*IOSR Journal of Applied Chemistry (IOSR-JAC)*
*e-ISSN: 2278-5736.Volume 13, Issue 12 Ser. II (December. 2020), PP 46-50*
*www.iosrjournals.org*

# Antioxidant capacity of *Calendula officinalis L.* flower extracts

Adriana Sales[a] Mirzeta Saletović[b],
*a Instituto de Química Analítica Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán.*
*Ayacucho 471. S. M. de Tucumán. CP 4000. Argentina*
*b Faculty of Natural Science and Mathematics, University of Tuzla, 75000 Tuzla Bosnia and Herzegovina*

***Abstract***
*Marigold (Calendula officinalis L.) is a plant from the family Asteraceae, traditionally used as a medicine for various inflammatory conditions of the digestive system, skin, irritated mucous membranes, and wound healing. The aim of this study is to determine the antioxidant capacity of Calendula officinalis L flower extracts sampled from the natural soil and soil with added Zn, Cu and Mn salts.*
*The flower of Calendula officinalis L. was collected under natural conditions and on salts-treated soil (Cu, Zn and Mn) in the area of Petrovo and Banovici, Bosnia and Herzegovina (BiH). The determination was performed by the FRAP (Ferric Reducing Antioxidant Power) method. The results of the study showed that marigold extracts grown in the open field, samples from Banovići, have the highest antioxidant capacity. While samples of marigold flowers grown on treated soil with salts of copper, zinc and manganese, samples from Petrovo, show less antioxidant capacity than samples in the open field.*

***Key Words:*** *Antioxidant capacity, aqueous extract, flower, Calendula officinalis L., FRAP.*

---

Date of Submission: 26-12-2020 Date of Acceptance: 08-01-2021

---

## I. Introduction

In the human body, free radicals are normally present, that is, atoms, molecules or ions with unpaired electrons that seek to react with similar ones to stabilize themselves.

Biochemical processes in the body lead to the formation of such free radicals and can keep them under control. However, when the free radicals formed exceed the amount of radicals required for normal physiological processes, due for example to some inadequate lifestyle habits, their concentration increases above average and they cause cellular damage, causing cellular oxidation.

Free radicals are formed by: thermolysis, electromagnetic radiation, redox reactions, enzymatic processes and chemical processes (**Acworth, I.N., 2003**). Antioxidants maintain the balance of prooxidant / antioxidant in the body, thus preventing the development of degenerative diseases that are associated with their harmful effects. An imbalance between them and the levels of the antioxidant defense system causes oxidative stress. Oxidative stress in physiological conditions is always greater than the capacity of antioxidant protection (**Irashad, 2002**). The enzymic antioxidants include superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT), monodehydroascorbate reductase (MDHAR), enzymes of ascorbate-glutahione (AsA-GSH) cycle such as ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Non-enzymatic antioxidants include: minerals (Se, Cu, Zn, and Mn as cofactors of antioxidant enzymes), vitamins C and E, carotenoids (primarily ß-carotene, luten and lutein), coenzyme Q10, cysteine, glutathione, polyphenols and albumin (**Šeatović, S., 2005**). Activation of $O_2$ may occur by two different mechanisms: (i) absorption of sufficient energy to reverse the spin on one of the unpaired electrons and (ii) stepwise monovalent reduction (Figure 1). In the former, $^1O2$ is formed, whereas in latter, O2 is sequentially reduced to $O2^{\bullet-}$, H2O2, and $^{\bullet}OH$ (**Fig. 1.**) **(Sharma. P., 2012).**

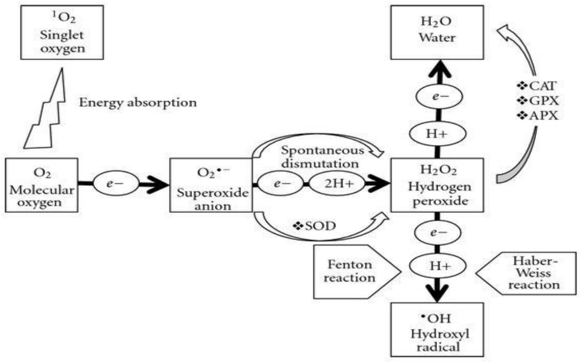


**Fig. 1.**

*Schematic representation of generation of reactive oxygen species (ROS) in plants. Activation of O2 occurs by two different mechanisms. Stepwise monovalent reduction of O2 leads to formation of O2* $^{\bullet -}$*, H2O2, and* $^{\bullet}$*OH, whereas energy transfer to O2 leads to formation of* $^1$*O2. O2*$^{\bullet -}$ *is easily dismutated to H2O2 either nonenzymatically or by superoxide dismutase (SOD) catalyzed reaction to H2O2. H2O2 is converted to H2O by catalase (CAT), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX).* ***(Sharma, P., 2012)***

The mobility of $Mn^{2+}$ in plants is relatively small, but still better than the mobility of copper and iron. Based on his role in the transport of substances, it represents one of the major metals in the life of plants. Participates in the activation of enzymes (enolase, carboxylase, MnSOD), in activation of the process of phosphorylation, carbohydrate metabolism, transmission and consumption of energy and photosynthesis. Most manganese accumulates in the leaf (**Marschener, H., 1995**). The plant tolerance to excess manganese depends on the plant species or genotype, a manganese excess as a result has oxidative stress of plant cells (**Horst, W. J., 1988)** Intimate interactions of Mn-nutrition and antioxidant metabolism exist since cytosolic CuZn-SOD and mitochondrial Mn-SOD activities increase under conditions of Mn-excess as well as Mn-starvation (**Shenker, et al., 2004**).

Copper is normally found only in protein-bound forms in cells, since as a free ion it may generate oxidative stress and cause serious damage to organic molecules. This means, the reactivity of copper that makes it so useful in redox reaction also makes it toxic. The principal mechanism of copper toxicity involves the Fenton reaction, characterised by metal catalysed production of hydroxyl radicals from superoxide and hydrogen peroxide. Although the biochemical response to copper in plants is increasingly well understood, the mechanism of copper tolerance in plants is still unknown. (**Ducic, T., 2005)**. An abrupt increase of $Zn^{2+}$ will be caused either by highly oxidative conditions or by failure of $Zn^{2+}$ homeostasis; therefore, its direct role in the antioxidant defense responses is unclear. $Zn^{2+}$ deficiency and $Zn^{2+}$ overload could contribute to oxidative stress. (**Sung Ryul Lee., 2018).** Antioxidant activity is achieved through nucleophilic phenolic groups to which free radicals are attached, while certain phenolic compounds can play the role of metal ion chelators, retaining antiradical activity even after the formation of complexes with the metal (**Afanas'ev I.B„ 1989**). In the last decades, plants especially from the Apiaceae and Lamiaceae families have been used in medicine, cosmetics and the food industry as extracts (soluble fractions derived from solvent extraction) or essential oils (volatile oils derived from steam distillation) (**Pateiro, M, 2018**) In literature, it has been reported that two-thirds of the world's plant population possesses medicinal effects (**Krishnaiah, et al., 2011**). Plant antioxidants — plant phenols encompass a wide range of substance and form one of the most numerous classes of secondary biomolecules. The basic structural elements that characterize them are: the presence of at least one aromatic ring, substituted with at least one hydroxyl group, free or modified: ether, ester and glycosidic.

The prevalence of plant phenolic compounds is very high, they are found in higher plant species, butit can also be found in lower plant species. Given the importance of consuming the marigold plant, the antioxidant capacity in plant extracts was examined in this paper.

Marigold (*Calendula officinalis*) belongs to the family of *Asteraceae,celandine.* It is seasonal aromatic plant. Marigold contains the following medicinal properties: essential oils, fatty oils, saponins, flavonoids, coumarins, bitter substances, mucus, resins, enzymes, organic acids, carotenoids. Flowers are commonly used as a source of essential oils and as a natural pigment. Marigold is considered to be an almost universal remedy for skin problems. It is therefore known as the "skin plant" because it is used both for external and internal use. For example, it prevents inflammation, enhances granulation, wound healing, etc. In Russia, it is used to make preparations that are used in the symptomatic therapy of some forms of tumors, and recently its anti-HIV properties have been discovered (**Kalvatchev, 1997**). Cromack (**1998)** lists different possibilities of using marigold: as a medicinal plant, in the cosmetics industry, in the production of natural dyes.

The high antioxidant potential of the *Asteraceae* family has been proven in previous research. Thus, plants of the *Asteraceae* family that are rich in phenolic compounds have been identified as source of natural antioxidants with potential application in medicine and pharmacy, and in the cosmetics and food industries. The aim of this study is to determine the antioxidant capacity of Calendula officinalis L flower extracts sampled from the natural soil and soil with added Zn, Cu and Mn salts.

**Fig. 2.** *a-Calendula officinalis L* complete plant.-b-its flower – right (own photo)

## II. Material And Methods

2.1. Plant material

*Calendula officinalis L* was grown on soil in natural conditions and on treated soil of the locality of Omazici, municipality Banovici and locality Kakmuz, municipality Petrovo, Bosnia and Herzegovina (BiH). The soil was treated with salt solutions: $CuSO_4.5H_2O$, $MnSO_4.H_2O$ and $ZnSO_4.7H_2O$. Concentrations of added salts are expressed in mg $Kg^{-1}$ and are shown in **Table 1**

**Table 1** Addition of Cu, Zn and Mn concentrations expressed in mg $Kg^{-1}$

| Nr. | Cu | Zn | Mn | Red.br. | Cu | Zn | Mn | Red br. | Cu | Zn | Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 3 | 0 | 35 | I | 3 | 2,5 | 0 | I | 0 | 2,5 | 35 |
| II | 3 | 2,5 | 35 | II | 3 | 2,5 | 35 | II | 3 | 2,5 | 35 |
| III | 3 | 5 | 35 | III | 3 | 2,5 | 70 | III | 50 | 2,5 | 35 |
| IV | 3 | 10 | 35 | IV | 3 | 2,5 | 150 | IV | 100 | 2,5 | 35 |
| V | 3 | 20 | 35 | V | 3 | 2,5 | 300 | V | 200 | 2,5 | 35 |

Flowers were picked every week, and twice a week during high temperatures and strong light. The plant material was cleaned, the damaged parts were removed and dried at room temperature. Representative samples were stored at the Faculty of Technology, University of Tuzla.

2.2. Herbal extracts

Extracts of dried marigold flower were prepared in an aqueous solvent. 1 g of plant material was transferred to a 100 mL flask and filled up to the mark with deionized water at 95 ° C (1% solution). Other research indicates that the process of preparation of extracts in hot (98 ° C) and cold (20 ° C) water is different, and that extracts prepared in hot water have twice the antioxidant capacity (**Ražić, 2005**). The content was left for 15 minutes. After extraction, filtration was performed through filter paper. The prepared extracts were stored for further testing.

2.3. FRAP method

All reagents used are of analytical grade. Aqueous solutions of 20 mM $FeSO_4.7H_2O$ in the range of 100-1000 μmol $L^{-1}$ were prepared for the construction of the calibration curve. From the prepared $FeSO_4.7H_2O$ solutions, 200 μL of standard solution was pipetted and 1800 μL of FRAP reagent was added and well mixed, incubated for 10 minutes at 37 ° C. After preparing the calibration curve, samples were measured by adding 0.2 mL of extract samples and 1.8 mL of FRAP reagent. A short time is given (10 minutes) in order to allow to react and form a colored complex, and absorbance at a wavelength of 593 nm is measured. The measurement was performed on a UV / VIS spectrophotometer "UV-mini 1240 SHIMADZU". The results were corrected by dilutions and expressed in μmol FeII $L^{-1}$.

All solutions were used on the day of preparation. The FRAP method was developed by Benzie and Strain (**Benzie, 1996**) in 1996. and modified in 1999.

## III. Results And Discussion

The results of antioxidant capacity of plant extracts sampled from soil treated with zinc salts are shown in **Table 2**

**Table 2** Results of AO extracts of Calendula officinalis sampled from soil treated with Zn

| AO [μmol FeII $L^{-1}$] | Banovići | Petrovo | AO [μmol FeII $L^{-1}$] | Petrovo |
|---|---|---|---|---|
| $Zn_{(0)}$ | 4038 | 6658 | $Zn_{(0)}$ | 7298 |
| $Zn_{(2,5)}$ | 5678 | 7778 | $Zn_{(7,5)}$ | 5388 |
| $Zn_{(5)}$ | 12628 | 8518 | $Zn_{(15)}$ | 4018 |
| $Zn_{(10)}$ | 8348 | 6258 | $Zn_{(30)}$ | 5878 |
| $Zn_{(20)}$ | 13218 | 9648 | $Zn_{(60)}$ | 5648 |

The results of antioxidant capacity of plant extracts sampled from soil treated with copper salts are shown in **Table 3.**

**Table 3** Results of AO extracts of Calendula officinalis sampled from soil treated with Cu

| AO [μmol FeII $L^{-1}$] | Banovići | Petrovo | AO [μmol FeII $L^{-1}$] | Petrovo |
|---|---|---|---|---|
| $Cu_{(0)}$ | 8448 | 6678 | $Cu_{(0)}$ | 4538 |
| $Cu_{(3)}$ | 16418 | 8108 | $Cu_{(9)}$ | 5608 |
| $Cu_{(50)}$ | 10648 | 6718 | $Cu_{(150)}$ | 7278 |
| $Cu_{(100)}$ | 6648 | 5578 | $Cu_{(300)}$ | 4518 |
| $Cu_{(200)}$ | 10438 | 7648 | $Cu_{(600)}$ | 4128 |

**Table 4** shows the results of the antioxidant capacity of plant extracts sampled from soil treated with manganese salts.

**Table 4** Results of AO in Calendula officinalis extract sampled from soil treated with Mn

| AO [μmol FeII $L^{-1}$] | Banovići | Petrovo | AO [μmol FeII $L^{-1}$] | Petrovo |
|---|---|---|---|---|
| $Mn_{(0)}$ | 9738 | 5808 | $Mn_{(0)}$ | 3738 |
| $Mn_{(35)}$ | 11578 | 5598 | $Mn_{(105)}$ | 3458 |
| $Mn_{(70)}$ | 4798 | 5508 | $Mn_{(210)}$ | 2558 |
| $Mn_{(150)}$ | 14948 | 4848 | $Mn_{(450)}$ | 3178 |
| $Mn_{(300)}$ | 10948 | 8148 | $Mn_{(900)}$ | 3698 |

The total value of antioxidant capacity in untreated (control) samples is shown in **Fig. 3.**

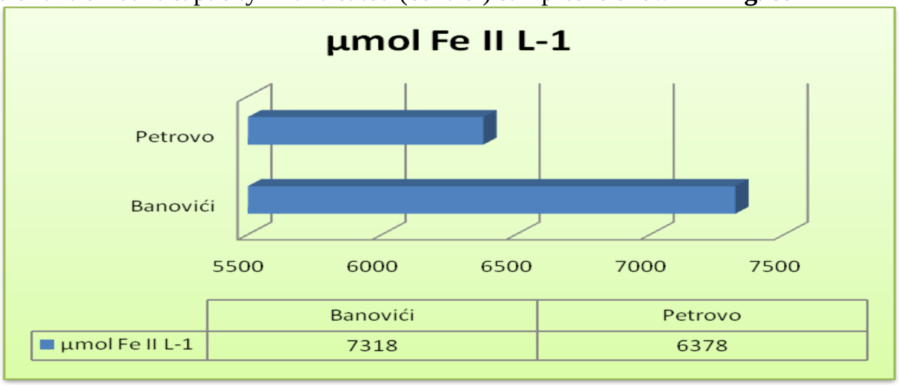


**Fig. 3.** Total antioxidant capacity of marigold flower in control samples

The values of antioxidant capacity in the tested plant extracts range from 2558-16418 μmol Fe II $L^{-1}$ of extract.

The value of antioxidant capacity increased to a certain limit of added salts Zn, Cu i Mn in soil, after which a further increase in salt led to a decrease in antioxidant capacity.

This can be explained by the fact that due to the high concentration of added salts in the soil, more salt is introduced through the roots and into the plant itself, and due to plant stress the plant itself produces more reactive oxygen species or free radicals than antioxidants.

Comparing the values of antioxidant capacity of plant extracts of *Calendula officinalis* flower and the dependence on the increasing concentration of added salts in the soil (from Zn (0) to Zn (20); from Cu (0) to Cu (200) and Mn (0) to Mn (300) ) we find that with increasing salt concentrations in the soil there is no linear increase in the antioxidant capacity of plant extracts. A possible reason is the prooxidant / antioxidant action of the metal. The value of antioxidant capacity of marigold flowers grown in untreated soil samples is as follows:

Banovići 7318 µmol Fe II $L^{-1}$ and Petrovo 6378 µmol Fe II $L^{-1}$. Since the aqueous extract was analyzed, not a pure substance, the obtained results confirm a significant antioxidant potential.

## IV. Conclusion

The results of the analysis show that the plant extracts sampled from the natural soil had lowest yield of antioxidant capacity compared with those from soil samples with added Zn, Cu and Mn salts.

Antioxidant capacity is growing to determined concentration of added Zn, Cu and Mn salts. After these concentrations, with the resumption the rise of salt, there is a gradual decrease in antioxidant capacity. Considering that the aqueous extract of *Calendula officinalis L* was analyzed and not the dry matter, obtained the results confirm a significant antioxidant potential.

Research into new natural sources of antioxidants is of great importance in the acquisition of new knowledge and finds its significant place in scientific research papers.

## References

[1]. Acworth, I.N., (2003). The Handbook of Redox Biochemistry. Eds. ESA, Inc.,Chelmsford, USA.
[2]. Afanas'ev, I. B., Dcrozhko A. I., Brodskii A. V., Kostyuk, V.A., Potapovitch, A.I., (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol*, 38, 1763-1769
[3]. Benzie, I.F., Strain, J.J., (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" The FRAP assay, *Anal. Biochem*, 239**,** 70–76.
[4]. Bessada, S. M. F., Barreira J C M and Oliveira M B P P (2015) Asteraceae species with most prominent bioactivity and their potential applications. A review, Ind Crop Prod, 76, 604-615, https://doi.org/10.1016/j.indcrop.2015.07.073
[5]. Cromack, H. T. H., Smith, J. M. *(1998). Calendula officinalis* – Production Potential and Crop Agronomy in Southern England. Industrial Crops and Products, 7, 223-229.
[6]. Ducic, T., Polle, A., 2005. Transport and detoxification of manganese and copper in plants. *Braz. J. Plant Physiol*, 17(1), ISSN 1677-9452, http://dx.doi.org/10.1590/S1677-04202005000100009
[7]. Horst, W. J., (1988). The physiology of manganese toxicity. In: Manganese in Soil and Plants, *Kluwer Academic Publishers*, Dordrecht, The Nedtherlands, 175-178.
[8]. Irashad, M., Chaudhuri, P.S., (2002). Oxidant-antioxidant System. Role and significance in human body. *Ind J Exp Biol*, 40, 1233-39.
[9]. Kalvatchev, Z., Walder, R., Garzaro, D., (1997). Anti-HIV activity of extracts from Calendula officinalis flowers. *Biomedicine & Pharmacotherapy*, 51,4, 76-180.
[10]. Krishnaiah, D., Sarbatly, R., Nithyanandam, R., (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioprod Process*, 89, 217-233
[11]. Marschener, H., (1995). Mineral Nutrition of Higher Plants. *Academic Press*, San Diego, CA, 889.
[12]. Pateiro, M., Barba, F.J., Domínguez, R., Sant' Ana, A.S., Khaneghahc, A.M., Mohsen, G., Gómez, B., Lorenzo, J.M., (2018). Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. *Food Res. Int*, 113, 156–166.
[13]. Ražić, S., Đogo, S., Slavković, L., (2005). Inorgaic analysis of herbal drugs. Part I Metal determination in herbal drugs originating from medicinal plants of the family *Lamiacae*. Belgrade. Serbia and Montenegro, 1347-1355.
[14]. Sharma, P., Bhushan Jha A., Shanker, Dubey R., Pessarakli, M., (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany Article*, ID 217037, https://doi.org/10.1155/2012/217037
[15]. Shenker, M., Plessner, O.E., Tel-Or, E., (2004) Manganese nutrition effects on tomato growth, chlorophyll concentration, and superoxide dismutase activity. *J. Plant Physiol*, 161 (2) 197-202.
[16]. Sung Ryul Lee., (2018). Critical Role of Zinc as Either an Antioxidant or a Prooxidant in Cellular Systems. Oxidative Medicine and Cellular Longevity. Article ID 9156285 https://doi.org/10.1155/2018/9156285
[17]. Šeatović, S., (2005). Galenika a.d. Institut, Centar za bazne tehnologije. Beograd.

Adriana Sales, et. al. " Antioxidant capacity of Calendula officinalis L. flower extracts." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 13(12), (2020): pp 46-50.