

Antioxidant Potential and Tyrosinase Inhibitory Properties of Quebecol as Natural Phenolic Compound

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ABSTRACT: In this paper, we investigated the antioxidant potential of Quebecol *in vitro* using several assays. These; Cu²⁺-Cu⁺ reducing, DPPH[•] scavenging, Fe³⁺-Fe²⁺ reducing, ABTS^{•+} scavenging. The IC₅₀ amounts of the Quebecol in the DPPH[•] and ABTS^{•+} methods were 14.78 and 5.38 µg mL⁻¹, respectively. Tyrosinase enzyme is a functional oxidase that is extensively divided in nature. It is the main enzyme in melanin synthesis and also is involved in designating the color of mammalian hair and skin. Recently, there has been a remarkable concern to study the inhibitory activity of the tyrosinase enzyme and some inhibitory molecules isolated from natural sources. For tyrosinase enzyme, Quebecol and kojic Acid (Standard) as natural phenols have IC₅₀ values of 4.64 and 9.28 µM.

Key words: Quebecol, antioxidant activity, tyrosinase, enzyme inhibition,

Doğal Fenolik Bileşik Olarak Quebecol'un Antioksidan Potansiyeli ve Tirozinaz İnhibitör Özellikleri

ÖZET: Yapılan bu çalışmada, Quebecol'un *in vitro* antioksidan özelliklerini farklı testler kullanarak araştırıldı. Bunlar; Cu²⁺-Cu⁺ indirgenme, DPPH[•] radikal giderme, Fe³⁺-Fe²⁺ indirgenme, ABTS^{•+} radikal giderme. DPPH[•] ve ABTS^{•+} testlerindeki Quebecol'un IC₅₀ değerleri sırasıyla 14.78 ve 5.38 µg mL⁻¹ elde edildi. Tirozinaz enzimi, doğada yaygın olarak bulunan fonksiyonel bir oksidazdır. Melanin sentezindeki ana enzimdir ve bu enzim aynı zamanda memeli kıllarının ve cildinin renginin belirlenmesinde önemli rol oynar. Son zamanlarda, tirozinaz enziminin ve bu enzimin doğal kaynaklardan izole edilen bazı inhibitör moleküllerin, aktivitesini incelemek için fazla çalışma bulunmamaktadır. Son zamanlarda, tirozinaz enziminin ve doğal kaynaklardan izole edilen bazı inhibitör moleküllerin inhibitör aktivitesini incelemek çok öneme sahiptir. Tirozinaz enzimi için, doğal fenoller olarak Quebecol ve kojik Asit (Standart), 4.64 ve 9.28 uM'lik IC₅₀ değerlerine sahiptir.

Anahtar Kelimeler: Quebecol; antioksidan aktivite; tirozinaz; enzim inhibisyonu

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INTRODUCTION

Recently, increasing concerns about food and nutrition have led to research efforts by the food and university industries to find new bioactive agents that are active in the human diet (Gülçin et al., 2005a). In addition, many of the foods used traditionally and familiarly with a molecular approach, such as foods, have been traded in Canadian handicrafts (especially in the province of Quebec) and maple syrup (Li and seeram, 2011; Ball, 2007). Lee and Siram have just recorded a new polyphenolic (Figure 1). The partridge is named. Natural phenolic compounds are naturally present, and the plant's secondary metabolites are present in almost all plant materials, such as plant-based foods. These compounds appear to be a complete part of the human and animal diets (Elmastaş et al., 2005; Gülçin et al., 2005b).

Nutritional tissues live (or have existed), and are also produced under constant oxidative stress from reactive oxygen species, free radicals, and peroxides as exogenous (light and heat) and endogenous (transport metals and H_2O_2). In fact, many of these tissues have developed antioxidant systems to control lipid oxidation catalysts, free radicals, secondary decomposition products, and oxidation mediators. Antioxidant molecules such as phenolic acids, carotenoids, flavonoids, and tocopherols, which can inhibit Fe^{3+} / AA oxidation, clear free radicals and act as reducing agents (Gülçin et al., 2005c; Gülçin, 2005).

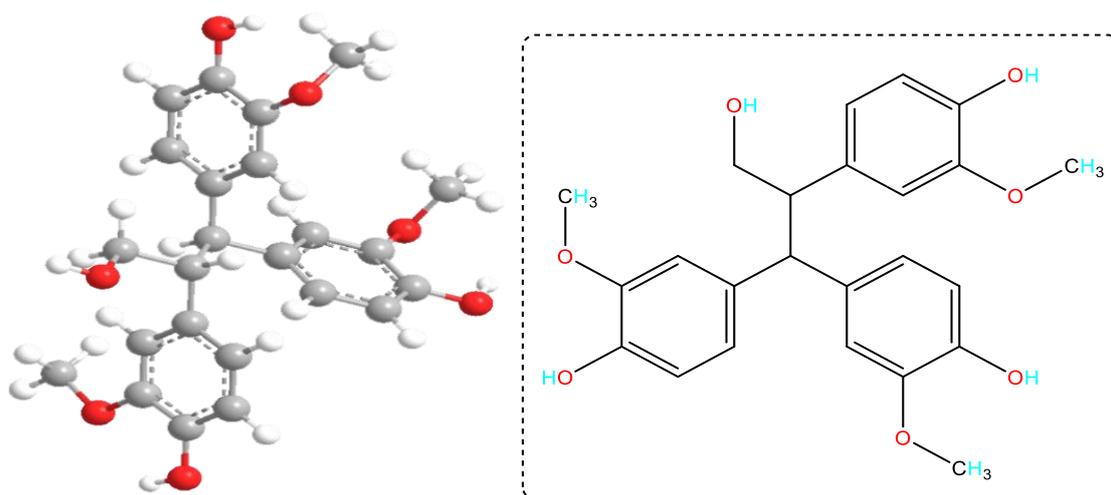


Figure 1. Structure of Quebecol compound

Melanogenesis is a physiological mechanism that leads to the production of melanin pigment, which plays an important role in preventing sunburn. However, the production of melanin in human skin is a major mechanism of light protection against ultraviolet light. Excessive return of epidermal pigments can cause a variety of high blood pressure disorders, such as age spots, melasma, and locations. Be actinic damage (Espin and Wichers, 1999; Mastumoto et al., 2018). As a copper enzyme, tyrosinase catalyzes two separate reactions waiting for melanin biosynthesis, L-tyrosine molecule hydroxylation to L-dopa, and oxidation of the L-dopa molecule to dopukinone, which is highly reactive and can also be automatically converted to melanin. Be. Therefore, regulating melanin synthesis by inhibiting tyrosinase is a common study topic in preventing high blood pressure (Zolghadri et al., 2019; Tanaka et al., 2019).

In this study, we have investigated the *in vitro* inhibition effects of Quebecol and kojic Acid (as Standard) as natural phenol on tyrosinase enzyme. The significant target of this paper, is compared its antioxidant activity to related standard molecules including BHT, BHA, trolox and α -tocopherol (for antioxidant activities).

MATERIALS AND METHODS

Antioxidant methods

Fe³⁺ reducing

To assess the ability of Fe₃ to reduce the composition of the partridge, the method of reducing Fe³⁺(CN⁻)₆ to Fe²⁺(CN⁻)₆ was utilized and also performed according to former studies (Gülçin et al., 2006a; Gülçin, 2006). According to the results, when the reduction ability decreases, the absorption of more values shows.

Cupric ions (Cu²⁺)

Mineral ions (Cu₂), which use the power measurement method, have been used as the second way to reduce the ability of cobblestones to combine. The ability to reduce Cu₂ was done conforming to the assay explained by Apak et al. (Apak et al., 2007) With low modifications and in accordance with previous studies (Gülçin, 2006; Elmastaş et al., 2006). Sampling was determined at 450 nm in 30 min.

DPPH[•] scavenging activity

DPPH inhibitory activity was determined from the composition of the particle as described earlier. Finally, the absorption amount was determined at 517 nm in the spectroscopy (Gülçin et al., 2006b).

ABTS^{•+} assay

ABTS radical cation was generated by the interaction of ABTS (7 mL⁻¹) and K₂S₂O₈ (2.45 mL⁻¹) [85] described previously [86,87]. This solution was diluted with methanol until the absorbance in the samples reached 0.750 - 0.05 at 734 nm. Then, 1 mL of ABTS[•] solution was supplemented to 3 mL of novel symmetric sulfamides and control solutions. The extent of decolorization is calculated as a percentage reduction of absorbance. ABTS radical inhibition activity from the composition of the particle utilizing the spectroscopic method described by Re et al. (Re et al., 1999) and was performed according to previous studies.

Tyrosinase enzyme activity

The inhibitory activity of the natural compound against tyrosinase was studied (Ishioka et al., 2019; Ros et al., 1993). The produced dopacrum values were determined by measuring the absorption rate at 475 nm using the VersaMaxTM display microplate (molecular devices, USA). Kojic acid (50µM) was used as a positive control.

RESULTS and DISCUSSION

Antioxidant results

Antioxidant compounds can be classified in several ways. Due to their activity, they can be classified as non-enzymatic and enzymatic antioxidants. These work by destroying and decomposing free radical molecules. Indeed, antioxidant enzymes convert dangerous oxidizing products into multi-stage mechanism and in the presence of manufacturers such as zinc, manganese, copper, and iron into multi-stage mechanism into water and hydrogen peroxide (H₂O₂) (Gülçin et al., 2004a). Some examples of non-enzymatic include vitamin E, vitamin C, carotenoids, and glutathione. The cells of the complete aerobic organism in nature have antioxidant defense systems such as antioxidant nutrients to repair or eliminate defective compounds. Antioxidant compounds can protect human cells from radicals and the effects of ROS (Gülçin et al., 2004b). Also, reactive oxygen species (RNS) and ROS can cause DNA

harm which can alter mutations. Antioxidant compounds are extensively utilized as food additives for protecting food oxidative reduction. ROS agents contain free radicals such as hydroxyl radicals, anion superoxide radicals, and non-radical types such as hydrogen peroxide and single oxygen (Gülçin et al., 2004c). Recently, the interest in studying natural antioxidants due to their use in food or pharmaceuticals to replace synthetic antioxidant compounds, which are limited due to their side effects like carcinogenicity, is of great interest in studying natural antioxidants. In this work, several measurements of various antioxidants based on various reaction processes were utilized to detect the strong antioxidant of the particle.

i. In Fe^{3+} (CN^-) reduction method 6, the reducing power of biologically active compounds indicates their electron donor capacity. The Fe^{3+} (CN^-) reduction method identifies 6 antioxidant effects of each molecule as a reduction in the ability to react (Gülçin, 2008). Quebecol compound had potent reducing activity using $\text{Fe}^{3+}(\text{CN}^-)_6$ reduction and with the standards (α -tocopherol, Trolox, BHT, and BHA). It can be observed in Table 1 and Figure 2 that the Fe^{3+} reducing capacity of Quebecol compound and the standard compounds were: BHA (2.418 ± 0.022 , r^2 : 0.9602) > BHT (1.930 ± 0.012 , r^2 : 0.9793) > Trolox (1.766 ± 0.013 , r^2 : 0.9638) > α -Tocopherol (1.512 ± 0.017 , r^2 : 0.9424) > Quebecol (1.164 ± 0.011 , r^2 : 0.9963) at the same concentration ($30 \mu\text{g mL}^{-1}$).

ii. CUPRAC is an affordable, simple, selective, fast, and stable and antioxidant that is useful for a extensive range of polyphenol molecules as well as for thiols, artificial antioxidant compounds (Gülçin et al., 2008). Mineral ions (Cu^{2+}) that reduce the strength of 30 micrograms per milliliter of the concentration of the cobblestone compound and the standard reducing agents are shown in Table 1 and Figure 2. Cupric ions (Cu^{2+}) reducing capacity of Quebecol compound and standards were: BHA (2.443 ± 0.027 , r^2 : 0.9834) > BHT (2.003 ± 0.004 , r^2 : 0.9966) > Quebecol (1.354 ± 0.018 , r^2 : 0.9703) > Trolox (1.208 ± 0.005 , r^2 : 0.9811) > α -Tocopherol (1.059 ± 0.182 , r^2 : 0.9072) at the same concentration ($30 \mu\text{g mL}^{-1}$).

iii. DPPH is based on measuring the ability to reduce antioxidant compounds compared to DPPH radicals (Köksal and Gülçin 2008). Table 2 and Figure 3 show half maximal radical scavenging concentrations (IC_{50}) of Quebecol and the reference radical scavenger agents like BHT, Trolox, BHA, and α -tocopherol. IC_{50} values were $8.63 \mu\text{g mL}^{-1}$ (r^2 : 0.9814) for BHT, $11.03 \mu\text{g mL}^{-1}$ (r^2 : 0.9783) for BHA, $23.04 \mu\text{g mL}^{-1}$ (r^2 : 0.9966) for α -Tocopherol, $18.24 \mu\text{g mL}^{-1}$ (r^2 : 0.9580) for Trolox, $14.78 \mu\text{g mL}^{-1}$ (r^2 : 0.9812) for Quebecol.

iv. As with DPPH free radical tracking activity, a lower value of IC_{50} indicates higher ABTS^+ activity. Another improved method for determining fine dust is ABT^{*+} activity. ABTS radicals are produced in an $\text{K}_2\text{S}_2\text{O}_8/\text{ABTS}$ system (Köksal et al., 2009). IC_{50} values for Quebecol and control agents like trolox, α -tocopherol, BHT, and BHA were $5.38 \mu\text{g mL}^{-1}$ (r^2 : 0.9710) for Quebecol, $4.06 \mu\text{g mL}^{-1}$ (r^2 : 0.9903) for BHT, $4.61 \mu\text{g mL}^{-1}$ (r^2 : 0.9618) for BHA, $6.80 \mu\text{g mL}^{-1}$ (r^2 : 0.9831) for Trolox, $9.52 \mu\text{g mL}^{-1}$ (r^2 : 0.9682) for α -Tocopherol. (Table 2 and Figure 3).

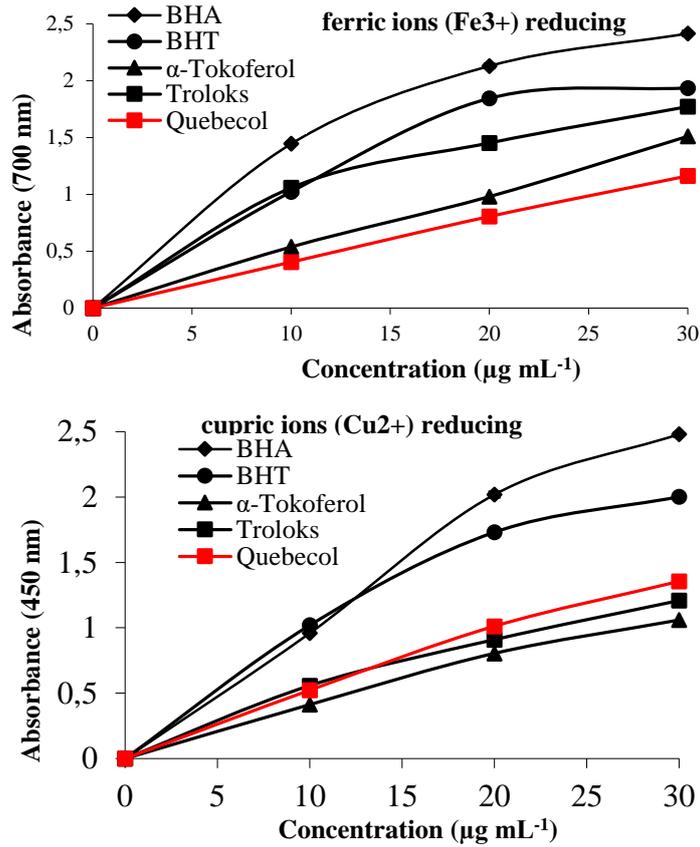


Figure 2. Reducing power graphs of ferric ions (Fe³⁺), and CUPRAK reducing capacity.

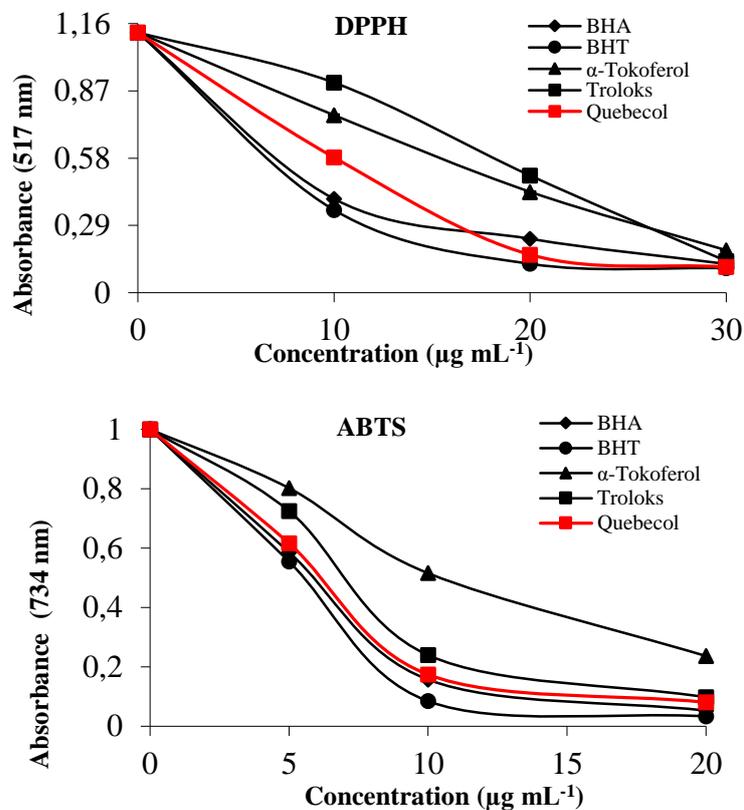


Figure 3. Radical scavenging graphs of DPPH· and ABTS⁺ methods.

Table 1. Determination of reducing power assays.

Antioxidants	Fe ³⁺ -Fe ²⁺ reducing		Cu ²⁺ -Cu ⁺ reducing	
	λ_{700}	R ²	λ_{450}	R ²
BHA	2.41±0.025	0.9602	2.443±0.027	0.9834
BHT	1.930±0.011	0.9793	2.001±0.002	0.9966
α -Tocopherol	1.510±0.015	0.9424	1.059±0.182	0.9072
Trolox	1.766±0.013	0.9638	1.208±0.003	0.9811
Quebecol	1.164±0.011	0.9963	1.354±0.018	0.9703

Table 2. Determination of IC₅₀ amounts of natural compound and standards for radical scavenging DPPH· and ABTS⁺.

Antioxidant Compounds	DPPH· scavenging	R ²	ABTS ⁺ scavenging	R ²
BHA	11.03	0.9783	4.61	0.9618
BHT	8.63	0.9814	4.06	0.9903
α -Tocopherol	23.04	0.9966	9.52	0.9682
Trolox	18.24	0.9580	6.80	0.9831
Quebecol	14.78	0.9812	5.38	0.9710

Enzyme results

Tyrosinase inhibitor compounds are utilized to therapy of hyperpigmentation diseases, skin whitening, and age spots, whereas tyrosinase activator compounds cause the enhance of melanogenesis that can protect the skin from UV harm (Wang et al., 2014). For tyrosinase enzyme, Quebecol and kojic Acid (Standard) as natural phenols have IC₅₀ values of 4.64 and 9.28 μ M (Table 1). For tyrosinase, IC₅₀ amounts of kojic acid as positive control and Quebecol the following order: Quebecol (4.64 μ M, r²: 0.9219) < kojic acid (9.28 μ M, r²: 0.9889). In addition to treating pigment disorders, tyrosinase inhibitors are also used to control insects and the food industry. Additionally, the tyrosinase enzyme has important roles in the developmental and defense functions of insects. Involved in sclerotization in insects, wound healing, melanogenesis, and parasite encapsulation. The importance and development of tyrosinase inhibitors have become an alternative method of pest control. Indeed, In the food, tyrosinase is crucial in controlling the economics and quality of vegetables and fruits (Hung et al., 2006; Chen et al., 2017).

Table 3. Tyrosinase inhibitory results of a natural compound

Natural Compounds	Tyrosinase Enzyme	
	IC ₅₀ (μ M)	R ²
Quebecol	4.64±0.62	0.9219
Kojic acid (as Standard)	9.28±1.15	0.9889

CONCLUSIONS

The decrease of DNA damage, chronic diseases, mutagenesis, growth, inhibition of pathogenic bacterial is mostly associated with the termination of radical molecules propagation in biochemical systems. The antioxidant potential is widely used as a feature for medicinal components. In this research, the antioxidant activity of Quebecol was compared to BHT, BHA, α -tocopherol, and Trolox. Quebecol as tyrosinase inhibitor compound can be attractive in medicinal industries and cosmetics as

depigmentation factors and also in agriculture and food industries as antibrowning molecule. Natural product compounds from animals, microbes, plants, and minerals have long been a traditional source for the therapy of human diseases. In the past decades, the study on natural product compounds for the pharmaceutical industry had reduced due to multiple challenges.

Conflict of interest statement

The author is no conflicts of interest.

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