



OXIDATIVE STRESS BIOMARKERS IN THE MUSCLE TISSUE OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) AFTER *IN VITRO* TREATMENT OF *SANSEVIERIA CAULESCENS* N.E.BR. EXTRACT

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The goal of this study was to assess *in vitro* the effect of buffer extract obtained from leaves of *Sansevieria caulescens* N.E.Br. on the 2-thiobarbituric acid reactive substances (TBARS) as lipid peroxidation biomarker, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity (TAC) in the muscle tissue of the rainbow trout. Our study suggests that the leaf *S. caulescens* extract have shown good antioxidant potential *in vitro* study after incubation with muscle tissue homogenate of rainbow trout. There were no significant changes for TBARS level as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins between values in control group and in the muscle tissue of rainbow trout after incubation with extracts from leaves of *S. caulescens*. Our results showed that extract of *S. caulescens* efficiently increased the total antioxidant capacity in muscle tissue by 46.6% ($p < 0.05$) due to inhibited the Fe²⁺/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. Taking into account existing experimental evidence, it is reasonable to assume that secondary plant metabolites, i.e. polyphenolic compounds in the extract of *S. caulescens* may contribute to the antioxidant activity. Further studies including both the use of other medicinal plants as food additives in aquaculture and the assessment of its antioxidant effects on various tissues are in progress.

Keywords: rainbow trout (*Oncorhynchus mykiss*), *Sansevieria caulescens* N.E.Br., 2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins, total antioxidant capacity

Introduction

The fishery is a source of income and social development, particularly in developing countries, playing a great role in food security and livelihood (FAO, 2016). Huge loss of production in aquaculture is occurring because of many reasons. Studies showed that almost fifty percent of

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production loss is because of diseases which are more severe in developing countries (Assefa et al., 2018). In recent years, to develop the alternative practices for disease management in aquaculture, attention was diverted to find novel drugs. Biological and chemical disease control strategies such as using probiotics, prebiotics, and medicinal plants are widely in use. Plant product application in aquaculture for disease control is one of the promising alternatives to antibiotics (Assefa et al., 2018). Plant-derived compounds act as a better antibacterial, antiviral, immunostimulant and antistress effect in fish and shellfish aquaculture (Anusha et al., 2014). Use of herbal therapy within animal production, as well as in the diet of commercial fishes has shown promise, in that it is natural and biodegradable and has antimicrobial activity against various pathogens, including those relating to fish (Valladão et al., 2015). The herbals having the characteristics of immunostimulants been able to increase the survival and reduce the pathogenic load against pathogenic challenge by improving the immune system in fishes (Anusha et al., 2014). On the other hand, many plant-derived compounds and medicinal plant products have been found to have non-specific immunostimulating properties in animals, of which more than a dozen have been evaluated in fin and shellfishes and, specifically, to prevent and control fish diseases (Galina et al., 2009). However, applying a new component as a drug in the fish diet requires more research on the effects on the physiological and health status of animals. Undoubtedly, a healthy diet is an important factor in the prevention of widespread various diseases in aquaculture. Therefore, the study of diet components such as dietary supplements, particularly drugs, is essential (Banaee et al., 2011).

Considering that almost all fish produced from aquaculture is for human consumption, residual drugs in fish products can affect people who consume them, and antimicrobials released into aquatic environments can select for resistant bacteria. Moreover, these antimicrobial-resistant bacteria, or their resistance genes, can be transferred to humans (Park et al., 2012).

In this study, attention was focused on *Sansevieria* Thunb., 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 (Watt and Breyer-Brandwijk, 1962; Chhabra et al., 1987; Khalumba et al., 2005; Staples and Herbst, 2005; Kiringe, 2006; Owuor and Kisangau, 2006; Takawira-Nyenya et al., 2014). Our previous study (Buyun et al., 2016; Tkachenko et al., 2017) have highlighted the antibacterial capacity of ten species of *Sansevieria* genus against *Staphylococcus aureus*. These plants have been screened in order to validate scientifically the inhibitory activity for microbial growth attributed to their popular use and to propose new sources of antimicrobial agents. The leaves of *Sansevieria canaliculata* Carrière, *S. trifasciata* Prain, *S. cylindrica* Bojer ex Hook., *S. parva* N.E.Br. (syn. *S. dooneri* N.E.Br.), *S. fischeri* (Baker) Marais, *S. kirkii* Baker, *S. aethiopica* Thunb., *S. metallica* Gérôme & Labroy, *S. caulescens* N.E.Br., *S. francisii* Chahin were used. Our results proved that the zones of inhibition ranged from 16 to 34 mm. Extracts from the leaves of *S. fischeri* and *S. francisii* were particularly active against tested organism (inhibition zones comprise up to 34 mm in diameter). 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 ranged from 25 to 31 mm). The ethanolic extracts of *S. canaliculata* and *S. trifasciata* showed less antimicrobial activities (diameters of inhibition zones ranged between 16 and 16.5 mm). 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* (Buyun et al., 2016; Tkachenko et al., 2017).

In our previous study, we also studied the antioxidant activity of extracts obtained from leaves of selected species from *Sansevieria* species against oxidative stress using equine erythrocyte suspension (Tkachenko et al., 2017). When erythrocytes were incubated with leaf extracts of various species from *Sansevieria* genus, the aldehydic derivatives level was significantly reduced by 13.6% ($p < 0.05$) for *S. forskaliana* extract. Moreover, all extracts (except *S. francisii* extract) reduced the formation of intracellular aldehydic derivatives of oxidatively modified proteins (OMP) in the extracts-treated erythrocytes, but these results were non-significant. Treatment by extracts of various *Sansevieria* species reduced the concentration of ketonic derivatives of OMP when compared to untreated erythrocytes. The most potent effect was demonstrated by the *S. canaliculata*, *S. forskaliana*, *S. aethiopica*, *S. cylindrica*, *S. metallica*, *S. hyacinthoides*, and *S. kirkii* compared to control samples (phosphate buffer) (16.1, 14.7, 13.4, 12.9, 12.9, 12.7, 12.1%, respectively). However, there were no significant changes in other extracts. The experimental evidence obtained in our previous study indicated that various species of *Sansevieria* genus are a rich source of compounds that manifest antioxidant activity and can effectively protect erythrocytes against oxidative-induced damage. Thus, *S. canaliculata*, *S. forskaliana*, *S. aethiopica*, *S. cylindrica*, *S. metallica*, *S. hyacinthoides*, and *S. kirkii* may be a valuable source of natural antioxidants that may potentially be recommended for applications in medicine and veterinary practice. According to the above-mentioned antioxidant mechanisms, extracts of various species from *Sansevieria* genus may inhibit the formation of protein carbonyl by scavenging free radicals formed *in vitro*. According to many supporting documents, it can be assumed that secondary plant metabolites, i.e. polyphenolic compounds in extracts of various species from *Sansevieria* genus extract may contribute to the antioxidant activity (Tkachenko et al., 2017).

Although antimicrobial activities of extracts obtained from the leaves of various species of *Sansevieria* genus were investigated so far (Aliero et al., 2008; Sheela et al., 2012; Kingsley et al., 2013; Buyun et al., 2016, 2017; Tkachenko et al., 2017), there is still much work to do, because studies regarding their total antioxidant defences as well as assessment of marker of lipid peroxidation under *in vitro* incubation with the muscle tissue of the rainbow trout (*Oncorhynchus mykiss* Walbaum) have not been undertaken yet.

Consequently, the aim of this study was to evaluate *in vitro* the effect of buffer extract obtained from leaves of *Sansevieria caulescens* on the 2-thiobarbituric acid reactive substances (TBARS) as lipid peroxidation biomarker, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the muscle tissue of the rainbow trout.

Materials and methodology

Collection of plant material

The leaves of *Sansevieria caulescens* plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine (Figure 1).



Figure 1 General view of *Sansevieria caulescens* N.E.Br. plant (Photo by Myroslava Maryniuk)

Preparation of plant extract

Freshly collected leaves were washed, weighted, crushed, and homogenized in 0.1M phosphate buffer (pH 7.4) (in proportion 1 : 19, w/w) at room temperature. The extract was then filtered and investigated for its antioxidant capacity. The extract was 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 ± 0.5 °C and pH 7.2–7.4. The dissolved oxygen level was about 9 ppm with additional oxygen supply, with a water flow of $25 \text{ L}\cdot\text{min}^{-1}$, 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.

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 an 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 ± 0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) in duplicate. The reactions were started by adding the tissue supernatant.

Experimental design

The supernatant of the muscle tissue was used to incubate with *S. caulescens* extract (in a ratio of 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. Oxidative stress biomarkers were studied in the incubated homogenate (control group and in samples with extract).

Determination of 2-thiobarbituric acid reactive substances (TBARS)

The level of lipid peroxidation was determined by quantifying the concentration of TBARS by Kamyshnikov (2004) for determining the malonic dialdehyde (MDA) concentration. Briefly, 2.1 mL of sample homogenate was added to 1 mL of 0.8% of 2-thiobarbituric acid (TBA), and 1 mL of 20% of trichloroacetic acid (TCA). The mixture was heated in a boiling water bath for 10 min. After cooling, the mixture was centrifuged at 3000 g for 10 min. The absorbance of the supernatant was measured at 540 nm. The concentration of MDA ($\text{nmol}\cdot\text{mg}^{-1}$ of protein) was calculated using $1.56 \cdot 10^5 \text{ mM}^{-1} \text{ cm}^{-1}$ as the extinction coefficient.

The carbonyl derivatives content of protein oxidative modification (OMP) assay

To evaluate the protective effects of the extract against free radical-induced protein damage, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the samples was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) and as modified by Dubinina and co-workers (1995). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Briefly, 1 mL of 0.1M DNPH (dissolved in 2M HCl) was added to 0.1 mL of the sample after denaturation of proteins by 20% trichloroacetic acid (TCA). After addition of the DNPH solution (or 2M HCl to the blanks), the tubes were incubated for a period of 1 h at 37 °C. The tubes were spun in a centrifuge for 20 min at 3000 g. After centrifugation, the supernatant was decanted and 1 mL of ethanol-ethylacetate solution was added to each tube. Following the mechanical disruption of the pellet, the tubes were allowed to stand for 10 min and then spun again (20 min at 3000 g). The supernatant was decanted and the pellet washed thrice with ethanol-ethylacetate. After the final wash, the protein was solubilized in 2.5 mL of 8M urea solution. To speed up the solubilization process, the samples were incubated in a 90 °C water bath for 10–15 min. The final solution was centrifuged to remove any insoluble material. The carbonyl content was calculated from the absorbance measurement at 370 nm and 430 nm, and an absorption coefficient $22.000 \text{ M}^{-1}\cdot\text{cm}^{-1}$. Carbonyl groups (nmol per mg of protein) were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehyde derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

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 (Galaktionova et al., 1998). 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 FeSO₄, 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 a 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 ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences (significance level, $p < 0.05$) was examined using the 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 a present study, we have studied the influence of extracts derived from leaves of *S. caulescens* on the TBARS level as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins and the total antioxidant capacity in the muscle tissue of rainbow trout after incubation with extract obtained from leaves of *S. caulescens* in *in vitro* culture. There were no significant changes for TBARS level between the value in the control group and in the muscle tissue of rainbow trout after incubation with extracts derived from leaves of *S. caulescens* (Figure 2).

It is considered that disturbed levels of reactive oxygen species (ROS) contribute to the pathology of many disorders and diseases. ROS and oxidative stress may cause cellular damage by directly and irreversibly damaging macromolecules such as proteins, membrane lipids, and DNA; another major cellular consequence of reactive oxygen species is the reversible modification of protein thiol side chains that may affect many aspects of molecular function (Terrill et al., 2013). The redox dysregulation and ROS do not just beget further reactive species but also drive antioxidant protein expression. The redox-regulated antioxidant response offers the possibility of controlling the extent of ROS available to damage proteins and to restore the intracellular redox potential (Griffiths et al., 2014). In our study, the level of aldehydic and ketonic derivatives of oxidatively modified proteins was non-significantly changed in muscle samples incubated with an extract obtained from the leaves of *S. caulescens* (Figure 2).

The total antioxidant capacity (TAC) is determines the ability of a tested material to neutralize oxygen-free radical specific form, irrespectively to the specific antioxidant activity of present antioxidants (Wang et al., 1997). Our results showed that extract of *S. caulescens* efficiently

increased the TAC level in muscle tissue by 46.6% ($p < 0.05$) due to inhibited the Fe^{2+} /ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level (Figure 2).

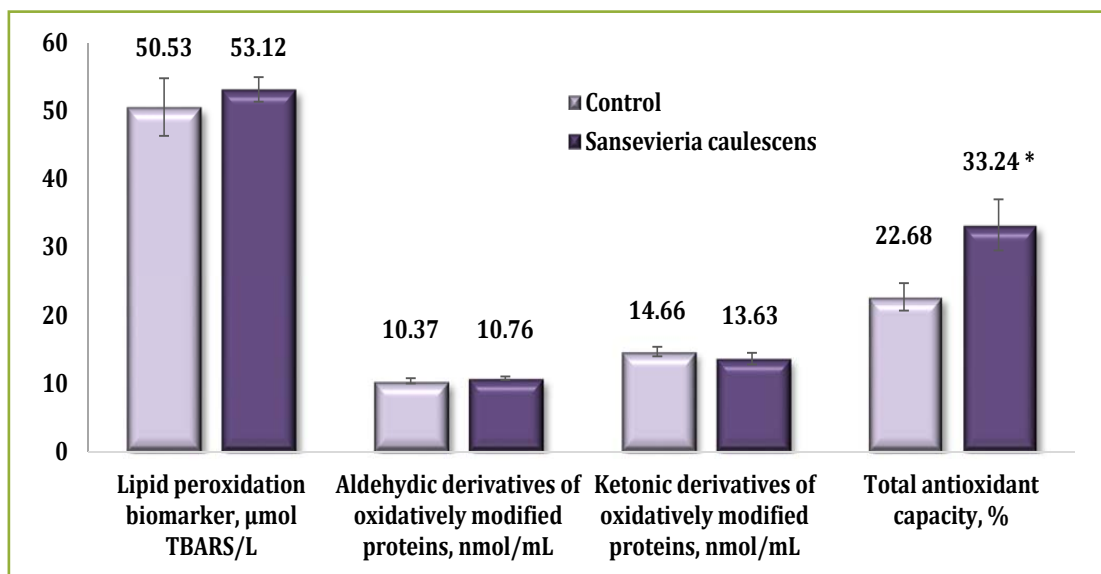


Figure 2 The level of 2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins and total antioxidant capacity in the muscle tissue of rainbow trout after incubation with extracts from leaves of *Sansevieria caulescens* ($M \pm m$, $n = 8$)

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

We suggested that high TAC value of muscle tissue is the result of a high content of by-products, i.e. alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins, carbohydrates etc. in *S. caulescens*. The antioxidant activities of most plant extracts can be traced to these bioactive constituents. For instance, various compounds belonging to the terpenoid and flavonoid groups are known to be biologically active (Grassmann, 2005; González-Burgos and Gómez-Serranillos, 2012; Bartikova et al., 2014). Due to their antioxidant behaviour terpenes have been shown to provide relevant protection under oxidative stress conditions in different diseases including liver, renal, neurodegenerative and cardiovascular diseases, cancer, diabetes as well as in ageing processes (González-Burgos and Gómez-Serranillos, 2012). The existing data indicate that monoterpenes and diterpenes, which are the main components of essential oils, act as allelopathic agents, attractants in plant-plant or plant-pathogen/herbivore interactions or repellants (Grassmann, 2005). Moreover, many sesquiterpenes biological activities (anti-inflammatory, antiparasitic and anti-carcinogenic activities) are based on antioxidant or pro-oxidant actions of sesquiterpenes. Structure, concentration, metabolism as well as the type of cells determine if sesquiterpene acts as anti-oxidant or pro-oxidant (Bartikova et al., 2014). On the other hand, the natural flavones, as well as some of their synthetic derivatives, have been shown to exhibit several biological activities, including antioxidant, anti-inflammatory, antitumor, anti-allergic, neuroprotective, cardioprotective and antimicrobial (Catarino et al., 2015). Also, flavonoids are found to have an effect on several

mammalian enzymes like protein kinases that regulate multiple cell signalling pathways and alterations in multiple cellular signalling pathways are frequently found in many diseases (Singh et al., 2014). Some flavones interfere in distinct oxidative-stress related events by directly reducing the levels of intracellular free radicals (hydroxyl, superoxide and nitric oxide) and/or of reactive species (e.g. hydrogen peroxide, peroxyxynitrite and hypochlorous acid) thus preventing their amplification and the consequent damage of other biomolecules such as lipids, proteins and DNA (Catarino et al., 2015). Flavones and flavonols re-establish the redox regulation of proteins, transcription factors and signalling cascades that are otherwise inhibited by elevated oxidative stress (Dajas et al., 2013). Flavones can also hinder the activity of central free radical-producing enzymes, such as xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase) or inducible nitric oxide synthase (iNOS) and can even modulate the intracellular levels of pro-oxidant and/or antioxidant enzymes (Catarino et al., 2015).

Indeed, the study of *S. roxburghiana* and *S. trifasciata* has revealed the presence of important compounds which were separated by thin layer chromatography (Kingsley et al., 2013). Preliminary phytochemical screening of the extracts of *S. trifasciata* plant showed the presence of alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins and carbohydrates (Anbu et al., 2009). Additionally, the methanolic extract of the whole plant of *S. trifasciata* has yielded 12 steroidal saponins, 10 of which are new constituents (Mimaki et al., 1996). Phytochemical analysis of the whole plant of *S. trifasciata* has resulted in the isolation of four new pregnane glycosides (Mimaki et al., 1997). Gas chromatographic analysis of the leaves revealed the presence of alkaloids, allcins, glycosides, and saponins (Ikewuchi et al., 2011). Pettit et al. (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* (Pettit et al., 2005). In addition, a new steroidal saponin from the leaves of *S. cylindrica* was isolated by Da Silva Antunes et al. (2003). The steroidal saponin showed no hemolytic effects in the *in vitro* assays and demonstrated inhibition of the capillary permeability activity (Da Silva Antunes et al., 2003). Accumulated evidence suggests that saponins have significant neuroprotective effects on attenuation of central nervous system disorders, such as stroke, Alzheimer's disease, Parkinson's disease, and Huntington's disease due to mechanisms of their neuroprotective function including antioxidant, modulation of neurotransmitters, anti-apoptosis, anti-inflammation, attenuating Ca²⁺ influx, modulating neurotrophic factors, inhibiting tau phosphorylation, and regeneration of neural networks (Sun et al., 2015).

Our study is in agreement with results obtained by other researchers. They have revealed hypoglycemic, hypolipidemic, immune-modulating, anti-inflammatory, ocular-, hepato-renal and cardio-protective potentials of other species of *Sansevieria* genus. Chigozie and Chidinma

(2013) have investigated the ability of an aqueous extract of the leaves of *S. liberica* to alter the hematology, plasma biochemistry and ocular indices of oxidative stress in alloxan-induced diabetic rats. Diabetes mellitus was induced by injection of alloxan (80 mg.kg⁻¹ body weight), via the tail vein. The extract was administered orally at 100, 200 and 300 mg.kg⁻¹ body weight (both to normal and diabetic rats), and metformin at 50 mg.kg⁻¹ body weight. Compared to test control, the treatment dose-dependently, significantly lowered ($p < 0.05$) ocular malondialdehyde content, atherogenic indices, red cell, total white cell and lymphocyte counts, mean cell hemoglobin concentration; and plasma levels of glucose, triglyceride, total-, very low density lipoprotein-, low density lipoprotein- and non-high density lipoprotein cholesterols, total, conjugated and unconjugated bilirubin, sodium, urea, blood urea nitrogen, as well as plasma activities of alkaline phosphatase, alanine and aspartate transaminases. However, the treatment significantly increased ($p < 0.05$) hematocrit, hemoglobin concentration, mean cell hemoglobin, and mean cell volume, neutrophil and monocyte counts, and plasma levels of high density lipoprotein cholesterol, potassium, chloride, calcium, bicarbonate and total protein, ocular ascorbic acid content and ocular activities of catalase and superoxide dismutase (Chigozie and Chidinma, 2013).

The leaves of *S. liberica* also possess anti-inflammatory effects. Chinasa et al. (2011) have evaluated the anti-inflammatory property of the leaves of *S. liberica* and to ascertain the toxicity and phytochemical profiles of the extract of the leaves. The crude extract was then fractionated into n-hexane fraction (HF), chloroform fraction (CF), ethyl acetate fraction (EF) and methanol fraction (MF). The crude extract and the fractions were screened for anti-inflammatory activity using egg albumen-induced paw (systemic) edema in rats as a measure of acute inflammation. The crude extract and the fractions significantly ($p < 0.05$) inhibited the development of paw edema induced by egg albumen in rats. The potency/activity of the crude extract and the fractions increased in the order HF > CE > MF > CF > EF, with the crude extract and HF at 400 mg.kg⁻¹ exhibiting inhibition comparable to that obtained with 5 mg.kg⁻¹ diclofenac sodium. Phytochemical screening of the crude extract and the fractions showed the presence of various bioactive substances such as alkaloids, saponins, flavonoids, terpenoids, steroids, glycosides, reducing sugars, tannins, resins, carbohydrates, proteins, acidic compounds, fats and oils (Chinasa et al., 2011).

The extract of the whole plant part of *S. roxburghiana* might possess some chemical constituents that are responsible for analgesic, cytotoxic and antioxidant activities. Roy et al. (2012) have investigated the crude methanolic extract of the whole plant part of *S. roxburghiana* and the possibility of analgesic, cytotoxic and antioxidant activities. The analgesic activity was assessed by acetic acid induced writhing test in mice. The cytotoxic activity was evaluated by brine shrimp lethality bioassay while the antioxidant effect was measured by 1, 1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) free radical scavenging assay. The ethyl acetate soluble fraction of the crude extract was found to have significant ($p < 0.001$) analgesic activity at the oral dose of 100 mg.kg⁻¹ body weight. The crude methanolic extract along with its all partitions revealed mild to moderate free radical scavenging activity (Roy et al., 2012).

Oxidative stress is considered to be an important component of various diseases. Identifying markers of oxidative stress has been the focus of many researchers as they have the potential

to act as an “integrator” of a multitude of processes that drive various kind of pathobiology (Ho et al., 2013). Therefore, in our opinion, any research assessing the biomarkers of oxidative stress has fundamental importance for the treatment of various diseases and disorders. On the other hand, plants with antioxidant potential may offer alternative therapeutic agents in the aquaculture industry.

Conclusions

Our study suggests that the *S. caulescens* leaf extract has shown good antioxidant potential *in vitro* study after incubation with muscle tissue homogenate of rainbow trout. There were no significant changes for TBARS level as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins between values in control group and in the muscle tissue of rainbow trout after incubation with extracts from leaves of *S. caulescens*. Nevertheless, our results showed that extract of *S. caulescens* efficiently increased the total antioxidant capacity in muscle tissue by 46.6% ($p < 0.05$) due to inhibited the Fe^{2+} /ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. Taking into account existing experimental evidence, it is reasonable to assume that secondary plant metabolites, i.e. polyphenolic compounds in the extract of *S. caulescens* may contribute to the antioxidant activity. In conclusion, the results of this study provide a new perspective on the use of various *Sansevieria* species as a medicinal plant to improve the antioxidant response of rainbow trout. 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. Finally, research needs to be focused on subjecting fish to these compounds to determine their effectiveness, stability, and impact both on the host and on the environment.

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