGACEAE) (ОГЛЯДОВА)

Маринюк М., Харченко И., Буюн Л., Національний ботанічний сад ім. М. М. Гришко НАН України

Ткаченко Г., Виташек М., Пазонтка-Ліпінські П., Осадовські З. Інститут біології та охорони середовища Поморського університету (Слупськ, Польща)

Метою дослідження було оцінювання в лабораторних умовах впливу буферних екстрактів, отриманих з листя різних видів з роду Сансевієрія за загальною антиоксидантною здатністю м'язової тканини райдужної форелі (*Oncorhynchus mykiss Walbaum*). Найпотужніший антиоксидантний ефект був продемонстрований для екстрактів *S. caulescens*, *S. suffruticosa*, *S. hyacinthoides*, *S. canaliculata*, *S. aethiopica*, *S. gracilis*, and *S. parva* порівняно з фосфатним буфером управління (46.6 %, 66.8 %, 77.3 %, 49.8 %, 71.1 %, 63.4 %, 39.4 %, відповідно). Проте суттєвих змін щодо інших скринингових екстрактів не помічено. Результати дослідження показали, що екстракти *S. hyacinthoides* і *S. эфиопика* ефективно підвищує рівень ТАС в м'язовій тканині. Результати даного дослідження дають нову перспективу використання різних видів *Sansevieria* в якості лікарської рослини для покращення антиоксидантної реакції райдужної форелі (*O. mykiss*). Проводяться подальші дослідження, в тому числі використання інших лікарських рослин в якості харчових добавок у аквакультурі та оцінка їх антиоксидантного впливу на різні тканини.

Ключові слова: райдужної форелі (*Oncorhynchus mykiss Walbaum*), загальна антиоксидантна ємність, аквакультура, сансевієрія thunb. (спаржа), сорти.