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Electrochemical Oxidation, Biological Evaluation, and Bioinformatics Approach for the Ethanolic Leaf Extract of *Melissa officinalis L.*

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In this work, the electrochemistry of the ethanolic leaf extract of *Melissa officinalis L.*, as an herbal medicine, was studied. The antioxidant activity of the plant was evaluated using the cyclic voltammetry technique. The results showed that this extract is oxidized at low potentials compared to quercetin, salicylic acid and gallic acid as standard synthetic antioxidants. It was also concluded that the increase in the antioxidant activity causes a decrease in E_p^A and an increase in the number of hydroxyl groups on the aromatic ring. The DPPH assay (free radical scavenging activity) was applied in order to estimate the antioxidant activity. The antioxidant activity of rosmarinic acid (as the main natural compound in this herb) against Cytochrome P450 3A4 (4D75), Myeloperoxidase (1DNW), and Thyosine (PBD ID: 3nm8) (reactive oxygen species (ROS) generating enzymes) was investigated through molecular docking studies. The interaction of rosmarinic acid with Thyosine exhibited the most inhibitory effect with -10.5 kJ mol^{-1} binding affinity value. The results indicated that the rosmarinic acid is bound exclusively to the binding sites of the ROS generating enzymes and has a significant role in counteracting the destructive effects of oxidative stress in the biological system. Finally, the electrochemical oxidation of the ethanolic leaf extract of *Melissa officinalis L.*, has been studied in the presence of captopril by cyclic voltammetry method in the biological pH range. The results showed that the electrochemically generated compounds from this herb can participate in the chemical reaction with captopril and reduce the concentration of the active form of this drug.

Keywords: Antioxidant activity, ROS generating enzymes, Captopril overdose, *Melissa officinalis L*

INTRODUCTION

Electrochemical evaluation is a useful method to determine antioxidant activity, regarding its application as a fast monitor of the antioxidant capacity of many organic compounds. The oxidation potentials measured by cyclic voltammetry (CV) are used to compare the antioxidant capacity of natural compounds in medicinal plants such as flavonoids, cinnamic acids, and phenolic acids [1-3].

On the other hand, the medicinal plants used in traditional medicine are one of the sources of antioxidants. Herbalists in 80% of the worldwide population employ herbal medicines in their primary health care [4]. Dietary antioxidants have an important protective role against free radicals in human organisms. Reactive oxygen species (ROS) or free radicals are produced in body by different endogenous systems with exposure to various pathological states or physiochemical conditions. For the normal conditions and appropriate physiological functions, the antioxidants and free radicals must be balanced [5-7]. In herbal products containing multiple components, there are herb-drug or herb-herb interactions, which are often complex and unpredictable. Several kinds of pharmacogenomic and pharmacokinetic interactions from herb-drug combinations have been characterized [8,9]. *Melissa Officinalis L.* (*lemon balm* or *Melissa*; *MOL*) is an

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edible perennial medicinal plant belonging to the family Lamiaceae, the name of which is derived from the Greek words "*Melissa*"and "*meli*" meaning bee and honey, respectively. Several medicinal applications have been recorded for this plant due to its safe exploitation [10,11]. On the other hand, captopril has been the first orally active angiotensin-converting-enzyme (ACE) inhibitor [12]. Captopril as a sulfhydryl-containing ACE inhibitor possesses a small half-life and necessitates several administrations a day. Furthermore, it is an ACE inhibitor, such as imidapril and enalapril, that decreases the pneumonia risk around one-third in comparison to calcium channel or beta-adrenergic receptor antagonists when they are applied to cure hypertension in patients with a history of stroke. Although captoprilis is metabolized in the liver, nearly all of the captopril and its metabolites are reduced through kidneys, mainly by tubular secretion. A reduction in dose interval of captopril or a decrease in its dose is advised for patients with decreased renal function [13-15].

Since the electrochemical oxidation is generally in parallel with cytochrome P450-catalyzed oxidation in liver microsomes, and the hepatic metabolism is the major route of endogenous clearance of captopril, it would be of special interest to study the electro-oxidation of ethanolic leaf extract of *Melissa officinalis* L. in the presence of captopril.

This study aims to investigate the electrochemistry of the ethanolic leaf extract of *Melissa officinalis* L. as an herbal medicine. To this end, the cyclic voltammetry technique and DPPH scavenging assay are used to assess the antioxidant activity of the plant. The molecular docking studies are performed to investigate the antioxidant activity of rosmarinic acid (as the main natural compound of the plant) against Cytochrome P450 3A4 (4D75), Myeloperoxidase (1DNW), and Thyosine (3nm8) (reactive oxygen species (ROS) generating enzymes). Finally, the electrochemical oxidation of the ethanolic leaf extract of *Melissa officinalis* L. is studied in the presence of captopril using the cyclic voltammetry method in the biological pH range.

EXPERIMENTAL

Materials and Apparatus

All the reagents and chemicals utilized in the present study were of analytical reagent grade and purchased from E. Merck (Darmstadt, Germany). They were applied without any further purification. Captopril tablet (25 mg) was obtained from Exir Pharmaceutical Co.

Preparation of the Ethanolic Leaf Extract of *Melissa Officinalis L*

Melissa officinalis L. leaves were collected from the University of Nahavand greenhouse. They were then washed and dried at room temperature for 72 h. After proper drying, the leaves were ground to a fine powder. In order to prepare the extract, 0.2 g of the powder was mixed with 50 ml of ethanol, then warmed to 30-40 °C, and finally maintained at room temperature for 15 h. The mixture was then filtered with Whatman filter paper number 1 and allowed to be cooled. This extract was considered as the ethanolic leaf extract of *Melissa officinalis L.*

Preparation of DPPH Radical and Captopril Solution

A 1 mM solution of DPPH radical (2,2-diphenyl-1-picrylhydrazyl) was prepared in methanol as the solvent. Also 5000 mg l^{-1} solution of captopril was prepared with solving 2 tablet of captoprile (each tablet contains 25 mg captopril) in the 10 ml 30% (v/v) solution of ethanol in water.

Cyclic Voltammetry

Voltammetric measurements were done with a Behpajoh model BHP-2065 potentiostat/galvanostat. A conventional three-electrode system was used to assemble the electrochemical cell. An Ag/AgCl/KCl (3 M) as the reference electrode (Metrohm), a platinum wire as the counter electrode, and a glassy carbon electrode as the working electrode were used. A Metrohm model 713 pH-meter was applied to adjust pH. Cyclic voltammetry assays carried out with 3 ml ethanolic leaf extract of *Melissa officinalis L.* and 7 ml of the buffer solution (phosphate buffer, c = 0.2 M, pH = 7.0). The voltammograms were analyzed for peak potentials (all reported *vs*. Ag/AgCl) and currents as described below.

The peak current ratios (I_{pC1}/I_{pA1}) were obtained through the following equation presented in Ref. [16].

$$I_{pc}/I_{pa} = (I_{pc})_0/I_{pa} + 0.485(I_{sp})_0/I_{pa} + 0.086$$

In this equation, $(I_{pc})_0$ represents cathodic peak current, and $(I_{sp})_0$ is the "*switching potential*" current concerning the zero current. I_{pc} and I_{pa} have their common meanings.

Molecular Docking Analysis

To assess the interactions between the main natural compound in the *Melissa officinalis L*. extract (rosmarinic acid) and the ROS generating enzymes (Cytochrome P450 3A4 (4D75), Myeloperoxidase (1DNW), and Thyosine (3nm8)), the molecular docking study was performed with AutoDock vina software [17]. The X-ray structures of 4D75, 1DNW and 3NM8 were obtained from the Protein Data Bank (PDB: https://www.rcsb.org). The ligand was modeled and optimized using Chem Bio Draw Ultra software (version: 16.0, Cambridge Soft) and Gaussian, respectively. The molecular docking study was performed with AutoDock Tools (ADT, version: 1.5.6) and Discovery Studio 4.5 Client software.

RESULTS AND DISCUSSION

Electro-oxidation of the Ethanolic Leaf Extract of *Melissa Officinalis L*

The evaluation of the quality consistency of *Melissa officinalis L.* was previously carried out using the simple and reliable HPLC method. For each chromatogram of the *Melissa officinalis L*. extract, around 50 peaks were observed, comprising 12 well-resolved characteristic peaks. Some of these peaks were attributed to phenolic acids including ferulic acid, chlorogenic acid, syringic acid, gallic acid, rosmarinic acid, and caffeic acid (Fig. 1). According to the statistical analysis, in the *Melissa officinalis L.* extract, gallic acid was at the lowest level, and rosmarinic acid was at the highest level [18].

The cyclic voltammogram of the leaf extract of *Melissa officinalis L.* (3 ml) in water (phosphate buffer, at the biological pH of 7.0, c = 0.2 M) is illustrated in Fig. 1 (curve a). According to Fig. 1, there is an anodic peak (A_1) and a cathodic peak (C_1), which are related to the oxidation of rosmarinic acid in the *Melissa officinalis L*. leaf extract to the corresponding product and *vice versa*, by a quasi-reversible process [19]. As can be observed, proportional to the increase of the potential sweep rate (Fig. 1, curves a, b, c, and d), the heights of the A_1 and C_1 peaks, and the peak current ratio (I_p^{C1}/I_p^{A1}) rise (Fig. 1. Inset). This observation revealed that A_1 and C_1 are counterpart and related together [19].

The cyclic voltammograms of gallic acid, quercetin, and salicylic acid as standard antioxidants were recorded, and the E_p^A values were used to determine the antioxidant activity [20,21]. A relationship between the oxidation potential in cyclic voltammetry and ferric reducing antioxidant power (FRAP) can be observed. An increase in the antioxidant activity caused a decrease in E_p^A and an increase in the number of hydroxyl groups on the aromatic ring [20,21]. Based on the results in Fig. 2, the *Melissa officinalis L*. leaf extract exhibited higher antioxidant activity compared with gallic acid, salicylic acid, and quercetin as standard synthetic antioxidants.

DPPH Radical Scavenging Activity

A DPPH free radical scavenging assay was applied to investigate the antioxidant activity (AA%) of the*Melissa officinalis* L. leaf extract (1). The DPPH radical scavenging activity was calculated based on the methodology presented by Brand-Williams *et al.* [22]. The extract reacted with the stable DPPH radical in methanol solution. The reaction mixture was obtained by adding 1 ml of the *Melissa officinalis* L. leaf extract, 2 ml of absolute methanol, and 1 ml of DPPH radical solution 1 mM prepared in methanol. The reduction of DPPH occurs when it reacts with an antioxidant compound with hydrogen-donating ability. After mixing 1 ml of the *Melissa officinalis* L. leaf extract with DPPH solution, the color changing (from dark violet to pale yellow (Fig. 4)) was measured [Absorbance (Abs)] at 517 nm with a UV-Vis spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA) (Fig. 5. curve b). The blank solution was prepared by mixing 3 ml of ethanol and 1 ml of the sample; while, the mixture of 3 ml of methanol and 1 ml of the DPPH radical solution was considered as the control solution (Fig. 5, curve a). The removal of absorbance peak at 517 nm is indicative of the reaction of DPPH radical with leaf extract of *Melissa officinalis L*. as a natural antioxidant (Fig. 5).

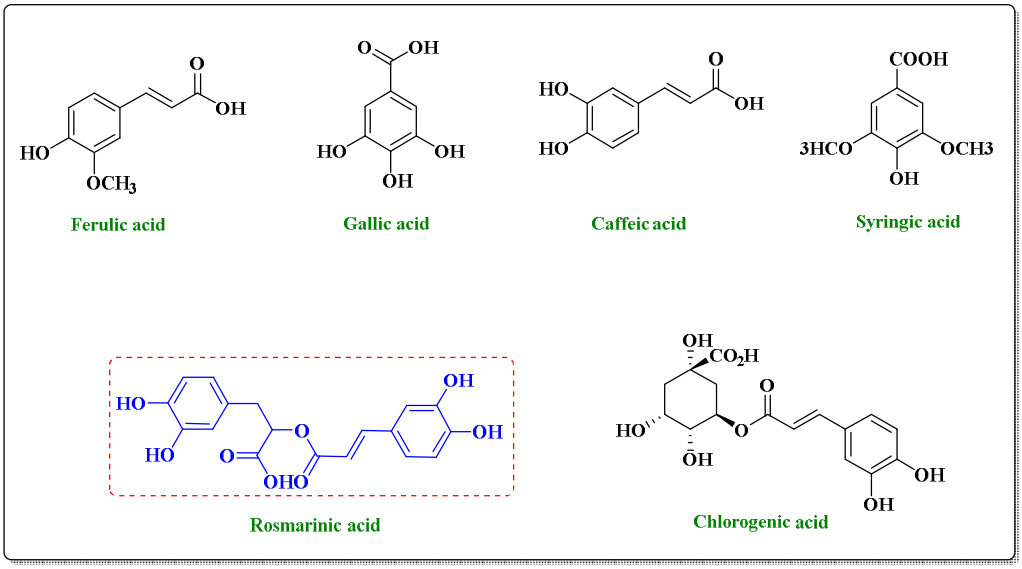


Fig. 1. The structure of various phenolic compounds in the *Melissa officinalis L.* leaf extract.

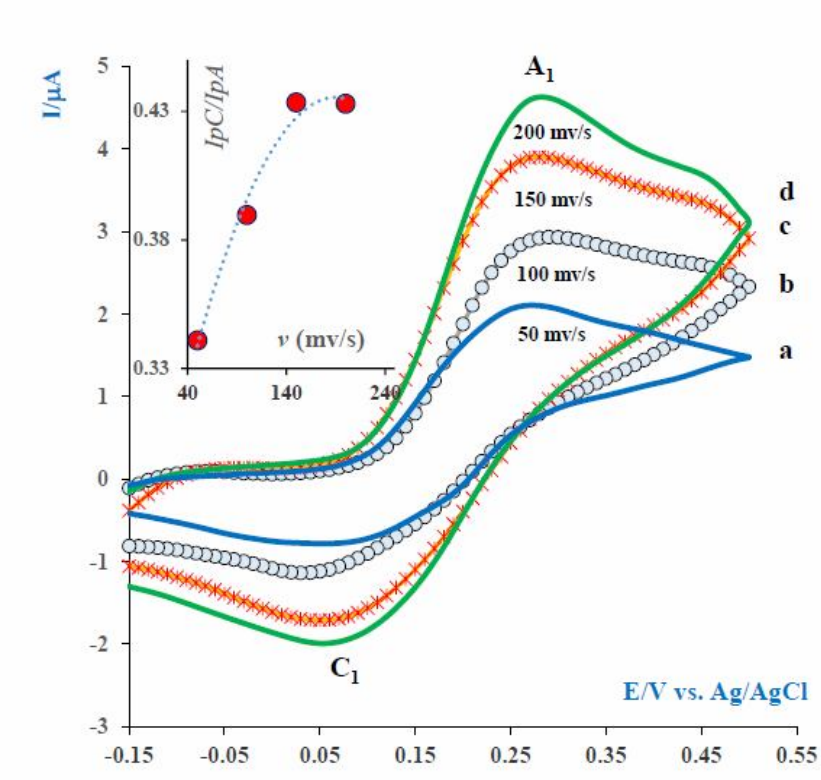


Fig. 2. Cyclic voltammograms of 3 ml of the *Melissa officinalis L.* leafextract at different scan rates of 50 (a), 100 (b), 150 (c), and 200 (d) mV s^{-1} on a glassy carbon electrode in an aqueous solution (phosphate buffer, c = 0.2 M, pH = 7.0). Inset: The relationship between the peak current ratio and scan rates. Scan rates: 50, 100, 150.

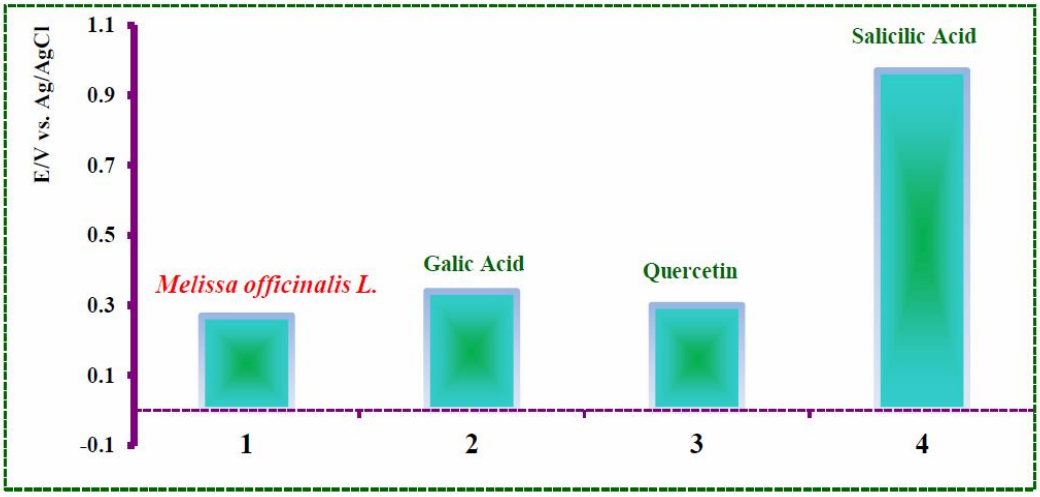


Fig. 3. Comparison of the antioxidant activity (E_p^A) of the *Melissa officinalis L.* leaf extract with gallic acid, salicylic acid, and quercetin.

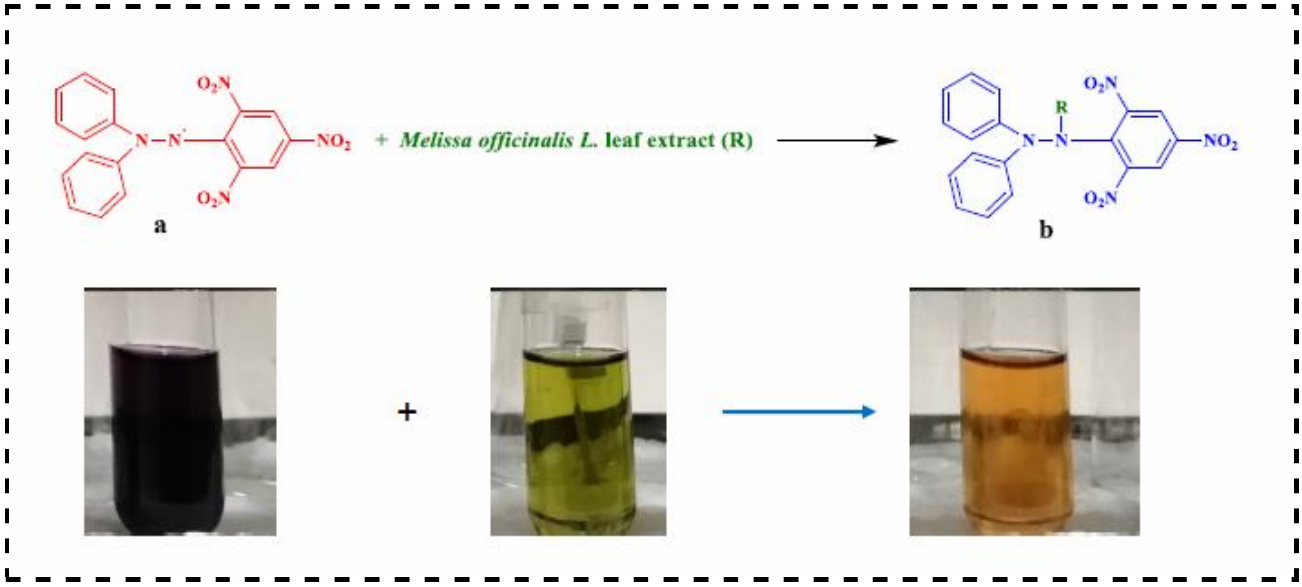


Fig. 4. Photographs of the solutions (a) DPPH$^{\bullet}$ radical, (b) the reduction of DPPH$^{\bullet}$ radical by the *Melissa officinalis L.* leaf extract.

Investigation of Antioxidant Activity Using Molecular Docking Studies

The biological activity of some enzymes can generate some reactive oxygen species (ROS) such as $OH^{\cdot}$, H_2O_2, and HOCl. The *Melissa officinalis L.* leaf extract with good antioxidant properties can counteract these oxidizing agents and prevent some disorders and diseases. The assessment of the antioxidant activity of rosmarinic acid (the main natural compound in the *Melissa officinalis L.* leaf extract) was carried out by molecular docking studies or ligand-protein

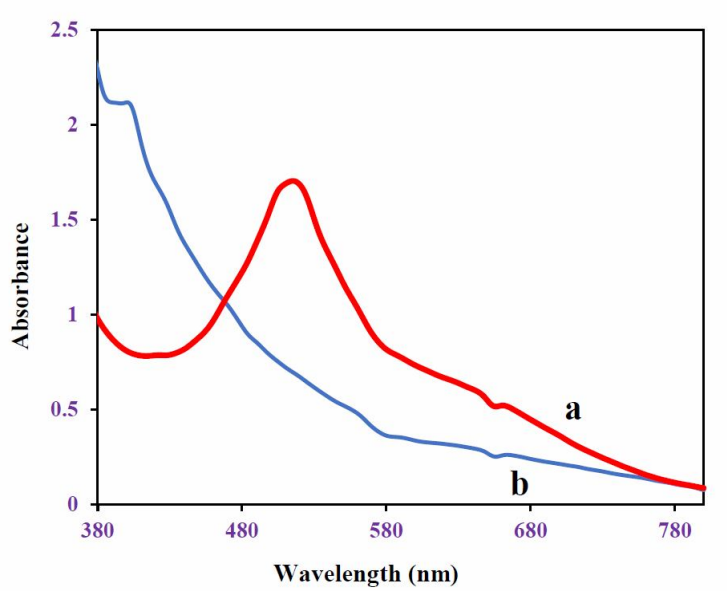


Fig. 5. UV-Vis. spectrum of (a) DPPH$^{\bullet}$, and (b) DPPH$^{\bullet}$ + the *Melissa officinalis L*. leaf extract.

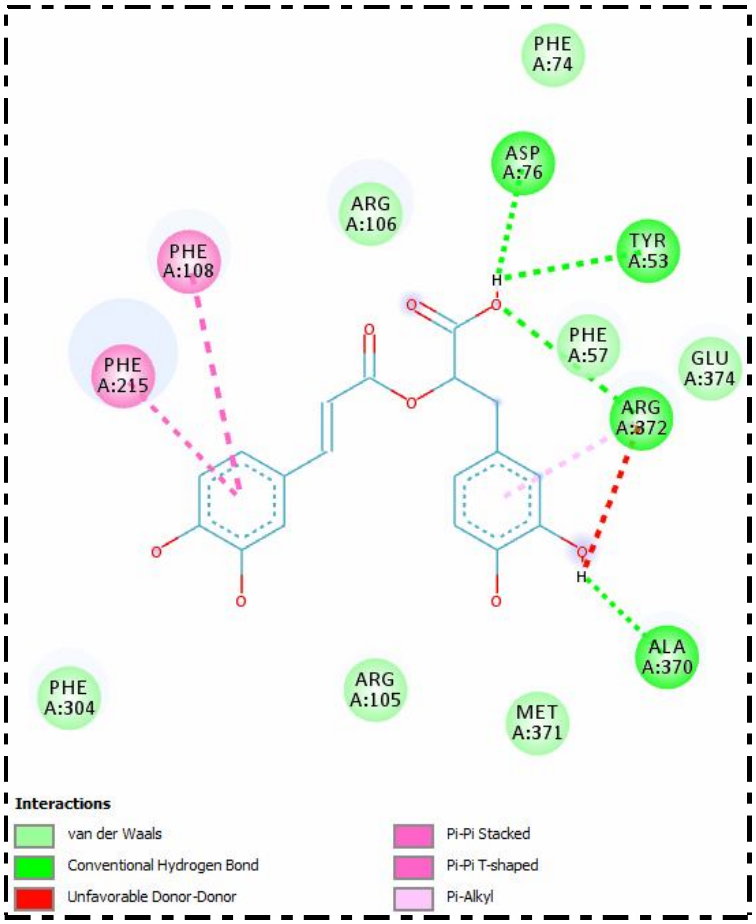


Fig. 6. 2D representation of the molecular study of rosmarinic acid-Cytochrome P450 3A4 (4D75) interacting complexes.

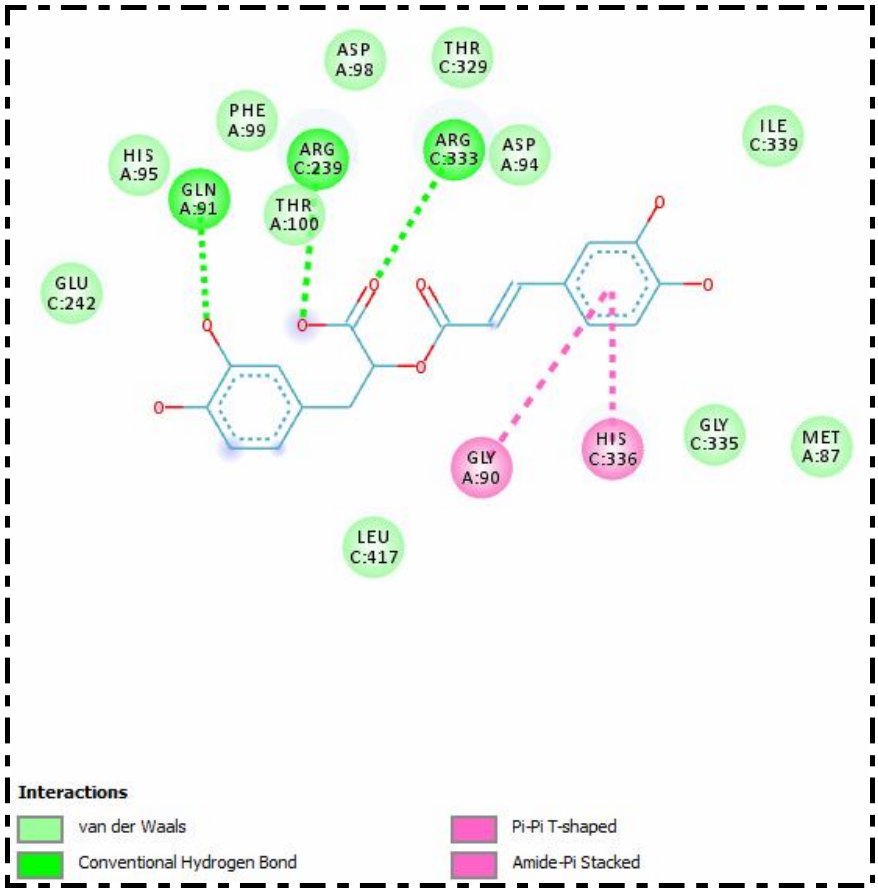


Fig. 7. 2D representation of the molecular study of rosmarinic acid-Myeloperoxidase (1DNW) interacting complexes.

interaction studies in the presence of three ROS generating enzymes including Cytochrome P450 3A4 (4D75), Myeloperoxidase (1DNW), and Thyosine (3nm8). According to the docking results (Figs. 6, 7 and 8), a wide variety of interactions such as pi-alkyl, pi-cation, amide-pi-stacked, unfavorable donor-donor, and pi-sigma can be observed in the interactions between rosmarinic acid and the receptors.

The results of the molecular studies and the obtained affinity values indicated that rosmarinic acid as a ligand had a positive effect on the stability and inactivation of the ROS generating enzymes, and consequently deactivatedthe oxidative stress process. The interaction of rosmarinic acid with Thyosine (PBD ID: 3nm8) exhibited the most inhibitory effect with -10.5 kJ mol^{-1} binding affinity value (Table 1).

Base on Fig. 8, the conventional hydrogen bonds are formed between OH moieties of catechol ring with ALA 40 residue and -COOH functional group with ALA 44 and TYR 267 residues. Amide-Pi stacked interaction is formed between GLY 43 and catechol ring. Other interactions such as Pi-cation, Pi-alkyl, alkyl, van der Waals, and unfavorable doner-doner further support the potential inhibition function of rosmarinic acid against ROS generation enzymes [23] (Table 1).

Electro-oxidation of the Ethanolic Leaf Eextract of *Melissa Officinalis L.* in the Presence of Captopril

Captopril containing a free sulfhydryl group is a drug that is applied to cure the hypertension and used as an angiotensin-converting enzyme inhibitor (Scheme 1) [12]. At high doses, captopril causes a dose-dependent reduction

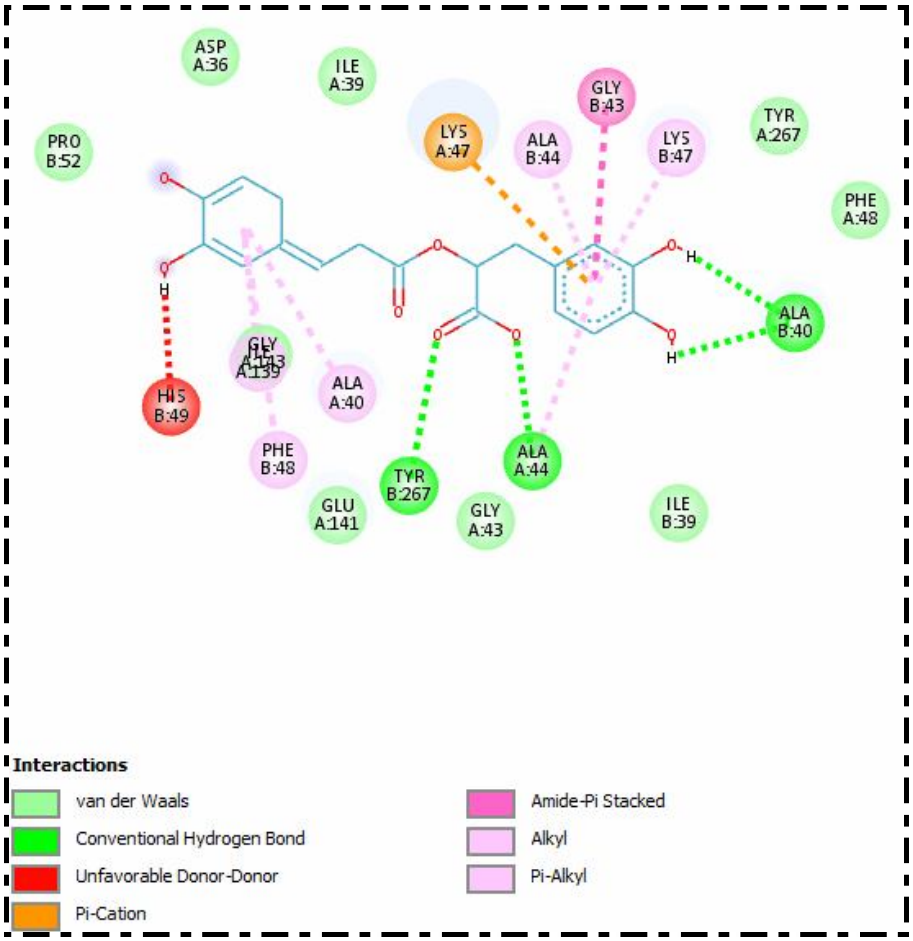
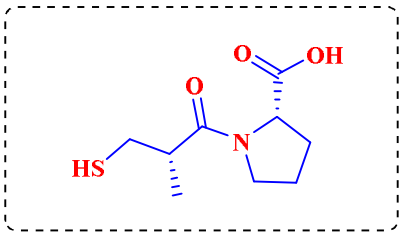


Fig. 8. 2D representation of the molecular study of rosmarinic acid-Thyosine (3NM8) interacting complexes.



Captopril

Scheme 1. The structure of captopril

of hepatic glutathione (GSH) and can disturb GSH and the relevant GSH enzymes.

The electrochemical oxidation is mostly in parallel with the cytochrome P450-catalyzed oxidation in the microsomes of the liver. The hepatic metabolism is the main route of endogenous clearance of captopril, so studying the electro-

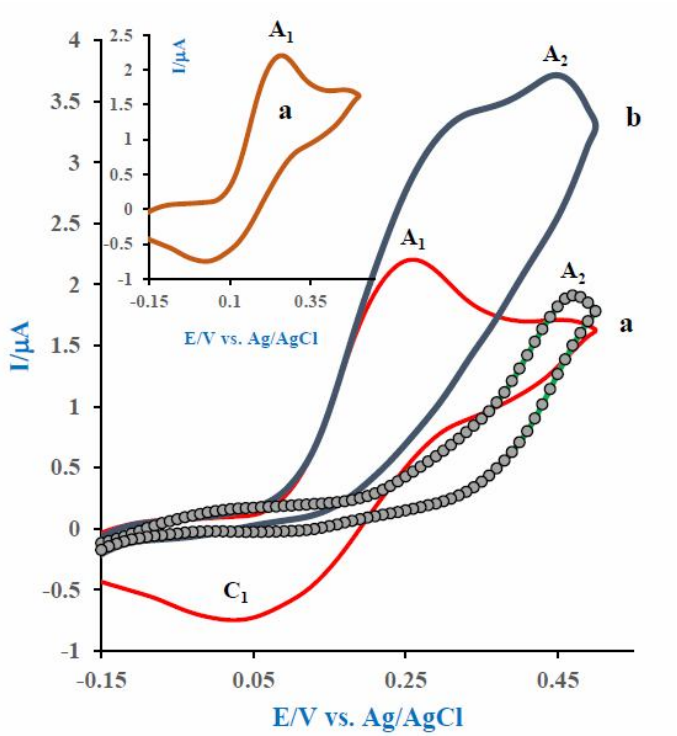


Fig. 9. (a) Cyclic voltammogram of 3 ml of the *Melissa officinalis L*. leaf extract (1), in the absence, and (b) in the presence of captopril (5000 mg l^{-1}: 2 tablets of 25 mg), and (c) captopril (500 mg l^{-1}) in the absence of 1 on a glassy carbon electrode in aqueous solution (phosphate buffer, c = 0.2 M, pH = 7.0). Scan rate: 50 mV s^{-1}. T = 25 ± 1°C.

oxidation of the ethanolic leaf extract of *Melissa officinalis* L. in the presence of captopril would be of interest. The oxidation of *Melissa officinalis* L.leaf extract (1) in the presence of captopril (3) (500 mg l^{-1}: 2 tablets of 25 mg) was investigated in some detail. The cyclic voltammogram resulted from 3 ml of the ethanolic solution of *Melissa officinalis* L. in the presence of captopril (5000 mg l^{-1}) is demonstrated in Fig. 2 (curve b), according which the cathodic peak (C_1) decreased, and the anodic peak (A_1) increased. The curve c in this figure is the cyclic voltammogram of captopril (3), and its oxidation can be seen in the anodic peak A_2.

More studies were conducted by changing the potential sweep rate in 3 ml of the *Melissa officinalis* L. leaf extract in the presence of captopril (3). The effect of the potential sweep rate on the cyclic voltammograms of 3 ml of the *Melissa officinalis L*. leaf extract in the presence of captopril (500 mg l^{-1}) is presented in Fig. 4. Based on the results in Fig. 4 (inset), the peak current ratio (I_p^{C1}/I_p^{A1}) is heavily dependent upon the potential sweep rate and rises with an increase in the sweep rate.

The following evidence supported (3) the presence of consequent chemical reactions of the compounds in the *Melissa officinalis L*. leaf extract with captopril: (a) Decreasing and disappearing I_p^{C1} throughout the reverse scan and increasing I_p^{A1} in the presence of captopril (Fig. 3, curve b). This fact revealed that the electrochemically generated compounds in the *Melissa officinalis L*. leaf extract were partly removed from the surface of the electrode by chemical reaction with captopril (3). (b) The reappearance of the cathodic peak (I_p^{C1}) at the high potential sweep rate (Fig. 10, curve e). Under these circumstances, at lower sweep rates, the peak current ratio (I_p^{C1}/I_p^{A1}) was approximately zero and rose with the increase in the potential sweep rate (Fig. 10, curves a-e). This indicated the departure from the intermediate reign and the arrival in the

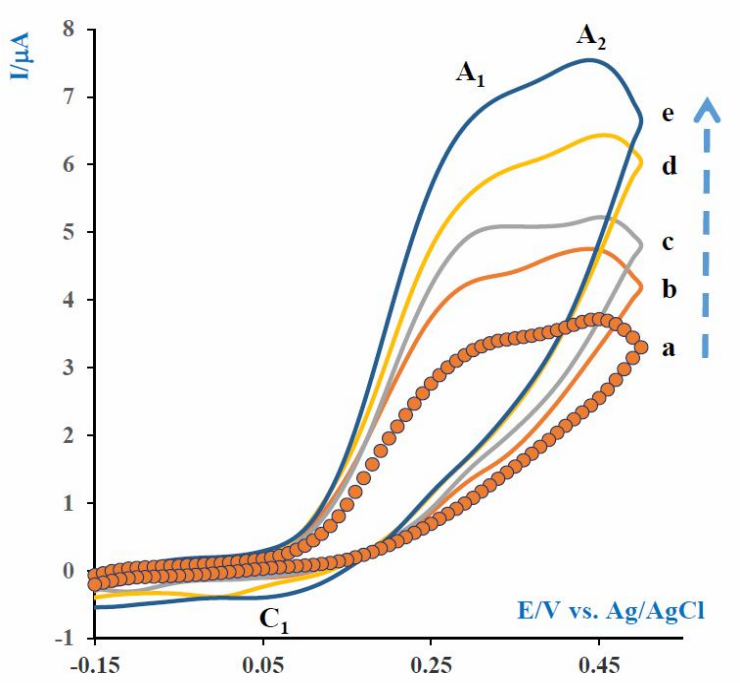


Fig. 10. Cyclic voltammogram of 3 ml of the *Melissa officinalis L.* leaf extract (1) in the presence of captopril (5000 mg l^{-1}) with various scan rates: 50 (a), 75 (b),100 (c), 150 (d), and 200 (e) mV s^{-1}on a glassy carbon electrode in aqueous solution (phosphate buffer, c = 0.2 M, pH = 7.0). T = 25 ± 1°C.

Table 1. The Results (Binding Affinity (kJ mol^{-1}) of Molecular Docking Studies of Rosmarinic Acid and Different Receptors (ROS Generating Enzymes)

Ligand receptor	Cytochrome P450 3A4 (4D75)	Myeloperoxidase (1DNW)	Thyosine (3nm8)
Rosmarinic acid	-8.1 kJ mol^{-1}	-8.1 kJ mol^{-1}	-10.5 kJ mol^{-1}

diffusion reign with increasing sweep rate.

The results revealed that the ethanolic leaf extract of *Melissa officinalis L.* can be applied as an antidote to captopril overdose before any clinical treatments. It can also be seen that there is synergy between the antioxidant activity and the treatment of captopril overdose in the ethanolic leaf extract of *Melissa officinalis L.*

CONCLUSIONS

The results of this investigation show that the ethanolic leaf extract of *Melissa officinalis L.* has high antioxidant activity in comparison with some of the synthetic antioxidants. Moreover, the components produced by the oxidation of *Melissa officinalis L.* leaf extract are effective antidotes to captopril overdose before starting clinical treatments. Theoretical investigations (molecular analysis) indicate that these natural compounds reduce the oxidizing effect of ROS generating enzymes in biological systems. To our knowledge, this is a new report of an effective herb-drug interaction in captopril overdose, particularly in patients with decreased renal function.

ACKNOWLEDGEMENTS

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