

## Biosynthesis of Ag Nanoparticle by *Peganum Harmala* Extract; Antimicrobial Activity and Ability for Fabrication of Quercetin Food Electrochemical Sensor

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In this study, we used *Peganum harmala* extract as a powerful reducing agent for biosynthesis of Ag nanoparticle (Ag-NP). The biosynthesized Ag nanoparticle was characterized using XRD and TEM methods. The results confirmed the synthesis of Ag nanoparticle with diameter ~ 10 nm. Then, the antibacterial activity of Ag nanoparticle was identified using *Streptococcus sp* as Gram-positive, and *E. coli* as Gram-negative bacteria, and the results showed good antibacterial activity. In addition, the Ag nanoparticle was used for modification of carbon paste electrode (CPE) in the presence of paraffin oil (PO), and n-hexyl-3-methylimidazolium hexafluoro phosphate (MIHP) as binder. The Ag-NP/PO/MIHP/CPE showed a powerful catalytic activity in accelerating the electrochemical reaction of quercetin. Due to this point, it is suggested as a powerful tool for determining quercetin in the concentration ranged from 0.01 to 550 µM with detection limit of 5.0 nM. The Ag-NP/PO/MIHP/CPE was successfully used for determination of quercetin in onion and hawthorn food samples.

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**Keywords:** Extract, Quercetin Electrochemical Sensor, Ag Nanoparticle, Modified Electrode *Peganum Harmala*

### 1. INTRODUCTION

Nano-based materials showed many advantages and different applications, due to unique properties and many powerful activities [1-7]. Therefore, many scientific studies focused on synthesized

nano-based materials and especially metal-based nanoparticles with different shapes and different properties [8-10]. Among them, biosynthesis methods showed more advantage compared to other synthesis methods such as combustion, CVD, chemical precipitation, and sole-gel, due to biocompatibility and low cost. Several different sources were suggested for biosynthesis of metal-based nanoparticles such as fungi, bacteria, and plant extract [11-15]. Application of plant extract for biosynthesis of metal-based nanoparticles showed many advantages such as easy operation and low cost [16-20]. Out of metal-based nanoparticles, the Ag nanoparticle showed many various applications due to having good antibacterial activity and high electrical conductivity [21-25]. Many research works reported synthesizing of Ag nanoparticles by biosynthesized methods, and showed different applications, especially antibacterial activity for this nanoparticle [26-30]. On the other hand, Ag nanoparticle has good electrical conductivity, and can be suggested as powerful mediator for fabrication of sensitive electrochemical sensors [31-33]. High over-potential and low redox signal in electrochemical determination are major problems for determination of food; also, drug and water pollutant compounds with unmodified electrode create a serious necessity for electrode modification [34-40]. To overcome this problem, nanomaterials having unique properties were suggested in different fields [41-48], and especially the electrochemical sensors [49, 50]. As a conductive material, Ag nanoparticle can be used to fabricate modified sensors.

Quercetin (Figure 1) is a natural flavonoid, which is broadly distributed in vegetables, fruits, and grains [51, 52]. On the other hand, the quercetin showed many different activities and properties for human health such as reducing the risk of cancer, reducing the risk of heart disease, relieving the allergy symptoms, and lowering high blood pressure [53]. Due to redox behavior of quercetin, the electrochemical methods are considered as useful strategies for determination of quercetin in different food samples [54-56]. Nevertheless, due to high overvoltage of quercetin, it is necessary to use modified electrode with conductive materials for improving limit of detection and selectivity of sensors [57-65].

Regarding this and in continuous of our group research works [66-70], we designed and made a new and sensitive electrochemical sensor entitle Ag-NP/PO/MIHP/CPE, and used it as electrochemical tool for determining the quercetin. The Ag-NP as a conductive material biosynthesized by *Peganum harmala* extract and antibacterial activity of this nanoparticle was checked, and the results showed that our sensor has good antibacterial activity, and can be used in biological samples. This is first quercetin electrochemical sensor that used Ag nanoparticle coupled with ionic liquid as green sensor in food sample analysis.

## 2. EXPERIMENTAL

### 2.1. Materials and instrument

Silver nitrate (for biosynthesis of Ag-NPs), graphite powder, n-hexyl-3-methylimidazolium hexafluoro phosphate, sodium hydroxide, quercetin hydrate, and paraffin oil were purchased in analytical grade from Sigma-Aldrich Company. Phosphoric acid and hydrochloric acid were purchased from Merck. *Streptococcus sp* and *E. coli* were used for antibacterial investigation. Cary 60 UV-Vis

spectroscopic machine was used to identify Ag-NPs. The electrochemical signals were recorded by Potentiostat/galvanostat machine (Ivium Vertex. One), connected to Ag/AgCl/KCl<sub>sat</sub>, Ag-NP/PO/MIHP/CPE, and Pt wire as reference, working, and counter electrodes.

## 2.2. Biosynthesis of Ag-NP using *Peganum harmala* extract

*Peganum harmala* (curry leaves) were collected in the seyed abosaleh village, Qaemshahr, Mazandaran Province, Iran. The *Peganum harmala* leaves were washed, and were followed by shade-drying at room temperature. The *Peganum harmala* leaves were then grinded using a blender to make fine powder. Ten gr of the obtained powder was mixed in 200 ml of distilled water, and then heated for 2.0 h at 120°C. In the final step, the extract was centrifuged for 15 min at 15,000 rpm, and was then filtered.

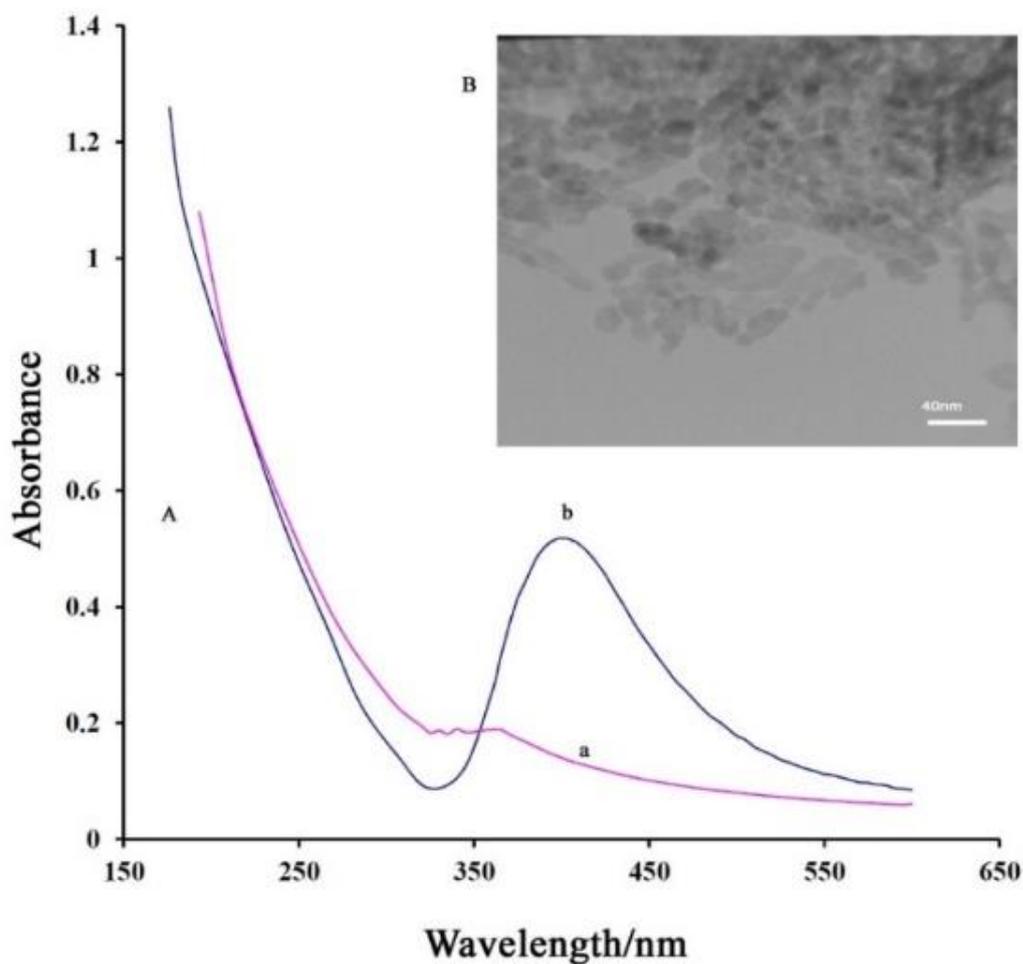
## 2.3. Fabrication of Ag-NP/PO/MIHP/CPE

The Ag-NP/PO/MIHP/CPE was fabricated by mixing 940 mg graphite powder + 60.0 mg Ag-NP in the presence of diethyl ether as solvent. After 15 min At 45 °C, the diethyl ether evaporated. Then, suitable amounts of MIHP+ paraffin oil were added as binders into pestle and mortar, and were hand-mixed for 45 min. The result paste was used as the sensor in the presence of a copper wire.

# 3. RESULTS AND DISCUSSION

## 3.1. Characterization and antibacterial activity of Ag-NPs

The Uv-Vis spectrum of Ag NO<sub>3</sub> (curve a) solution, and Ag-NPs (curve b) are presented in Figure 1A. As can be observed, the Ag salt showed no absorption band in wavelength 200-700 nm. In similar condition, the Ag-NPs solution showed an absorption band ~400 nm that is relative to plasmon resonance Ag-NPs. The obtained results is similar to previous reported data by different group for biosynthesized of Ag nanoparticle by plant extract [71, 72]. On the other hand, the TEM image of synthesized Ag nanoparticle showed a spherical shape with a diameter of 10.0 nm (Figure 1B). The growth of *Streptococcus sp.* as Gram positive and *E. coli* as Gram-negative bacteria, were completely inhibited after treatments by all Ag-NPs synthesized *Peganum harmala* extracts at OD600 condition (see table 1). Results confirmed good antibacterial activity of Ag-NPs. The antibacterial results relative to Ag nanoparticle are consistent with previous report by Soliman et al. [73].



**Figure 1.** A) UV-Vis absorption spectra of silver salt (a) and silver nanoparticles (b). B) TEM image of silver nanoparticle

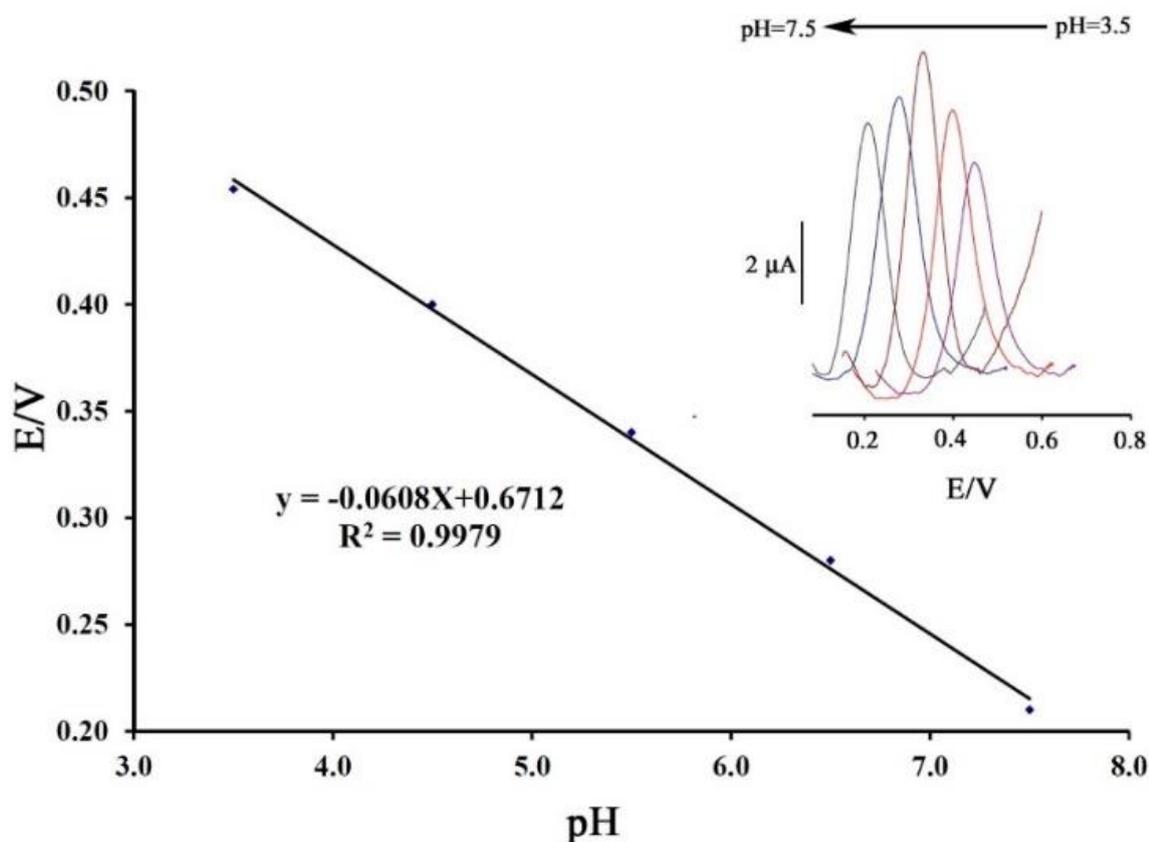
**Table 1.** Effect of synthesized Ag-NPs by *Peganum harmala* extract on microbial growth

Organism	OD600
<i>Streptococcus sp.</i>	0.0
<i>E. coli</i>	0.0

### 3.2. Electrochemical behavior of quercetin at surface of Ag-NP/PO/MIHP/CPE

Oxidation signal of 100.0  $\mu\text{M}$  quercetin was recorded at surface of Ag-NP/PO/MIHP/CPE, using differential pulse voltammetric method at pH ranged from 3.5 to 7.5 (Figure 2 inset).

The oxidation signal of quercetin showed linear relationship between potential and pH with equation  $E_{pa} = 0.0608 \text{ pH} + 0.6712$ , which is acceptable for an redox reaction with equal value of electron and proton (Figure 2).

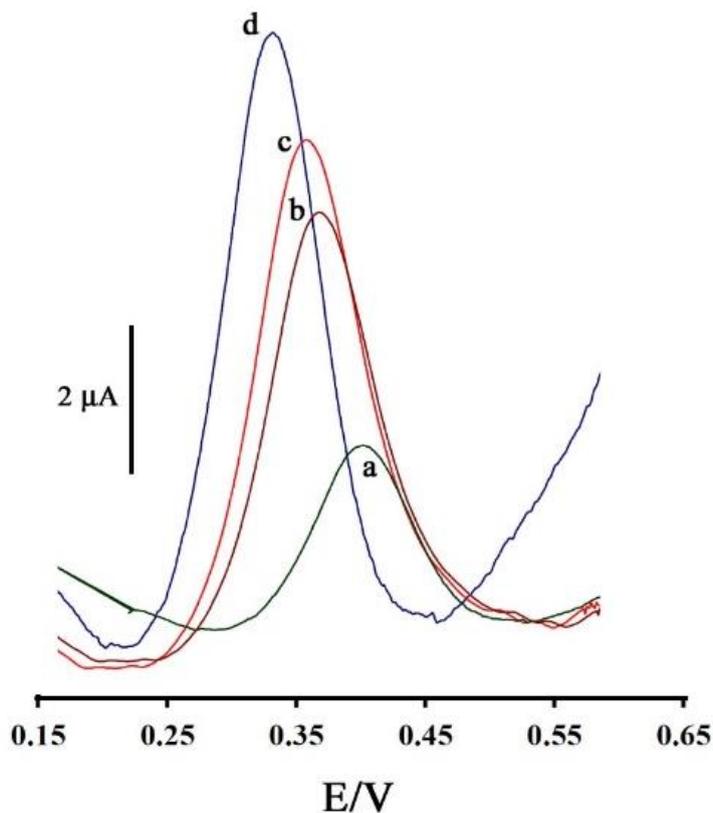


**Figure 2.** E-pH curve for electrooxidation of 100.0  $\mu\text{M}$  quercetin at surface of Ag-NP/PO/MIHP/CPE. Insert) DP voltammogram 100.0  $\mu\text{M}$  quercetin at surface of Ag-NP/PO/MIHP/CPE in the pH range 3.5-7.5.

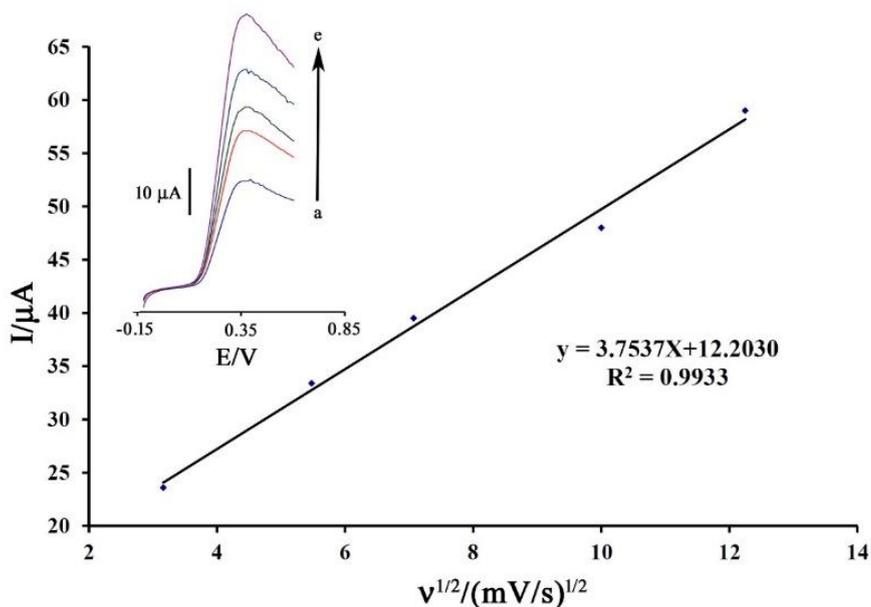
The obtained results are consistent with previous reports for equal value of electron and proton in electro-oxidation mechanism of quercetin [74].

The DP voltammograms of 100.0  $\mu\text{M}$  quercetin was at surface of CPE (curve a), Ag-NP/PO/CPE (curve b), PO/MIHP/CPE (curve c), and Ag-NP/PO/MIHP/CPE (curve d). As shown in figure 3, by moving of CPE to Ag-NP/PO/MIHP/CPE, and in the presence of 100.0  $\mu\text{M}$  quercetin, the oxidation signal of quercetin increased from 2.32  $\mu\text{A}$  up to 7.7  $\mu\text{A}$ , and at the same time, oxidation potential of quercetin decreased from 403 mV to 334 mV.

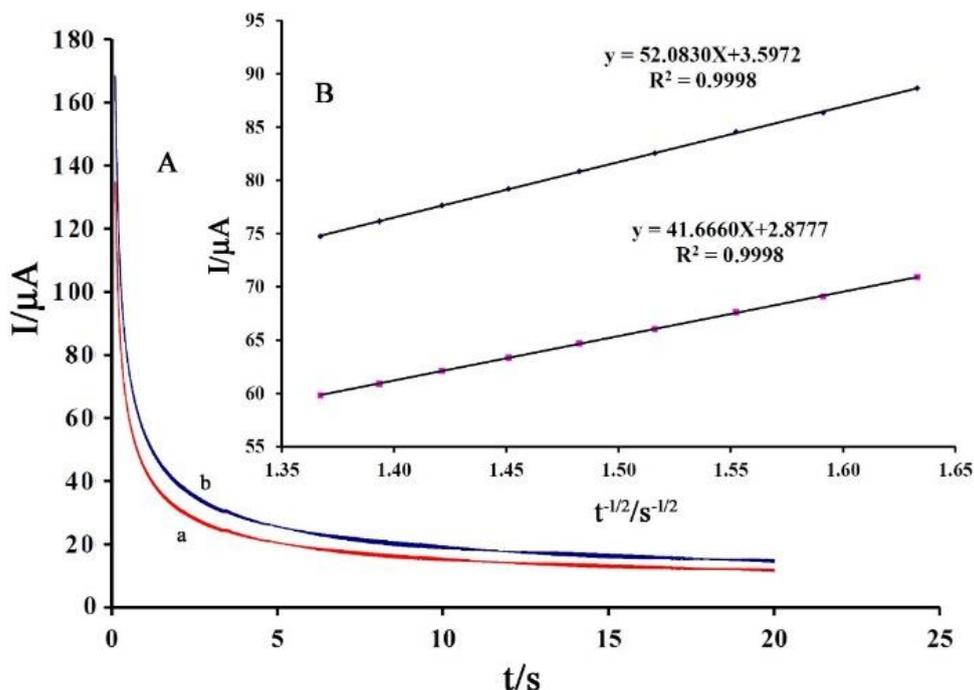
This improvement confirmed excellent modification of CPE with Ag-NP and MIHP as two conductive mediators. Previous papers reported the conductivity of Ag nanoparticle for amplification of electrochemical sensor and the obtained results in this study is similar to previous report papers [75].



**Figure 3.** DP voltammograms of 100.0  $\mu\text{M}$  quercetin at surface of CPE (curve a), Ag-NP/PO/CPE (curve b), PO/MIHP/CPE (curve c) and Ag-NP/PO/MIHP/CPE (curve d).



**Figure 4.**  $I-v^{1/2}$  curve for electro-oxidation of 500  $\mu\text{M}$  quercetin at surface of Ag-NP/PO/MIHP/CPE. Inset) Linear sweep voltammogram of 500  $\mu\text{M}$  quercetin at scan rate a) 10.0; b) 30.0; c) 50.0; d) 100.0 and e) 150.0 mV/s.

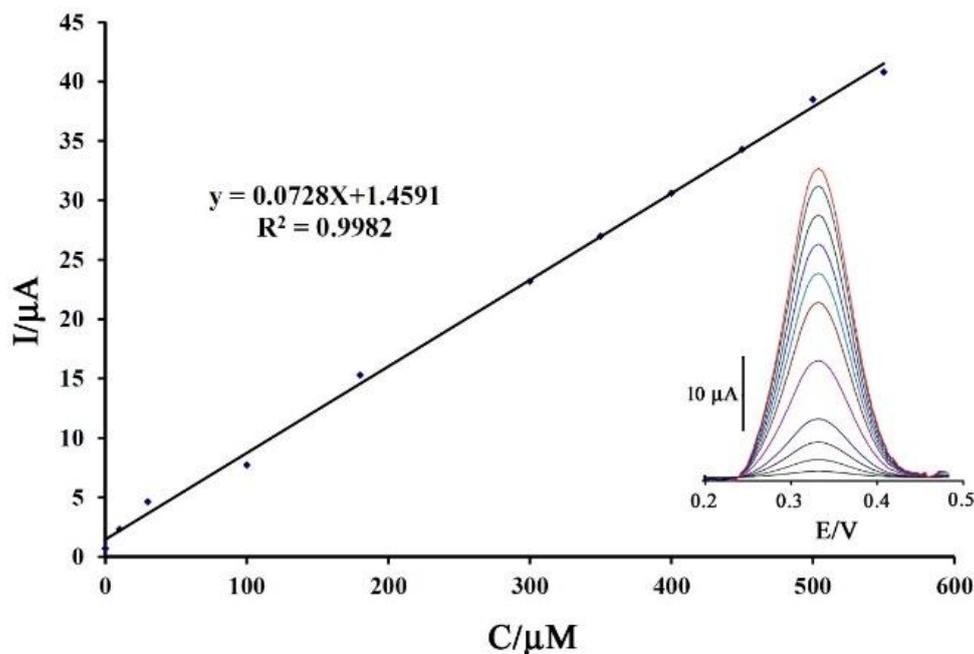


**Figure 5.** (A) Chronoamperograms obtained at the Ag-NP/PO/MIHP/CPE in the presence of (a) 300 and (b) 500  $\mu\text{M}$  quercetin at pH 7.0. (B) Plots of  $I$  vs.  $t^{-1/2}$  obtained from chronoamperograms.

The linear relationship between oxidation signal of quercetin at surface of Ag-NP/PO/MIHP/CPE and  $v^{1/2}$  are displayed in figure 4, and this point confirmed the diffusion process or electro-oxidation of quercetin at surface of Ag-NP/PO/MIHP/CPE. According to reported papers, the direction relation between current and  $v^{1/2}$  confirm a diffusion process for electroactive materials [76-80].

In this study, the value of diffusion coefficient ( $D$ ) of quercetin was determined by recording chronoamperograms of 300  $\mu\text{M}$  and 500  $\mu\text{M}$  at surface of Ag-NP/PO/MIHP/CPE using applied potential 0.5 V (Figure 5A). By the use of Cottrell equation showed in Figure 5B and slopes of them, the value of diffusion coefficient was determined to be  $1.55 \times 10^{-5} \text{ cm}^2/\text{s}$ .

Stability and repeatability of the Ag-NP/PO/MIHP/CPE were checked using DP voltammetric measurements of 100.0  $\mu\text{M}$  quercetin. The repeatability of Ag-NP/PO/MIHP/CPE was investigated by nine successive DP determination of 100.0  $\mu\text{M}$  quercetin and relative standard deviation was calculated to be 3.11%. Stability of Ag-NP/PO/MIHP/CPE was evaluated by storing developed sensor for 45 days, and also by DP determination in different days. According to the obtained results, the oxidation peak of quercetin had no change and decreased 8%, which confirms good stability.



**Figure 6.** Standard calibration curve for quercetin at concentration range 0.01-550.0  $\mu\text{M}$ . Insert) DPVs for electro-oxidation of quercetin in concentration range 0.01-550.0  $\mu\text{M}$  at surface of Ag-NP/PO/MIHP/CPE.

**Table 2.** The analytical data reported by different electrochemical sensors for determination of quercetin

Electrode	Mediator	LDR ( $\mu\text{M}$ )	LOD ( $\mu\text{M}$ )	[Ref]
Glassy carbon electrode	Graphene Nanosheets	0.006-10.0	0.0039	[81]
Glassy carbon electrode	Molecularly imprinted polymer based on polypyrrole film+ graphene oxide	0.6-15.0	0.04	[82]
Glassy carbon electrode	Lowerlike $\text{Co}_3\text{O}_4$ nanoparticles	0.5-330.0	0.1	[83]
Carbon nanotube paste electrode	Copper microparticles	4.98-38.5	0.543	[84]
Carbon paste electrode	Ag-NP and MIHP	0.01-550	0.005	This work

Interference of some biological samples such as vitamin B<sub>9</sub>, glucose, and alanine, and some cation and anions  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  were checked in the presence 50.0  $\mu\text{M}$  quercetin at surface of Ag-NP/PO/MIHP/CPE. The results confirmed that 500 folds of above mentioned compounds have no interference in determination of quercetin.

The Ag-NP/PO/MIHP/CPE showed a linear dynamic range 0.01-550.0  $\mu\text{M}$  with detection limit 5.0 nM, for determination of quercetin using differential pulse voltammetric methods (See Figure 6).

These values of detection limit and linear dynamic range for determination of quercetin using suggested sensor is better than of previous reported papers. This point confirm that synergic effect of Ag-NPs and MIHP created a good condition for sensitive determination of quercetin (see table 2).

### 3.3. Real sample analysis

The ability of Ag-NP/PO/MIHP/CPE in determining quercetin in food sample was checked using standard addition method. The hawthorn and onion were selected as real samples, and standard addition results are shown in table 3. The recovery data between 98.36-101.32% confirm powerful ability of sensor in determination of quercetin in real samples.

**Table 3.** Investigation of Ag-NP/PO/MIHP/CPE for determination of quercetin in food samples

Sample	Quercetin added ( $\mu\text{M}$ )	Quercetin expected ( $\mu\text{M}$ )	Quercetin found ( $\mu\text{M}$ )	Recovery%
onion	---	---	1.12 $\pm$ 0.02	---
	5.00	6.12	6.02 $\pm$ 0.03	98.36
hawthorn	---	---	1.03 $\pm$ 0.02	---
	5.00	6.03	6.11 $\pm$ 0.8	101.32

## 4. CONCLUSION

In the present study, we investigated the ability of *Peganum harmala* extract for biosynthesis of Ag-NPs. The Ag-NPs showed good antibacterial activity for *Streptococcus sp* as Gram-positive, and *E. coli* as Gram-negative bacteria. On the other hand, Ag-NPs was used for fabrication of Ag-NP/PO/MIHP/CPE, and send showed high performance ability for electrocatalytic determination of quercetin with detection limit of 5.0 nM. Finally, the Ag-NP/PO/MIHP/CPE was successfully used for determination of quercetin in different food samples such as hawthorn and onion.

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