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Research Article

Structure-Antioxidant Capacity Relationships of the Dihydroxy Derivatives of Benzaldehyde and Benzoic Acid

María Teresa Moreno, Rafael Estévez Brito, José Miguel Rodríguez Mellado*

Department of Physical Chemistry and Applied Thermodynamics, Institute of Fine Chemistry and Nanochemistry (IUIQFN), University of Córdoba, Rabanales Campus, Córdoba E-14014, Spain
E-mail: jmrodriguez@uco.es

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Abstract: In this paper, the antioxidant capacities (AOC) of the dihydroxybenzaldehyes (DHB) and dihydroxybenzoic acids (DHBA) are determined by two single electron transfer (SET) assays, namely CUPRAC and e-CUPRAC (CUPric Reducing Antioxidant Capacity), and a SET + hydrogen atom transfer (HAT) assay: 2,2-diphenyl-1-picrylhydrazyl (DPPH). It is found that the oxidation potential (E_{OX}) determined by voltammetry can be used as a structural parameter in the same way as the HOMO energy. From the dependencies of the AOC with E_{OX}, it follows that only the electron-donor derivatives of DHB and DHBA exhibit antioxidant activity, which means that the number of hydroxyl groups, two for all molecules, is not the only factor related to the AOC. A relationship between the position of the hydroxyl groups and the AOC is shown. Moreover, the value of the AOC also has a contribution of dissociation of the hydroxyl groups.

***Keywords*:** HOMO energies, dihydroxybenzoic acids, dihydroxybenzaldehydes, electrochemical oxidation, antioxidant capacity, CUPRAC, DPPH, structure-reactivity relationships

1. Introduction

In metabolic reactions in the presence of oxygen, that is, the aerobic part of metabolism, energy is produced in the form of adenosine triphosphate (ATP) from electron transfer reactions.[1] Free radicals that are present in the body, generated endogenously or exogenously, cause metabolic oxidation-reduction reactions, in many cases, unwanted.[2] Among these radicals that are found, the so-called reactive oxygen species (ROS) include radicals such as alkoxyl ($RO^{\bullet}$), peroxyl ($ROO^{\bullet}$), hydroxyl ($HO^{\bullet}$), superoxide ($O_2^{\bullet-}$) and nitric oxide ($NO^{\bullet}$). ROS can damage proteins, lipids, carbohydrates, polyunsaturated fatty acids, or nucleic acids, causing DNA damage and leading to mutations or cancer. Such species are involved in so-called oxidative stress, initiating oxidative modification of lipids, carbohydrates, proteins, DNA, etc. Oxidative stress is implied in many diseases,[3,4] being part of a redox imbalance depending on the amount of antioxidants present in the organism. Antioxidants react with ROS, reducing their concentration and mitigating their undesirable effects. Synthetic antioxidants have been proposed,[5-8] although research on natural antioxidants is also of great interest.[5]

Polyphenols, secondary metabolites with -OH groups in their structure produced by higher plants, are widely present in their tissues, from roots to fruits,[8-10] have beneficial effects on many diseases such as cardiovascular disease, neurodegenerative diseases or cancer.[9] This class of compounds includes gallic acid derivatives and phenolic

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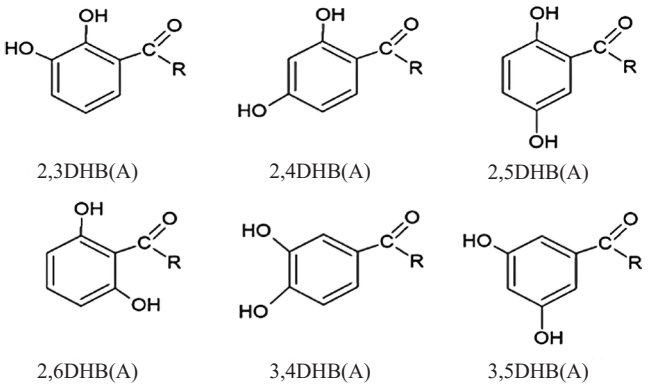
benzaldehydes, and have antimicrobial activity.[11-14] These substances can act as antifungal agents, either alone or in combination with other chemicals,[15-17] and as anticancer agents.[18-20] In addition, such compounds exhibit antioxidant activity[13,16,21-25] and several investigations have been conducted exploring the relationship between their antioxidant activity and their structures.

The possible relationship between structure and antioxidant activity has been tested on many occasions for different chemicals, such as flavonoids and phenolic acids,[26,27] polyphenols[28] and benzoic acid derivatives.[29-31] The compounds investigated do not usually have a common structure except, of course, the aromatic ring and the presence of one or more -OH groups. In these reports, flavonoids, phenylpropenoic acids, mono-di and triphenols, etc. are mixed, although some exceptions to this are found.[30]

The complete group of dihydroxybenzaldehydes (DHB) and dihydroxybenzoic acids (DHBA) has been studied in a previous work from the point of view of the structure-activity relationship.[31] In this paper, the relation between structure and chemical reactivity for these compounds is shown. The relationships between highest occupied molecular orbital (HOMO) energies and pK values of acid dissociation were established by means of the oxidation potentials obtained from voltammetric measurements. It is known that the reduction potentials of species in the solution can be related to their lowest unoccupied molecular orbital (LUMO) energies, while oxidation potentials can be related to the HOMO energies.[32-35] By making measurements under the same experimental conditions, that is, same pH, same buffer solution composition and same ionic strength value, the differences between the peak potential values of the voltammetric oxidation peak of the dissociated and non-dissociated species were obtained. The existence of relationships between these differences and the pK values was demonstrated, the correlation observed for aldehydes being more robust than that observed for acids.

So, the aim of this paper is to use the oxidation potential (E_{OX}) determined by voltammetry as structural parameter to show the occurrence of correlations between the HOMO energies and the antioxidant capacities of dihydroxy derivatives of both DHB and DHBA, exploring the role of the positions and dissociation state of the -OH groups in the aromatic ring.

The structures of the investigated compounds are shown in Scheme 1.



Scheme 1. Structures of the compounds studied. For DHB, R = H; for DHBA, R = OH

2. Materials and methods

Chemicals used were of analytical quality. DHBs and DHBAs (97%) were form Sigma-Aldrich, except for 2,6-DHB (ChemBo Pharma, 98%) and 2,6-DHBA (Merck, 98%). 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a free radical (95%). Neocuproine (98%), $CuCl_2$ (99%), $Cu(NO_3)_2$ (98%) and methanol (99.8%) were from Sigma-Aldrich. Ethanol (99.8%) was from Merck. Buffer components as phosphoric acid (85%), ammonium acetate (98%) and sodium hydroxide (pellets, ≥ 97%) were from Sigma. The water used for the preparation of solutions was obtained by using a Millipore system, having a resistivity of 18.2 MΩ·cm at 298 K. To avoid decomposition, dihydroxy derivative solutions were stored at low temperature (≤ 278 K) in the dark.

UV measurements were made with a double-beam spectrophotometer from Perkin-Elmer (model Lambda 750S). The cuvettes (Hanna) were of 1 cm path-length and the absorbances were measured at room temperature.

Electrochemical measurements were performed on an Autolab PGSTAT101 equipped with NOVA 1.8 software and thermostated glass cells of 15 mL volume. The working electrode was a glassy carbon electrode, GCE (IJCambria) with a geometrical area of 7.5 mm^2. As reference electrode, a Metrohm Ag/AgCl/3M KCl (model 6.0733.100) was used. The auxiliary electrode used was a platinum rod. A stream of purified nitrogen was passed for 10 to 12 minutes through the solutions to prevent unwanted reactions on the electrode caused by the presence of molecular oxygen.

The protocol for the CUPRAC (CUPric Reducing Antioxidant Capacity) assays was as follows: 1.0 mL of 1 M ammonium acetate buffer was used for all measurements, with the pH adjusted to 5.5 with NaOH. The final volume was 4.1 mL. In all cases, 1.0 mL of a 0.01 M $CuCl_2$ aqueous solution and 1.0 mL of a 7.5 mM ethanolic solution of neocuproine were taken. The volumes of each test solution were variable, to obtain different final concentrations, and water was added to make up the final volume. To make a calibration curve, the test solution was a 2.5×10^{-4} M ethanolic Trolox solution. This calibration curve allows to obtain the values of antioxidant capacities in Trolox equivalents. The samples thus prepared were incubated in the dark for 60 min. The UV-V is spectra of the samples showed maximum absorbance at 450 nm. This absorbance was measured for each sample at room temperature after incubation. To correct the matrix effect, the absorbance of a blank assay was subtracted from these values.

DPPH is an assay in which, due to a radical scavenging reaction, a given antioxidant provokes the decrease of the DPPH absorption band, which has a maximum absorbance ranging from 515 to 518 nm. The EC_{50}, or efficient concentration, is the quantity of a given antioxidant capable of decreasing the DPPH radical concentration to the 50% of the initial value. The inverse of EC_{50}, ARP, or anti-radical power, is a measurement of the antioxidant capacity. The protocol for the DPPH assays is as follows: for a final volume of 10 mL, 9.0 mL of a 0.56 mM DPPH methanolic solution were added, as well as variable volumes of dihydroxy derivatives solutions, and completing the 10 mL with water. The samples were incubated in the dark at 298 K for 90 min. The absorbance was measured at 517 nm to obtain the final DPPH concentration after incubation.[36,37] From the variation of this absorbance with the reactant concentration, the EC_{50} was measured for each derivative.

The protocol for the e-CUPRAC assay is as follows[35]: to remove possible organic matter, the glassy carbon electrode was immersed in 1:3 diluted chromic mixture for 30 seconds. To remove traces of metallic copper, the glassy carbon electrode was immersed in 1:3 diluted aqua regia for another 30 seconds. After cleaning with ultrapure water, the electrode surface was successively polished with 0.25 μm diamond paste, 0.3 μm alumina slurry and 0.05 μm alumina slurry. Residues after polishing were removed by sonication for 3 minutes in a water bath. The electrodeposition of Cu was made at -0.3 V using a solution 1×10^{-3} M in $Cu(NO_3)_2$ and 0.5 M in HNO_3. The addition of an antioxidant to a phosphate-buffered solution (PBS) at pH 7.0 decreases the intensity of the voltammetric reduction peak. From this decrease, the antioxidant capacity (AOC) of the antioxidant is obtained.[38]

3. Results and discussion

HOMO energies of the species in solution can be related to their oxidation,[32-35] provided that the calculated energies correspond to the molecules that are actually present in the solution. The HOMO energies for the non-dissociated molecules of DHBA in solution were found in reference.[30] In the case of DPPH measurements, methanolic solutions are used, and the DHBA are not dissociated, but for CUPRAC, aqueous solutions of pH 5.5 (with a certain amount of ethanol used to solubilize neocuproine) are used. So, the undissociated forms are found at these pH values, which are much lower (by at least two units) than the lowest pK of the molecule, which corresponds to the dissociation of the -COOH group to $-COO^-$ ion. These pK values are around 3[31] and therefore the oxidation potentials must be measured at $pH \leq 1$. Figure 1 shows the correlation obtained for the oxidation potentials measured in a 1.0 M $HClO_4$ solution and the HOMO energies of DHBA.

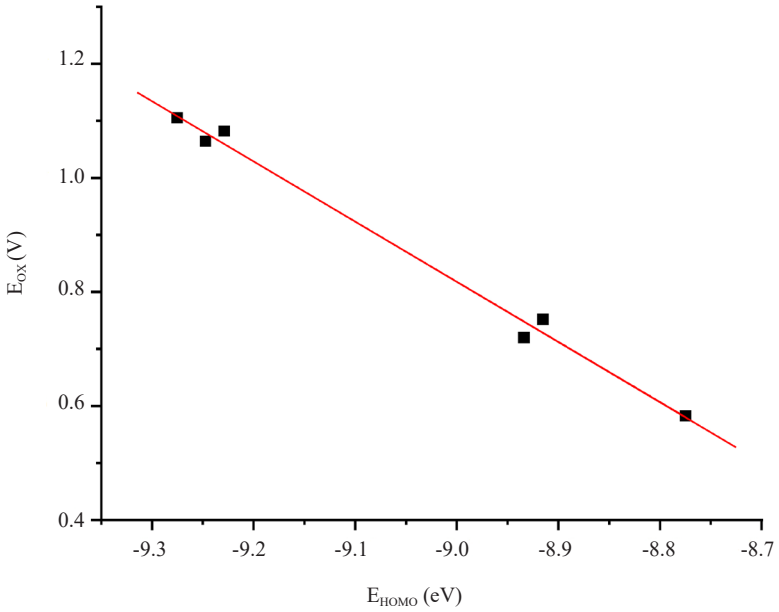


Figure 1. Plot of the oxidation peak potentials of 1 mM DHBA in 1 M $HClO_4$ vs. HOMO energies obtained from reference[22]

Molecules presenting the highest E_{HOMO} values, 2,3-, 2,5- and 3,4-DHBA are better electron-donor and have lower E_p values, whereas the rest of the molecules (those presenting the lowest E_{HOMO} value) are better electron-acceptor and have higher E_p values.[30]

The above results indicate that the oxidation potential can be used as a structural parameter in the same way as the HOMO energy. Thus, the E_p values obtained in 1.0 M $HClO_4$ solution will be used in the following discussion for the analysis of the DPPH results, and those obtained at pH 5.5 for that of the CUPRAC measurements.

The EC_{50} of all investigated compounds were measured by DPPH making five independent measurements for each compound. In addition, the EC_{50} of Trolox was also measured. Trolox equivalents of the AOC were calculated from the EC_{50} of Trolox (ECT) and the EC_{50} of each antioxidant (ECA) as: AOC = ECT/ECA. These values are shown in Figure 2.

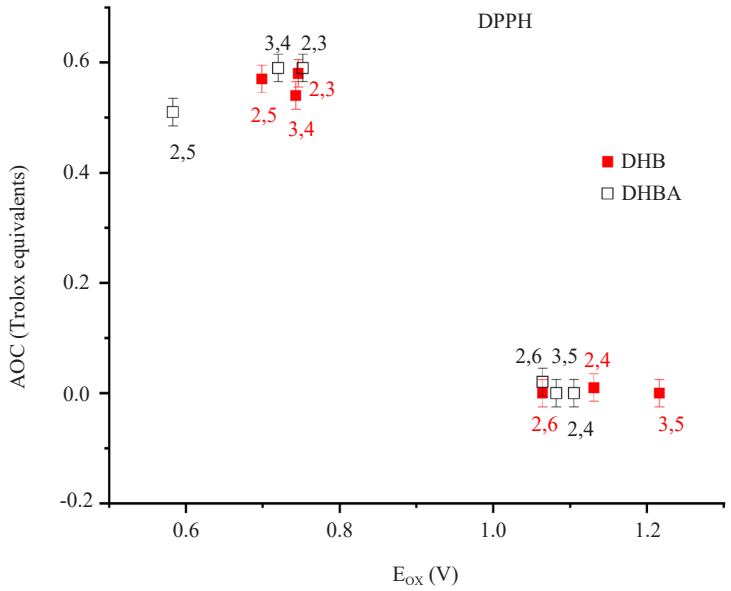


Figure 2. Plot of AOC measured by DPPH given in Trolox equivalents vs. the oxidation peak potentials of 1 mM compounds in 1 M $HClO_4$

As can be seen, electron-acceptor compounds show no antioxidant activity, while electron-donor compounds exhibit AOC values that prove their antioxidant activity. For this group of compounds, AOC values were very similar, regardless of the type of compound (acid or aldehyde) and the positions of the -OH groups.

Figure 3 presents the AOC in Trolox equivalents measured by CUPRAC making five independent measurements for each compound. The values of abscissa axis correspond to the peak potentials measured at pH 5.5 under the same conditions as CUPRAC, but in the absence of neocuproine.

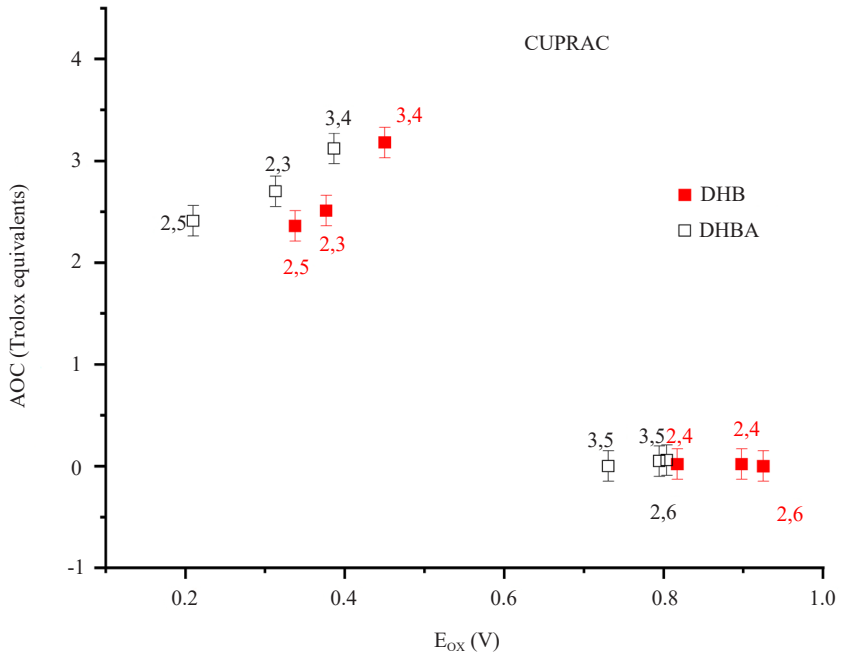


Figure 3. Plot of AOC measured by CUPRAC given in Trolox equivalents vs. the oxidation peak potentials of 1 mM compounds at pH 5.5

In this figure, the same behaviour of electron acceptors and donors is observed, but there is a trend for the latter, unlike what happens with DPPH. Although there are few points, it seems that the AOC for CUPRAC is related to the oxidation potential and, consequently, to the HOMO energy. This relation can be explained by considering that the CUPRAC assay is based on a single electron transfer (SET) mechanism[39]: only the transfer of one electron is involved in the reaction and, consequently, HOMO energies must be especially important in the antioxidant activity because these energies are directly related to the ability of the molecule to exchange an electron. Since the pK values of the carboxylic groups are less than 3.8,[30] the measured antioxidant capacity corresponds to the molecules that have the -COOH group dissociated, not to the neutral molecules but in contrast to the DPPH measurements.

With respect to the results given in Figure 2, in the DPPH assay, the AOC is measured from the extent to which the DPPH radical reacts with other radicals. This reaction is a combination of SET and HAT (hydrogen atom transfer) mechanisms, which explains the absence of correlation found for the group of electron-donor compounds.

Figure 4 shows the AOC in Trolox equivalents measured by e-CUPRAC (making five independent measurements for each compound) versus the peak potentials measured at pH 7.0 in an aqueous media (PBS buffer).

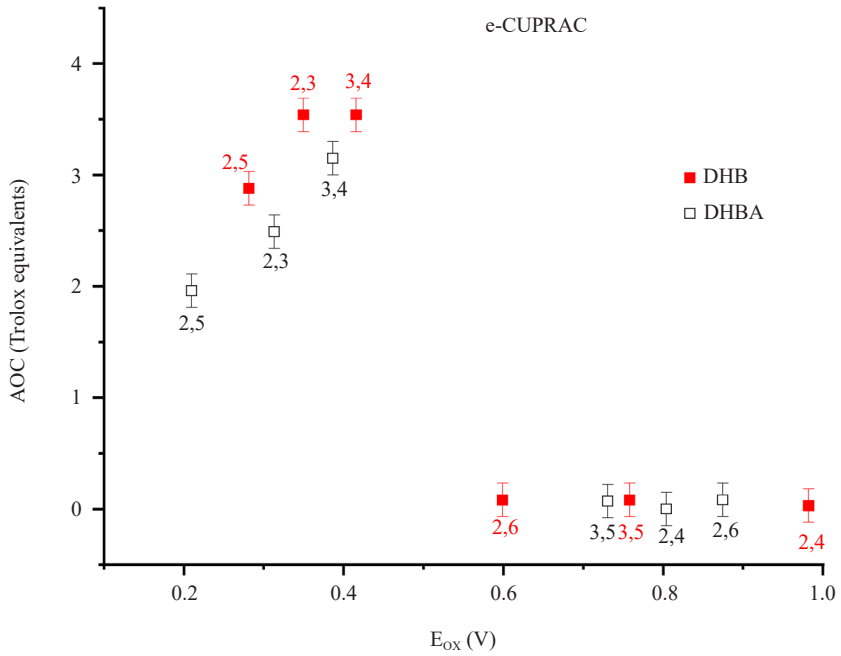


Figure 4. Plot of AOC measured by e-CUPRAC given in Trolox equivalents vs. the oxidation peak potentials of 1 mM compounds in 0.1 M PBS buffer

e-CUPRAC is a SET method that evaluates antioxidant activity in a very similar way to CUPRAC,[31] but in this case, the AOC is measured at a pH value for which the dissociation state of the molecules is different, especially for the aldehydes. As stated above, the pK values of the carboxylic groups are less than 3.8; in addition, the values of the second pK, corresponding to the dissociation of an -OH group, are less than 8.2. This means that at pH 7, the predominant forms of the acids are the monoanions corresponding to the carboxylate derivatives. On the other hand, the pK_1 values of the DHBs range from 6.5 to 8.4 (2,5-DHB), this meaning that at pH 7, there is a significant proportion of molecules containing a dissociated -OH group. The AOC values of the electron-donor DHBs are higher than those of electron-donor DHBAs. This is the opposite of CUPRAC's findings and can be explained by the SET mechanisms involved. The antioxidant activity measured by these assays is related to the release of one electron from the hydroxyl groups. So, it is evident that is easier to capture an electron from the anion $-O^-$ than from the neutral -OH group. In consequence, the antioxidant capacity of DHBs is enhanced at pH 7 with respect to those of DHBAs, as previously sketched for 2,4-DHB and 2,5-DHB.[24] No other comparison with previously reported studies can be made, because, as stated in the introduction, the sets of compounds investigated in the literature under the viewpoint of antioxidant-capacity structure relationship are heterogeneous, and do not usually have a common structure as is the case of DHB and DHBA reported in this paper.

4. Conclusion

The E_{OX} can be used as a structural parameter in the same way as the HOMO energy. From the dependencies with E_{OX} of the AOC measured by DPPH (a SET + HAT assay), CUPRAC and e-CUPRAC (two SET assays) methods, it follows that only electron-donor derivatives of DHB and DHBA exhibit antioxidant activity, which means that AOC is not uniquely related to the number of -OH groups present in the molecules (two in all cases). SET assays show that there is a relationship between the position of the hydroxyl groups and the AOC, and that the dissociation of the hydroxyl groups is a contributing factor to the value of the AOC.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Bottje, W. G. *Poult. Sci.* **2019**, *98* (10), 4223-4230.
[2] Di Meo, S.; Venditti, P. *Oxid. Med. Cell. Longevity* **2020**, *20*, 1-32.
[3] Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D.; et al. *Clin. Interventions Aging* **2018**, *13*, 757-772.
[4] Forman, H. J.; Zhang, H. *Nat. Rev. Drug Discovery* **2021**, *20*, 689-709.
[5] Gülçin, I. *Arch. Toxicol.* **2012**, *86*, 345-391.
[6] Aljohani, E. T.; Shehata, M. R.; Alkhatib. F.; Alzahrani, S. O.; Abu-Dief, A. M. *Appl. Organomet. Chem.* **2021**, *35* (5), 1-24.
[7] Aljohani, F. S.; Abu-Dief, A. M.; El-Khatib, R. M.; Al-Abdulkarim, H. A. Alharbi, A.; Mahran, A.; Khalifa, M. E.; El-Metwaly, N. M. *J. Mol. Struc.* **2021**, *1246*, 1-15.
[8] Abdel-Moneim, A. -M. E.; Shehata, A. M.; Alzahrani, S. O.; Shafi, M. E.; Mesalam, N. M; Taha, A. E.; Swelum, A. A.; Arif, M.; Fayyaz, M.; Abd El-Hack, M. E. *J. Anim. Physiol. Anim. Nutr.* **2020**, *104*, 1851-1866.
[9] Vauzour, D.; Rodriguez-Mateos, A.; Corona, G.; Oruna-Concha, M. J.; Spencer, J. P. E. *Nutrients* **2010**, *2* (11), 1106-1131.
[10] Lima, G.; Vianello, F.; Corrêa, C.; Campos, R.; Borguini, M. *Food Nutr. Sci.* **2014**, *5* (11), 1065-1082.
[11] Alvarado-Martinez, Z.; Bravo, P.; Kennedy, N. -F.; Krishna, M.; Hussain, S.; Young, A. C.; Biswas, B. *Antibiotics* **2020**, *9*, 1-6.
[12] Hossain, M. A.; Park, H. -C; Lee, K. -J.; Park, S. -W.; Park, S. -C.; Kang, J. *BMC Microbiol*. **2020**, *20*, 118.
[13] Rodríguez-Vaquero, M. J.; Vallejo, C. V.; Aredes-Fernández, P. A. *Open J. Pharmacol. Pharmacother.* **2020**, *5* (1), 1-6.
[14] Oliveira, E. F.; Yang, X.; Basnayake, N.; Huu, C. N.; Wang, L.; Tikekar, R.; Nitin, N. *J. Photochem. Photobiol., B* **2021**, *214*, 1-9.
[15] El-Nagar, A.; Elzaawely, A. A.; Taha, N. A.; Nehela, Y. *Agronomy* **2020**, *10* (9), 1-23.
[16] Silva, B.; Souza, M. M.; Badiale-Furlong, E. *J. Sci. Food Agric.* **2020**, *100* (3), 1256-1264.
[17] Nehela, Y.; Taha, N. A.; Elzaawely, A. A.; Xuan, T. D.; Amin, M. A.; Ahmed, M. E.; El-Nagar, A. *J. Fungi* **2021**, *7* (8), 1-26.
[18] Yip, E. C. H.; Chan, A. S. L.; Pang, H.; Tam, Y. K.; Wong, Y. H. *Cell Biol. Toxicol.* **2006**, *22* (4), 293-302.
[19] Lin, H. -H.; Chen, J. -H.; Huang, C. -C.; Wang, C. -J. *Int. J. Cancer* **2007**, *120* (11), 2306-2316.
[20] Sankaranarayanan, R.; Valiveti, C. K.; Dachineni, R.; Kumar, D. R.; Lick, T.; Bhat, G. J. *Mol. Med. Rep.* **2020**, *21*, 20-34.
[21] Velika, B.; Kron, I. *Free Radicals Antioxid.* **2012**, *2* (4), 62-67.
[22] Arteaga, J. F.; Ruiz-Montoya, M.; Palma, A.; Alonso-Garrido, G.; Pintado, S.; Rodríguez-Mellado, J. M. *Molecules* **2012**, *17* (5), 5126-5138.
[23] Palma, A.; Montoya, M. R.; Arteaga, J. F.; Mellado, J. M. R. *J. Agr. Food Chem.* **2014**, *62* (3), 582-589.
[24] Brito, R. E.; Mellado, J. M. R.; Montoya, M. R.; Palma, A.; Rodríguez-Amaro, R.; Mayén, M. *C. R. Chim.* **2017**, *20* (4), 365-369.
[25] Spiegel, M.; Kapusta, K.; Kołodziejczyk, W.; Saloni, J.; Zbikowska, B.; Hill, G. A.; Sroka, Z. *Molecules* **2020**, *25* (13), 1-15.
[26] Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radical Biol. Med.* **1996**, *20* (7), 933-956.
[27] Giacomelli, C.; da Silva Miranda, F.; Gonçalves, N. S.; Spinelli, A. *Redox Rep.* **2004**, *9* (5), 263-269.
[28] Kurek-Górecka, A.; Rzepecka-Stojko, A.; Górecki, M.; Stojko, J.; Sosada, M.; Świerczek-Zięba, G. *Molecules*

2014, *19* (1), 78-101.
[29] Fadilah, F.; Arsianti, A.; Yanuar, A.; Andrajati, R.; Paramita, R. I.; Purwaningsih, E. H. *Orient. J. Chem.* **2018**, *34* (5), 2656-2660.
[30] Kalinowska, M.; Golebiewska, E.; Swiderski, G.; Meczynska-Wielgosz, S.; Lewandowska, H.; Pietryczuk, A.; Cudowski, A.; Astel, A.; Swisłocka, R.; Samsonowicz, M.; et al. *Nutrients* **2021**, *13* (9), 1-33.
[31] Brito, R. E.; Aguilar, M. R.; Moreno, M. T.; Amaro, R. R.; Mellado, J. M. R. *J. Electroanal. Chem.* **2019**, *847* (15), 113235.
[32] Pullman, B.; Pullman, A. *Quantum Biochemistry*; Interscience: New York, 1963.
[33] Liu, Y.; Liu, M. S.; Jen, A. K. -Y. *Acta Polym.* **1999**, *50* (2-3), 105-108.
[34] Hwang, S. -W.; Chen, Y. *Macromolecules* **2002**, *35* (14), 5438-5443.
[35] Dance, I. *Inorg. Chem.* **2006**, *45* (13), 5084-5091.
[36] Abu-Dief, A. M.; Abdel-Rahman, L. H.; Shehata, M. R.; Abdel-Mawgoud, A. A. H. *J. Phys. Org. Chem.* **2019**, *32* (12), 1-18.
[37] Abu-Dief, A. M.; El-khatib, R. M.; El Sayed, S. M.; Alzahrani, S.; Alkhatib, F.; El-Sarrag, G.; Ismael, M. *J. Mol. Struct.* **2021**, *1244*, 1-14.
[38] Moreno, M. T.; Brito, R. E.; Mellado, J. M. R. *Biointerface Res. Appl. Chem.* **2021** (in press).
[39] Santos-Sánchez, N. F.; Salas-Coronado, R.; Villanueva-Cañongo, C.; Hernández-Carlos, B. Antioxidant Compounds and Their Antioxidant Mechanism. In *Antioxidants*; Shalaby, E., Ed.; IntechOpen: London, U.K., 2019; pp 1-28.