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Ultrasonically-Extracted Marine Polysaccharides as Potential Green Antioxidant Alternatives †

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Abstract: Marine-extracted sulfated polysaccharides (SPs) have been the subject of myriad research since they are considered an eco-friendly source of biologically active compounds. Meanwhile, food and pharmaceutical industries urgently produce natural sugar substitutes and antioxidants as alternatives to synthetic ones which are associated with cytotoxicity and safety issues. This study assesses the potential of using marine SPs obtained via the ultrasonic-assisted extraction of different marine species, to utilize them as antioxidant sugar substitutes. The carbohydrate, total phenolic contents and antioxidant activities, were measured for the SPs extracts of the algal species of *Ulva lactuca, Jania rubens* and the marine plant mangrove *Avicennia marina*. These SPs were structurally elucidated by Fourier Transform Infrared (FTIR) spectroscopic and high-performance liquid chromatography (HPLC) analyses. The results revealed that SPs' highest yield percent was obtained from *Ulva lactuca*, 5.50 ± 0.25%. The SPs of *Avicennia marina* had the highest carbohydrate content, 44 ± 1% and antioxidant activity, 78.85 ± 0.06 at the 100 µg/mL concentration and 89.50 ± 0.21 at the 250 µg/mL concentration. Meanwhile, the highest phenolic content was exhibited by algal SPs obtained from *Jania rubens*, 132.60 ± 2.50 mgGa/g. Results also showed that the antioxidant potential of *Jania rubens* and *Avicennia marina* can be owed to their high glucose content. This work emphasizes the need to consider sulfated polysaccharides from marine sources for their antioxidant activity and to correlate it with their monosaccharide content to determine the effect of reducing sugar concentration on the antioxidant activity.

Keywords: sulfated polysaccharides; antioxidant activity; ultrasonic-assisted extraction; marine algae; mangrove leaves

1. Introduction

Marine organisms have recently gained traction owing to their rich content of bioactive compounds. These organisms are mainly classified into six kingdoms including bacteria, protozoans, algae (seaweeds), fungi, plants like mangroves, and animals [1]. Some marine organisms are edible like sea lettuce (*Ulva spp.)* [2] and fruits of mangroves [3], and can, hence, be used in nutraceuticals after considering food safety measures. The antioxidants extracted from mangroves, particularly in the form of herbal drugs, play an essential role in protecting from free radical-induced injury [4].

Same applies for the antioxidants extracted from green algae (*Ulva lactuca*) which could be used in food supplements [5].

The extracts of these marine species contain polyphenolic compounds and polysaccharides that contribute to the antioxidant activity exhibited by the extracts [6]. As such, sulfated polysaccharides, especially fucans extracted from brown seaweeds, could be used as sources of natural antioxidant alternatives with potential food industry applications [7]. The search for natural antioxidants as alternatives to synthetic ones is of great interest, recently, due to their potential as economic, safe and non-toxic compounds [8,9]. These species are also edible, and after appropriate processing, they could be used as antioxidant supplements.

In the current study, sulfated polysaccharides (SPs) were extracted from the different marine species of green algae, red algae, and mangrove leaves. These extracts were tested for their carbohydrate and phenolic contents then examined for their structure by Fourier Transform Infrared (FTIR) Spectroscopy. The sugar content, which was analyzed by high-performance liquid chromatography (HPLC), was then correlated to antioxidant activity, tested by 1-Diphenyl-2-picrylhydrazyl (DPPH) in order to assess the potential of these extracts as alternative natural antioxidants.

2. Materials and Methods

Ulva lactuca green algae and *Jania rubens* red algae were collected from Alexandria city coast (31°19′ N 30°03′ E) and were immediately brought to the laboratory in sterile plastic bags containing seawater in order to prevent evaporation. Fresh mangrove leaves (*Avicennia marina* (Forssk.) Vierh were collected in winter of 2017 from Ras Mohammed National Park at the Aqaba Gulf in Sinai. Dr. Mohamed Massed Hejazi recognized the plants' voucher samples at the Botany Herbarium Laboratory of the Department of Marine Science, Suez Canal University, Ismailia, Egypt. All marine samples were then washed with fresh water, air dried, ground into powder and stored before extraction at −20 °C (Figure 1).

For the extraction of sulfated polysaccharides, ethanol (96%) was purchased from the International Company for Supplies and Medical Industries (Giza, Egypt). For chemical analyses, phenol (BDH, England), sulfuric acid 98% (Penta, Prague, Czech Republic), and Folin and Ciocalteu's phenol reagent (Loba Chemie, Colaba, Mumbai, India) were used. For antioxidant activity, 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH), gallic acid (Ga), and ascorbic acid (AscA), were all purchased from Sigma-Aldrich (USA).

Ten grams of algal powders were soaked in 200 mL of distilled water and placed for four hours in the ultrasonicator (VWR® sonicator model 150HT) at 60 °C for *Ulva lactuca* and at room temperature (RT) for *Jania rubens.* Meanwhile, the same ratio of mangrove leaves powder and distilled water were mixed for 15 min at RT in an ultrasonicator (Branson 2510 sonicator, model 2510DTH, Hampton, NH, USA). As per reported literature, red algae and mangroves contained thermolabile metabolites, hence extraction under high temperature was avoided. All marine suspensions were filtered through a cheese cloth. The filtrates were separately dialyzed, collected and added to ethanol in the proportion of 1:3/3.5 (*v*/*v*) as previously reported [10,11] and then the supernatants (SPs) were collected. Extraction was performed in triplicate.

The respective total carbohydrate and phenolic contents of the extracted marine SPs were determined using glucose and gallic acid as standards, as described in our previous studies [5,12].

FTIR spectroscopy was used to determine the functional groups of the SPs of each marine species. In this regard, the powdered SPs were mixed with potassium bromide to form 1-mm pellets. Analysis was carried out using a Nicolet 380 Thermogravimetric Analysis/Fourier Transform Infrared (TGA/FTIR) spectrometer for a range of 500 to 4000 cm^{-1}.

High-performance liquid chromatography (HPLC) was performed to estimate the monosaccharide content of these SPs as previously outlined [10,11].

The free radical scavenging activity of the marine SPs was measured using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay following Blois's method [13] with minor modifications.

The radical scavenging activity was determined using equation 1 in which A_R is the absorbance of the pure DPPH while As is the absorbance of the sample SPs in the presence of DPPH.

$$\textbf{\textit{Radical Scavenging Activity}}\ (\%) = \left[\frac{A_R - As}{A_R}\right] * 100 \quad (1)$$

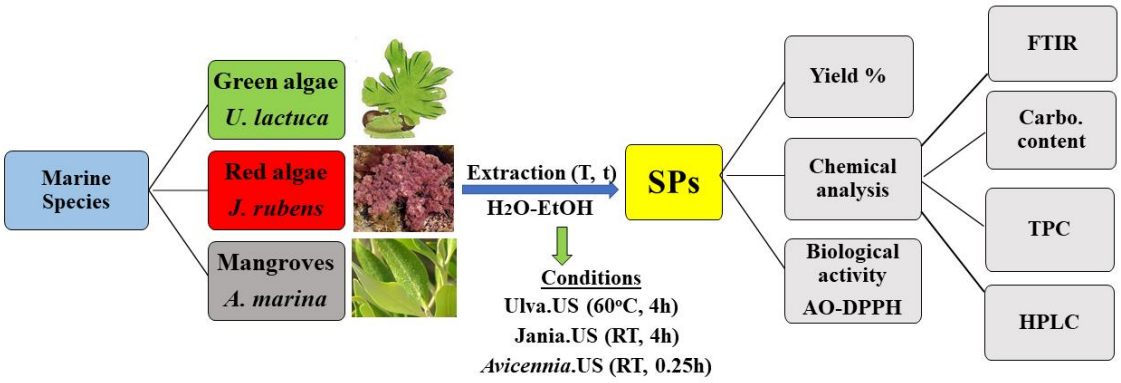

Figure 1. Schematic outline of the work conducted in this study.

3. Results and Discussion

The ultrasonic-assisted extraction of SPs from *U. lactuca (GU-SPs)*, *J. rubens (RJ-SPs)* and *A. marina (M-SPs)* produced SPs yields of 5.50 ± 0.25, 0.36 ± 0.04 and 3.52 ± 0.94%, respectively. As shown in Figure 2a, the green and red algal SPs of *GU-SPs* and *RJ-SPs* constituted 7.70 ± 0.10 and 2.60 ± 0.30% carbohydrates, respectively, while the mangrove SPs exhibited a much higher carbohydrate content of 44.0 ± 1.00%. Figure 2b depicts the phenolic contents in mgGA/g which amount to 60.60 ± 7.90 for *GU-SPs*, 132.60 ± 2.50 for *RJ-SPs* and 50.62 ± 0.10 for *M-SPs*. The red algae SPs possess the highest phenolic content, whereas those of the green algae have comparable content to that of the mangrove SPs.

Table 1 shows the structural elucidation of the extracted SPs by FTIR. The FTIR spectra (Figures S1–S3) of *GU-SPs* and *M-SPs* show bands at 3400, 1625-1450, 1260, 1109 and 963 cm^{-1} that correspond to the stretching vibration of the hydroxyl (O-H) group, the bending vibration of the C-O of uronic acids, (S=O) sulfate ester bonds, acidic polysaccharides and glycosidic bonds, respectively [14,15]. On the other hand, spectra of the red algal *RJ-SPs* exhibit all the previously mentioned bands with the exception of the sulfate ester band at 1260 cm^{-1}; however, the presence of sulfate is confirmed by the appearance of the bands corresponding to the galactose sulfate groups at 850 cm^{-1}.

The sugar contents of the extracted marine SPs were measured by HPLC and their values were compiled in Table 2. Results reveal that *GU-SPs* has glucuronic acid as the most dominant sugar, possibly due to the high temperature (60 °C) used for its extraction and which could have led to the oxidation of glucose and galactose fractions to glucuronic acid. As for *RJ-SPs*, it is primarily composed of glucose (94.04%), as a result of the ultrasonic-aided conversion of galactose to glucose [10]. On the other hand, *M-SPs* consisted mainly of glucose and galactose with 24.51 and 17.46%, respectively, probably due to its shorter extraction duration and consequently less exposure time to the ultrasonic waves that can convert galactose to glucose [11].

Table 1. Functional groups of RJ-SPs, GU-SPs and M-SPs.

Wavelength, cm^{-1}	Functional Groups	RJ-SPs	GU-SPs	M-SPs
3500–3400	OH group	√	√	√
1600–1420	Uronic acid and phenolic groups	√	√	√
1260–1258	Ester Sulfate group	×	√	√
1088–1012	Acidic polysaccharide	√	√	√
963–927	Glycosidic linkage	√	√	√

850–845	Galactose sulfate group	√	√	×

Table 2. Constituents of sugars by mole%, for RJ-SPs, GU-SPs and M-SPs.

Types of Monosaccharides	RJ-SPs	GU-SPs	M-SPs
Glucose	94.04	6.55	24.51
Galactose	0.10	3.53	17.46
Glucuronic acid	0.16	89.92	7.65
Xylose	2.14	NA	1.29
Mannose	3.51	NA	0.16

To assess their potential as antioxidants, the marine SPs used in this study were tested for their antioxidant activities at two different concentrations; 100 and 250 µg/mL. Ascorbic acid was used as a reference standard and it antioxidant profile is given in Figure S4. The results, depicted in Figure 2c, show that the highest % DPPH scavenging activity was exhibited by *M-SPs* of mangrove yielding 79.85 ± 0.06% and 89.50 ± 0.21% at the two applied concentrations, respectively. Glucose is the principal sugar constituent in *RJ-SPs* and *M-SPs* which might have contributed to the higher antioxidant activities of these extracts relative to *GU-SPs*.

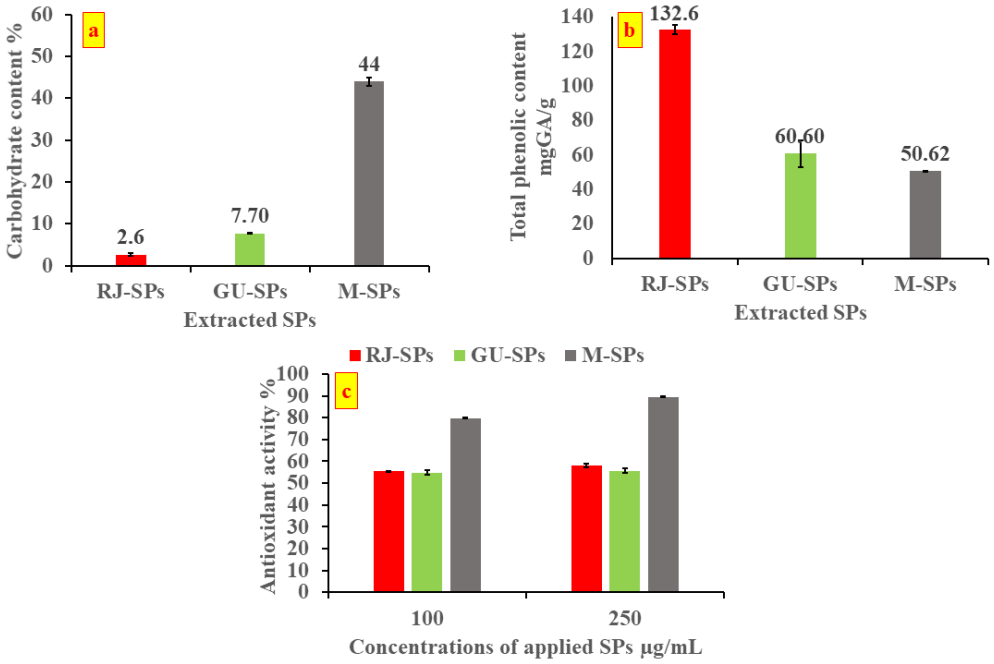

Figure 2. Carbohydrate (**a**), total phenolic (**b**) contents and antioxidant activity (**c**) of RJ-SPs, GU-SPs and M-SPs.

4. Conclusions

Sulfated polysaccharides of the marine organisms *U. lactuca*, *J. rubens* and *A. marina*, were extracted using ultrasonication. SPs of *U. lactuca* showed the highest yield; 5.50 ± 0.25% of all marine species being studied. Meanwhile, the SPs of *A. marina* exhibited the highest carbohydrate content; 44 ± 1%. The SPs of *J. rubens* were characterized by the highest phenolic content; 132.6 ± 2.5 mg GA/g. However, SPs of *A. marina* showed the highest antioxidant activity at the two applied concentrations indicating that its SPs could be utilized as antioxidant alternatives. As per the HPLC analysis, the SPs of *J. rubens* and *A. marina* have glucose as their major sugar constituent comprising 94.04% and 24.51%, respectively, which implies that this monosaccharide could be partially responsible for the high antioxidant activities of these SPs. The current study suggests that sulfated polysaccharides

extracted from marine organisms can be utilized as natural antioxidant alternatives in diet supplements or nutraceuticals.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figures S1–S3: FTIR of RJ-SPs, GU-SPs and M-SPs, Figure S4: the % DPPH scavenging activity for ascorbic acid.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Abdullah, S.A.; Omar, H.H.; Bahabri, F.S. Health Benefits of Edible Seaweeds and Their Nano-Applications. *J. Am. Sci.* **2020**, *16*, 40–72.
2. Wiseman, J. *SAS Survival Handbook*, Harvill Secker, London, UK, 1986.
3. Chiavaroli, A.; Sinan, K.I.; Zengin, G.; Mahomoodally, M.F.; Sadeer, N.B.; Etienne, O.K.; Cziáky, Z.; Jekő; J; Glamocilja, J.; Sokovic, M.; et al. Identification of Chemical Profiles and Biological Properties of *Rhizophora racemosa* G. Mey. Extracts Obtained by Different Methods and Solvents. *Antioxidants* **2020**, *9*, 533.
4. Thatoi, H.N.; Patra, J.K.; Das, S.K. Free radical scavenging and antioxidant potential of mangrove plants: A review. *Acta Physiol. Plant.* **2014**, *36*, 561–579.
5. El Azm, N.A.; Fleita, D.; Rifaat, D.; Mpingirika, E.Z.; Amleh, A.; El-Sayed, M.M.H. Production of bioactive compounds from the sulfated polysaccharides extracts of *Ulva lactuca*: Post-extraction enzymatic hydrolysis followed by ion-exchange chromatographic fractionation. *Molecules* **2019**, *24*, 2132.
6. Jime'nez-Escrig, A.; Jime´nez-Jime´nez, I.; Pulido, R.; Saura-Calixto, F. Antioxidant activity of fresh and processed edible seaweeds. *J. Sci. Food Agric.* **2001**, *81*, 530–534.
7. Rez, P.R.; Ahrazem, O.; Leal, J.A. Potential Antioxidant Capacity of Sulfated Polysaccharides from the Edible Marine Brown Seaweed *Fucus vesiculosus*. *J. Agric. Food Chem*. **2002**, *50*, 840–845.
8. Duan, X.-J.; Zhang, W.-W.; Li, X.-M.; Wang, B.-G. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chem.* **2006**, *95*, 37–43.
9. Wijesekara, I.; Yoon, N.Y.; Kim, S.K. Phlorotannins from *Ecklonia cava* (Phaeophyceae): Biological activities and potential health benefits. *Biofactors* **2010**, *36*, 408–414.
10. Essa, H.; Fleita, D.; Rifaat, D.; Samy, S.; El-Sayed, M. Towards optimizing the conventional and ultrasonic-assisted extraction of sulfated polysaccharides from marine algae. *IOP Conf. Ser. Mater. Sci. Eng*. **2018**, *464*, 1–11.
11. Essa, H.L.; Abdelfattah, M.S.; Marzouk, A.S.; Guirguis, H.A.; El-Sayed, M.M.H. Nano-Formulations of Copper Species Coated with Sulfated Polysaccharide Extracts and Assessment of Their Phytotoxicity on Wheat (*Triticum aestivum* L.) Seedlings in Seed Germination, Foliar and Soil Applications. *Appl. Sci.* **2020**, *10*, 6302.
12. Fleita, D.; El-Sayed, M.; Rifaat, D. Evaluation of the antioxidant activity of enzymatically-hydrolyzed sulfated polysaccharides extracted from red algae; Pterocladia capillacea. *LWT Food Sci. Technol.* **2015**, *63*, 1236–1244.
13. Blois, M.S. Antioxidant determination by the use of a stable free radical. *Nature* **1985**, *181*, 1199–2000.
14. El-Rafie, H.M.; El-Rafie, M.H.; Zahran, M.K. Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. *Carbohydr. Polym*. **2013**, *96*, 403–410.
15. Ananthi, S.; Balaji Raghavendran, H.R.; Sunil, A.; Gayathri, V.; Ramakrishnan, G.; Vasanthi, H.R. In vitro antioxidant and in vivo anti-inflammatory potential of crude polysaccharide from *Turbinaria ornata* (Marine Brown Alga). *Food Chem. Toxicol*. **2010**, *48*, 187–192.

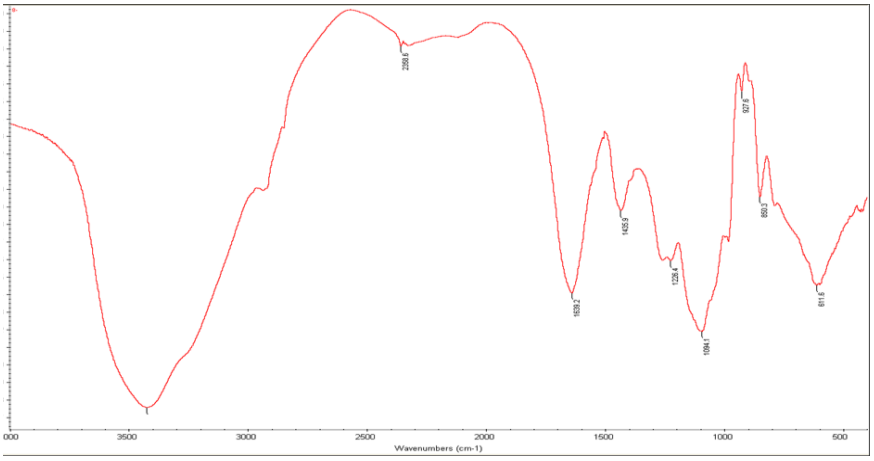

Figure S1. FTIR of GU-SPs.

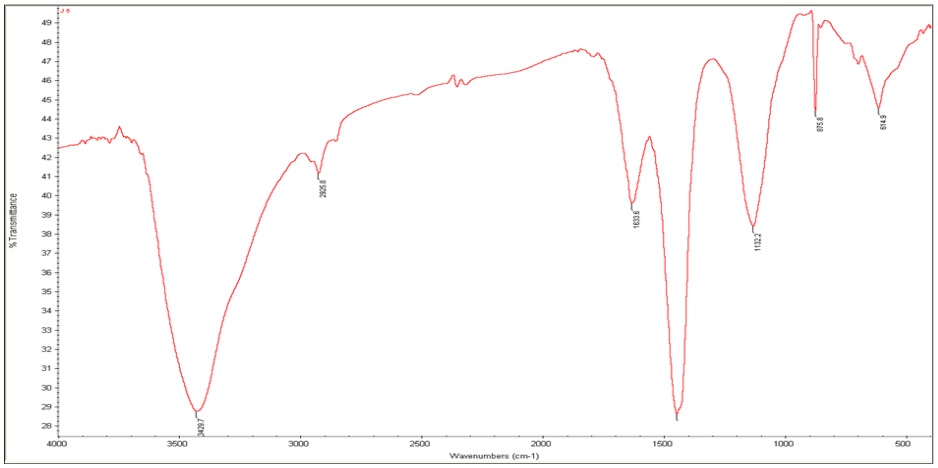

Figure S2. FTIR of RJ-SPs.

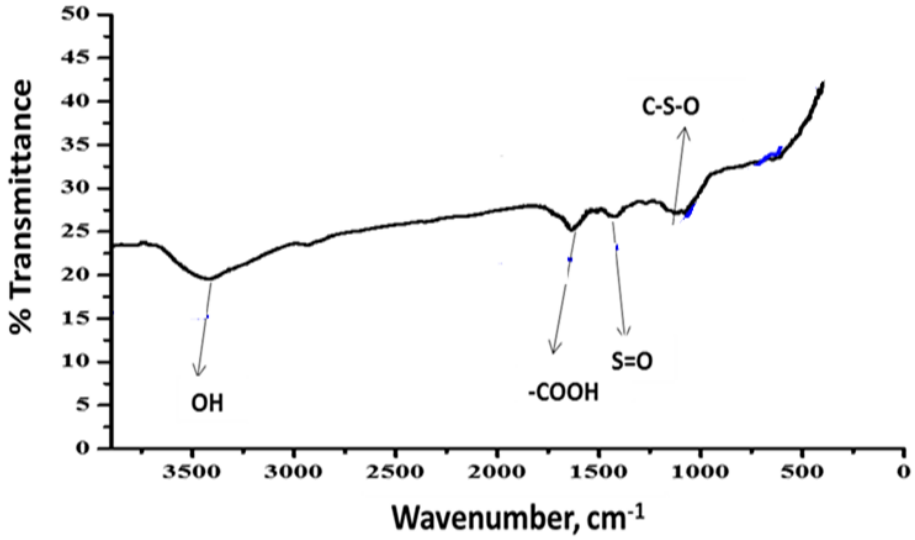

Figure S3. FTIR of M-SPs.

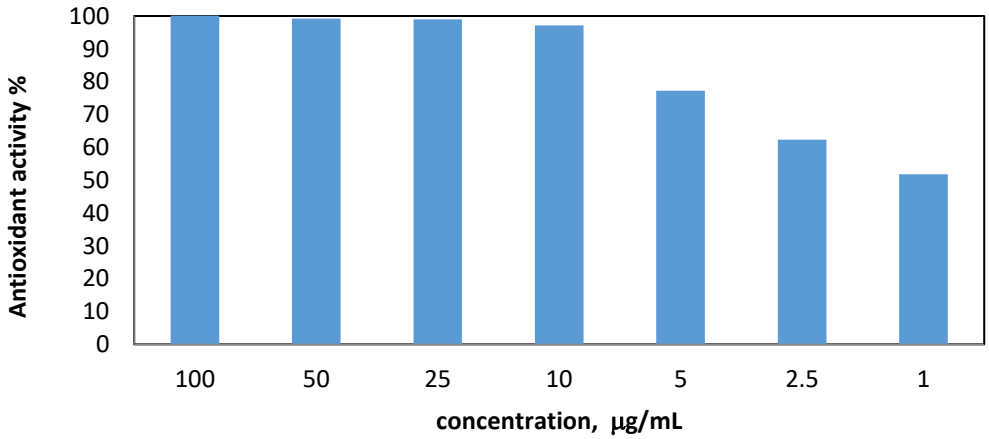

Figure S4. Ascorbic acid antioxidant activity.