1 **Determination of antioxidant activity of spices and their active principles by**

2 **Differential Pulse Voltammetry**

3 by

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7 **ABSTRACT**

8 The anodic oxidation of mercury in presence of hydrogen peroxide in DPV
9 (Differential Pulse Voltammetry) was used to determine the antioxidant (AO) character of
10 radical scavengers. Hydroperoxide radical is formed at the potentials of the oxidation peak on
11 mercury electrodes, such radical reacting with the antioxidants in different extension. The
12 parameter C_{10} (antioxidant concentration at which the peak area decreases in a 10%) is used to
13 measure the scavenging activity of the individual antioxidants. To establish the scavenging
14 activity of antioxidant mixtures as a whole it was selected the parameter, μ_{10} as the reverse
15 V_{10}, being V_{10} the volume necessary to decrease the peak area in DPV in a 10%. The higher
16 μ_{10} values correspond to higher scavenging activity. The studies have been extended to
17 aqueous extracts of some species. The results may be useful in explaining the effect of spices
18 over in vitro and in vivo studies.

19 **KEYWORDS:** Hydroperoxide radical; radical scavengers; antioxidant activity; spices;

20 cinnamon; clove; numteg, oregano; thyme.

21 **INTRODUCTION**

22 Oxidative stress is defined as the excessive production of reactive oxygen species
23 (ROS) and/or deficiency of the antioxidant cellular defence system. The ROS play a major
24 role in causing antioxidant stress and damage to DNA, proteins and lipids[1]. Free radical
25 mediated cell damage has been implicated in the pathology of various human chronic diseases
26 such as Alzheimer's, Parkinson's, Crohn's, and certain cancers[2,3]. In addition to endogenous
27 antioxidant systems, a diet rich in antioxidant food products also protects DNA and increases
28 resistance against oxidative stress. Spices may offer many health benefits and have been
29 proven to counteract oxidative stress over *in vitro* and *in vivo* systems[4,5].

30 Primary antioxidants (prooxidants) prevent the formation of free radicals[6], particularly
31 reactive oxygen species (ROS). Secondary antioxidants (antioxidants with radical scavenging
32 activity) interrupt the propagation of free radicals, inhibit the generation of ROS, and prevent
33 the metabolic activation of carcinogens[7,8]. Tertiary antioxidants repair the damage from free
34 radicals or eliminate damaged molecules[9,10].

35 Direct and indirect methods have been proposed to evaluate the antioxidant activity of
36 natural products[11]. Electrochemical measurements are a rapid proof to test the antioxidant
37 capacity of many organic molecules[12,13] and to study the interaction between the antioxidant
38 and ROS, as was observed for dihydropyridines and electrogenerated superoxide radical[14,15].

39 Oxidation potentials of the peaks appearing in cyclic voltammetry have been used for
40 comparison of the antioxidant action of compounds such as phenolic acids, flavonoids,
41 cinnamic acids etc.[16-18]. The electrode more used was the glassy carbon electrode, GCE.
42 Similarly, cyclic voltammetric measurements have been applied to the determination of
43 antioxidant capacity of wines[17-18]. The variation of the oxidation potential with the pH values
44 can be provided, and measurements made at different pH values can be compared.
45 Concentration is critical to the success of the technique. The area under the first voltammetric

46 peak is a good estimate of phenols concentration with low oxidation potential and can be
47 related to the antioxidant activity of the sample of wine. Another advantages are: there is no
48 need to measure the antioxidant capacity specific to each component, the sample preparation
49 is simple and fast and the sensitivity of the technique is sufficient for determination at
50 physiological concentrations of antioxidants. Low oxidation potentials are associated with a
51 greater facility of a given molecule for the electrodonation i.e., to act as an antioxidant, with
52 good correlation with other techniques such as the scavenging of the $DPPH^{\bullet}$ radical[19].

53 The radical scavenging ability given by the $DPPH^{\bullet}$ assay was related to the reduction
54 potential of $DPPH^{\bullet}$. Radicals as $ROO^{\bullet}$ and $OH^{\bullet}$, exhibit much higher formal potentials than
55 $DPPH^{\bullet}$ and can react with species with high oxidation potentials for which it is not possible to
56 determine the antioxidant activity by the DPPH assay. There are some accepted methods for
57 ROS, like fluorimetric assay for hydrogen peroxide[20], ESR/EPR method and so on[20-24].

58 The anodic oxidation reaction of hydrogen peroxide was used in a pioneering research
59 to determine the antioxidant character of compounds in foods[25-29], but some experimental
60 problems lead to revise this method[30]. Recently Gorjanovic et al. and Potkonjak et al.[31-33]
61 have developed polarographic chriteria for antioxidant activity.

62 Sužnjević and coworkers[25] assessed that the anodic wave of H_2O_2 observed on
63 mercury electrodes is due to the oxidation of Hg to Hg^{++} and the subsequent formation of a
64 mixed complex with HO_2^- and OH^- ions, as previously reported by Kikuchi and Murayama[34].
65 This lead to revise the approach made in reference 30.

66 On the other hand, spices are commonly used to enrich flavour and aroma in cooking
67 and to preserve food, and also as components of a healthy diet. They are traditionally thought
68 to have beneficial effects on health and specific spices are used in traditional medicine as part
69 of therapeutic formulations. Pharmacological studies on spices revealed a wide range of

70 biological activities, including, antioxidant, anti-inflammatory, anti-tumor,
71 immunomodulatory and antiradical activities[35-37]. Their antioxidant constituents are identified
72 as the responsible of the health-promoting properties of spices, such as anti-atherosclerotic,
73 anti-cancer, and anti-inflammatory activities[38].

74 The aim of this work was, first, to revise the DPV method under the light of the
75 oxidation mechanism on mercury electrodes; second to apply the studies to potentially food
76 antioxidants, namely a number of bioactive compounds found in spices and condiments; and,
77 third, to extend these studies to mixtures of antioxidants and to aqueous extracts of some
78 species.

79 The compounds studied are mainly active principles of spices, seasonings or drugs,
80 belonging to the family of low molecular weight antioxidants, being aromatic phenolics and
81 non-phenolics, or cyclic and acyclic non-aromatic compounds: eugenol, carvacrol,
82 cinnamaldehyde, thymol, sesamol, 3-hydroxycoumarin, salicylaldehyde, limonene, geraniol,
83 vanillin, cinnamic acid, α- and β-pinene, 2,4- and 2,5-dihydroxybenzaldehydes and gallic
84 acid. The spices studied contain one or more of the above compounds.

85

86 **MATERIALS AND METHODS**

87 *Materials*

88 3-Hydroxycoumarin, carvacrol, vanillin and gallic acid were purchased from Aldrich
89 (or Sigma-Aldrich) and the rest of chemicals were Merck analytical grade reagents. All the
90 reactants were used without further purification. Thyme, cinnamon, oregano, clove and
91 nutmeg were purchased in a grocery store and were of the Mercadona S.A. trademark.

92 Solutions of 0.1 M in both sodium carbonate and phosphoric acid at pH = 10.5 were
93 used as supporting electrolytes. The aqueous solutions were prepared with ultrapure water

94 type I (resistivity 18.2 MΩ.cm at 25°C) obtained from a Millipore Simplicity® system. The
95 ionic strength was adjusted to 0.5 M with solid KNO_3 and the *pH* was adjusted with solid
96 NaOH. Antioxidants were dissolved in ethanol and the stock solution concentrations were
97 $5x10^{-3}$ M. These solutions were stored in darkness at 277 K to avoid decomposition.

98 Aqueous extracts of thyme, cinnamon, oregano, clove and nutmeg were obtained by
99 adding 250 mg of the spice to 100 mL of deionized water, heating for five min at 95 ºC, and
100 filtering at room temperature. The DPPH assays of these filtrates were made directly, but they
101 were tenfold diluted for the H_2O_2 experiments. In this last case, the data reflected in the
102 results and discussion section show the results corrected for the dilution.

103 *Methods*

104 *Electrochemical measurements*

105 The concentration of hydrogen peroxide in the cell was $5x10^{-4}$ M and the percentage of
106 ethanol in the cell was 30%. Because the change in the ethanol content modifies the DPV
107 peak area[30], solutions were prepared with a fixed amount (6.9 mL) of supporting electrolyte,
108 100 μL of $5x10^{-2}$ M H_2O_2, variable volumes, V_{AO}, of the stock solution of antioxidant in
109 ethanol and completing the total volume with $(3–V_{AO})$ mL of pure ethanol. So, it was
110 necessary to prepare a separate solution for each concentration of antioxidant and the samples
111 were not gradually added to the solution because the undesirable decrease produced by the
112 presence of ethanol, which depends on the ethanol content. The 100 μL H_2O_2 were added
113 after the solutions were purged with purified nitrogen. For the experiments made with the
114 aqueous extracts of spices, no ethanol was added, the overall volume being completed with
115 water, and the measurements being referred to the signal obtained for H_2O_2 in the absence of
116 ethanol.

117 Measurements were made on a CHI650A electrochemical workstation from IJCambria
118 coupled to an EF-1400 controlled growth mercury electrode from BAS instruments, in the
119 HMDE mode. The Hg drop area was 6.70×10^{-3} cm^2. The temperature was kept at 298±0.1 K.
120 All potentials were measured against an $Ag|AgCl|KCl_{sat}$ electrode (BAS MF-2052). A
121 platinum counter electrode BAS MW-1034 was used. The parameters selected in the
122 differential pulse voltammetry (DPV) were: pulse amplitude 0.05 V, pulse width 0.05 s and
123 pulse period 0.2 s.

124 The reproducibility of the measurements was ensured by repeating the experiments and
125 the standard deviations of the data were less than 5%.

126 *Spectrophotometric measurements. $DPPH^{\bullet}$ radical scavenging assay*

127 The maximum wavelength of the UV□visible absorption band of the $DPPH^{\bullet}$ is 515 nm
128 and the action of an AO causes the decrease of this band or its eventual disappearing through
129 the reactions to give DPPH and $AO^{\bullet}$. The amount of antioxidant required to decrease the
130 initial concentration of $DPPH^{\bullet}$ to 50% (efficient concentration or EC_{50}) is a measurement of
131 the antioxidant activity. The reverse value, namely anti-radical power, $ARP = 1/EC_{50}$, should
132 be higher as the antioxidant is more efficient.

133 UV measurements were made on a Genesys 10 UV spectrophotometer from Thermo
134 Electron Corporation with quartz cuvettes of path-length 1.0 cm.

135 Different concentrations of antioxidants were added to $DPPH^{\bullet}$ methanolic solution. The
136 initial $DPPH^{\bullet}$ concentration was $6\text{x}10^{-5}$ M. The $DPPH^{\bullet}$ concentration in the reaction medium
137 was calculated from a calibration curve with the equation:

$$Abs_{515\ nm} = 12.195 \text{ x } c_{DPPH} - 0.0137$$

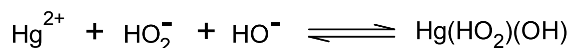
138 as determined by linear regression.

139 For the aqueous extracts of the spices, the ARP was referred to weight of antioxidant
140 required to decrease the initial concentration of $DPPH^{\bullet}$ to 50%, W_{50}, expressed in mg, being
141 the ARP value $ARP = 1/W_{50}$, in mg^{-1}.

142

143 **RESULTS AND DISCUSSION**

144 Kikuchi and Murayama[34] proposed that the anodic wave of H_2O_2 observed on mercury
145 electrodes is due to the oxidation of Hg to Hg^{++} and the subsequent formation of a mixed
146 complex with HO_2^- and OH^- ions:



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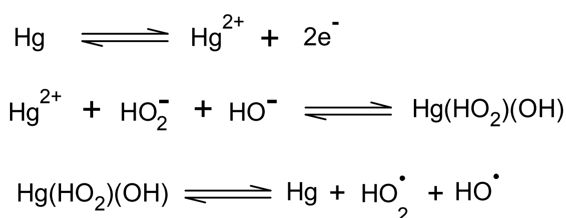
148 The formation of this complex was assessed by Sužnjević and coworkers[25] by titration
149 of H_2O_2 with $HgCl_2$ in basic solutions. However, if the reactions shown in the above scheme
150 were the only processes occurring at the electrode, no effect of the addition of antioxidants on
151 the oxidation wave could be observed, because the antioxidants do not react with the mercury
152 ions in the absence of hydrogen peroxide at least in the experimental conditions of
153 polarographic and voltammetric experiments[39].

154 It could be claimed that antioxidants act by reacting with the dissociated hydrogen
155 peroxide and thus decreasing the concentration of the complex, this producing a decrease in
156 the polarographic limiting current (or in the DPV peak current) that can be related to the
157 antioxidant activity. However, this implies that the antioxidant must react with H_2O_2 in the
158 solution. Experiments were made to check this possibility. As can be seen in figure 1, linear
159 sweep cyclic voltammograms of solutions of 0.5 mM the antioxidant sesamol were recorded
160 at pH 10.5 in the absence and in the presence of increasing concentrations of hydrogen
161 peroxide.

162 No effects on the oxidation wave were observed even at H_2O_2 concentrations 10 times
163 higher than sesamol. In addition, the peak current of the oxidation peak of sesamol remained
164 constant during 30 min, even at the maximum peroxide concentration used. So, no reaction
165 occurs involving these two reactants and in conclusion, additional reactions must be
166 considered.

167 Since the presence of radicals is needed to give an interaction with the antioxidants,
168 the only possibility is that in the range of potentials of the oxidation peak on mercury
169 electrodes such radicals are formed. It is evident that the electrode must play an important role
170 on the electro-oxidation. This role could be viewed as the occurrence of adsorbed species on
171 the electrode, the Gibbs energy of the adsorption processes decreasing the oxidation potential
172 of hydrogen peroxide. In consequence, the simplified reaction scheme given in reference 30
173 must involve adsorbed species.

174 Another approach to the problem is to assume that radicals are produced after the
175 formation of the mercury complex, probably through intramolecular electron transfers:



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177 In this scheme, the last reaction must occur at trace levels in the absence of radical
178 scavengers. The addition of increasing amounts of antioxidants must cause a shifting to the
179 right of the last step by reaction with the radicals, and, consequently, the shifting to the right
180 of the overall process. In this case, the addition of antioxidants must increase the limiting
181 current of the dc wave (or the peak current of the DPV peak), in contradiction with the
182 experimental findings.

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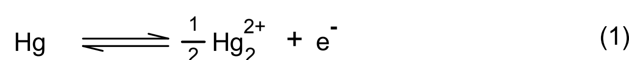
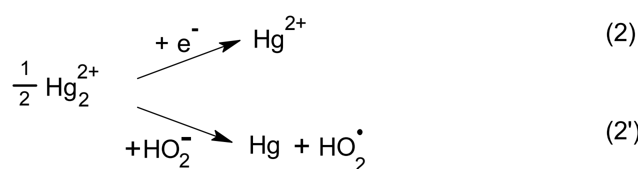
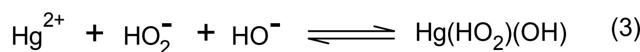
It is known from decades ago[40] that the anodic oxidation of the mercury involves both the Hg(II) and Hg(I) ions in equilibrium with the complexes formed with chloride or bromide ions present in the solution. This takes place at potentials 0.1 to 0.2 V more positive than the peak here analyzed. This behavior is common to the redox processes of metal ions, that is, the electron transfers are one-electron and the redox state of the ions increase or decrease in one unity[41]. So, it seems reasonable to think that the Hg(I) ions are formed after the first one-electron transfer and the following reactions could take place:







In the absence of AO, the reaction 2' takes place only at trace levels. When a radical scavenger is added, this species reacts with the hydroperoxyde radical produced in reaction 2' and decreases the available concentration of the Hg(I) ion to give Hg(II) ion and, consequently, the oxidation current. This scheme explains all the experimental results obtained. First, the DPV peak area decreases as the antioxidant concentration increases from the value corresponding to a two-electron transfer (Hg oxidation to mercuric ions through mercurous ions followed by the complex formation) to the value corresponding to an EC one-electron transfer (Hg oxidation to mercurous ions followed by hydroperoxyde radical and subsequent reaction with AO), that is, to a value not less than a 50% of the original area. Second, the half width of the DPV peak changes from the value corresponding to a two-electron process, to the value corresponding to an EC one-electron process, as was experimentally found[30].

203 The I-E relationships for the above-described process can be obtained by using the
204 convective diffusion approximation and the steady-state conditions and the Nernst diffusion-
205 layer approximation, but the equations for the limiting currents as a function of the AO
206 concentration and the equilibrium and rate constants of reaction 2' are complex and cannot be
207 linearized. It is evident that the rate of such reaction must increase as the rate constant
208 increases, and the decrease in limiting current (or in the area of DPV peak) must be higher at
209 lower AO concentrations.

210 So, the approach of Gorjanovic et al. and Potkonjak et al.[31-33] seems appropriate for
211 the evaluation of the scavenging activity of antioxidants. In these papers, the slope of the
212 linear part of the curve obtained by plotting percentage of decrease of polarographic current
213 vs. the amount of individual compounds or volume of complex samples was used to express
214 AO activity. This slope was found to be the most relevant and accurate way to express AO
215 activity of samples with large differences in activity and was finally chosen as AO activity
216 criteria. High correlations were reported between the slope and the content of total phenolics
217 and AO activity determined by standard spectrophotometric assay such as FRAP, ABTS and
218 DPPH.

219 DPV is a differential technique, this implying that the parameter proportional to the
220 concentration of the species that is oxidized or reduced at the electrode is the peak area[40].
221 Conversely as the polarographic limiting current, the peak current depends on the reaction
222 mechanism, at the same concentration, pH, temperature and ionic strength. The half-width of
223 the DPV peak changes with the AO addition, this implying that there is a change in the
224 oxidation mechanism, and so, the change of intensity does not reflect only the dependence
225 with the AO concentration, but also this change in half-width. So, the parameter to be tested is
226 the DPV peak area.

227 Figure 2 (A, B) shows the decrease of the peak area of the DP voltammograms of the
228 DPV oxidation peak obtained for H_2O_2 in mercury electrodes after the addition of increasing
229 amounts of several antioxidants.

230 Figure 2 (C, D) shows the decrease of the peak area as a function of the added volume
231 for two selected antioxidants and starting from different concentrations of antioxidant, to
232 study the dependence on this parameter. From these graphs, it follows that the dependence of
233 the decrease with the added volume is roughly linear at low percentages of decrease, and is
234 proportional to the initial antioxidant concentration. So, this slope can be used as an AO
235 activity criterion in the same way as in references 31-33. The calculated slopes are given in
236 table 1.

237 To perform the experiments, different concentrations of antioxidants were used, because
238 the more active antioxidants decrease the current below the 10% at very low volumes added.
239 So, if this is the case, the essays were repeated after dilution of the antioxidant, this allowing
240 the addition of higher volumes and thus permitting a more accurate determination of the C_{10},
241 that is, the antioxidant concentration at which the area of the H_2O_2 oxidation peak decreases
242 in a 10%. The 10% of decrease falls in the linear range of the plots shown in figure 2.

243 As stated in a previous work[30] the results obtained by using $1/C_{10}$, ARP or voltammetric
244 oxidation potentials (E_P) are quite different. Table 1 and figure 3 illustrate this. In this table,
245 the ARP values of the previously essayed compounds have been taken from reference 30 (the
246 α- and β-pinene have been tested for this work) and the E_P values were determined again for
247 this work, being the same as found previously[30] as is expected.

248 From table 1 it follows that there are compounds that do not exhibit ARP or E_P
249 measurable values, but their scavenging activity can be evaluated using $1/C_{10}$. A part of these
250 compounds consists of antioxidants of low scavenging activities. Other compounds as 3-

251 hydroxycoumarin, 2,4-dihydroxybenzaldehyde or cinnamaldehyde showed very low ARP
252 values and high oxidation potentials, but the corresponding $1/C_{10}$ values are high, this
253 indicating a high scavenging activity. In other cases as carvacrol and vainillin or eugenol and
254 sesamol, the ARP and the oxidation potentials have similar values, but the $1/C_{10}$ values are
255 quite different. In fact, no evident correlation between ARP and $1/C_{10}$ nor between E_p and
256 $1/C_{10}$ has been found.

257 It is known[19] that both ARP and oxidation potentials measures the capacity of a given
258 compound to react with the $DPPH^{\bullet}$ radical. The $1/C_{10}$ parameter is related to the interaction of
259 the antioxidant with the ROS (hydroperoxide radicals).

260 The method can be easily extended to mixtures of *n* antioxidants because in this case the
261 reaction 2' will consist of *n* parallel reactions. In this case, the goal is to establish the
262 scavenging activity of the mixture as a whole, not the individual antioxidant activities. Since
263 the added volume is proportional to the concentrations, the volume necessary to decrease the
264 area of the H_2O_2 oxidation peak in a 10%, V_{10}, must be related with the scavenging activity
265 (more precisely, the reverse of V_{10}), equivalent to C_{10}. In this work $\mu_{10}=1/V_{10}$ (expressed in
266 mL^{-1}) is selected to express the antioxidant activity. In this way, the higher μ_{10} values, the
267 higher scavenging activity. This parameter can be also used with individual antioxidants.
268 Table 1 also lists the μ_{10} values corresponding to the antioxidants in the experiments made at
269 concentrations of 5 mM.

270 Figure 3 shows the DP voltammograms of the H_2O_2 oxidation before and after the
271 addition of increasing volumes of four antioxidant mixtures. Binary and ternary mixtures of
272 antioxidants of high, medium and low μ_{10} have been investigated. The concentrations were
273 chosen to cover different proportions of compounds having low and high AO activities. As
274 the volume added was increased, the DP peak decreases and at a high enough volume of

275 mixture was added, the area of the peak becomes one-half of the original area (data not
276 shown).

277 Figure 4 shows the dependence of the peak area of the DP voltammograms of the H_2O_2
278 oxidation after the addition of increasing volumes of antioxidant mixtures, relative to the area
279 of the peak in the absence of antioxidant, for the same mixtures as figure 3.

280 From these data, the μ_{10} values can be obtained. Table 2 shows these values for a
281 collection of mixtures of four antioxidants, mainly binary combinations.

282 The scavenging activities of the mixtures must be related, evidently, to the scavenging
283 activities of the antioxidants used. So, an antioxidant having high scavenging activity but in
284 low concentration could contribute to the overall activity of the mixture in less grade than
285 other antioxidant having lower activity, but present in great concentration. Since the
286 assumption in this work is that μ_{10} values are related with the scavenging activity, “expected"
287 μ_{10} values have been calculated for each mixture from the individual μ_{10} values given in table
288 1 and taking into account the concentration of each compound. The results of such
289 calculations are given in figure 5. The graph shows a correlation between experimental and
290 calculated values. More precisely, there is a proportionality between both values, being the
291 slope obtained from the correlation 1.06, that is, close to unity. This indicates that μ_{10}
292 parameter is a measure of the scavenging activity, being additive, i.e., the overall μ_{10} value of
293 a mixture is constituted by the sum of the μ_{10} values of the individual antioxidants.

294 Moreover, the proportionality shown in figure 6 indicates that there are not synergistic
295 effects in the mixtures, at least for the investigated mixtures, but this conclusion must be
296 checked with a wider range of antioxidant mixtures.

297 The measurements were extended to the aqueous extracts of five spices. Figure 6 and
298 table 3 summarize the results. As can be seen, at low values of decrease, the plot shown in

299 figure 3 is roughly linear. From these experiments, μ_{10} values have been calculated for each
300 extract and have been gathered in table 3. In the table are also given the ARP values obtained
301 from the DPPH$^{\bullet}$ experiments but not the oxidation potentials, because the voltammograms
302 were complex and showed more than one oxidation peak.

303 In general, the sequence of μ_{10} values is related to the contents in antioxidants of the
304 different spices, but it is important to note that such contents correspond to essential oils, not
305 to aqueous extracts[42] and the proportions must vary from an extraction mode to another.
306 Nevertheless, when the spices are used as food condiments act essentially as aqueous extracts
307 rather than non-aqueous essential oils.

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422

423 **Figure Captions**

424 **Figure 1.** Linear-sweep cyclic voltammograms on a glassy carbon electrode (IJCAMBRIA
425 38.5 mm^2) of 1 mM sesamol at pH 10.5 and different amounts of $5x10^{-2}$ M H_2O_2 solution
426 added to 10 mL final volume in the cell.

427 **Figure 2.** Dependencies of the DPV area of H_2O_2 $5x10^{-4}$ M after the addition of increasing
428 amounts of antioxidants, relative to the area of the peak in the absence of antioxidant.
429 (a) AO concentration: 5 mM. ▲ Gallic acid; ◀ Eugenol; ▶ 3-Hydroxycoumarin; ■ 2,4-
430 Dihydroxybenzaldehyde; ▼ Carvacrol; ◆ Cinnamaldehyde; ● 2,5- Dihydroxybenzaldehyde.
431 (b) AO concentration: 5 mM. ◀ Thymol; ▶ Vainillin; ■ Cinnamic acid; ▼ Salicylaldehyde;
432 ◆ Sesamol; ▲ Limonene; ● Geraniol.
433 (c) Gallic acid. Concentrations: △ 1 mM; □ 3 mM; ○ 5 mM
434 (d) Carvacrol. Concentrations: △ 1 mM; □ 3 mM; ○ 5 mM

435 **Figure 3.** Dependencies of the reverse of the antioxidant concentration that decreases in a
436 10% the DPV area of H_2O_2 (in mM), with the slope of the percentage of decrease vs. volume
437 added (see text).

438 **Figure 4.** DPV voltammograms of $5x10^{-4}$ M H_2O_2 and different amounts of antioxidants
439 mixture solution. pH = 10.50. 30% ethanol in the medium. AO mixtures: A) 2.5 mM eugenol
440 + 2.5 mM limonene; B) 0.5 mM eugenol + 0.75 mM thymol + 0.75 mM sesamol; C) 0.5 mM
441 eugenol + 0.75 mM thymol + 2 mM limonene; D) sesamol 2.5 mM + limonene 2.5 mM.

442 **Figure 5.** Dependencies of the DPV area of H_2O_2 $5x10^{-4}$ M after the addition of increasing
443 amounts of antioxidants mixture solution, relative to the area of the peak in the absence of
444 antioxidant. AO mixture: ■ 2.5 mM eugenol + 2.5 mM limonene; ▲ 0.5 mM eugenol + 0.75
445 mM thymol + 2 mM limonene; ● sesamol 2.5 mM + limonene 2.5 mM; ▼ 0.5 mM eugenol
446 + 0.75 mM thymol + 0.75 mM sesamol.

447 **Figure 6.** Correlation between experimental and calculated values of the μ_{10} parameter (see
448 text) for the antioxidant mixtures shown in table 2.

449 **Figure 7.** Dependencies of the DPV area of H_2O_2 $5x10^{-4}$ M after the addition of increasing
450 amounts of aqueous extracts of spices, relative to the area of the peak in the absence of
451 antioxidant.

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452 **Table 1.** Values of $1/C_{10}$ (antioxidant concentrations in $mmol \cdot L^{-1}$), Slopes of the plots of the
453 decrease of the peak area versus the added volume, ARP and Oxidation potentials vs.
454 Ag/AgCl/KCl (3M) electrode for the antioxidants studied. Last column corresponds to μ_{10}
455 values at concentrations of 5 mM.

Antioxidant	Slope[a]	$1/C_{10}$ (mM^{-1})	ARP[b]	E_p(mV)[c]	μ_{10}/mL^{-1}
Gallic acid	0.440	102.108	8.5	274	45.53
Eugenol	0.259	68.243	5	411	25.59
3-Hydroxycoumarin	0.250	53.214	--	763	24.77
2,4-Dihydroxybenzaldehyde	0.147	40.400	--	841	20.04
Carvacrol	0.165	35.924	0.12	522	17.76
Cinnamaldehyde	0.128	26.639	--	588	16.48
α-Pinene	0.103	22.260	0.10	--	9.93
2,5- Dihydroxybenzaldehyde	0.054	19.688	17.5	202	11.20
Thymol	0.08	18.685	0.78	529	9.10
Vainillin	0.067	14.580	0.11	584	8.80
Cinnamic acid	0.059	14.470	--	--	6.59
Salicylaldehyde	0.053	12.271	--	860	5.76
Sesamol	0.056	12.048	5.5	343	5.76
β-Pinene	0.035	9.435	--	--	4.28
Geraniol	0.026	5.383	--	--	4.05
Limonene	0.021	5.233	--	--	2.33

456 [a] ±1% = average of slope confidence interval; [b] ±0.5% = average of ARP confidence
457 interval; [c] Confidence interval for Ep values was always lower than ±4 mV

458 **Table 2.** μ_{10} values for mixtures of antioxidants. Numbers under each compound correspond

459 to Concentrations in mM for the mixture in question.

Mixture number	Eugenol	Limonene	Sesamol	Thymol	Vainillin	μ_{10}/mL^{-1}
1	2.5	2.5	0	0	0	14.57
2	0	0.2	0	0.8	0	1.77
3	0	0.6	0	0.4	0	1.32
4	0	0.4	0	0.6	0	1.25
5	0	1.75	0	0	3.25	7.36
6	1	4	0	0	0	7.10
7	1	2	0	1.5	0	9.31
8	1	2	0	0	0	8.30
9	0	4	0	0	1	5.50
10	0	2.5	2.5	0	0	4.67
11	0.5	0	0.75	0.5	0	2.75

460 ±1% = average of μ_{10} confidence interval

461 **Table 3.** V_{10} and μ_{10} values, ARP and antioxidants found in the extracts for aqueous extracts
462 of spices[40].

Spice	V_{10} (µL)	μ_{10} (mL^{-1})	ARP (mg^{-1})	Main antioxidants	
Oregano	23.1	43.29	5.25	p-cymene Thymol Carvacrol	12% 5% 49%
Clove	32.4	30.86	16.23	β-Caryophyllene Eugenol Acetyl-eugenol	7% 83% 8%
Thyme	51.7	19.34	21.81	p-Cymene Linalool Thymol	27% 6% 46%
Cinnamon	56.8	17.61	16.02	β-Caryophyllene Cinnamaldehyde Eugenol	5% 68% 7%
Numteg	219.2	4.56	10.40	α-Pinene β-Pinene Sabinene Terpinen-4-ol	21% 15% 19% 4%

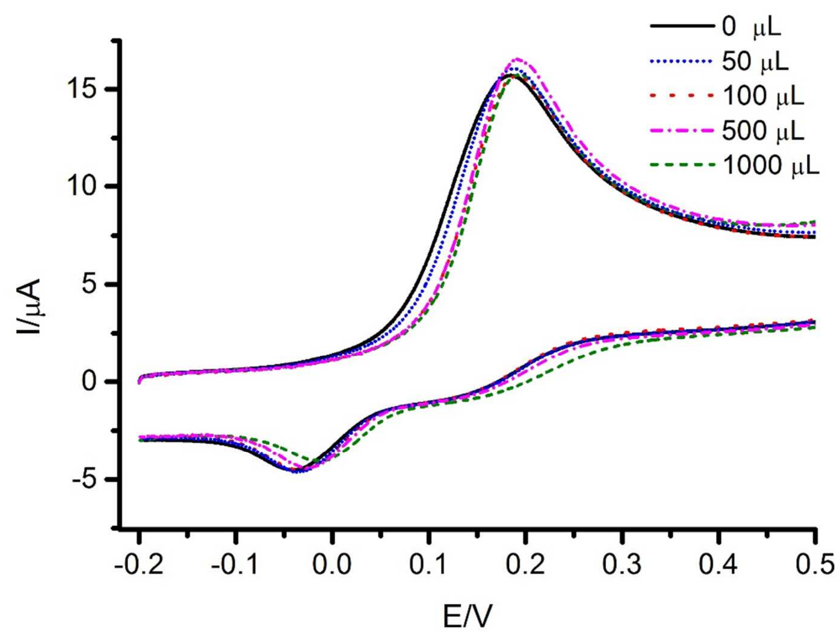

Figure 1

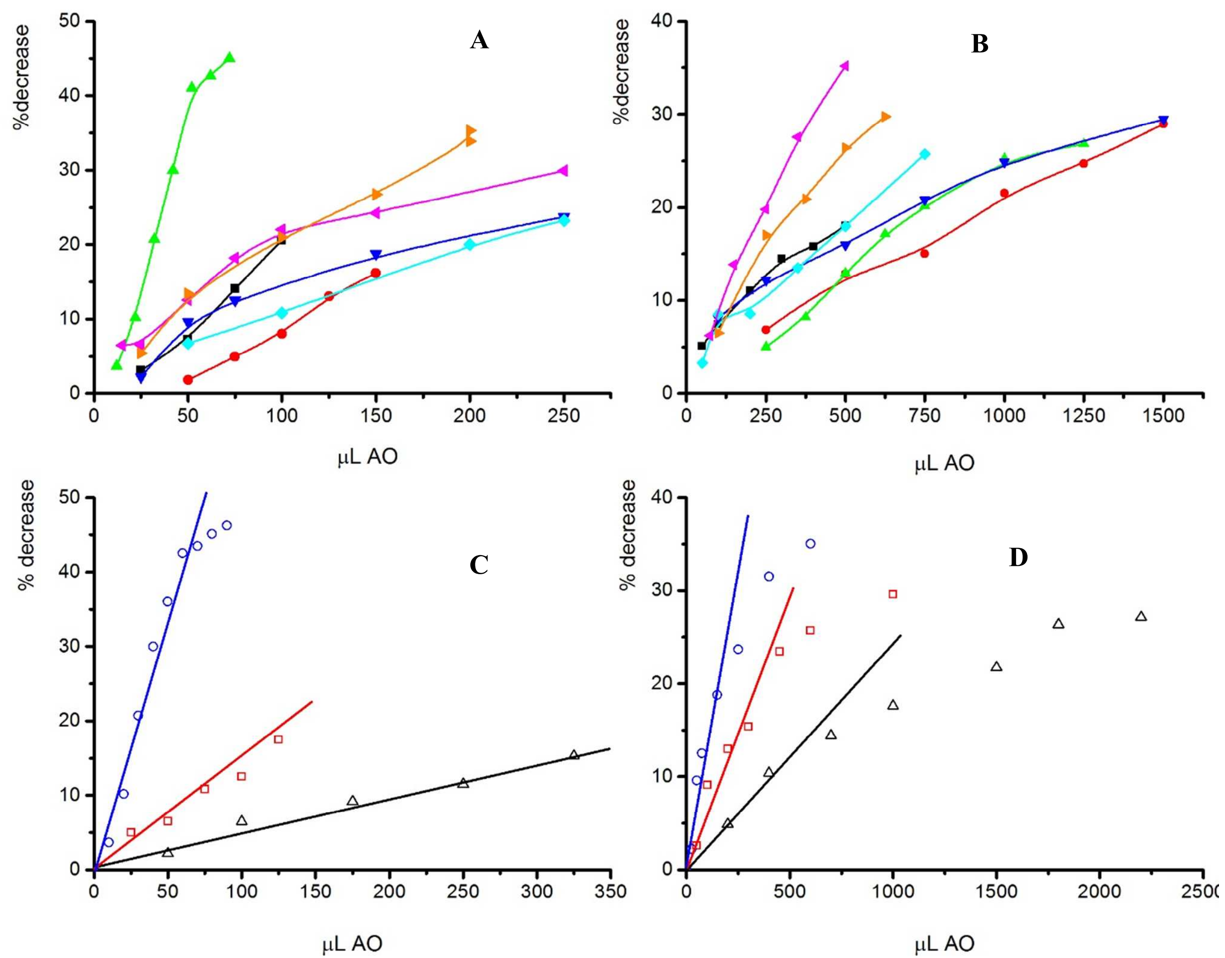

Figure 2

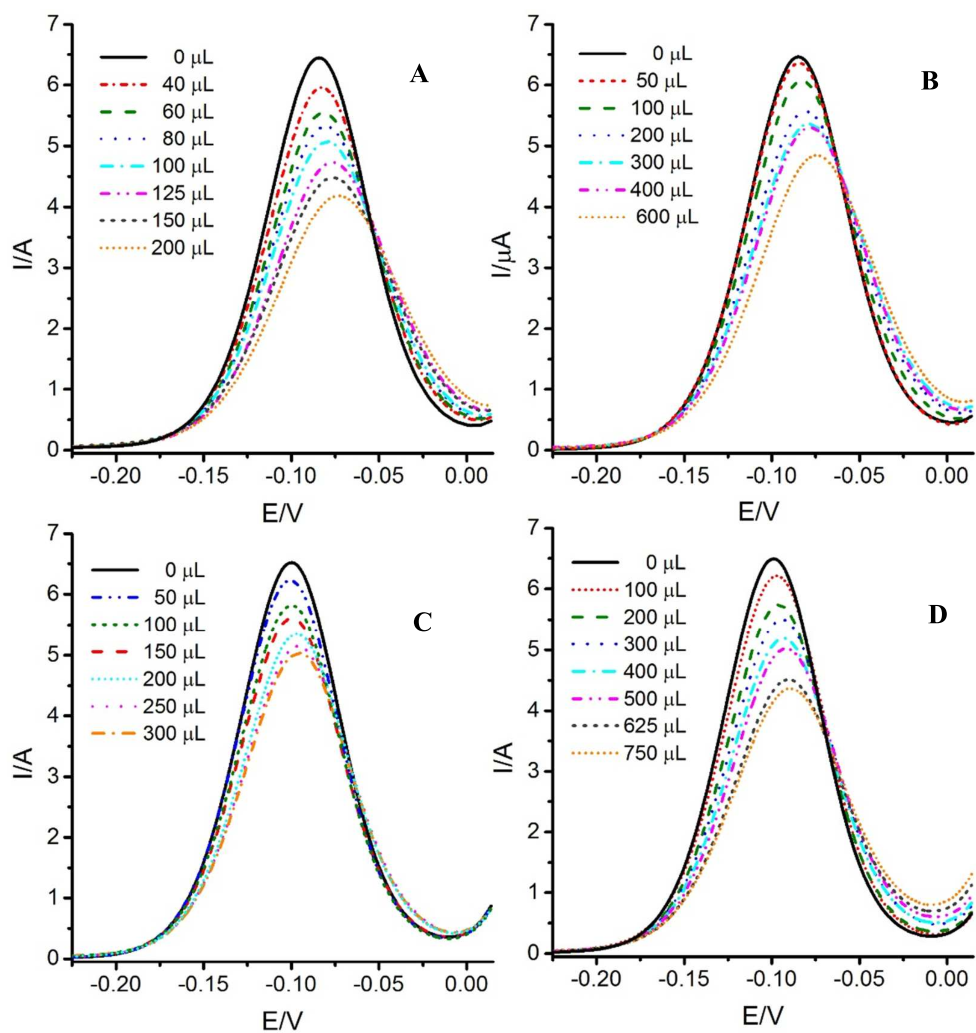

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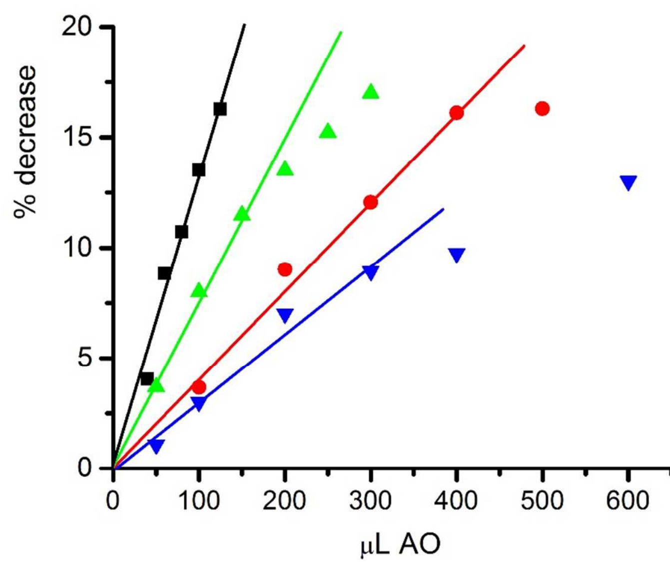
Figure 3



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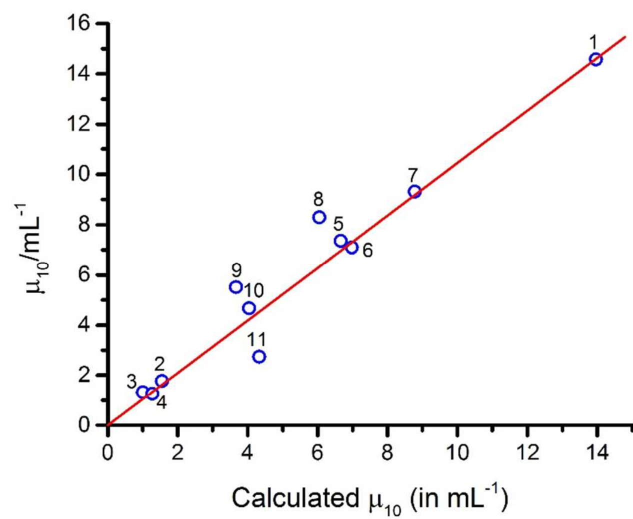

Figure 5

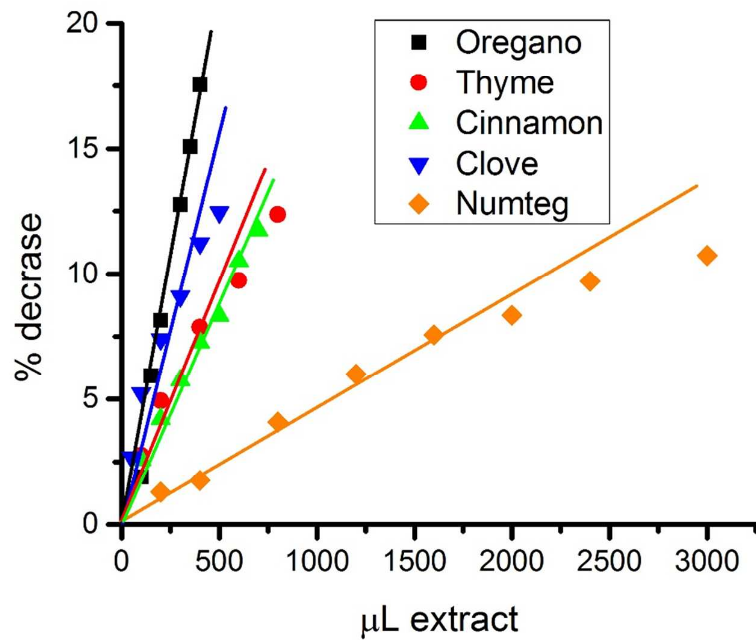

Figure 6

TOC