

## Application of poly(3,4-ethylenedioxythiophene)/manganese Dioxide Composite-Modified Electrode for Sensitive Electrochemical Detection of Luteolin

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In this work, the electrochemical determination of luteolin using poly(3,4-ethylenedioxythiophene)/manganese dioxide nanocomposite modified glassy carbon electrode (PEDOT/MnO<sub>2</sub>/GCE) is reported. PEDOT/MnO<sub>2</sub> nanocomposite has been proposed via a simple two-step deposition approach. Electrochemical results revealed that PEDOT/MnO<sub>2</sub> showed enhanced electrocatalytic performance toward luteolin, which might be attributed to good conductivity and accelerated electron transfer of PEDOT and good catalytic effect of MnO<sub>2</sub>. Experimental conditions, including pH values and scan rates were optimized. In optimal conditions, a good linear relationship was found between oxidation peak currents and the concentration of luteolin in 5.0 nM-7.0 μM range. The detection limit was calculated to be 1.8 nM at a signal-to-noise ratio of 3. Finally, the applicability for luteolin determination in real samples was successfully demonstrated.

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**Keywords:** Poly(3,4-ethylenedioxythiophene); Manganese dioxide; Two-step deposition ; Luteolin

### 1. INTRODUCTION

Luteolin, 3,4,5,7-tetrahydroxyflavone, is one of the typical flavonoids that function as a crucial role in human health, has received increasing interest in modern society because of its positive health effects, such as antioxidant, anticancer, immune-modulation, anti-inflammatory, anti-allergic, and properties of suppressing hyperactive immune systems [1-3]. It is also a promising agent in clinic that

used in hepatitis, cardiovascular disease, ophthalmology, etc. Therefore, it is of great significance to develop reliable methods for the determination of luteolin.

Up to now, several methods have been developed for trace amount of luteolin analysis, such as capillary zone electrophoresis [4], high performance liquid chromatography [5], and gas chromatography [6]. However, these methods need high operating cost, expensive instruments and well trained technicians to operate the measurements, which are not suitable for routine assay with a large amount of samples. Contrary to these methods, electrochemical techniques provide a cheap, portable and sensitivity techniques for the determination of luteolin, which is superior to pre-mentioned techniques. For example, Wang et al. fabricated an electrochemical sensor based on metal-organic frameworks cooperate with graphite oxide for the detection of luteolin [7]. Ibrahim et al. constructed a novel sensor based on  $\text{In}_2\text{O}_3$  nanoparticles modified glassy carbon paste electrode for sensitive electrochemical determination of luteolin [8]. Wu et al. depicted novel nickel redox polymer for electrochemical sensing of luteolin [9].

In recent years, metal and metal oxide nanoparticles have been investigated extensively in construction various electrochemical sensor [10-12]. There are three important functions for their wide use in electroanalysis, including the excellent catalytic properties that decrease apparent activation energy and alleviate overpotential of some important reactions in electrochemical analysis [13], the high surface-to-volume ratio that increase absorbing site [14], and the good conductivity properties that provide electrochemical reversibility for redox reactions [15]. For instance, Li et al. fabricated porous gold for electroanalysis of glucose using hydrogen bubble dynamic template [16]. Polsky et al. constructed Pt nanoparticles functionalized nucleic acid for electrochemical detection of  $\text{H}_2\text{O}_2$  [17]. Though these noble metal nanoparticles showed excellent electrocatalytic performance, their widespread applications are limited by their high cost. To date, low-cost transition metal oxide materials including  $\text{Fe}_3\text{O}_4$  [18],  $\text{Co}_3\text{O}_4$  [19], NiO [20] and  $\text{MnO}_2$  [21] have been explored as effective electrode materials for their excellent performance and relatively low environmental footprint.

Manganese dioxide ( $\text{MnO}_2$ ) is generally considered to be the most promising transition metal oxides for the next generation of electrochemical sensor because of its high catalytic properties, natural abundance, low cost and environmental friendliness [22].  $\text{MnO}_2$  nanoparticles as sensing elements could be immobilized on working electrode surface by kinds of methods, including chemical covalent bonding, physical adsorption and so on. Compared to these complicated, cumbersome chemical and physical methods, electrodeposition technology has the merit of easy preparation, well-reproducible and good repeatability. In order to make full use of the advantages and compensate for the poor conductivity of  $\text{MnO}_2$ , several conducting nanomaterials are employed to combine with  $\text{MnO}_2$  to form novel conducting composites for various applications [24].

Poly(3,4-ethylenedioxythiophene) (PEDOT), a new polythiophene derivate, has the merits of excellent electrochemical activity, low band gap, excellent environmental stability, high electric conductivity, and transparency in the doped state [25-29]. Improvement have been proved in the previous works [30,31], synergistic properties like enhancement in electrochemical catalysis and electrical conductivity have shown based on PEDOT/ $\text{MnO}_2$  nanocomposite. PEDOT and  $\text{MnO}_2$  nanocomposite have been explored in the field of energy storage [32-34]. However, to the best of our knowledge, the electrochemical detection of luteolin based on this composite has not been reported.

In this report, PEDOT/MnO<sub>2</sub> composite was prepared by two-step electrodeposition technology according to the previous reports [35,36]. PEDOT has good conductivity and accelerated electron transfer between oxidation and reduction state of luteolin since the detection procedure is a reversible response. In addition, the good catalytic effect of MnO<sub>2</sub> can decrease apparent activation energy, causing a significant increase in sensitivity for the detection of luteolin [37]. Thanks to the synergistic properties of PEDOT and MnO<sub>2</sub>, the obtained sensor exhibited high sensitivity toward luteolin. The detection limit was calculated to be 1.8 nM and the linear was in the range of 5.0 nM-7.0 μM. Also, the applicability of this fabricated sensor in real sample was successfully demonstrated.

## 2. EXPERIMENTAL

### 2.1 Chemicals and reagents

Stock solution of luteolin (0.01 M) was prepared in absolute ethyl alcohol and stored at 278-281 K, phosphate buffer solution (PBS) were prepared with 0.1 M NaH<sub>2</sub>PO<sub>4</sub>·2 H<sub>2</sub>O and 0.1 M Na<sub>2</sub>HPO<sub>4</sub>·12 H<sub>2</sub>O, 3,4-ethylenedioxythiophene (EDOT) and luteolin were obtained from Aldrich and Aladdin respectively, MnCl<sub>2</sub> was purchased from Shanghai Vita Chemical Plant. All these reagents were received in analytical grade and used without further purification. Doubly deionized water was used in the whole experiments.

### 2.2 Apparatus

The electrochemical measurements were carried out on a CHI660D electrochemical workstation (Shanghai, China) with conventional three-electrode system. A bare glassy carbon electrode (GCE,  $\Phi = 3\text{mm}$ ) or modified GCE was used as the working electrode, a platinum foil electrode as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode.

### 2.3 Preparation of PEDOT, MnO<sub>2</sub> and PEDOT/MnO<sub>2</sub> nanocomposite modified GCEs

Prior to modification, GCE was polished using 0.05 μM alumina slurry on a chamois leather. After rinsed with doubly distilled water, the electrode was sonicated with absolute ethanol and doubly distilled water for 5 min, respectively. Then the obtained GCE was used in subsequent experiment.

Prior to modification, all the solutions were purged with pure nitrogen for 5 min. Cyclic voltammetry was used to electrochemically deposited PEDOT on the GCE with a sweep rate of 100 mV s<sup>-1</sup> from -0.9 to 1.1 V potential scanning window at room temperature for 10 cycle in a fresh solution containing 0.01 M EDOT and 0.01 M LiClO<sub>4</sub> [35].

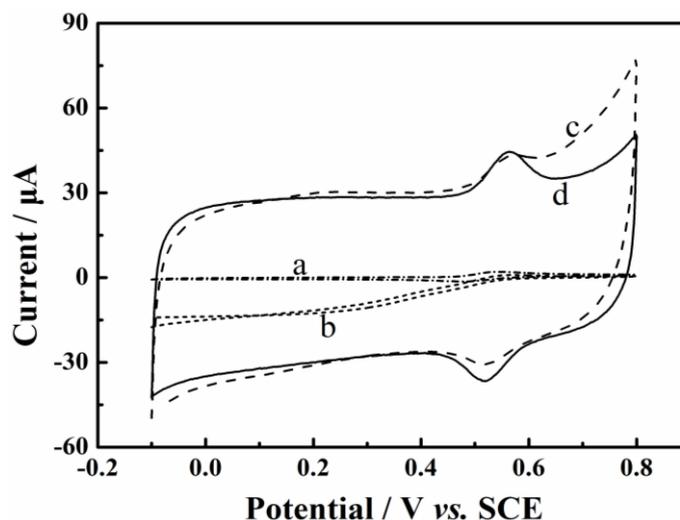
MnO<sub>2</sub> was electrochemically deposited on the GCE by cyclic voltammetry with a constant potential of +0.6 V for 50 s a quiescent ammonium buffer solution (pH 9.2) containing 0.2 mM MnCl<sub>2</sub>, which is consistent with previous report [36].

PEDOT/MnO<sub>2</sub> was fabricated through two-step deposition method. First, PEDOT film was deposited on bare GCE. And then MnO<sub>2</sub> film was deposited on PEDOT film modified electrode. After deposition, these working electrodes were washed with distilled water and used in subsequently experiment.

### 3. RESULTS AND DISCUSSION

#### 3.1 Electrochemical behaviors of luteolin at various electrodes

The electrochemical behavior based on bare GCE, PEDOT/GCE, MnO<sub>2</sub>/GCE, and PEDOT/MnO<sub>2</sub>/GCE for determination of luteolin (100 μM) were studied via cyclic voltammetry in 0.1 M PBS (pH 3.0). As shown in Fig. 1, a pair of reversible redox peaks correspond to the process of electrochemical oxidation and reduction of luteolin, were found on all electrodes, which proved a reversible process in this electrochemical reaction. It can be observed that electrochemical oxidation of luteolin on bare GCE and MnO<sub>2</sub>/GCE showed weak peak. While at the PEDOT/GCE, the corresponding anodic peak current remarkably increased, which might be attributed to the accelerated electron transfer and good conductivity of PEDOT on the electrode. Compared to PEDOT/GCE and MnO<sub>2</sub>/GCE and bare GCE, PEDOT/MnO<sub>2</sub>/GCE showed higher electrochemical activity towards the redox of luteolin, which can be attributed to increased surface area of modified electrode and synergistic effect of PEDOT and MnO<sub>2</sub> enhanced adsorption ability of luteolin.

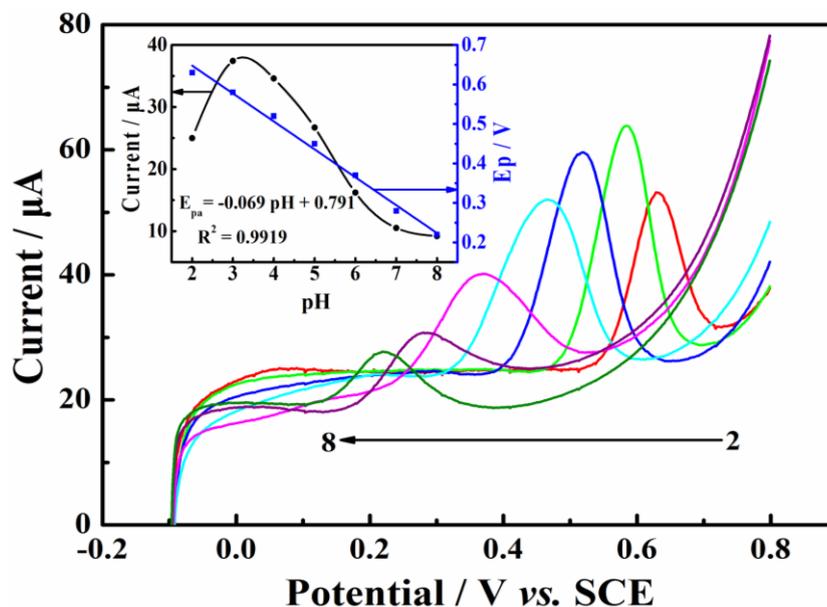


**Figure 1.** Cyclic voltammograms bare GCE (a), MnO<sub>2</sub>/GCE (b), PEDOT/GCE (c), PEDOT/MnO<sub>2</sub>/GCE (d) in 0.1 M PBS (pH 3.0) containing 100 μM luteolin. Scan rate: 50 mV/s

#### 3.2 Effect of pH values

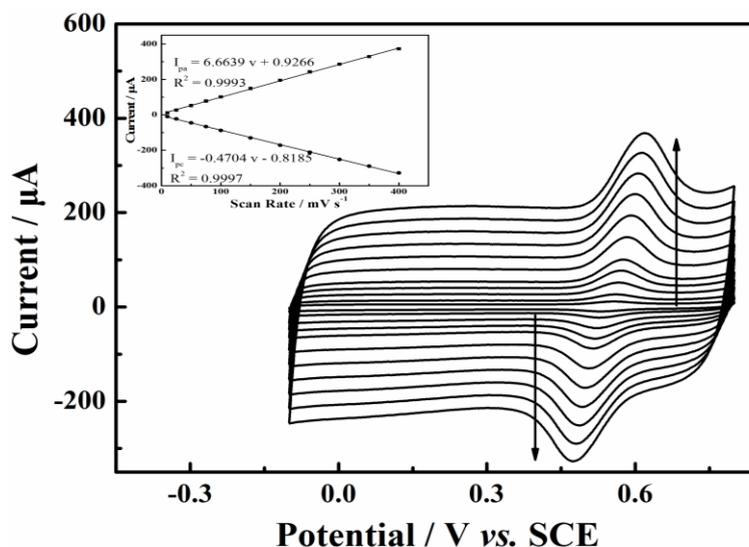
It is vital to select a proper pH value since the pH value is essential to get better sensitivity. Thus, the effect of pH values on the redox reaction of luteolin were investigated in 0.1 M PBS from

2.0 to 8.0. As shown in Fig. 2, the response current of luteolin increased and reached maximum at 3.0 when the pH value shift from 2.0 to 3.0, and then decreased with pH value further increased. pH 3.0 was chosen for the subsequent analytical experiments considering the sensitivity for determining luteolin.



**Figure 2.** Cyclic voltammograms of PEDOT/MnO<sub>2</sub>/GCE in 0.1 M PBS with different pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0) containing 100 μM luteolin. Scan rate: 50 mV s<sup>-1</sup>. Inset: effect of pH values on the anodic peak potentials and anodic peak currents of luteolin (100 μM).

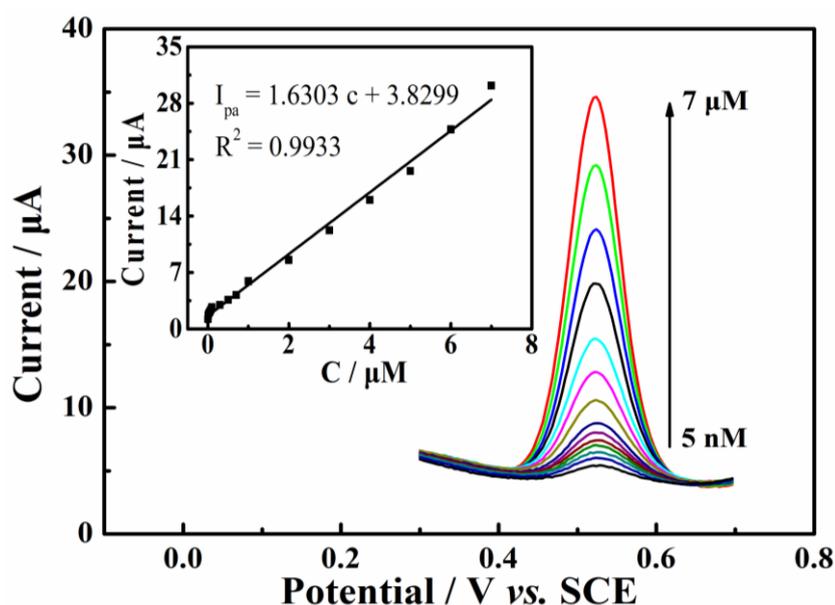
### 3.3 Effect of scan rate



**Figure 3.** Cyclic voltammograms of PEDOT/MnO<sub>2</sub>/GCE with different scan rate: 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400 mV s<sup>-1</sup> containing 100 μM luteolin. Inset: Linear relationship of redox peak current I versus the natural logarithm of v.

The anodic and cathodic response with different scan rates were used to investigate the kinetics process of luteolin (100  $\mu\text{M}$ ) in 0.1 M PBS (pH 3.0) on PEDOT/MnO<sub>2</sub>/GCE by cyclic voltammetry. As can be observed in Fig. 3, the redox peaks potentials shifted to more negative direction and the peak separation also began to increase with increased scan rate from 10 to 400  $\text{mV s}^{-1}$ , which clarified the limitation caused by charge transfer kinetics. In addition, the cathodic peak currents ( $I_{pc}$ ) and anodic peak currents ( $I_{pa}$ ) increased linearly with increased scan rates, corresponding to the following equation:  $I_{pc} (\mu\text{A}) = 0.5863 v (\text{mV s}^{-1}) + 7.0643$  ( $R^2 = 0.9997$ ) and  $I_{pa} (\mu\text{A}) = 6.6639 v (\text{mV s}^{-1}) + 0.9266$  ( $R^2 = 0.9993$ ), respectively. These results indicated that the electrochemical behavior of luteolin at PEDOT/MnO<sub>2</sub>/GCE was an adsorption-controlled electrode process.

### 3.4 Electrochemical determination of luteolin.



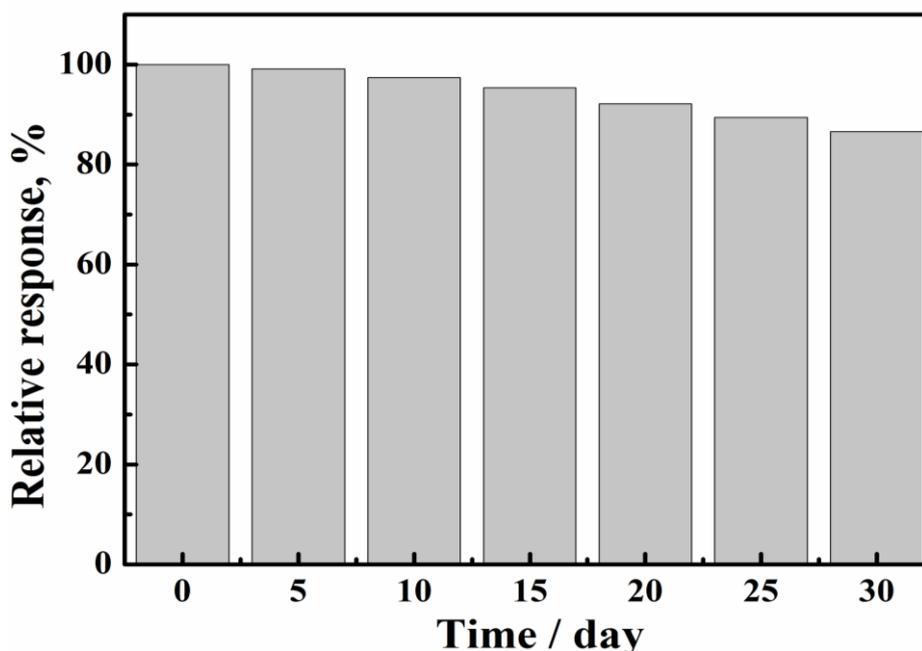
**Figure 4.** DPVs of luteolin in PBS (pH 3.0) at PEDOT/MnO<sub>2</sub>/GCE. Inset: plot of  $i$  vs. concentration of luteolin.

Under optimized experimental conditions, differential pulse voltammetry (DPV) was used for the quantification of luteolin at PEDOT/MnO<sub>2</sub>/GCE. It can be seen from Fig. 4 that the stripping peak current increased linearly with increasing luteolin concentration from 5.0 nM to 7.0  $\mu\text{M}$  and the corresponding linear regression equation was represented as  $I_{pa} = 1.6303 c + 3.8299$  with a correlation coefficient of 0.9933. The detection limit was calculated to be 1.8 nM for luteolin, which was lower than that of  $2.0 \times 10^{-7}$  M at poly(diallyldimethylammonium chloride)-functionalized graphene-carbon nanotubes hybrid/ $\beta$ -cyclodextrin composite film modified GCE [38],  $2.0 \times 10^{-8}$  M at Poly(diallyldimethylammonium chloride) functionalized graphene-multiwalled carbon nanotubes hybrid materials modified GCE [39],  $1.0 \times 10^{-8}$  M at graphene and hydroxyapatite modified GCE [40], and  $1.99 \times 10^{-9}$  M at In<sub>2</sub>O<sub>3</sub> nanoparticles modified glassy carbon paste electrode [8]. The low detection limit could be attributed to the synergistic reaction of PEDOT and MnO<sub>2</sub>, in which PEDOT showed good conductivity and MnO<sub>2</sub> displayed good catalytic effect. Therefore, by combination of the

advantages of good analytical performances and simple preparation procedure, the proposed PEDOT/MnO<sub>2</sub> electrode is promising for the development of effective luteolin sensor.

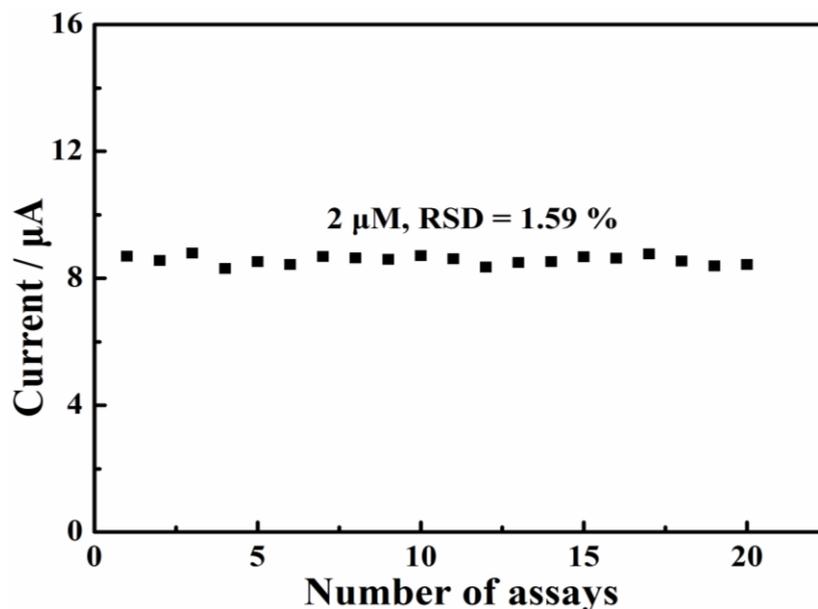
### 3.5 Stability, reproducibility and interferences of PEDOT/MnO<sub>2</sub>/GCE

The long-term stability of PEDOT/MnO<sub>2</sub>/GCE was tested by changes in response currents for detection of 2.0 μM luteolin daily over consecutive 30 days after it's storage in air at room temperature. The relative standard deviation (RSD) of precious test was calculated to be 6.98%, which confirmed its excellent long-term stability. Another parameter, reproducibility is important for precise analytical measurements, hence, 2.0 μM luteolin was successively measured for 20 times under identical conditions. The RSD was calculated to be 1.80%, indicating the good reproducibility of the proposed method.



**Figure 5.** Long-term storage stability of response current at PEDOT/MnO<sub>2</sub>/GCE in 0.1 M PBS (pH 3.0) containing 2.0 μM luteolin for 30 successive days.

The interference of a variety of compounds for the detection of luteolin at the PEDOT/MnO<sub>2</sub>/GCE were investigated to evaluate the selectivity of PEDOT/MnO<sub>2</sub>/GCE. In this experiment, 2.0 μM luteolin and interference ions, including 50-fold concentration of SO<sub>4</sub><sup>2-</sup>, Al<sup>3+</sup>, Br<sup>-</sup>, Mg<sup>2+</sup>, NO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>, and Cl<sup>-</sup>, 10-fold concentration of vitamin B<sub>6</sub>, vitamin B<sub>2</sub>, glucose, uric acid, starch, and saccharose did not interfere significantly during luteolin determination, which ensured the selectivity of this proposed sensor.



**Figure 6.** Reproducibility of response current at PEDOT/MnO<sub>2</sub>/GCE in 0.1 M PBS (pH 3.0) containing 2.0 μM luteolin for 20 successive assays.

### 3.6 Real sample analysis

To evaluate its applicability in real sample, the fabricated PEDOT/MnO<sub>2</sub>/GCE was used for the determination of luteolin in capsule of *Lamiophlomis rotata* Kudo in PBS (pH 3.0). Two capsules of *Lamiophlomis rotata* Kudo were accurately weighed and grinded into powder in a mortar. Typically, absolute ethanol was added into precise amount of obtained powder and then dissolved, sonicated and centrifuged. The solution was determined using a temporary calibration plot. The recoveries of results were from 98.3% to 103.5%, indicated that the present electrode could be efficiently applied for luteolin detection in real samples.

**Table 1.** Determination of luteolin in the extraction of *Lamiophlomis rotata* Kudo.

| Samples | Added (nM) | Expected (nM) | Found (nM) | Recovery (%) |
|---------|------------|---------------|------------|--------------|
| 1       | 0          | -             | 2.04       | -            |
| 2       | 5.00       | 7.04          | 7.29       | 103.5        |
| 3       | 10.00      | 12.04         | 12.39      | 102.9        |
| 4       | 15.00      | 17.04         | 16.75      | 98.3         |

## 4. CONCLUSION

In summary, a simple two-step deposition method for preparing PEDOT/MnO<sub>2</sub> nanocomposite modified electrode was successfully proposed and the modified electrode was used for the detection of luteolin. PEDOT/MnO<sub>2</sub> exhibited excellent electrochemical activity towards the redox of luteolin due to the synergistic reaction of PEDOT and MnO<sub>2</sub>, in which PEDOT accelerated electron transfer and

compensated for the poor conductivity of MnO<sub>2</sub>, and MnO<sub>2</sub> enhanced the catalytic activity of PEDOT. The modified electrode showed high sensitivity and wide linear range for the detection of luteolin. Also, the good long-term stability and selectivity were also successfully demonstrated. Finally, the applicability in real sample was also verified.

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