Department of Pharmaceutical Biology[1], Dahlem Centre of Plant Sciences, Freie Universität, Berlin; WALA Heilmittel GmbH[2], Bad Boll/Eckwälden, Germany

SOD activity and extremophilicity: a screening of various plant species

E. Woith[1], F. Stintzing[2], M. F. Melzig[1,*]

Received March 2, 2017, accepted May 5, 2017

**Corresponding author: Prof. Dr. Matthias F. Melzig, Freie Universität Berlin, Department of Pharmaceutical Biology, Königin-Luise-Str. 2+4, 14195 Berlin, Germany*
melzig@zedat.fu-berlin.de

Pharmazie 72: 490–496 (2017) *doi: 10.1691/ph.2017.7493*

All aerobes are dependent on enzymatic and non-enzymatic antioxidants to withstand the presence of reactive oxygen species (ROS). Superoxide dismutase (SOD) is a part of the enzymatic antioxidant system. It is one of the most important antioxidant enzymes, enabling organisms to survive in an oxygen containing atmosphere. A disorder in the oxidative and antioxidative balance can be associated with the occurrence of diseases in human organisms. Little data exist on the relevance of SOD in plants. Moreover, it is not known whether there is any association between a plant´s origin and its SOD activity. Our screening of 27 different plant species was intended to expose whether there is a connection. The highest SOD activities were found for extremophile plants. Especially the Crassulaceae *Aeonium haworthii* Salm-Dyck ex Webb & Berthel. and *Crassula multiflora* Schönland & Baker f. were highly active. Nevertheless, we did not find unambiguous evidence for a correlation between extremophilicity and SOD activity.

1. Introduction

In general, reactive oxygen species (ROS) are important in physiological intracellular messaging or in defense against diseases (Bae et al. 1997; Apel and Hirt 2004). Elevated concentrations of ROS, however, can cause oxidative stress. Oxidative stress is the shift of physiological steady state between oxidant and antioxidant processes in favor of oxidants (Sies 1991; Gill and Tuteja 2010). It can cause various damages on cell-structures and thus malfunction of the cell (Chopra and Wallace 1998; Matés et al. 1999).

It is thought that superoxide dismutase (SOD) is one of the most important enzymes enabling organisms to survive the presence of molecular oxygen (McCord et al. 1971). Because of the high importance of SOD for aerobes, we focused on this agent as a representative of the enzymatic antioxidant system.

The disproportionation of superoxide ($O_2^{\cdot-}$) can be summarized into Eq. (1) (McCord and Fridovich 1969).

$$2O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2 \quad (1)$$

Different SOD isozymes can be classified by their metal co-factor. The following metal ions have been found in the active sites of SODs: copper and zinc (CuZnSOD), manganese (MnSOD), iron (FeSOD) and nickel (NiSOD) (Alscher et al. 2002; Manaa et al. 2014; Sheng et al. 2014).

The dismutation of $O_2^{\cdot-}$ is very similar in all isozymes. It follows a so called "ping-pong-mechanism" as shown in Fig. 1 for a CuZnSOD, selected as a representative. Since the oxidation number of the metal co-factor switches back and forth, it is called "ping-pong-mechanism" (Sheng et al. 2014). Different investigations have shown that SOD activity can be induced by environmental factors, such as UV light (Jenkins 2009; Rybus-Zając and Kubiś 2010), low temperature (Spychalla and Desborough 1990; Nayyar and Chander 2004), salt stress (Manaa et al. 2014), heavy metals (Gratão et al. 2006), or paraquat (Alscher et al. 2002). In this study, we evaluated whether extremophile plants show higher SOD activities compared to plants originating from tempered climate zones (in this investigation plants are called "extremophile" when they originate from a cold zone, tropical or subtropic zone, or from great altitude).

An imbalance between ROS and antioxidants plays a role in the pathogenesis in a row of human diseases such as ischemia/reperfusion injury, atherosclerosis, neurodegenerative diseases, and cancer (Matés et al. 1999). Danno et al. (1984) showed that SOD reduces the occurrence of sunburn cells in mice, when injected either before or after UV-irradiation. Topical application of SOD containing creams possibly bears beneficial effects for the skin, protecting it from oxidative damage. Thus, SOD could be a promising agent in prevention and therapy of distinct skin disorders, e.g. photoaging, sunburn, and cancer (Benyahia et al. 1996; Chen et al. 2016; Ichihashi et al. 2003; Mizushima et al. 1991; Wenk et al. 2001; Zhao et al. 2001).

Abbreviations:
BCA bicinchoninic acid;
FCR Folin Ciocalteu´s reagent;
NSA normalized SOD activity;
ROS reactive oxygen species;
SAPM SOD activity per gram plant material;
SOD superoxide dismutase;
WST water-soluble tetrazolium;
X xanthine;
XO xanthine oxidase.

2. Investigations and results

SOD activities are presented as two different values. On the one hand, we estimated SOD activity per gram plant material (SAPM). On the other hand, we determined the "normalized SOD activity" (NSA). We defined NSA as SOD activity per mg total protein. SAPMs of the 27 investigated plants ranged from 0.5-297 µKat/g. NSAs did not scatter as broad as SAPMs. They ranged from 0.08-1.25 µKat/mg total protein. SOD activities are shown in Fig. 2.

Further we investigated the induction of SOD activity by UV stress and cold. Therefore, we irradiated *Mesembryanthemum crystallinum* L. with UV light (254 and 366 nm of wavelength) over 8 days for one hour per day. Irradiance was 600 µW/cm² at 254 nm and 1100 µW/cm² at 366 nm, in the adjusted average-distance of 17 cm (UV-lamp was received from CAMAG, Muttenz, Switzerland). Another plant was stored in the dark at 4 °C. For control a third plant was stored at room temperature. Determination of SOD

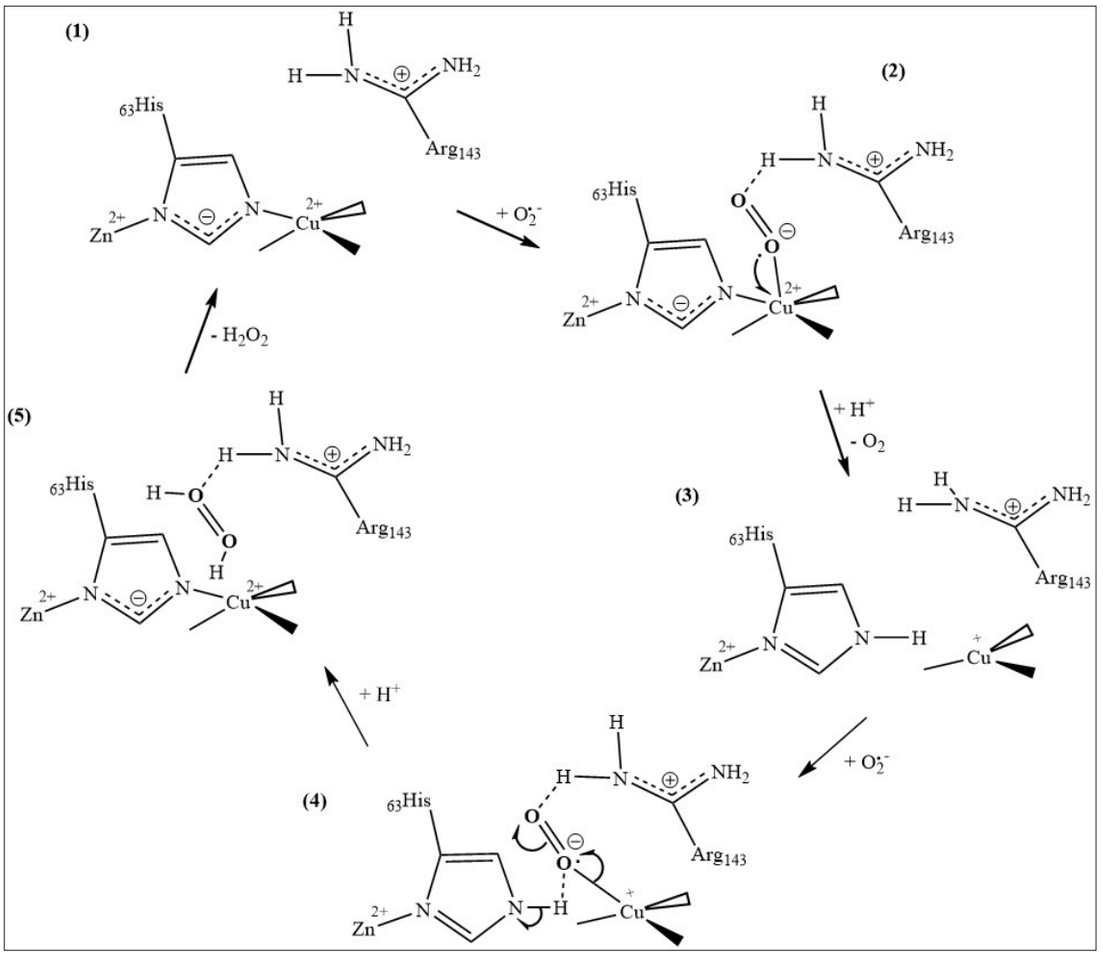

Fig. 1: Mechanism of superoxide scavenging by CuZnSOD, modified after (Tainer et al. 1983; Hart et al. 1999): **(1)** resting state (oxidized form)- active site empty **(2)** superoxide enters active site and reduces Cu(II) to Cu(I) under formation of oxygen **(3)** resting state (reduced form) **(4)** second superoxide enters active site and oxidizes Cu(I) to Cu(II) **(5)** formation and release of hydrogen peroxide

activity was accomplished on day 9. In order to test whether the rise of SOD activity was statistically significant, we conducted an one-sided Mann-Whitney U test ($p \leq 0.05$). SOD activities increased approximately 2.5 fold after UV-irradiation and 1.5 fold when stored cold. The results are shown in Fig. 3.

M. crystallinum was selected as a representative extremophile plant. Pharmaceutical or dermocosmetic formulations of *M. crystallinum* show antioxidant and antibacterial properties and benefit skin hydration. Therefore, they are of therapeutical interest (Schario et al. 2014). Further, due to the low content of phenolic compounds (Falleh et al. 2009), interactions to SOD Assay Kit were negligible (see also 3.1).

3. Discussion

3.1. Interactions between SOD Assay Kit and phenolic compounds

It is known that flavonoids inhibit xanthineoxidase (Iio et al. 1985). Further phenols can stabilize unpaired electrons in their π-electron-cloud (Rice-Evans et al. 1996). Both mechanisms would lead to false positive results for SOD activity. For this reason, we investigated the influence of rutoside (Sigma Aldrich, St. Louis, USA) and quercetin (Carl Roth, Karlsruhe, Germany) on SOD Assay Kit-WST (water-soluble tetrazolium). We found that final rutoside- or quercetin-concentrations of 1-4 µM interfered slightly with the SOD Assay Kit-WST. Thus, we defined three categories for the plants: category A - no interference expected (final phenol-concentration less than 1 µM, calculated as rutoside), category B - interference negligible (final phenol-concentration between 1 and 4 µM) and category C - interference very likely (final phenol-concentration higher than 4 µM). Categories of the investigated plants are shown in the Table. Since we could not find any correlation between phenol-content and determined SOD activity, we expect our data of category A and B-plants to be very reliable, whereby SOD activities of plants in category C rather show a "superoxide scavenging capacity".

3.2. Adaptations on abiotic stress in plants

As already mentioned, some environmental factors can trigger the rise of SOD activity. In our study, intensified UV-irradiation and cold storage resulted in increased levels of SOD activity. This is probably due to the fact that UV-irradiation (Hideg and Vass 1996; Rybus-Zając and Kubiś 2010; Nawkar et al. 2013; Yokawa et al. 2016) and cold (Spychalla and Desborough 1990; Sala 1998; Nayyar and Chander 2004) cause higher amounts of ROS and thus oxidative stress in plants. Plant cells seem to respond to this oxidative stress by upregulation of SOD expression.

Being sessile, plants have developed a set of physiological adaptation mechanisms to cope with stress, among which is SOD (Dat et al. 2000; Gill and Tuteja 2010). Spychalla and Desborough (1990) could show that cold storage of potato tubers induces not only SOD activity, but also the content of α-tocopherol and catalase activity, while the content of soluble proteins decreased. In response to UV-irradiation, plants can produce higher amounts of non-enzymatic antioxidants (e.g. flavonoids and carotenoids (Middleton and Teramura 1993; Solovchenko and Schmitz-Eiberger 2003)), as

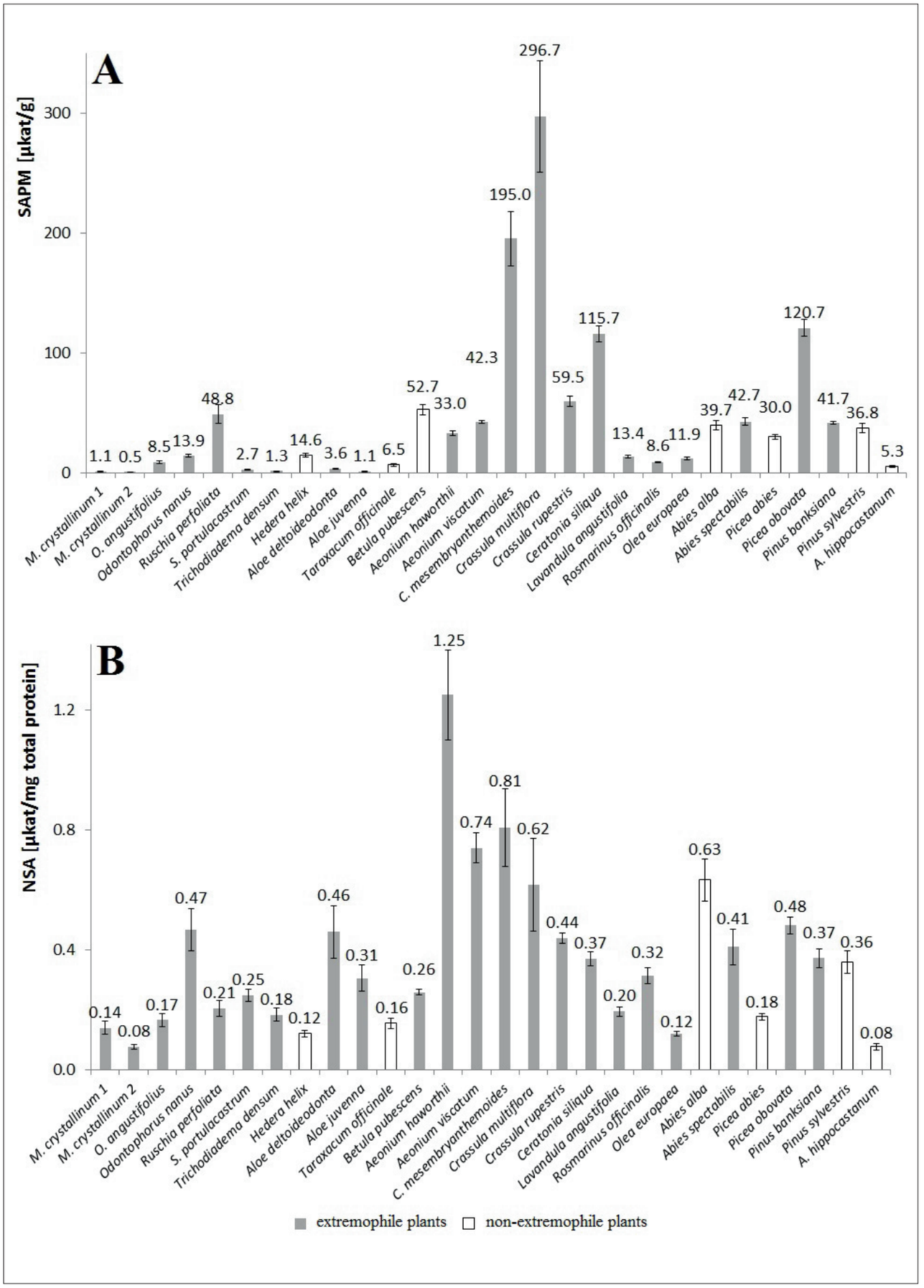

Fig. 2: Mean SAPM (A) and NSA (B) ± standard deviation (n= 9); 1- South Africa, 2- Tenerife

Table: Plant material - species, family, place of collection, and category of potential interaction of phenols to SOD assay Kit-WST

Plant	Family	Place of collection	Accession number*	Category **
Mesembryanthemum crystallinum L. [e]	*Aizoaceae* Martinov	1- South Africa (culture) 2- Tenerife (wild)	-	B
Odontophorus angustifolius L. Bolus [e]	*Aizoaceae* Martinov	Botanical Garden Berlin	134-77-74-80/1	B
Odontophorus nanus L. Bolus [e]	*Aizoaceae* Martinov	Botanical Garden Berlin	213-16-90-10/1	C
Ruschia perfoliata Schwantes [e]	*Aizoaceae* Martinov	Botanical Garden Berlin	134-83-74-80/1	B
Sesuvium portulacastrum (L.) L. [e]	*Aizoaceae* Martinov	Botanical Garden Berlin	304-04-95-30/1	B
Trichodiadema densum Schwantes [e]	*Aizoaceae* Martinov	Botanical Garden Berlin	134-97-74-80/1	B
Hedera helix L. [n]	*Araliaceae* Juss.	Institute of Pharmacy	-	B
Aloe deltoideodonta Baker [e]	*Asphodelaceae* Juss.	Botanical Garden Berlin	125-40-74-80	A
Aloe juvenna Brandham & S. Carter [e]	*Asphodelaceae* Juss.	Botanical Garden Berlin	124-01-04-80/1	A
Taraxacum officinale L. [n]	*Asteraceae* Bercht. & J. Presl	Instute of Pharmacy, Berlin	-	B
Betula pubescens Ehrh. [n]	*Betulaceae* Gray	Institute of Pharmacy, Berlin	-	B
Aeonium haworthii Salm-Dyck ex Webb & Berthel. [e]	*Crassulaceae* J. St.-Hil.	Botanical Garden Berlin	124-03-94-30/1	A
Aeonium viscatum Bolle [e]	*Crassulaceae* J. St.-Hil.	Botanical Garden Berlin	233-02-11-30/1	A
Crassula mesembryanthemoides D. Dietr. [e]	*Crassulaceae* J. St.-Hil.	Botanical Garden Berlin	125-97-74-80/1	A
Crassula multiflora Schönland & Baker f. [e]	*Crassulaceae* J. St.-Hil.	Botanical Garden Berlin	001-32-74-80/1	A
Crassula rupestris L. [e]	*Crassulaceae* J. St.-Hil.	Botanical Garden Berlin	061-62-74-80/1	A
Ceratonia siliqua L. [e]	*Fabaceae* Lindl.	Cyprus (wild)	-	B
Lavandula angustifolia Mill. [e]	*Lamiaceae* Martinov	Cyprus (wild)	-	B
Rosmarinus officinalis L. [e]	*Lamiaceae* Martinov	local supermarket	-	B
Olea europaea L. [e]	*Oleaceae* Hoffmanns. & Link	Cyprus (wild)	-	C
Abies alba Mill. [n]	*Pinaceae* Spreng. ex Rudolphi	Botanical Garden Berlin	100-63-74-80	B
Abies spectabilis (D. Don) Spach [e]	*Pinaceae* Spreng. ex Rudolphi	Botanical Garden Berlin	309-22-85-10/247	B
Picea abies (L.) H. Karst. [n]	*Pinaceae* Spreng. ex Rudolphi	Botanical Garden Berlin	181-52-74-80/302	B
Picea obovata Ledeb. [e]	*Pinaceae* Spreng. ex Rudolphi	Botanical Garden Berlin	181-64-74-80	B
Pinus banksiana Lamb. [e]	*Pinaceae* Spreng. ex Rudolphi	Botanical Garden Berlin	060-02-99-10/101	B
Pinus sylvestris L. [n]	*Pinaceae* Spreng. ex Rudolphi	Botanical Garden Berlin	182-01-74-80/255	B
Aesculus hippocastanum L. [n]	*Sapindaceae* Juss.	private garden	-	C

[e] species evaluated as extremophile
[n] species evaluated as non-extremophile

* plant species without accession number of the Botanical Garden Berlin were confirmed by comparison to the herbaria of the Institute of Pharmacy, Freie Universitaet Berlin and Botanical Museum Berlin

**category A – no interference expected

**category B – interference negligible

**category C – interference very likely

well as antioxidant enzymes like ascorbate peroxidase, glutathione reductase, and peroxidase (Rao et al. 1996). Plants are also known to respond to abiotic conditions by expressing heat-shock proteins and chaperones. Such abiotic conditions can be e.g. elevated or decreased temperature, as well as drought or osmotic stress (Vierling 1991; Boston et al. 1996; Waters et al. 1996; Wang et al. 2004).

3.3. SOD activity and extremophilicity

We determined that SOD activities varied widely between distinct species. Further, especially depending on the habitat, SOD activity can variegate in one and the same plant as well, as we could show for *M. crystallinum*. This plant´s SOD activity can rise about 2.5 fold when irradiated with UV light. This demonstrates that the presented data are one snapshot of a fluctuating biologic system.
In this study, the highest SOD activities were found for plants of the genera *Aeonium* Webb & Berthel. and *Crassula* L. (*Crassulaceae* J. St.-Hil.). Although we evaluated these genera as extremophile, we could not find a clear association between extremophilicity and the SOD activity. This conclusion is reinforced by the fact that some extremophile species (e.g. *M. crystallinum*, *Sesuvium portulacastrum* (L.) L. and *Trichodiadema densum* Schwantes) yielded comparatively low values - even lower than in typical species of the tempered zone like *Hedera helix* L., *Betula pubescens* Ehrh. or *Taraxacum officinale* L. In our collected data, we did not recognize any consistent systematics between a plant's origin and its SOD activity. Further work is needed to clarify the relevance of SOD in plants.

4. Experimental

4.1. Plant material

Leaves of 27 different plant species, belonging to 11 families were investigated. We screened aqueous extracts of the plant material for SOD activity and total protein. Methanolic extracts were screened for content of phenols. The analyzed plant species are listed in the Table with their family, place of collection, and categories of potential interaction of phenols to SOD assay Kit-WST. The evaluations, whether the species were evaluated as extremophile or non-extremophile, are also indicated.

4.2. Extract preparation

Aqueous extracts were prepared in triplicates by freezing leaves in liquid nitrogen and grounding in a nitrogen cooled mortar and pestle. Then 25±2 mg powdered plant material were passed into reaction tubes. Subsequently, 1.0 ml of TRIS-buffer (50 mM TRIS + 1 mM EDTA, pH 7.8 adjusted with HCl) was added. TRIS was received from Merck, Darmstadt, Germany; EDTA was provided by Carl Roth, Karlsruhe, Germany; and HCl by VWR, Darmstadt, Germany.
For determination of phenols, a methanolic extract was prepared from 5 g powdered plant material in 200 ml methanol by boiling for 60 min under reflux. Crude extract was concentrated in rotavapor (IKA, Staufen, Germany), filtered into volumetric flask, and volume adjusted to 50 ml.

4.3. SOD activity

SOD activities were screened in the aqueous extracts of 27 different plant species listed in the Table. For screening, we used the SOD Assay Kit-WST (Donjindo,

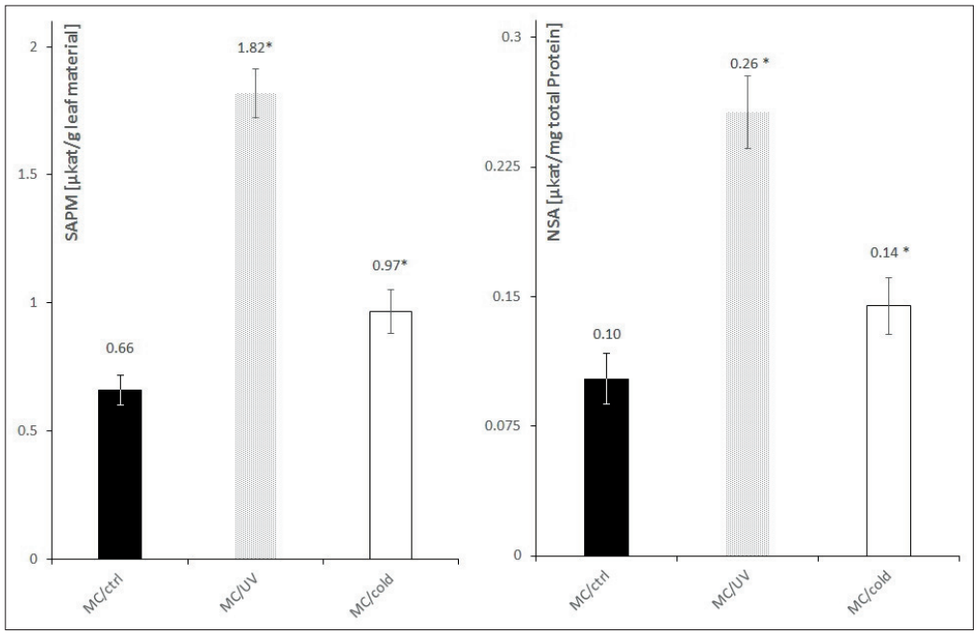

Fig. 3: Mean SAPM and NSA ± standard deviation (n= 9) of *Mesembryanthemum crystallinum* (MC) in untreated plant (ctrl), after UV-irradiation (UV) and cold storage (cold); * significant rise of SOD activity (one-sided Mann-Whitney U test, p≤ 0.05)

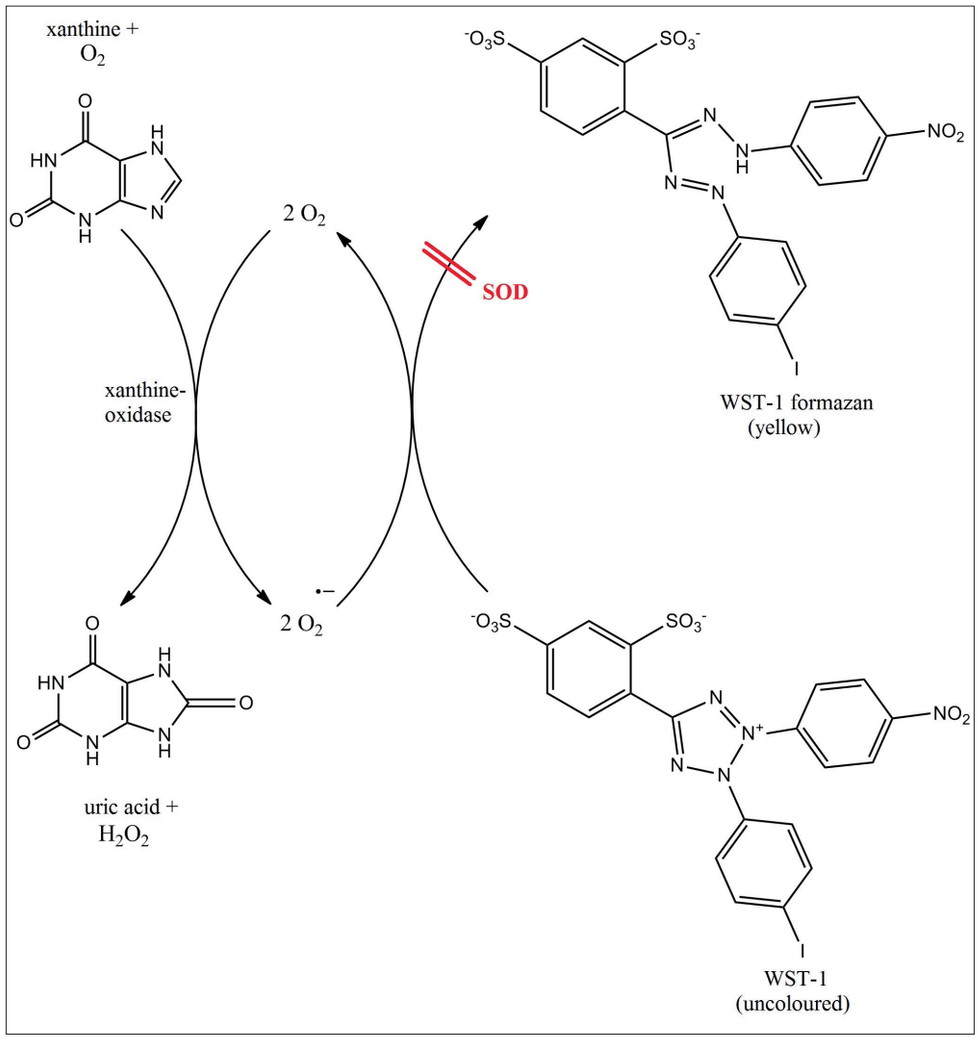

Fig. 4: Mechanism of SOD Assay Kit-WST, modified after (Dojindo-Laboratories 2011)

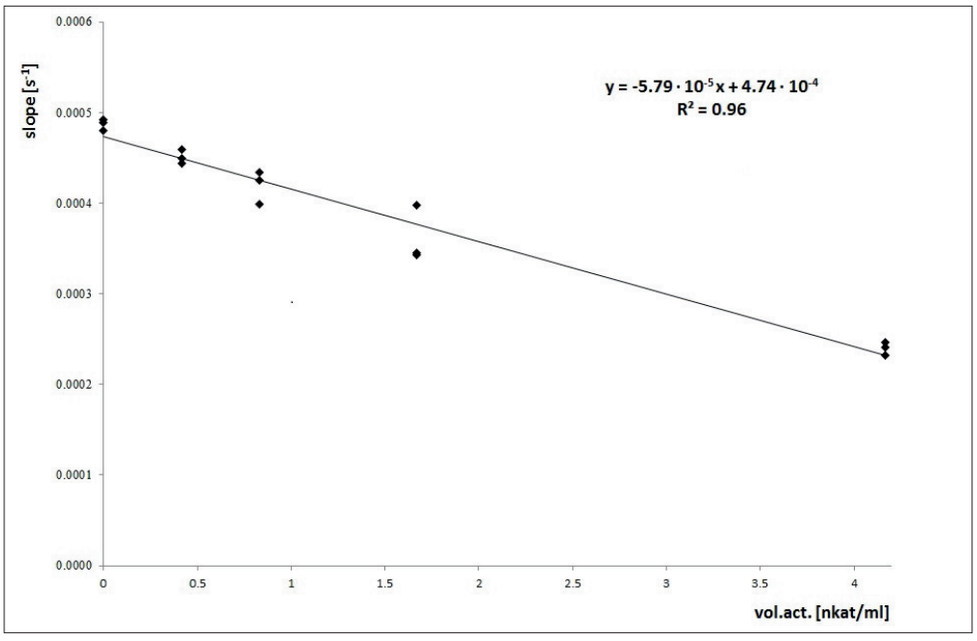

Fig. 5: Calibration of SOD Assay Kit-WST

Kumamoto, Japan) with SOD from bovine erythrocytes as standard (Sigma Aldrich, St. Louis, USA). The kit uses xanthine/xanthine oxidase (X/ XO) to generate superoxide. $O_2^{\cdot-}$ reduces uncolored WST-1 to the yellow WST-1 formazan dye. SOD inhibits this reduction by scavenging $O_2^{\cdot-}$. The mechanism of SOD Assay Kit-WST is shown in Fig. 4.

For calibration, SOD standard was diluted with TRIS-buffer to volume-activities between $4.17 \cdot 10^{-4}$ µkat/ml and $4.17 \cdot 10^{-3}$ µkat/ml. Dilution of aqueous plant extracts was adjusted into calibration range by preliminary tests. Measurement was performed on 96-well microplates (Carl Roth, Karlsruhe, Germany), absorbance at 450 nm was detected every minute by a Tecan Infinite F200 Microplate Reader (Tecan Group, Maennedorf, Switzerland), temperature was set to 37 °C. Reaction volumes were added as specified in the kit´s manual (Dojindo-Laboratories 2011). Measurement was terminated after 16 minutes (optimization of measuring time was performed in preliminary tests). Calculation of SOD activities was conducted by plotting slopes against concentrations, as shown in Fig. 5. All solutions were surveyed in triplicates, for blank we exchanged enzyme working solution with TRIS-buffer.

4.4. Protein content

For determination of protein content, we used Pierce bicinchoninic acid (BCA) assay kit (Thermo Fisher, Waltham, USA). All measurements were performed as specified in distributor´s manual (Thermo-Scientific 2013). For calibration, we used bovine serum albumin as standard (Carl Roth, Karlsruhe, Germany).

4.5. Total phenols

To determine the phenolic content of the plant material we used Folin Ciocalteu´s reagent (FCR), received from Sigma Aldrich (St. Louis, USA). We adapted the method to our lab conditions, after various templates (Singleton and Rossi 1965; Ainsworth and Gillespie 2007; Cicco et al. 2009; Agbor et al. 2014). As standard we used rutoside in concentrations between 25 and 750 µM. FCR was diluted one to ten with demineralized water. To avoid precipitation of FCR we diluted methanolic solutions and extracts in equal volumes of demineralized water. A solution of 7.5% sodium carbonate in distilled water (w/v) was added to start the reaction. 210 µl reaction volume was composed by 20 µl sample + 40 µl diluted FCR + 150 µl sodium carbonate solution. Microplates were shaken and then incubated at room temperature in the dark for 30 min. Absorbance was measured with microplate reader at 620 nm. All solutions were investigated in triplicates, for blanks FCR was replaced by demineralized water.

Acknowledgment: We are grateful to the employees of the Botanical Garden Berlin for the accommodating supply of the majority of the investigated plant material.
This work was supported by WALA Heilmittel GmbH.

Conflicts of interest: None declared.

References

Agbor GA, Vinson JA, Donnelly PE (2014) Folin-ciocalteau reagent for polyphenolic assay. Int J Food Sci Nutr 3: 147-156.
Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using folin-ciocalteu reagent. Nat Protoc 2: 875-877.
Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53: 1331-1341.
Apel K, Hirt H (2004) Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55: 373-399.
Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, Rhee SG (1997) Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. J Biol Chem 272: 217-221.
Benyahia B, Campana F, Perdereau B, Gez E, Fourquet A, Magdelenat H (1996) Effects of superoxide dismutase topical treatment on human skin radiofibrosis: a pathological study. Breast J 5: 75-81.
Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and protein folding in plants. Plant Mol Biol 32: 191-222.
Chen X, Liu S, Rao P, Bradshaw J, Weller R (2016) Topical application of superoxide dismutase mediated by HIV-TAT peptide attenuates UVB-induced damages in human skin. Eur J Pharm Biopharm 107: 286-294.
Chopra S, Wallace HM (1998) Induction of spermidine/spermine N1-acetyltransferase in human cancer cells in response to increased production of reactive oxygen species. Biochemical Pharmacology 55: 1119-1123.
Cicco N, Lanorte MT, Paraggio M, Viggiano M, Lattanzio V (2009) A reproducible, rapid and inexpensive folin-ciocalteu micro-method in determining phenolics of plant methanol extracts. Microchem J 91: 107-110.
Danno K, Horio T, Takigawa M, Imamura S (1984) Role of oxygen intermediates in UV-induced epidermal cell injury. Journal of Investigative Dermatology 83: 166-168.
Dat J, Vandenabeele S, Vranová E, van Montagu M, Inzé D, van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57: 779-795.
Dojindo-Laboratories (2011). SOD Assay Kit- WST: Technical Manual. Kumamoto (Japan).
Falleh H, Ksouri R, Oueslati S, Guyot S, Magné C, Abdelly C (2009) Interspecific variability of antioxidant activities and phenolic composition in mesembryanthemum genus. Food Chem Toxicol 47: 2308-2313.
Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48: 909-930.
Gratão PL, Pompeu GB, Cardoso PF, Lea PJ, Azevedo RA (2006) Plant antioxidant responses to toxic elements. Curr Top Biochem Res 8: 41-70.
Hart PJ, Balbirnie MM, Ogihara NL, Nersissian AM, Weiss MS, Valentine JS, Eisenberg D (1999) A structure-based mechanism for copper-zinc superoxide dismutase. Biochemistry 38: 2167-2178.
Hideg É, Vass I (1996) UV-B induced free radical production in plant leaves and isolated thylakoid membranes. Plant Sci 115: 251-260.
Ichihashi M, Ueda M, Budiyanto A, Bito T, Oka M, Fukunaga M, Tsuru K, Horikawa T (2003) UV-induced skin damage. Toxicology 189: 21-39.
Iio M, Moriyama A, Matsumoto Y, Takaki N, Fukumoto M (1985) Inhibition of xanthine oxidase by flavonoids. Agric Biol Chem 49: 2173-2176.
Jenkins GI (2009) Signal transduction in responses to UV-B radiation. Annu Rev Plant Biol 60: 407-431.
Manaa A, Mimouni H, Terras A, Chebil F, Wasti S, Gharbi E, Ahmed HB (2014) Superoxide dismutase isozyme activity and antioxidant responses of hydroponically cultured lepidium sativum l. to NaCl stress. J Plant Interact 9: 440-449.
Matés JM, Pérez-Gómez C, de Castro IN (1999) Antioxidant enzymes and human diseases. Clin Biochem 32: 595-603.
McCord JM, Fridovich I (1969) Superoxide dismutase- an enzymic function for erythrocuprein (hemocuprein). J Biol Chem 244: 6049-6055.

McCord JM, Keele BB, Fridovich I (1971) An enzyme- based theory of obligate anaerobiosis: The physiological function of superoxide dismutase. Proc Natl Acad Sci U S A 68: 1024-1027.
Middleton EM, Teramura AH (1993) The role of flavonol glycosides and carotenoids in protecting soybean from ultraviolet-B damage. Plant Physiol 103: 741-752.
Mizushima Y, Hoshi K, Yanagawa A, Takano K (1991) Topical application of superoxide dismutase cream. Drugs Exp Clin Res 17: 127-131.
Nawkar G, Maibam P, Park J, Sahi V, Lee S, Kang C (2013) UV-induced cell death in plants. Int J Mol Sci 14: 1608-1628.
Nayyar N, Chander S (2004) Protective effects of polyamines against oxidative stress induced by water and cold stress in chickpea. J Agron Crop Sci 190: 355-365.
Rao MV, Paliyath G, Ormrod DP (1996) Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of arabidopsis thaliana. Plant Physiol 110: 125-136.
Rice-Evans CA, Miller NJ, Pagangea G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20: 933-956.
Rybus-Zając and Kubiś J (2010) Effect of UV-B radiation on antioxidative enzyme activity in cucumber cotyledons. Acta Biol Cracoviensia Ser Bot 52: 97-102.
Sala JM (1998) Involvement of oxidative stress in chilling injury in cold-stored mandarin fruits. Postharvest Biol Technol 13: 255-261.
Scharioo M, Lünnemann L, Stroux A, Reisshauer A, Zuberbier T, Blume-Peytavi U, Garcia Bartels N (2014) Children with dry skin and atopic predisposition: daily use of emollients in a participant-blinded, randomized, prospective trial. Skin Pharmacol Physiol 27: 208-216
Sheng Y, Abreu IA, Cabelli DE, Maroney MJ, Miller A-F, Teixeira M, Valentine JS (2014) Superoxide dismutases and superoxide reductases. Chem Rev 114: 3854-3918.
Sies H (1991) Oxidative stress: From basic research to clinical application. Am J Med 91: 31-38.
Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16: 144-158.
Solovchenko A, Schmitz-Eiberger M (2003) Significance of skin flavonoids for UV-B-protection in apple fruits. J Exp Bot 54: 1977-1984.
Spychalla JP, Desborough S (1990) Superoxide dismutase, catalase and alpha-tocopherol content of stored potato tubers. Plant Physiol 94: 1214-1218.
Tainer JA, Getzoff ED, Richardson JS, Richardson DC (1983) Structure and mechanism of copper, zinc superoxide dismutase. Nature 306: 284-287.
Thermo-Scientific (2013). Instructions: Pierce BCA Protein Assay Kit. Rockford (USA).
Vierling E (1991) The roles of heat shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 42: 579-620.
Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9: 244-252.
Waters ER, Lee GJ, Vierling E (1996) Evolution, structure and function of the small heat shock proteins in plants. J Exp Bot 47: 325-338.
Wenk J, Brenneisen P, Meewes C, Wlaschek M, Peters T, Blaudschun R, Ma W, Kuhr L, Schneider L, Scharffetter-Kochanek K (2001) UV-induced oxidative stress and photoaging. Curr Probl Dermatol 29: 83-94.
Yokawa K, Kagenishi T, Baluška F (2016) UV-B induced generation of reactive oxygen species promotes formation of BFA-induced compartments in cells of arabidopsis root apices. Front Plant Sci 6: 1162.
Zhao Y, Xue Y, Oberley TD, Kiningham KK, Lin S-M, Yen H-C, Majima H, Hines J, StClair D (2001) Overexpression of manganese superoxide dismutase suppresses tumor formation by modulation of activator protein-1 signaling in a multistage skin carcinogenesis model. Cancer Res 61: 6082-6088.