

# Kinetics and antiradical activity of natural and synthetic phenolic compounds by DPPH method: a comparative study

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## Abstract

Phenolic compounds are highly bioactive compounds which are widely present in plants (fruits and vegetables) and natural beverages (tea, coffee, wine etc.). Biological effects associated with phenolic compounds include antioxidant, anti-carcinogenic, anti-inflammatory and antibacterial activities which contribute to various health benefits. Although phenols are mainly natural compounds, there are also phenolic compounds, with high antioxidant activity, obtained by synthetic pathways, both natural and synthetic phenols being used in food and beverage, pharmaceutical and cosmetic industries.

The aim of the present study is to assess by comparison the antioxidant activities of natural and synthetic phenolic compounds. Therefore, one natural phenolic compound (caffeic acid) and two synthetic phenols (butylated hydroxyanisole and propyl gallate) are evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH·) assay and consist of determination of antioxidant compounds capacity to scavenge the synthetic free radical, DPPH·, and calculating the reaction rates between phenolic compounds and DPPH·. For each compound, ethanolic solutions of 1 mM, 0.2 mM, 0.1 mM, 0.05 mM are prepared, and their antiradical activities are determined in the presence of 1mM ethanol solution of DPPH· by UV-VIS spectrophotometry. Propyl gallate showed the highest antiradical activity up to thirty seconds. The highest DPPH· reaction rate of 2.4 μM/s was obtained for caffeic acid solution at 1mM for the time range up to one minute of reaction.

**Keywords:** natural and synthetic phenolic compounds, antioxidant activity, DPPH· method, reaction rate, kinetics

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## 1. Introduction

Polyphenols are phenolic compounds with high antioxidant activities which are mostly occurring in fruits, vegetables and various beverages obtained from natural sources (coffee, tea, wine, beer, natural juices) [1-3]. In addition to antioxidant activity, phenolic compounds have other biological activities such as anti-carcinogenic, anti-inflammatory and antibacterial activities, which are related to a series of health benefits. Hence, phenolics reduce risks of several disorders and chronic diseases (cancer, diabetes, Alzheimer's, Parkinson, cardiovascular diseases, hypertension), improve lipid and glucose

metabolism, have an inhibition effect on ageing process and on viruses and bacteria (e.g., Influenza virus, *Streptococcus pneumoniae*, *Shigella dysenteriae*, *Escherichia coli*, human immunodeficiency virus, *Helicobacter pylori*, *Staphylococcus aureus* etc.) [4-7]. Due to the aforementioned biological properties, polyphenolic compounds are mainly used in food and beverage, cosmetics and pharmaceutical industries [8,9].

Although phenolic compounds are mostly found in natural sources, there are also phenolic compounds obtained by synthetic pathways and used as additives or bioactive compounds in food and

beverages, cosmetic, pharmaceutical and personal care products, food packaging material such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tert-butylhydroquinone (TBHQ) etc. [10-12].

The capacity to scavenge radicals and to suppress oxidative processes in alimentary products and human body are attributed to both synthetic and natural phenolic compounds. Due to a several safety issues ascribed to synthetic phenolic compounds (e.g. BHT, TBHQ –tumor promoters), it has been an increasing interest of using natural antioxidants as alternatives to synthetic antioxidants [1,12,13].

In this paper, antioxidant properties of two synthetic phenolic compounds (BHA, PG) are evaluated in comparison to antioxidant activity of one natural phenolic compound (caffeic acid) by DPPH· method, wherein antioxidant activities and reaction rates between antioxidants and DPPH· are determined and analyzed.

## 2. Materials and Method

### 2.1. Materials and equipment

The ethanol (96%, v/v) used for the preparation of DPPH· and phenolic compound solutions was provided from Chimreactiv S.R.L. and Atochim S.R.L. The free radical DPPH· (2,2-diphenyl-1-picrylhydrazyl) and phenolic compounds, i.e. caffeic acid (CA, >98%), butylated hydroxyanisole (BHA, >98.5%) and propyl gallate (PG, >98%) were obtained from Sigma Aldrich. The measurements were performed using a Camspec M501 Scanning UV/Visible Spectrophotometer (Camspec Ltd.) and a quartz cuvette of 1 cm.

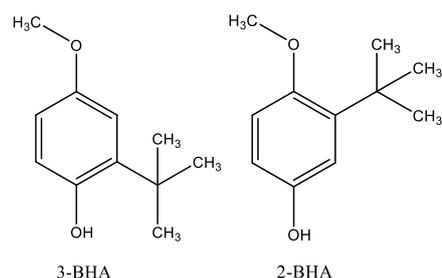
### 2.2. Antioxidant assessment by DPPH· method

Antiradical evaluation of BHA, PG and CA was performed by DPPH· method using UV-VIS spectrometry. Solutions of each phenolic compound in ethanol were prepared at four different concentrations (1, 0.2, 0.1 and 0.05 mM). Measurements consist of monitoring the absorbance for each antioxidant solutions in the presence of DPPH· solution in ethanol during 15 minutes. For every measurement, 2 mL of ethanol and 0.5 mL of antioxidant solution were mixed in the presence of 0.5 mL of DPPH· solution (1 mM) and introduced in UV-VIS spectrophotometer.

## 3. Results and discussion

### 3.1. Antioxidant activity of phenolic compounds

BHA is a monohydroxylic phenolic compound commercially used as an antioxidant and preservative (food additive E320) in food or cosmetics and also as an oxidizing agent in food packaging materials. Antiradical activity of butylated hydroxyanisole is given as a result of its capacity to inhibit oxidation reactions such as oxidation of fats, vegetable oil and vitamin A. It is a white waxy solid and usually consists of a mixture of 2 isomers (Figure 1): 3-*tert*-butyl-4-hydroxyanisole (3-BHA) and 2-*tert*-butyl-4-hydroxyanisole (2-BHA). It is resistant at high temperatures and it is reported that it might cause carcinogenic effect on human health and cytotoxic effects, information based on animal experiments. BHA possess the ability to scavenge free radicals by donating hydrogen of the only hydroxyl group and forming a free stable radical [12,14-16].



**Figure 1.** Chemical structures of 3-*tert*-butyl-4-hydroxyanisole (3-BHA) and 2-*tert*-butyl-4-hydroxyanisole (2-BHA)

Propyl gallate (Figure 2) is a propyl ester of 3,4,5-trihydroxybenzoic acid (gallic acid) and is industrially obtained by esterification gallic acid with propanol. It is widely used in cosmetics and in personal care products as an antioxidant and aroma ingredient, but also preservative (food additive E310) and antioxidant in food containing lipids, oils, food packaging materials. As in the case of BHA, PG has an inhibitory effect on oxidation reaction of fats and as well, it inhibits the oxidation of monoterpenes, aldehydes and ketones in essential oils. Several studies have associated PG with toxic effects (carcinogenesis promoter), but at low concentration PG is considered a safe antioxidant. PG contains three hydroxyl groups which involve an antiradical scavenging activity being capable of

transferring the hydrogen from hydroxyl group to a radical compound [11,12,17,18].

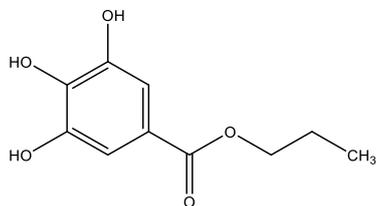


Figure 2. Chemical structure of propyl gallate (PG)

Caffeic acid (Figure 3) is hydroxycinnamic acid (3-(3,4-dihydroxyphenyl)-2-propenoic acid), a natural antioxidant compound occurring rarely in its free form and mostly as derivate esters (e.g., caffeic acid phenethyl ester, chlorogenic acids). Natural sources of caffeic acid include but not limited to vegetable oils (sunflower oil, olive oil), cereals (e.g., wheat, sorghum), coffee, blackberries, cranberries, cherries, spinach, etc. Caffeic acid possesses two hydroxyl groups and its antioxidant properties are associated with a series of health benefits such as anti-carcinogenic and anti-inflammatory effects. [12,19,20].

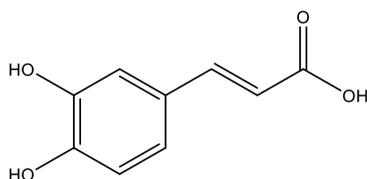


Figure 3. Chemical structure of caffeic acid

Antiradical properties of CA, BHA and PG are evaluated by DPPH· assay. DPPH· is a free stable synthetic radical with a free pair of electrons which is reacting with antioxidant compounds by accepting a hydrogen atom from these compounds. During the reaction between DPPH· and antioxidant compounds, the color is changing from purple to yellow which is the confirmation of radical scavenger activity of antioxidant compounds. Using the UV-VIS spectrophotometer, the absorbance of DPPH· ethanol solution is monitored at wavelength of 517 nm for an established period. As the reaction between DPPH· and the antioxidant compound occurs, the absorbance of DPPH· is decreasing due to the consumption of DPPH· during the reaction.

As mentioned before, caffeic acid, butylated hydroxyanisole and propyl gallate possess antioxidant activity, being capable of transferring a hydrogen atom of hydroxyl group to a free radical as represented in Figure 4.

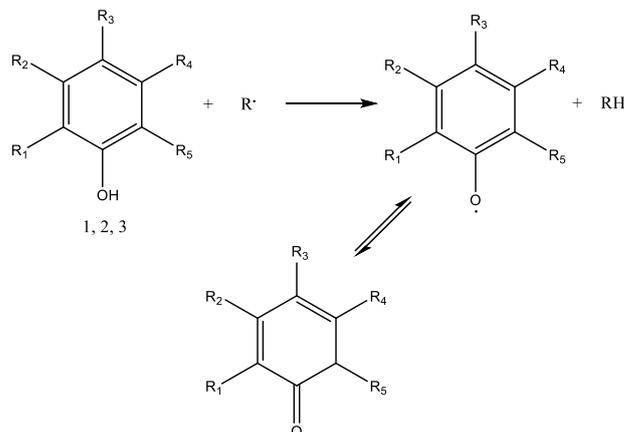


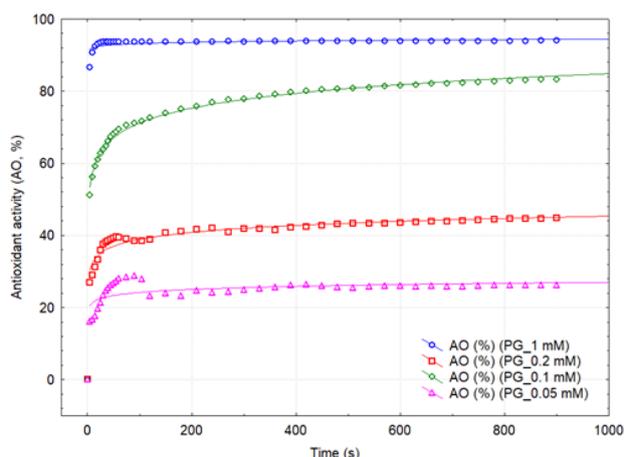
Figure 4. The reaction of antioxidant compounds with a radical compound (R·), where compound 1 represents caffeic acid (R<sub>1</sub>: OH, R<sub>3</sub>: -CH=CH-COOH, R<sub>2,4,5</sub>: H), compound 2 is propyl gallate (R<sub>1,2</sub>: OH, R<sub>3,5</sub>: H, R<sub>4</sub>: -COOC<sub>3</sub>H<sub>7</sub>) and compound 3 is 3-*tert*-butyl-4-hydroxyanisole (R<sub>1</sub>: -C(CH<sub>3</sub>)<sub>3</sub>, R<sub>2,4,5</sub>: H, R<sub>3</sub>: -OCH<sub>3</sub>)

Antioxidant activities (AO, %) of CA, BHA and PG ethanolic solutions of 1, 0.2, 0.1 and 0.05 mM are determined based on absorbance values by the following equation (Eq. 1):

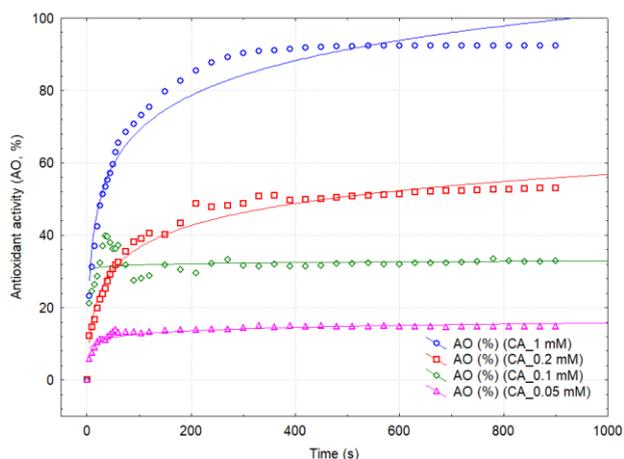
$$AO (\%) = 100 \cdot \left(1 - \frac{Abs(t)}{Abs(t_0)}\right) \quad (\text{Eq. 1})$$

- Abs(*t*) – represents the absorbance at the time *t*;
- Abs(*t*<sub>0</sub>) is absorbance at the time *t* = 0 s of the reactions between DPPH· and phenolic compounds.

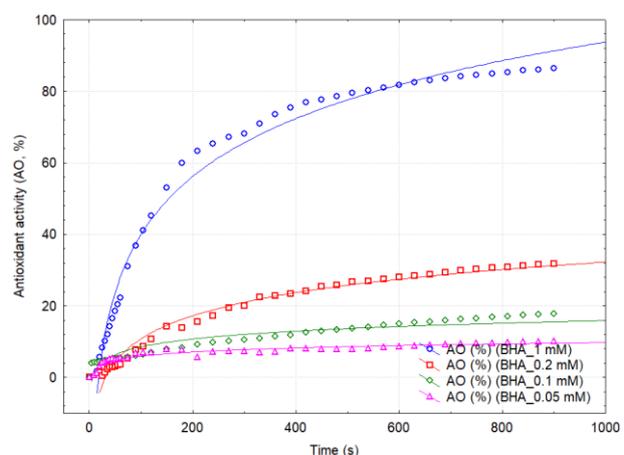
Further, the variations of antioxidant activities of each phenolic compound (CA, BHA, and GP) at various concentrations of phenolic compounds in ethanol (1, 0.2, 0.1 and 0.05 mM) vs. time (s) are represented in Figures 5-7.



**Figure 5.** Antioxidant activities (%) of PG solutions prepared at various concentrations (PG\_1 mM – blue, PG\_0.2 mM – green, PG\_0.1 mM – red, PG\_0.05 mM – pink), determined by DPPH· method



**Figure 6.** Antioxidant activities (%) of CA solutions prepared at various concentrations (CA\_1 mM – blue, CA\_0.2 mM – green, CA\_0.1 mM – red, CA\_0.05 mM – pink), determined by DPPH· method



**Figure 7.** Antioxidant activities (%) of BHA solutions prepared at various concentrations (BHA\_1 mM – blue, BHA\_0.2 mM – green, BHA\_0.1 mM – red, BHA\_0.05 mM – pink), determined by DPPH· method

The behavior of AO over time determined for caffeic acid is different in comparison with AO over time determined for propyl gallate and butylated hydroxyanisole. Hence, for a better understanding of antioxidant activity behavior of every phenolic compound, Table 1 presents the values of antioxidant activity for CA, PG and BHA at various time periods ( $t = 1'$ ,  $t = 3'$ ,  $t = 5'$  and  $t = 15'$ ).

**Table 1.** Antioxidant activities (AO, %) of CA, PG and BHA solutions prepared at various concentrations (1, 0.2, 0.1 and 0.05 mM) at 1, 3, 5 and 15'; RSD < 5%

Code	AO (%) (@ 1')	AO (%) (@ 3')	AO (%) (@ 5')	AO (%) (@ 15')
PG_1 mM	93.72	93.68	93.77	93.98
PG_0.2 mM	69.52	72.02	77.89	83.32
PG_0.1 mM	39.50	41.18	41.91	44.86
PG_0.05 mM	27.99	23.27	24.79	26.27
CA_1 mM	65.55	82.60	90.30	92.41
CA_0.2 mM	32.49	43.30	48.82	53.08
CA_0.1 mM	37.12	30.55	31.67	32.95
CA_0.05 mM	13.16	13.85	14.37	14.81
BHA_1 mM	22.19	60.00	68.21	86.46

The best antioxidant activity is associated with sample PG\_1mM, so that AO for PG\_1 mM reached the value of approximately 94% after almost 30" followed by an insignificant variation between 30 and 900". At the same concentration, the antioxidant activity of caffeic acid and butylated hydroxyanisole is increasing from 65.55% and 22.19% at  $t = 1'$  to 92.41% and 86.46% at  $t = 15'$ , respectively. Even if the antioxidant activity variations of CA and BHA during the 15' are higher than antioxidant activity variation of PG, the antioxidant activities of CA, PG and BHA are similar after 15' of evaluation. Also, the antioxidant activities are increasing with the increasing of initial concentration of phenolic compounds.

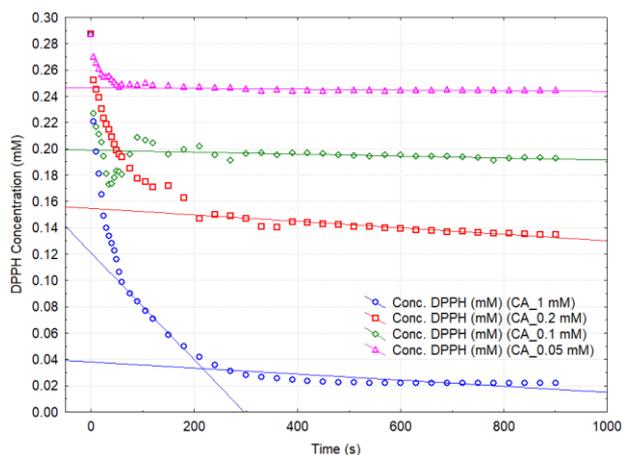
### 3.2. Reaction rate of phenolic compounds with DPPH·

Kinetic studies of reactions between phenolic compounds (CA, PG and BHA) with DPPH· are performed by determining the reaction rate of DPPH· with phenolic compounds for three pseudolinear ranges corresponding to the following time intervals: 0-60", 1-3' and 3-15'. These ranges were established by using statistical analysis of DPPH· concentration values over time. The concentration of DPPH· over time, in the presence of antiradical compounds, is calculated with a calibration curve determined for solutions of DPPH·

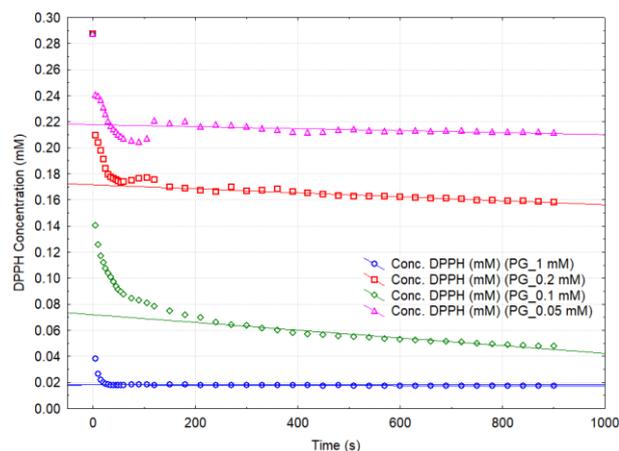
in ethanol at various concentrations in a previous study [21]. The starting concentration of DPPH· used for absorbance measurement before mixing of reagents and ethanol is 1 mM for each measurement and the initial concentration of every antioxidant compound is ranging from 1 mM to 0.05 mM (1, 0.2, 0.1 and 0.05 mM). The reaction rate is calculated using Eq. 2:

$$\bar{v} = - \frac{\Delta c_{DPPH\cdot}}{\Delta t} \quad (\text{Eq. 2})$$

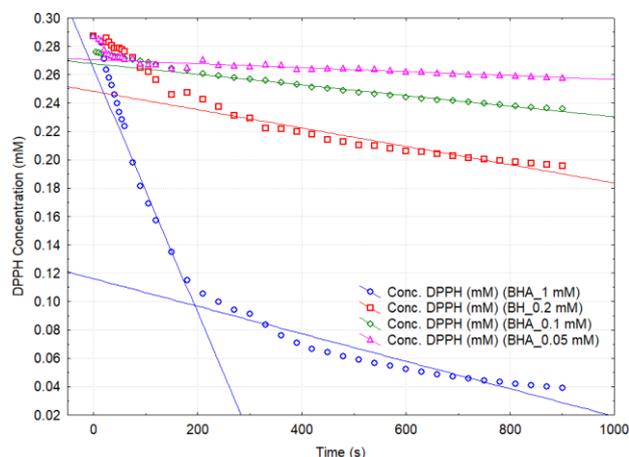
Further, the variation of DPPH· concentration in time in the presence of caffeic acid, propyl gallate and butylated hydroxyanisole are presented in Figures 8-10.



**Figure 8.** Variation of DPPH· concentration in time in the presence of CA – ethanolic solutions of 1mM – blue, 0.2 mM – red, 0.1 mM – green and 0.05 mM – pink



**Figure 9.** Variation of DPPH· concentration in time in the presence of PG – ethanolic solutions of 1 mM – blue, 0.2 mM – red, 0.1 mM – green and 0.05 mM – pink



**Figure 10.** Variation of DPPH· concentration in time in the presence of BHA – ethanolic solutions of 1 mM – blue, 0.2 mM – red, 0.1 mM – green and 0.05 mM – pink

As shown in Figures 8-10, the concentration of DPPH· has been reached the lowest value (0.018 mM) when ethanolic solution of propyl gallate of 1 mM concentration was added at t = 60". After this time, the concentration of DPPH· decreased very slowly and reached a value of 0.017 mM at t = 15'. In the presence of BHA\_1mM and CA\_1 mM, the concentration of DPPH· reached the minimum value of 0.022 mM and respectively 0.039 mM after 500" and 884", respectively. Considering the variation of DPPH· concentration over time, the reaction rates of free radical with antioxidant compounds were calculated in Table 2.

**Table 2.** Mean DPPH· reaction rates ( $\bar{v}_{1-3}$ ,  $\mu\text{M/s}$ ) of the CA, PG and BHA solutions prepared at various concentrations (1, 0.2, 0.1 and 0.05 mM) on various time ranges (0-60", 1-3' and 3-15', respectively); RSD < 5%

Code	$\bar{v}_1$ ( $\mu\text{M/s}$ )	$\bar{v}_2$ ( $\mu\text{M/s}$ )	$\bar{v}_3$ ( $\mu\text{M/s}$ )
PG_1 mM	1.9	-	0.001
PG_0.2 mM	1.9	0.1	0.03
PG_0.1 mM	1.2	0.05	0.015
PG_0.05 mM	1	0.1	0.008
CA_1 mM	2.5	0.4	0.023
CA_0.2 mM	1.3	0.2	0.025
CA_0.1 mM	1.3	0.07	0.007
CA_0.05 mM	0.5	0.02	0.003
BHA_1 mM	1.2	0.9	0.097
BHA_0.2 mM	-	0.3	0.065
BHA_0.1 mM	0.2	0.07	0.037
BHA_0.05 mM	0.3	0.06	0.014

Taking into account that the concentration of DPPH· showed the lowest value in the presence of PG\_1mM at t = 1', it is expected that the reaction rate calculated for free radical in the presence of

PG\_1 mM sample over the range of 0-60'' to be the highest. Still, the best reaction rate was calculated for DPPH· in the presence of CA\_1mM and this may be explained by the fact that the minimum concentration of DPPH· in the presence of PG\_1mM was almost reached at t = 30'' and after this moment the reaction rate was nearly 0 μM/s. Reaction rates corresponding to DPPH· in the presence of butylated hydroxyanisole showed the lowest values for the range 0-60'' and the highest values for the second and the third range comparing to reaction rates of DPPH· in the presence of caffeic acid and propyl gallate. For example, the reaction rate between DPPH· and BHA associated with sample BHA\_0.05 mM is 0.3 μM/s for the interval 0-60'' and the reaction rate between DPPH· and BHA associated with sample BHA\_1 mM is 0.90 μM/s for range of 1-3' and respectively 0.97 μM/s for range of 3-15'.

#### 4. Conclusion

Antioxidant activity of natural (caffeic acid) and synthetic (propyl gallate and butylated hydroxyanisole) phenolic compounds at various concentrations (1 mM, 0.2 mM, 0.1 mM and 0.05 mM) were determined by DPPH· assessment for 15 minutes, followed by a kinetic study of reaction between antioxidant compounds and the a free radical compound. Propyl gallate at a concentration of 1 mM showed the highest antioxidant activity and reached the maximum value after only 30 seconds. Antioxidant activities of caffeic acid and butylated hydroxyanisole are lower than antioxidant activities of propyl gallate determined for the range of 0-15 minutes but at 15 minutes 1 mM concentrations of propyl gallate, caffeic acid and butylated hydroxyanisole showed similar values of antioxidant activities. The reaction rates of phenolic compounds with the free radical were calculated for three pseudolinear intervals and are significantly decreasing from the first to the last time range. The highest reaction rate is associated with caffeic acid at 1mM (2.4 μM/s) for first interval (0-60 seconds) and the lowest reaction rates are corresponding to sample of propyl gallate at 1 mM for second and third intervals. As a consequence of reaction occurring between antioxidants and DPPH·, the latter reached a concentration of approximately 0.02 mM in the presence of the sample of propyl gallate and caffeic acid at 1 mM.

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