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RESEARCH PAPER

Electrochemical Determination of Gallic Acid in *Camellia sinensis* (L.) Kuntze, *Viola odorata* L., *Commiphora wightii* (Arn.) Bhandari, and *Vitex agnus-castus* L. by MWCNTs-COOH Modified CPE

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ABSTRACT

Gallic acid (GA) is the main phenolic antioxidant which has been subjected of many studies because of its important biological properties including anticancer, anti-inflammatory and antimicrobial activities as well as free radicals scavenger and cardiovascular diseases protector. Hereupon, fabricating a selective and sensitive sensor for GA detection and measurement is an important issue. In this paper, a carboxylated MWCNTs modified carbon paste electrode (MWCNTs-COOH/CPE) was successfully fabricated and employed for GA determination. Activating the carboxylic sites of the MWCNTs carried out in nitric acid solution in an ultrasonic bath and further studied by field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy. The electrocatalytic oxidation of GA at the MWCNTs-COOH/CPE surface was studied by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. The GA presented a high electrochemical response on MWCNTs-COOH/CPE at pH 2 in comparison with the CPE. This sensor showed a linear response range of 0.33 - 196 µM and a detection limit of 17.2 nM (S/N = 3). Furthermore, the designed MWCNTs-COOH/CPE was successfully applied as an electrochemical sensing system for GA determination in extracts of *Camellia sinensis* (L.) Kuntze, *Viola odorata* L., *Commiphora wightii* (Arn.) Bhandari, and *Vitex agnus-castus* L., with the estimated amount of 11.4, 8.9, 11.91 and 2.9 mg L^{-1} GA in each plant extract, respectively.

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INTRODUCTION

Free radicals are generally reactive oxygen species (ROS) which at high concentration will induce oxidative stress and injure cell structures such as lipids, proteins, and DNAs [1]. Also, they are important in the development of many auto-immune and neurodegenerative diseases, cardiovascular malfunction, cancers, cataracts, aging and rheumatism which seriously threaten human health. Antioxidants could prevent, delay and repair damages caused by ROS through inhibiting the propagation of free radical reactions [2].

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Gallic acid (3, 4, 5-trihydroxybenzoic acid; GA) is the most important phenolic antioxidant which acts as reducing agent, hydrogen donator and singlet oxygen quencher [3] and owes antimicrobial, anti-inflammatory, antimutagenic and anticarcinogenic activities. GA present in fruits, nuts, grapes, and plants [4] such as *Camellia sinensis* (L.) Kuntze (*C. sinensis*) [5], *Viola odorata* L. (*V. odorata*) [6], *Commiphora wightii* (Arn.) Bhandari (*C. wightii*) [7] and *Vitex agnus-castus* L. [8]. Also, GA is used as a reference-standard for total polyphenol content estimation [9].

C. sinensis (green tea) native to China and Southeast Asia is kind of flowering plants of *Theaceae* family. Several studies have shown that green tea confer significant protection against Parkinson's, alzheimer and cancer [10], reduce high cholesterol [11], owing antidiabetic effects [12], antibacterial [13], anti-inflammatory [14], anti-HIV [15] and antioxidant activities [16]. Most methods used for GA detection in *C. sinensis* are electrochemical [17] and HPLC [18].

V. odorata (sweet violet) from *Violaceae* family is native to Europe and Asia. It has been used to remedy hypertension, anxiety, and insomnia [19]. This plant also has diuretic, laxative [20], antibacterial [21], lung-protective [22] and high antioxidant activities [23]. To the best of our knowledge, there is no report of the electrochemical method for GA detection in *V. odorata*. Gontova et al. published an HPLC method in order to quantify phenolic capacity of *V. odorata* [24].

C. wightii (*Commiphora mukul*) known as Indian bdellium-tree is a flowering plant in the family *Burseraceae*. It could be found in Northern Africa to Central Asia, but is most common in Northern India. It is known to have antioxidant [25], and anti-inflammatory [26] activities. Also, it is used as a natural treatment for heart diseases [27]. Muguli et al. used HPLC method for determination of GA in *C. wightii* [28], as we know there is not electrochemical report on GA determination.

V. agnus-castus also called *Vitex* is native to Mediterranean Europe and Central Asia region. It is a medicinal plant contained *flavonoids* [29]. *Vitex*'s fruits and leaves have been shown antioxidant [30], antimicrobial [31] and anti-inflammatory [32] activities. Sarikurkcu et al. used GC-MS method for study antioxidant activity of *V. agnus-castus* [33]. Also, we have not found an electrochemical method determining GA in this plant.

In recent years, several methods have been used for determination of GA and its derivatives include high performance liquid chromatography (HPLC) [34], mass spectrometry [35], flow injection analysis [36], resonance light scattering [37] and capillary zone electrophoresis [38]. These methods require expensive equipment need expensive equipment, complicated function, and toxic organic solvents usage, lead to limit of their application. In contrast, there is an increasing interest on electrochemical methods [39] based on modified electrodes as sensitive, selective and low-cost analytical techniques for phenolic compound detection and various types of nanostructures have been used as surface modifiers [40-42]. Among the modifiers of the electrode surface, MWCNTs due to unique electronic, thermal and mechanical properties are most commonly used in biomedical and engineering applications [43]. But, MWCNTs have poor solubility in a large number of solvents. The defects on MWCNTs created by oxidants can be stabilized by bonding with carboxylic or hydroxyl groups, which help to stabilize the aqueous MWCNTs-COOH suspension [44].

In this study, we report an electrochemical sensor for rapid determination of GA in plants extracts, using differential pulse voltammetric (DPV) technique based on a carbon paste electrode modified with carboxylated multi-walled carbon nanotubes (MWCNTs-COOH/CPE). The MWCNTs-COOH significantly improves the oxidation peak current of GA.

MATERIALS AND METHODS

Gallic acid was purchased from Sigma-Aldrich. MWCNTs, (diameter: 30-50 nm; length 20 µm) were purchased from Times nano Co. (Chengdu, China) with 95% purity. Pure fine graphite powder and paraffin oil (Merck, Germany) used as binding agents in graphite pastes. Ethanol (≥ 99.9 %) and nitric acid (65%) were purchased from the Merck. Dipotassium hydrogen phosphate and potassium dihydrogen phosphate, from Merck, were used to prepare phosphate buffer solution (PBS) with a concentration of 0.2 M and different pH values (2.0 to 5.0) which used as the supporting electrolyte for electrochemical quantification. All aqueous solutions were prepared in doubly distilled water. The extracts of *V. odorata* and *C. wightii* were purchased from the traditional medicine department of Barij Essence Pharmaceutical in Kashan, Iran. Pills of *V. agnus-castus* obtained from Pursina Pharmaceutical Company in Tehran, Iran. *C. sinensis* tea bag received from Lipton Company in Qazvin, Iran. All the other used chemicals and reagents were of analytical grade.

Apparatus

Electrochemical measurements were performed using a computerized potentiostat/galvanostat (model SAMA 500, Isfahan, Iran), and conventional three-electrode system with the modified carbon paste as the working electrode, a platinum rod as

the auxiliary electrode, and a saturated Ag/AgCl electrode as the reference electrode. The used electrochemical techniques are cyclic voltammetry (CV) and differential pulse voltammetry (DPV). IR spectra were determined on a Nicolet Magna series FTIR 550 spectrometer using KBr pellets in the range of 400-4000 cm^{-1}. The XRD patterns were obtained from a diffractometer of Philips Company equipped with a Cu Kα anode (λ=1.54 Å) in the 2θ range from 10 to 80˚. The field emission scanning electron microscopy (FESEM) images of the electrode surface were recorded using an electronic microscope (ZEISS, Sigma VP-500, Germany). Other instruments such as water bath ultrasonic (model Eurosonic 4D, Euronda, Montecchio Pre-calcino (Vincenza), Italy) and vacuum filtration system (mixed cellulose ester membrane filter with a pore size of 0.45 µm) were employed in this research.

Carboxylation of MWCNTs

MWCNTs-COOH was prepared using the reported method by Karimi et al. [45]. In brief, 20 mg of MWCNTs was added to 30 mL nitric acid solution (35%) and then ultrasonicated at 40 °C for 6 h which was periodically relaxed for at least 10 min after each 20 min sonication. After that, the black suspension was filtered with vacuum filtration equipped with mixed cellulose ester membrane filter and washed thoroughly with distilled water until neutral pH achieved. The residue dried under the infrared lamp.

Preparation of the MWCNTs-COOH/CPE

MWCNTs-COOH/CPE was prepared by a simple procedure, first 0.004 g of the MWCNTs-COOH were dispersed in 2 ml ethanol using an ultrasonic bath over 10 minutes, then 0.5 g of graphite powder added to the mixture and after drying at room temperature, 6 drops of paraffin oil added, and resultant paste packed into a syringe (2 mm diameter and 10 mm deep). Electrical contact was made by pushing a copper wire through the center of the back mixture. In order to achieve a new surface, CPE surface vertically polished on a piece of waxed paper.

Sample preparation

In order to prepare extracts, 0.25 g of *V. agnus-castus* powder added to 20.0 mL of doubly distilled water:ethanol (30:70) at 25 °C. Also, aqueous *V. odorat*, *C. wightii*, and *C. sinensis* diluted (20x) with doubly distilled water:ethanol (30:70) at 25 °C. Samples subject at ultrasound treatment at a constant frequency of 35 kHz for 30 min at room temperature. The solutions were filtered through a Whatman no. 1 filter paper into a 25 mL volumetric flask and were kept at 4 °C for further use.

RESULTS AND DISCUSSION

Characterization of MWCNTs-COOH

In order to investigate the surface functional groups formed on MWCNTs, FTIR spectra of pristine MWCNTs (Fig. 1-A) and MWCNTs-COOH (Fig. 1-B)

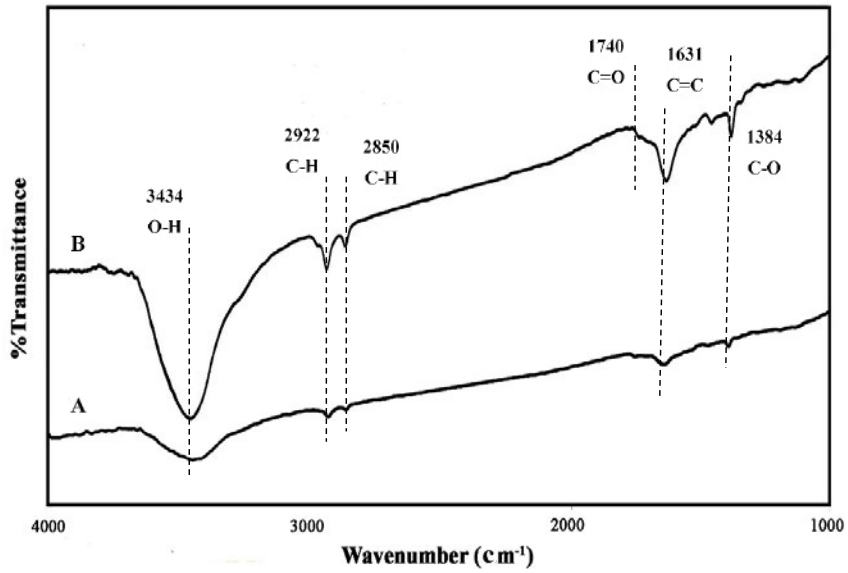

Fig. 1. FT-IR spectra of: A) pristine MWCNTs, and B) MWCNTs-COOH

in the range 4000–1000 cm^{-1} were recorded. For the pristine MWCNTs, the peak at 1629 cm^{-1} can be attributed to the (C=C) stretching, which indicates the graphite structure of MWCNTs [46].

Characteristic peaks of MWNTs-COOH observed at 1384 and 3434 cm^{-1} can be assigned to C-O and OH stretching vibrations of the carboxylic acid group, respectively [47]. The peak at 1631 cm^{-1} (C=C) indicates the graphite structure of MWCNTs-COOH, a new peak around 1740 cm^{-1} in the spectra of MWCNTs-COOH can be assigned to C=O stretching of the carboxylic acid groups [47]. The peaks at 2850 and 2922 cm^{-1} can be attributed to asymmetric and symmetric CH_2 stretching [46]. As compared with the FT-IR spectrum of pristine MWCNTs, results suggest that the carboxylic acid groups have been successfully introduced onto the surfaces of MWCNTs by acid treatment.

Fig. 2 shows XRD patterns of the pristine MWCNTs (Fig. 2-A) and MWCNTs-COOH (Fig. 2-B). The pristine MWCNTs samples revealed the presence of two diffraction peaks at 2θ values of 25.94° and 44.42° attributed to the graphite structure (002) and (100) planes of the MWCNTs, respectively [48]. No drastic change in the position of characteristic peaks of MWCNTs-COOH was observed, which suggests that MWCNTs are maintained with their original structure after the functionalization process with the carboxylic acid groups.

SEM images for MWCNTs-COOH, CPE and MWCNTs-COOH/CPE are shown in Fig. 3. The acid treatment can fragment the MWCNTs and it can be seen that the MWCNTs-COOH are shorter in length (Fig. 3-A) [47]. The surface of CPE is shown in (Fig. 3-B), a layer of irregular and isolated flakes of graphite powder present on the surface of CPE. After MWCNTs-COOH were added to the paste matrix, it can be seen that MWCNTs-COOH were distributed on the surface of the electrode with special three-dimensional structure (Fig. 3-C), indicating that the MWCNTs-COOH were successfully modified on the CPE and observed morphology was in agreement with previous works [49-51].

Electrochemical behaviour of GA on MWCNTs-COOH/CPE

In order to study the electrocatalytic activity (behaviour) of GA on MWCNTs-COOH/CPE, DPV technique was used due to its low background currents. Fig. 4 shows the DPVs of different electrodes recorded in 0.2 M PBS, pH 2.0 at the scan rate of 0.148 Vs^{-1}. As shown in Fig. 4-a, in the absence of GA no obvious oxidation peak observed indicating that MWCNTs-COOH has no response in the absence of GA and the background current is very low. In the presence of GA (33 µM) two anodic

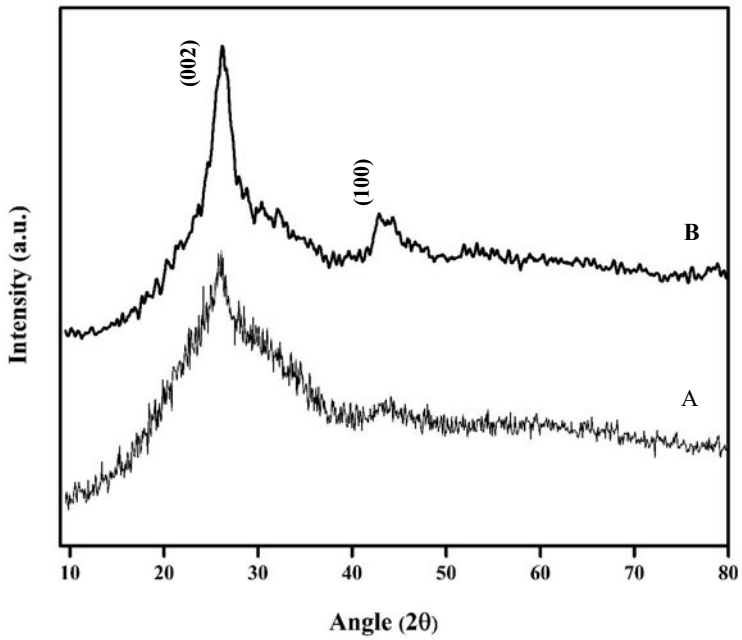

Fig. 2. XRD spectra of: A) pristine MWCNTs, and B) MWCNTs-COOH

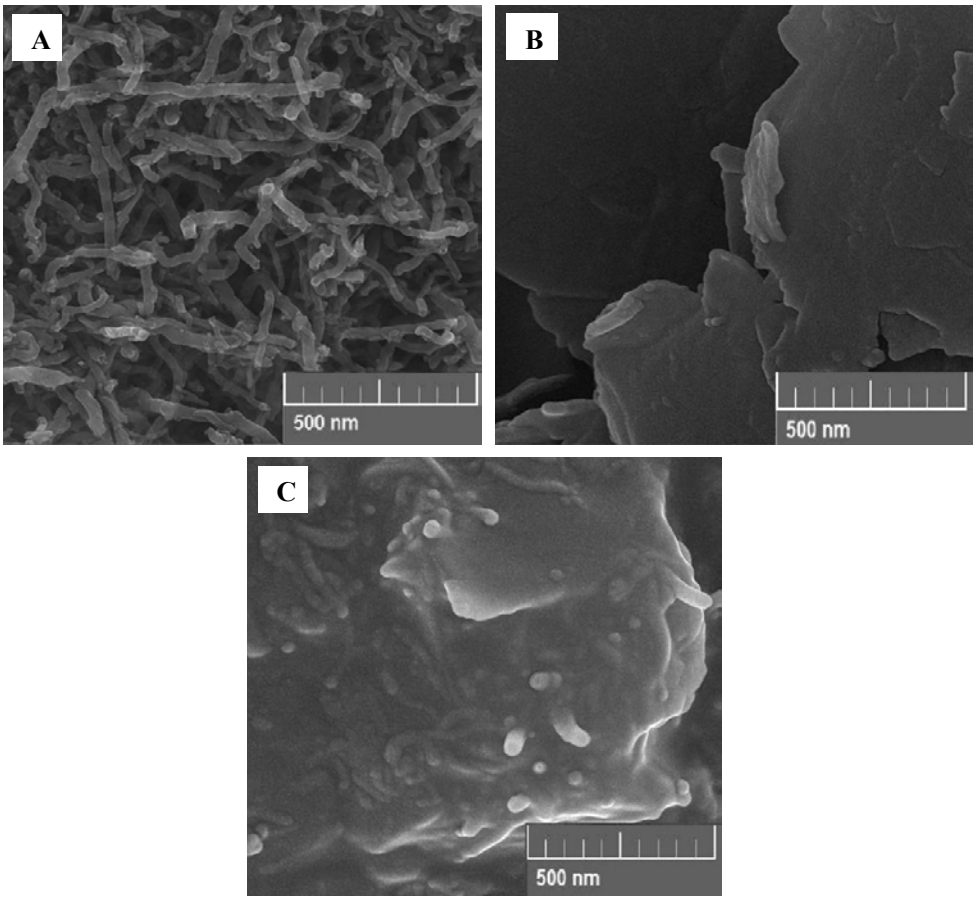

Fig. 3. SEM images of: A) MWCNTs-COOH, B) CPE, and C) MWCNTs-COOH/CPE

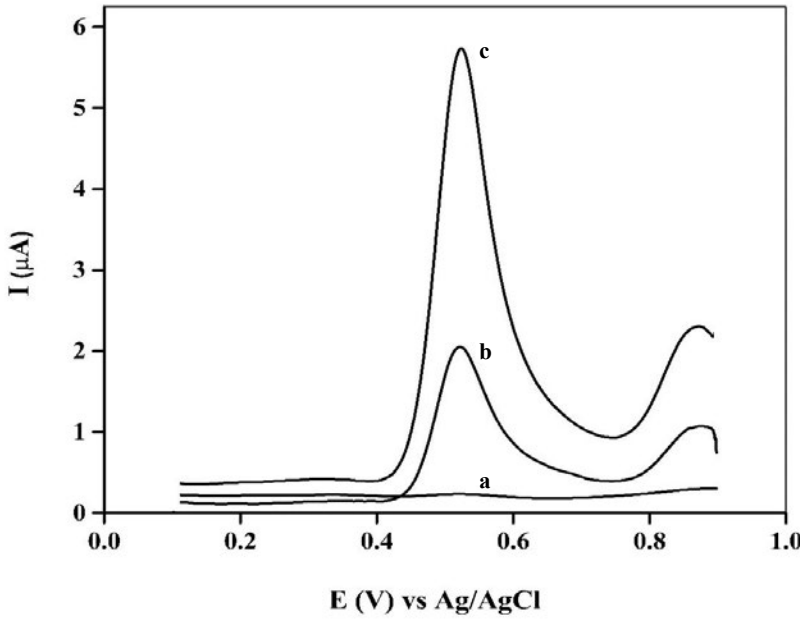

Fig. 4. DPVs of a) bare CPE in 0.2 M PBS (pH 2.0), b) bare CPE, and c) MWCNTs-COOH/CPE in 0.2 M PBS (pH 2.0) containing 33 μM GA at a scan rate of 0.148 Vs^{-1}

peaks around 0.5 and 0.8 V appeared on CPE (Fig. 4-b) and MWCNT-COOH/CPE (Fig. 4-c). The first peak is originated from semiquinone radical formation, followed by its oxidation to the quinone form (second peak) [52]. The second peak is very poor in comparison with the first one; hence we focused on the first oxidation peak for study GA oxidation. The response of MWCNTs-COOH/CPE toward electrooxidation of GA increases 3 times compared with the CPE. The enhanced catalytic activity of MWCNTs-COOH/CPE is due to unique properties such as high electrical conductivity and large surface area of MWCNTs.

The process of GA oxidation is affected by protonation reactions [42]. Thus, the influence of pH value of supporting electrolyte solution on the oxidation of GA at the MWCNTs-COOH/CPE surface studied using DPV. Through the increase in pH value from 2.0 to 5.0 at a scan rate of 0.148 Vs^{-1}, the peak current is reduced and the peak potential slightly shifted to more negative values, because the oxidation process of GA is related to H^+ ions of buffer solution [41]. The highest peak current was obtained at a pH of 2.0 (the results not shown here). Therefore, further studies were carried out at this pH value of the buffer solution.

Effect of potential scan rate

The effect of potential scan rate on MWCNTs-COOH/CPE in the electrooxidation of GA was studied by recording the CVs of a 33 µM GA in 0.2 M PBS, (pH 2.0) at scan rates of 0.027, 0.037, 0.047, 0.067, 0.087, 0.118, and 0.148 Vs^{-1} (Fig. 5). The oxidation peak currents of GA increased with increasing the potential scan rates and the anodic peak potential was shifted to the negative side. Inset shows that the oxidation peak current of GA was linear with the scan rate (υ), and the regression equation was I_p = 0.0071 υ + 1.0239 (I_p: µA, υ: mVs^{-1}, R^2 = 0.997), indicating that the oxidation of GA at MWCNTs-COOH/CPE was controlled by the adsorption process [53].

Quantification of GA in MWCNTs-COOH/CPE

The DPVs of different concentrations of GA from 0.33 to 190 µM on MWCNTs-COOH/CPE is shown in Fig. 6. The sharp oxidation peaks observed and the peak current increased with increasing the concentration of GA. The calibration curve was made by plotting the current density of anodic peak versus the concentration of GA. The result was two range of linear calibration plots of low concentration (0.33 to 19.9 µM; Fig. 6-A) and

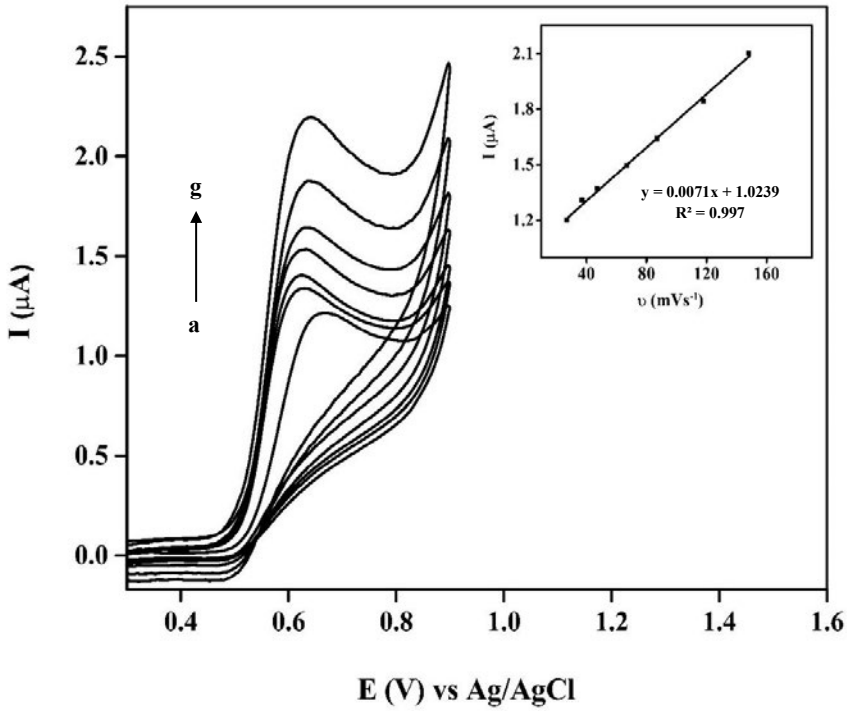

Fig. 5. CVs of MWCNTs-COOH/CPE in 0.2 M PBS (pH 2.0) containing 33 µM GA at scan rates of 0.027, 0.037, 0.047, 0.067, 0.087, 0.118 and 0.148 Vs^{-1} (a to g). Inset: the relationship between anodic peak current vs. potential scan rate

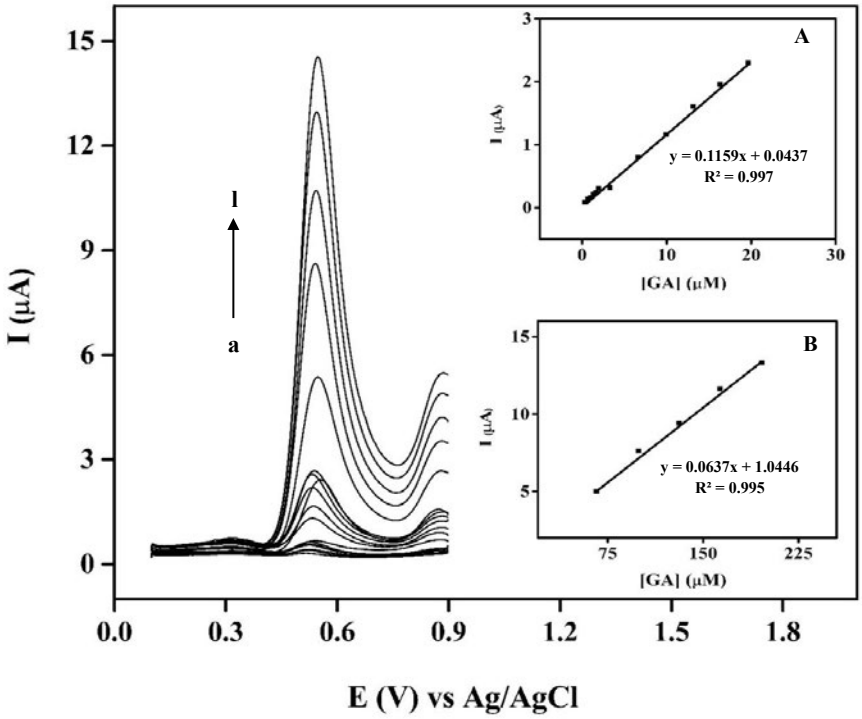

Fig. 6. DPVs of MWCNTs-COOH/CPE in 0.2 M PBS (pH 2.0) containing 0.33, 0.66, 0.99, 1.31, 1.96, 3.3, 6.6, 9.9, 13.1, 19.6, 66, 99, 131, 163 and 196 µM of GA at scan rate of 0.148 Vs^{-1}. Insets: relationship between the oxidation peak current and concentrations of GA in the range of A) 0.33 to 19.6 µM, and B) 66 to 196 µM

Table 1. Comparative results of the electrochemical GA detection on various electrodes

Electrode	Nanomaterial	Linear range (M)	Detection limit (M)	Ref.
GCE	Au microclusters/sulfonate functionalized graphene	5.0×10^{-8} - 8.0×10^{-6}	10.7×10^{-9}	54
CPE	Molecularly imprinted polymer–MWCNT	0.12×10^{-6} - 38.0×10^{-5}	47.0×10^{-9}	55
CPE	Graphene nanosheets	3.0×10^{-5} - 1.5×10^{-4}	1.1×10^{-7}	56
GCE	AgNP/delphinidin	0.6×10^{-6} - 6.2×10^{-4}	0.28×10^{-6}	57
GCE	Polyepinephrine	1.0×10^{-6} - 20.0×10^{-6}	6.63×10^{-7}	58
CPE	MWCNTs-COOH	3.3×10^{-7} - 1.9×10^{-4}	17.2×10^{-9}	This work

high concentration (66 to 190 µM; Fig. 6-B). The regression equation of the calibration plot for high concentration of GA (CGA) was Ip (µA) = 0.0637 CGA (µM) + 1.0446, (R^2 = 0.995) and for low concentration of GA was Ip (µA) = 0.1159 CGA (µM) + 0.0437, (R^2 = 0.997) with the detection limit (S/N=3) of 17.2 nM. In Table 1 the ability of the proposed sensor for GA determination with some other sensors has been compared. As results of the comparison, this work suggests a simple, inexpensive, sensitive, and reliable sensor for GA determination and the proposed biosensor can be used as an analyzer device for estimation of total polyphenols of plant samples.

Real sample analysis

The presence of GA has been reported in *C. sinensis*, *V. odorata*, *C. wightii*, and *V. agnus-castus*. Standard addition method used for determination of GA in plant extracts. The DPVs of the standard addition method for the determination and measurement of GA in *C. sinensis*, *V. odorata*, *C. wightii*, and *V. agnus-castus* are shown in Figs. 7 to 10, respectively. Clear oxidation peak of each sample in the absence of GA appeared around 0.5 V (curve a in Figs. 7 to 10). After adding a certain amount of GA standard solution, the peak current at the same oxidation potential increased (curves b to e). Insets show the calibration curves resulted

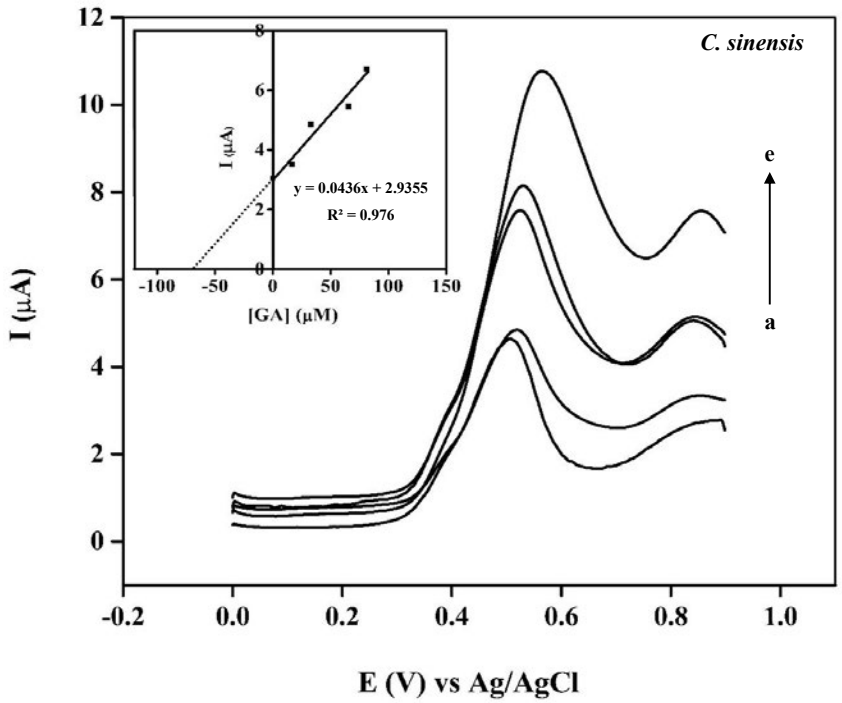

Fig. 7. DPVs of MWCNTs-COOH/CPE in 0.2 M PBS (pH 2.0) containing a) appropriate amounts of *C. sinensis* and b-e) increasing amounts of GA standard solution at a scan rate of 0.148 Vs^{-1}. Inset shows the corresponding standard addition curve for determination of GA in *C. sinensis*.

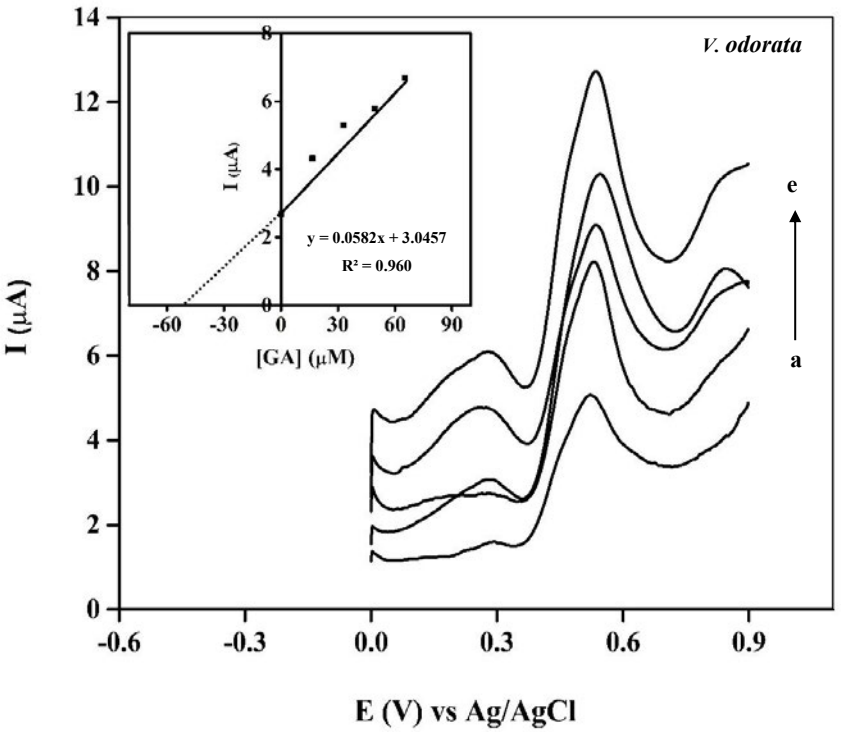

Fig. 8. DPVs of MWCNTs-COOH/CPE in 0.2 M PBS (pH 2.0) containing a) appropriate amounts of *V. odorata* and b-e) increasing amounts of GA standard solution at a scan rate of 0.148 Vs^{-1}. Inset shows the corresponding standard addition curve for determination of GA in *V. odorata*.

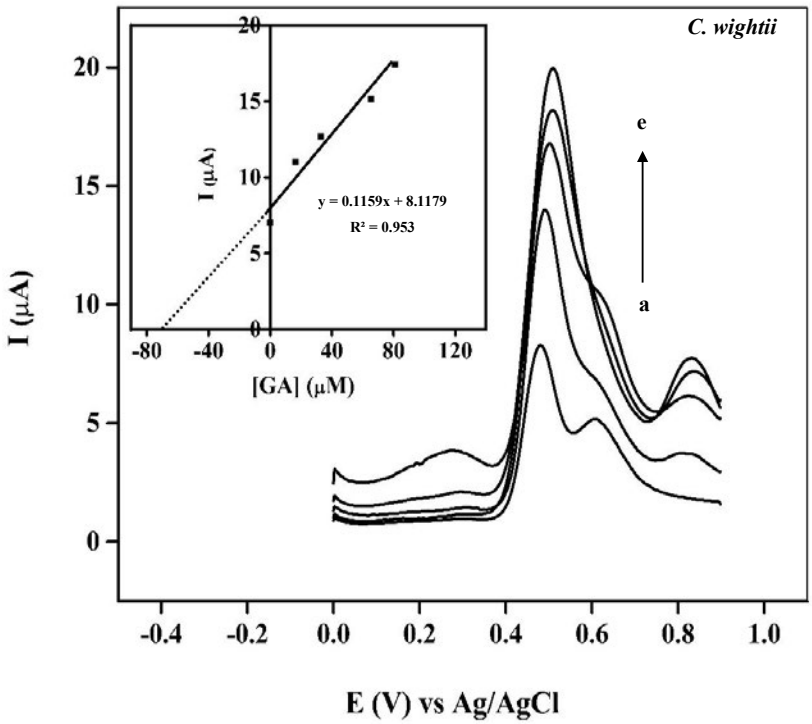

Fig. 9. DPVs of MWCNTs-COOH/CPE in 0.2 M PBS (pH 2.0) containing a) appropriate amounts of *C. wightii* and b-e) increasing amounts of GA standard solution at a scan rate of 0.148 Vs^{-1}. Inset shows the corresponding standard addition curve for determination of GA in *C. wightii*.

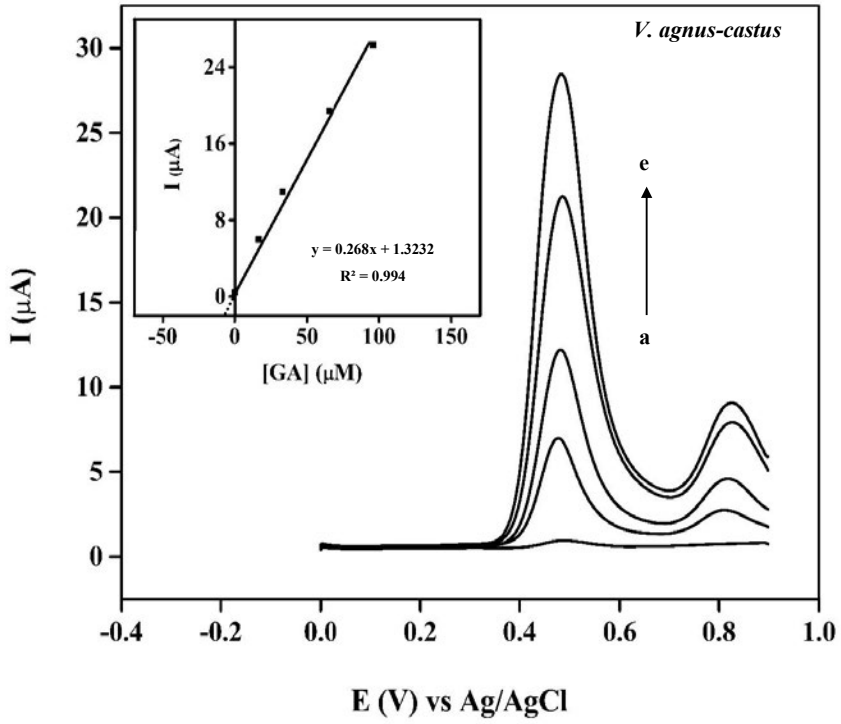

Fig. 10. DPVs of MWCNTs-COOH/CPE in 0.2 M PBS (pH 2.0) containing a) appropriate amounts of *V. agnus*-castus and b-e) increasing amounts of GA standard solution at a scan rate of 0.148 Vs^{-1}. Inset shows the corresponding standard addition curve for determination of GA in *V. agnus-castus*.

from standard edition DPVs of each sample. By extrapolating the calibration curve to y=0, the content of GA in extracts of *C. sinensis*, *V. odorata*, *C. wightii*, and *V. agnus-castus* estimated to be 11.4, 8.9, 11.91 and 2.9 mg L^{-1}, respectively.

CONCLUSION

In this paper, the MWCNTs-COOH/CPE was fabricated with a simple and low-cost method than used for fast, sensitive, and selective GA determination. Via Cyclic voltammetry and differential pulse voltammetry techniques, the MWCNTs-COOH/CPE represented great electrochemical effects toward GA oxidation. A good linear relationship of peak currents with the concentrations of GA ranging from 0.33 to 196 µM and a low detection limit of 17.2 nM (S/N = 3) were obtained. Then, the sensor was successfully applied for the determination of GA content in *C. sinensis*, *V. odorata*, *C. wightii*, and *V. agnus-castus* extracts using the standard addition method.

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CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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