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Original Research

Electrochemical Sensing Platform Based on the Modified Carbon-Ceramic Electrode Using Multiwalled Carbon Nanotubes (MWCNTs) Through the Sol-Gel Process: Application in Sensitive Determination of Morin as a Common Flavonoids

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Abstract

Background: Morin is one of the most common flavonoids which found in nature, plants and vegetable-derived foods and Brazilian yellow tree is a source for its extraction. Because of hydroxyl group existence, Morin represents better antioxidant properties and have great significance in bio electrochemistry-based investigations.

Methods: In this research work, an electrochemical-based sensing platform was designed based on a modified carbon-ceramic electrode (CCE) through a sol-gel process with carbon nanotubes for the investigation of the electrochemical behavior of Morin. Various electrochemical techniques have been used for the analytical investigation of the proposed sensing platform including cyclic voltammetry (CV), differential pulse voltammetry (DPV), and anodic stripping voltammetry (ASV) methods. Furthermore, the morphological investigation was efficiently performed for the demonstration of electrode surface characteristics.

Results: The obtained results showed that the anodic peak currents increased linearly in a concentration range of 0.7–3 μM, where detection limit is equal to 0.27 μM for DPV, and the linear range is considered to be 0.6-4 μM where there is a lower detection limit of 0.3 μM for ASV. Such a biosensing assay can also prove a great potential for the determination of Morin in dry tea.

Conclusion: As clearly demonstrated, Morin as one of the flavonoids which is a penta hydroxy flavone that is 7-hydroxy flavonol bearing three additional hydroxy substituents at positions 2′ 4′ and 5. There are many conventional analytical methods for determination of Morin. However, electrochemical-based sensing assay are cheaper, simpler and more economical. Therefore, in this research work, we have used electrochemical-based techniques applying CCE modified MWCNTs and appropriate analytical features have been obtained.

Introduction

Flavonoids were discovered in the 1930s and were initially considered vitamins (vitamin P) for their effect on capillary permeability, that way, by investigation on ascorbic acid, it was found that flavonoids are very important in animal life and they are natural dyes and vitamins.[1,2] When considering the plants, there are flavonoids which are considered as an extensive polyphenolic compound group. Dietary components such as tea, olive oil, vegetables and fruits contain them. In addition to their physiological role in plants, they can possess anticarcinogenic, antiviral, anti-inflammatory and anti-allergic activities. Morin (3,5,7, 2′,4′- pentahydroxy flavone) is a flavonoid and it is dispersed in plants and foods of plant origin.[3] Antioxidant properties are the characteristics of Morin, such as chemopreventive agents against carcinogenesis in vitro and in vivo,[4] modulator of the activities of some metabolic enzymes including cytochrome P450,[5] deactivator of free radical generating enzymes, a chelator of some metal ions in oxy radical formation[6] and oxy radical scavenger (inhibits lipid peroxidation). Consequently, the highly sensitive determination of Morin and its conjugated metabolites have gained significant importance,[7] and further investigations are required to identify the actual cause of the loss of flavonoids in dried leaves and seaweeds.[8,9]

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Various analytical methods have been applied to determine Morin, such as electrochemical methods,[3,10-12] HPLC,[13,14] and spectroscopy methods.[15,16] Conventional analytical methods have numerous drawbacks. For instance, some limitations are considered for HPLC methods, which contain the use of lengthy analysis, toxic solvent and hard operation for compound analyses particularly pharmaceutical and food samples.[17] On another hand, in spectroscopic techniques, considering the application of Fourier transform spectroscopy in the near UV and visible regions have multiplex disadvantage,[18] as the simultaneous measurement of all spectral intervals is a drawback as the photon noise from the flux at each spectral interval contributes to the noise of each signal. Thus, a small signal may be buried in the noise from a large signal(s).[19] According to the above-mentioned drawbacks, significant attention for developing low-cost and portable analytical electrochemical sensing assays was enlarged during the past few decades.[20,21] Due to the food forbidden additives and lower drug concentrations in real samples, increasing the selectivity of electrochemical sensors is highly needed.[22,23] A platform with interesting and novel properties is generated by the surface modification of the electrode.[24-26] On the electrode surface, the modification agents exist in the shape of thick coatings, monolayers or electroactive thin films. Fabricating the surface-modified electrodes is an advanced method which can figure conductive materials into practical electrodes suitable for biological environments. In order to prepare greatly rough inorganic silica films, the carbon-ceramic electrode (CCE) was successfully employed.[27,28]

Carbon nanotubes (CNTs) can be used in many places such as the storage of hydrogen,[29] batteries[30] as well as developing electrochemical sensing assays.[21,31] Furthermore, their great stability, low resistance and great accessible surface area[32-34] indicate that CNTs are appropriate materials for electrodes in electrochemical double-layer capacitors, which are being studied extensively.[35-38] Multi-wall carbon nanotubes (MWCNTs) have some benefits in contrast to the single-wall ones. The MWCNT structure is stiffer than the single-wall ones, particularly in compression. The inters hell spacing close to that of the graphite interlayer spacing of 3.35 Å[39] is generally maintained by them. In comparison to single walled carbon nanotubes (SWCNTs), another benefit of MWCNTs is the easy achievement of large-scale synthesis when operating multiple enhanced chemical vapor deposition methods.[40] It should be noted that MWCNTs are more readily available and less expensive in comparison to SWCNTs.[41]

In this study, the modified CCE with CNTs was used for the selective determination of Morin in tea samples. To attain this purpose, the mentioned electrode was first fabricated through the sol-gel process. For the coating of thin films on complex shapes, the sol-gel process is very well adapted.[42] In order to maximize the specific surface area and increase the permeability of the film, the porosity and pore size of these films are controlled. Additionally, the condensation of appropriate monomeric precursors and low-temperature hydrolysis and its suitable inclusion of organic moieties that cannot endure the high temperatures are involved for the sol-gel process.[43] Then, the modified CCE was prepared by casting the MWCNTs on fabricated electrodes. Eventually, the modified electrode has been used effectively for determining Morin hydrate in the real sample of dry tea.

Materials and Methods

Materials

Graphite powder and MTMOS (methyl trimethoxy silane) have been acquired from Merck and utilized without any purifications. Morin and other reagents were of analytical grade from Merck. Aldrich has been selected to buy multi-walled carbon nanotubes with a length of 1-10 μm and a wall number of 3-15. The sterilized, deionized and distilled water has been utilized in all solution preparation. The supporting electrolyte has been considered to be the phosphate buffer solution (PBS 0.1 M, pH=6).

Apparatus

Using Autolab PGSTAT 30 electrochemical analysis system and GPES 4.9 software package (Eco Chemie, The Netherlands), the electrochemical experiments have been performed. The applied three-electrode system has been consisted of a platinum wire as the auxiliary electrode, an saturated calomel electrode as the reference electrode and a CCE with 3 mm diameter as the working electrode. A room temperature has been used for all experiments where the dissolved oxygen has not been removed.

Preparation of CCE through sol-gel process

By mixing 0.1 mL hydrochloric acid (0.1 M), 0.9 mL methanol and 0.6 mL of MTMOS, the silica sol solution has been prepared and then stirred for 5 minutes in order to obtain a homogeneous gel solution. Such an ormosil has been mixed with a 300-mg graphite powder for 5 minutes. A Teflon tube (with 3 cm length and 3 mm in diameter and the length of composite in the tube was about 8 mm) has been used to add the mixture and it has been dried for 48 hours at room temperature. The mechanical polishing with 800, 2000, 2500, 3000 grit polishing papers has respectively been used to polish the surface of all electrodes. In order to yield shiny surfaces, the electrodes have been rinsed comprehensively with water. A contact has been made between the copper wire and the other end. Provided the electrical contact.

Preparation of MWCNTs-modified electrode

MWCNTs were pretreated by reflowing in HNO_3-H_2SO_4 (volume ratio 1:1) mixture for 2 hours at 55°C and then 3 hours at 80°C, washed with distilled water, and dried in a vacuum oven.[44] 12 mg of MWCNTs was dispersed in DMF with the ultrasonic striping to form a black solution. The 10 μL of this solution was dropped on the polished

sol-gel-based CCE surface and dried for 12 hours at room temperature.

Preparation of tea samples

For the preparation of tea samples, 1 g of dry tea was mixed with 28 mL acetone at first. Then, the upper solution was removed and dried by gas (N_2). Subsequently, the remained solid phase was solved in 25 mL methanol. The obtained solution was applied as a real sample for the determination of Morin as well.

Result and Discussion

MWCNTs characterization

Scanning electron microscopy (SEM) was used for the investigation of MWCNT-modified CCE electrode surface morphology, and the results were shown in Figure 1. Figure 1a and 1b represents the bare sol-gel electrode and MWCNT-modified CCE. The mentioned figure demonstrates that MWCNTs film is satisfactory for modifying the surface of the CCE electrode.

Electrochemical-behavior of Morin

Figure 2a demonstrates the voltammogram of the bare CCE in presence of a 5 mL phosphate buffer solution (pH=6). Figure 2b shows the cyclic voltammetry (CV) of the CCE electrode in the presence of 1×10^{-4} M Morin in 5 mL PBS (pH=6). Moreover, Figure 2c shows the CV of CCE modified with MWCNTs in the absence of Morin, and Figure 2d is about MWCNT/CCE in presence of 1×10^{-4} M Morin. The obtained voltammograms clarify that deposition of MWCNTs on the electrode surface increases the electron transfer rate and the surface area.

Optimization of experimental parameters

Effect of amount of modifier (MWCNTs)

Figure 3a shows the effects of MWCNTs amount on the oxidation peak current of Morin. Accordingly, increasing the amounts of modifier on the surface of the modified CCE from 2 to 7 mg (dispersed in 5 mL DMF) results in the maximum peak current. This is due to the accumulation of the MWCNTs on the surface of the modified CCE. When increasing the concentration of MWCNTs from 7 to 12 mg, the corresponding peak current decreases. Therefore, a value of 7 mg/5 mL of the modifier was selected for the optimum concentration modifier.

Effect of pH

To study the pH effect, solutions with various pHs have been prepared from 2 to 8 (in presence of 0.1 M phosphate buffer solution and 0.1 mM Morin). In Figure 3b, CVs of CCE modified with MWCNTs, for different amounts of pHs have been demonstrated. Accordingly, by increasing pH, oxidation peaks of Morin were moved to low positive potentials, as shows that, in proton involved oxidation of Morin. Figure 3c is about the changes in peak current according to different pHs, which shows pH=6 is optimum. Furthermore, Figure 3d demonstrates the linear relation of potential and pH amounts.

Investigation of the scan rate

Investigation of the scan rate is very important in electrochemical studies because it gives useful information from the mechanism of the electrochemical reaction and involved electrons in the speed determinant stage. For investigation of scan rate, as clear in Figure 4, the voltammograms of 0.1 mM Morin in scan rates from 20 to 200 mV/s have been reported. Figure 4a shows that with increasing the scan rate, peak potential oxidation of Morin gradually moved to positive potentials. In an electrochemical process performed on the surface of a modified electrode, the peak currents of the voltammogram increases as the potential scan rate increases. It is important to consider that, the peak current intensity is linear with scan rate (υ) and the second root of ($\upsilon^{1/2}$) for non-diffusion and controlled by diffusion electrode processes. As shown in Figures 4b and 4c, the intensity of peak currents for electrode processes has a linear dependency with both υ and the square root of scan rate potential ($\upsilon^{1/2}$). Subsequently, according to their linearity, the electrode process is controlled by non-diffusion processes.

On the other hand, one method for the prediction of the number of involved electrons in the speed determinant stage is to draw TOEFL. Figure 4d demonstrates the voltammograms of the modified electrode for 0.1 mM Morin. This figure clearly shows that in the potential of

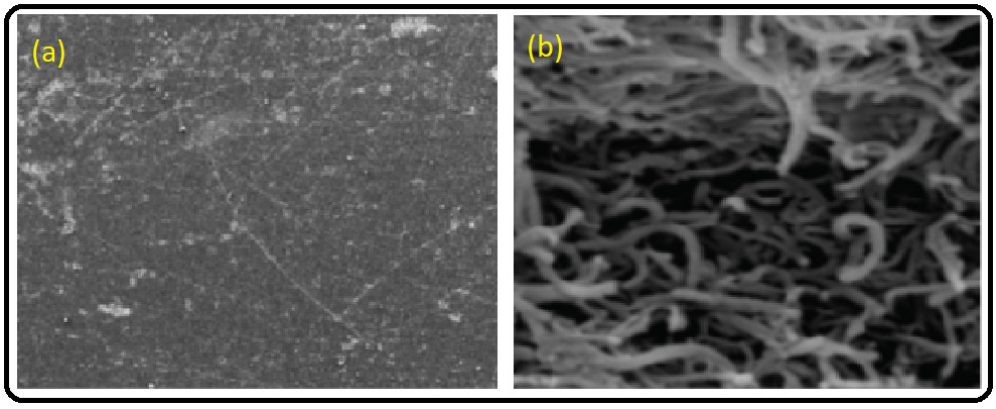

Figure 1. SEM images of the bare CCE electrode (a) and modified CCE with MWCNT (b).

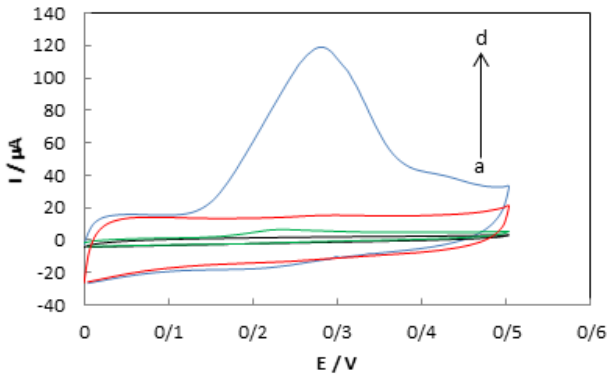

Figure 2. CVs of the bare CCE in the absence of Morin (a), in presence 1 ×10^{-4} M Morin (b), and modified CCE in the absence of Morin (c), and the modified CCE in presence 1×10^{-4} M Morin (d), in presence 0.1 PBS (pH=6).

0.195 to 0.211 V, dependency of log (I) vs E is linear which the obtained slope can be applied for calculating the number of involved electrons in the speed determinative stage at the electro-oxidation reaction ($n\alpha$). According to equation 1, nα for electro-oxidation of Morin was calculated 1 .

2.3 RT/ $n\alpha$ (1- α)F = TOEFL slop (Equation 1)

DPV voltammograms of Morin

To improve sensitivity and reduce the detection limit of Morin, the effect of increasing the concentration of Morin on the anodic peak current by the differential pulse voltammetry (DPV) method was investigated. To attain this aim, DPVs with various concentrations of Morin

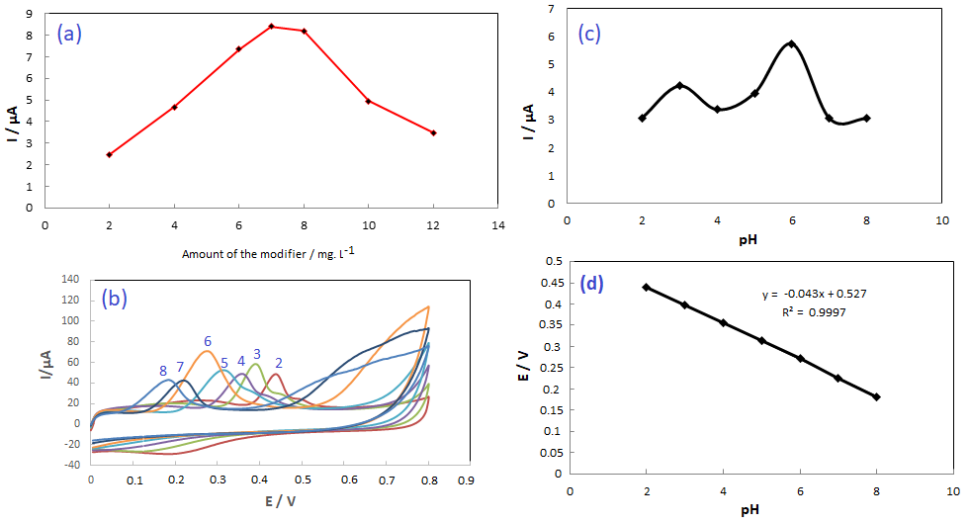

Figure 3. Changes in anodic peak current in the presence of 0.1 mM Morin at the CCE modified with 2 to 12 mg/5 mL in presence of 0.1 M PBS (pH=6) (a) cyclic voltammograms of CCE modified with MWCNTs, for different pHs (b) changes in peak currents again various pH amounts for oxidation of Morin in presence of 0.1M PBS at the CCE modified with MWCNTs (c). Linear dependency of anodic peak current against pHs at CCE modified with MWCNTs (in the presence of 0.1 M PBS and 0.1 mM Morin).

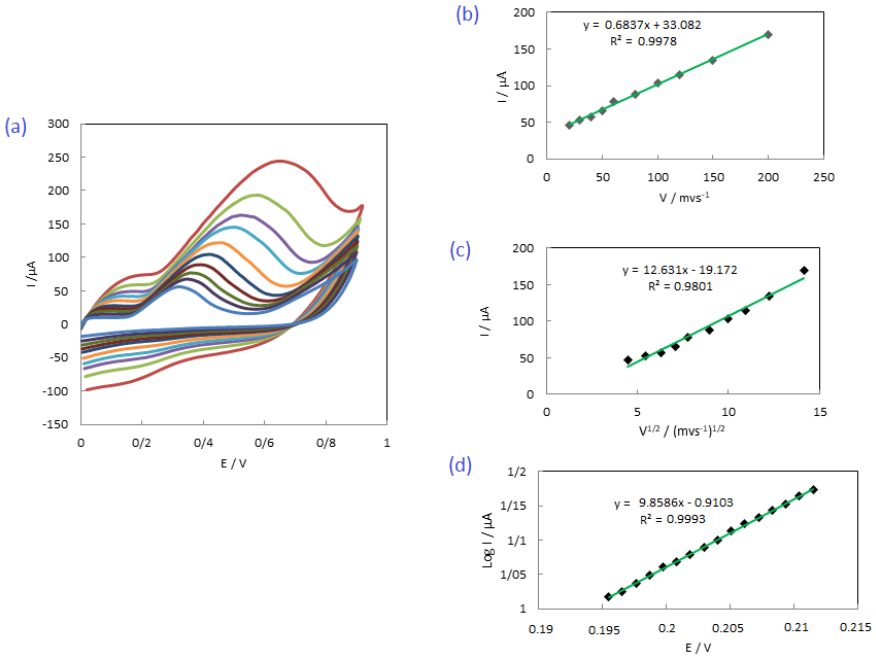

Figure 4. (a) cyclic voltammogram of CCE modified with MWCNTs for 0.1 mM Morin in 0.1 M PBS (pH=6) at various scan rates (20, 30, 40,50,60,100,120,150,200 mV/s) and (b) Plot of Ip (µA) vs υ ½ (V$^{1/2}$. s$^{-1/2}$), (c) Ip (µA) vs υ (m. s^{-1}), (d) the TOEFL plot according to dependency of peak current vs E for 0.1 mM Morin in 0.1 M PBS.

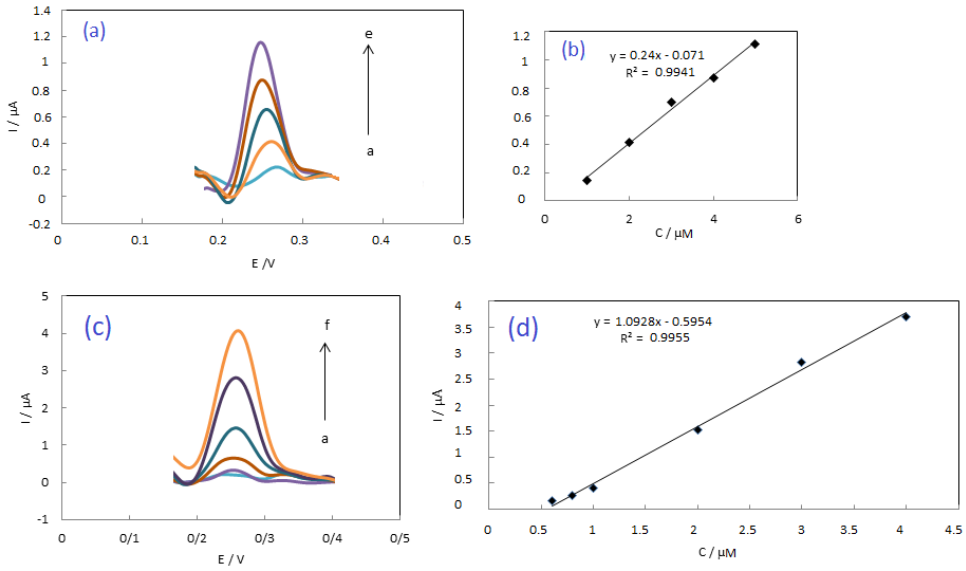

Figure 5. (a) DPVs of MWCNT modified electrode in 0.1 M PBS in the concentration of 1 to 5 µm of Morin. (b) changes of DPV peak currents in terms of concentration of Morin. (c) voltammograms of ASV for adsorption on CCE modified in 0.1 M PBS with pH=6 in concentrations of 0.6, 0.8, 1, 2, 3, 4. (b) changes of ASV on the concentration of Morin based on obtained voltammograms.

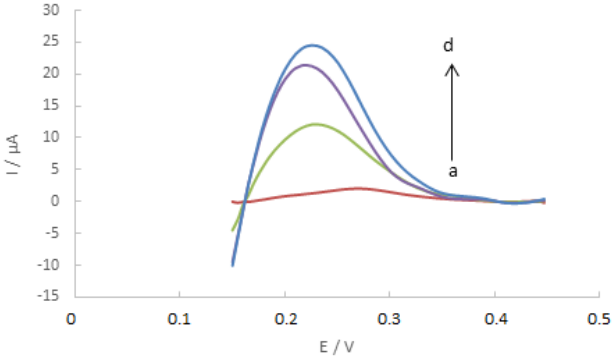

Figure 6. The voltammograms on the surface of The MWCNTs/CCE modified electrode in the absence of (a) and the presence of Quercetin at half the amount of Morin (b) and the presence of Quercetin equal to the amount of Morin (c) and the presence of Quercetin at twice the amount of Morin (d).

in a concentration range of 1 to 5 µm using MWCNTs modified CCE in 0.1 M PBS (pH=6) have been achieved (Figure 5a and 5b). As can be seen, the anodic peak currents increase with increasing Morin concentration, this is done in such a way that at low concentrations all the Morin sit on the electrode, and the current increases with increasing Morin concentration. However, at high concentrations, some of the analyte in the solution settles on the surface of the electrode and the rest remains inside the solution which results in the saturation of the electrode surface, and subsequently, the increase in peak currents stops. This fact is due to how the process is controlled by adsorption. According to the stated optimum conditions, linear range (LR), limit of detection (LOD), and R^2 have been equal to 0.7– 3 µM, 0.27 µM, and 0.9941 respectively.

Anodic stripping voltammetry

In this step, the anodic stripping voltammetry (ASV) method was used to investigate the Morin on the modified electrode (Figure 6c and 6d). In the voltammetric method, potential and time were applied to the device by anodic stripping and stirring the solution containing the analyte and the carrier electrolyte, perform the precipitation operation at the desired time and potential, and then the adsorption process was used to measure the desired analyte. According to the stated optimum conditions, LR, LOD, and R^2 have been equal to 0.6-4 µM, 0.3 µM, and 0.9955 respectively.

Interference studies

Because Morin is a group of flavonoids, there may be interference from other flavonoids such as Quercetin, and also other compounds such as uric acid and ascorbic acid may cause interference in other environments. According to the performed research works and also because Morin has the property of forming complexes with metals, so interference was observed by metal cations such as Zn^{2+}, Pb^{2+} and Cu^{2+}. However, as mentioned, Quercetin is always present along with Morin, and the main interference can be Quercetin. To investigate the Quercetin-induced disturbance according to Figure 6 , voltammograms have been obtained in the absence of (a) and the presence of quercetin at half the amount of Morin (b) and the presence of quercetin equal to the amount of Morin (c) and the presence of quercetin at twice the amount of Morin (d).

Conclusion

As clearly demonstrated, Morin as one of the flavonoids which is a pentahydroxy flavone that is 7-hydroxy flavonol bearing three additional hydroxy substituents at positions 2’ 4’ and 5. It has a role as an antioxidant, a metabolite, an antihypertensive agent, a neuro protective agent, an anti-inflammatory agent, an antineoplastic agent and an antibacterial agent. There are many conventional analytical methods for determination of Morin. However, electrochemical-based sensing assay are cheaper, simpler and more economical. Therefore, in this research work, we have used electrochemical-based techniques applying

CCE modified MWCNTs. Carbon nanotubes have been utilized not only for enlarging the surface but also for increasing the electrochemical signal and enlarging the rate of electron transfer. The achieved outcomes showed a detection limit of 0.27 µM for DPV and 0.3 µM for ASV. According to the achieved outcomes, it was possible to verify that the sensing assay has the potential for being desirably utilized in determination of Morin in dry tea samples.

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Authors' Contribution
SM: Writing and preparing of original draft; MRM: Supervision, reviewing and editing; KAZ: Supervision, reviewing and editing; HS: Writing and preparing of original draft, reviewing and editing.

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Ethics Issues
Not applicable.

Conflict of Interest
The author(s) declare that they have no competing interests.

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