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Electrochemical Determination of Rosmarinic Acid in Edible Flowers Using Ionic Liquid Modified Electrode

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The current research focused on developing a nanocomposite of ionic liquid/CNTs paste electrode (IL/CNTs-PE) for use as a precise and sensitive electrochemical sensor to quantify rosmarinic acid (RA) as phenolic acid in a prepared sample of Lavandula angustifolia. Structural analyses using XRD and SEM revealed that IL was successfully incorporated on the surface of CNTs in the IL/CNTs composite. Electrochemical studies with CV and DPV demonstrated that combining ILs and carbon CNTs improves the electrocatalytic activity, selectivity, and sensitivity of IL/CNTs-PE to determine RA. The results showed a stable linear range of 0 to 680 μ M and a sensitivity of 0.0951 μ A/ μ M were achieved, with detection limits as low as 15 nM. The results of determining RA in prepared real samples from Lavandula angustifolia showed low RSD values (4.33 to 5.14%) and high recovery values (98.75 to 99.50%), indicating that the IL/CNTs-PE results were accurate and valid for determining RA level in a prepared sample from an extracted liquid of flowers.

Keywords: Ionic Liquid; Nanocomposite; CNTs; Phenolic Acids; Rosmarinic Acid; Lavandula Angustifolia; Edible Flowers; Electrochemical Technique

1. INTRODUCTION

One of the main classes of plant phenolic compounds is phenolic or phenol carboxylic acids, which are a type of phytochemical known as a polyphenol [1]. These compounds formed by the substitution of hydrogen atoms on benzene rings by a carboxylic acid group and at least one hydroxyl, are abundant in cereals, legumes, oilseeds, fruits, vegetables, beverages, and herbs [2, 3]. These phytochemical compounds are bioactive molecules with antioxidant and anti-inflammatory properties that influence a plant's physiology, ecology, and development [4, 5]. Phenolic acids are easily absorbed through the intestinal tract's walls [6]. They may be advantageous to human health because they function

as antioxidants, preventing cellular damage caused by free-radical oxidation reactions. When consumed regularly promote anti-inflammatory conditions in the human body. Furthermore, when combined with vitamin C and E-containing skincare, phenolic acid doubles antioxidant protection [7, 8].

Rosmarinic acid (RA, C₁₈H₁₆O₈) is a phenolic carboxylic acid derived from several Lamiaceae species primarily responsible for anti-inflammatory and antioxidant activity. Lavender (Lavandula angustifolia) is a wild, edible, and nutritious food, and research has shown that the extract obtained from Lavandula angustifolia is high in antioxidant bioactives, particularly RA [9]. Research suggests that RA may help treat inflammatory conditions like arthritis, asthma, and atopic dermatitis [10, 11]. Due to RA's effectiveness, it must be identified in pharmaceutical and food samples, and numerous studies have been performed to enhance the sensing performance using high-performance liquid chromatography [12], capillary electrophoresis [13], NMR spectroscopy [14], coulometry [15], spectrophotometry [16], FT-IR spectroscopy [17], gas chromatography–mass spectrometry [18] and electrochemical sensors [19-26]. However, due to insufficient precision and sensitivity, these sensors require further modification [27-29]. Nanostructure-based electrochemical sensors have demonstrated commendable precision and sensitivity for RA determination in actual food and plant samples [30-32]. Furthermore, these sensors are quick, low-cost, and easy to use. The current study's goal is to create a nanocomposite of ionic liquid/CNTs paste electrodes that can be used as a precise and sensitive electrochemical sensor to quantify RA in a prepared sample of Lavandula angustifolia.

2. EXPERIMENT

2.1. Preparation of the modified electrodes

To make the IL/CNTs paste electrode (IL/CNTs-PE) [33, 34], 0.5 g CNTs (99%, Guangzhou Hongwu Material Technology Co., Ltd., China) and 0.8 mL paraffin oil (Northern Mongolia Pulis Chemical Co., Ltd., China) were ultrasonically mixed with 6 mL diethyl ether (99%, Fisher Scientific International, Inc., USA). Following that, 0.5 mL of n-octyl-pyridinium hexafluorophosphate (Sigma-Aldrich) was added as an ionic liquid to the obtained mixture. To obtain a homogeneous wet paste, the resulting mixture was mechanically blended for 15 minutes. The paste was then placed in a glass tube and electrically contacted (with copper wire). To obtain the new surface of the modified electrode in electrochemical measurements, an excess of the paste can be pushed out of the tube, polished on soft paper, and rinsed with water. As a control and comparison, CNTs-PE were prepared in the same manner but without IL. Carbon paste electrode (CPE) was created by combining graphite powder (99.99%, Huixian Wanda Graphite Mould Factory, China) in a 7:3 ratio with paraffin oil. After fully blending, the mixture was placed in a glass tube, and an electrical contact was established at the end of the tube using a copper wire.

2.2. Preparation of a real sample of flower

Lavandula angustifolia samples weighing 0.5 kg were collected in the Yili region of Xinjiang, China. Lavandula angustifolia puree was combined with 150 mL of ethanol. After 20 minutes of sonication at room temperature, the mixture was chilled for 24 hours before being ultrasonically blended for 15 minutes at room temperature. The mixture was then filtered, and the liquid extracts were used to make 0.1 M PBS.

2.3. Characterization instruments

Electrochemical analyses were carried out on an electrochemical workstation (Xiamen Tob New Energy Technology Co., Ltd., China) in 0.1M phosphate buffer solution (PBS) with pH 7.4 as the supporting electrolyte in a conventional three-electrode electrochemical cell with a modified electrode (CPE or IL/CNTs-PE or CNTs-PE) as the working electrode and Ag/AgCl (3 M KCl) as the reference electrode. K₂HPO₄ (99%, Sigma Aldrich) and KH₂PO₄ (98.0%, Sigma Aldrich) were mixed in equal parts to make the electrolyte. The crystallography of synthesized nanomaterials was characterized using a Bruker D8 X-ray diffractometer (XRD), and scanning electron microscopy (SEM, FEI Siri200) was used to characterize the nanomaterial's morphological information.

3. RESULTS AND DISCUSSION

3.1. Structural analyses



Figure 1. The XRD spectra of powders of IL/CNTs-PE and CNTs-PE.

Figure 1 depicts the XRD spectra of IL/CNTs-PE and CNTs-PE powders. Both IL/CNTs-PE and CNTs-PE XRD spectra show two diffraction peaks of (002) and (100) crystallographic planes of CNTs at $2\theta = 26.04^{\circ}$ and 43.79° , respectively, corresponding to the sp2 hexagonal graphite structure in

CNTs [35, 36]. As can be seen, after the addition of IL to the CNTs structure, the intensity of the (002) and (100) crystallographic peaks decreases, and an additional peak at $2\theta = 2.59^{\circ}$ and 5.08° is observed, which is attributed to the pyridinium of IL according to previous reports [37, 38], indicating successful incorporation of IL on the surface of CNTs in the IL/CNTs composite [39, 40].

Figure 2 shows SEM images of the IL/CNTs-PE and the CNTs-PE. Both SEM images show a network of tangled tubular CNT structures with highly effective surface area and porosity. The diameter of the tubular structure on the IL/CNTs-PE and CNTs-PE surfaces is 76 and 62 nm, respectively, indicating that the diameter of the tubular structure on the IL/CNTs is increased toward the CNTs because the IL covers the CNTs uniformly [41]. Furthermore, IL prevents CNT agglomeration by preventing direct contact between CNT particles [42-44].



Figure 2. SEM images of the (a) CNTs-PE and (b) IL/CNTs-PE.

3.2. Electrochemical studies

Figure 3 depicts CV curves for CPE, CNTs-PE, and IL/CNTs-PE at applied potentials ranging from -0.20 V to 0.60 V at a scan rate of 50 mVs⁻¹ in 0.1 M PBS (pH 7.4) electrolyte solution. CV curves were recorded in an electrochemical cell before and after adding RA molecules. It is discovered that none of the recored CV curves have a peak before the addition of RA solution to the electrochemical cell. Following the addition of 15 M RA solution, anodic peaks at 0.31 V, 0.20 V, and 0.16 V appear in the CV curves of CPE, CNTs-PE, and IL/CNTs-PE, respectively, indicating that the peak can be related to the redox mechanism associated with the oxidation of the catechol moieties, as shown in Figure 4 [45-47]. RA as a hidroxycinnamic acid derivative is structurally an ester of caffeic acid with 3,4-dihydroxyphenyl lactic acid, thus having two electroactive catechol moieties, which are able to neutralize free radicals by an electron/proton donor mechanism [45, 48].On the other hand, the o-dihydroxyphenyl groups of RA can enter the internal cavity of IL which catalyzes the aromatic compounds, and this would form aryloxy radicals from oxidation of RA in the solution to the o-quinone [49-51].

According to the results, IL/CNTs-PE has a higher electrocatalytic current and a lower oxidation potential than CPE and CNTs-PE. CNTs with rolled-up sheet graphene porosity and hollow structure, a

large specific surface area, and a large number of edge plane-like defect sites that provide strong interaction with target molecules and can improve electrocatalytic activity and charge transfer rate [52-54]. CNTs' porous surface and smaller blocks allow more material to diffuse around them over time [55-57]. Because of their high ionic conductivity, ILs as green media can provide well-dispersed CNTs and increase conductivity, resulting in a lower percolation threshold that promotes the outcome of electrocatalytic reactions [42, 58, 59]. Because ILs on the surface of carbon CNTs can act as a suitable charge transfer bridge to facilitate electron transfer efficiency by reducing charge transfer resistance, the combination of ILs and carbon CNTs improves electrocatalytic activity via simple ion exchange reactions [60, 61]. According to studies, ILs have a large number of caves within their molecular structure, which is responsible for their ability to hold more charges [60, 62] and improves the electrochemical responses of IL/CNT-PE. The improved electrochemical response of RA on IL/CNT-PE suggests a synergetic effect of ILs and CNTs. Based on the successful incorporation of IL on the surface of CNTs in an IL/CNT composite, the findings are consistent with the XRD and SEM analyses. As a result, additional electrochemical studies on IL/CNTs-PE were carried out.



Figure 3. CV curves CPE, CNTs-PE and IL/CNTs-PE at applied potentials between -0.20 V to 0.60 V in potential scanning rate of 50 mVs⁻¹ in 0.1 M PBS (pH 7.4) electrolyte solution before and after addition 15 μ M RA molecules in electrochemical cell.



Figure 4. The mechanism associated with the oxidation of the catechol moieties RA [45, 46].

Figure 5 shows the DPV curves and calibration graph of IL/CNTs-PE at applied potentials ranging from 0.00 V to 0.42 V at a scanning rate of 50 mVs⁻¹ after repeated injections of 40 μ M RA

solutions in an electrochemical cell containing 0.1 M PBS (pH 7.4). The results show that the DPV peak current of IL/CNTs-PE increases after each injection of 40 M RA solution, indicating a significant response of IL/CNTs-PE to RA. The calibration graph shows a stable linear range of 0 to 680 μ M and a sensitivity of 0.0951 μ A/ μ M, with detection limits (S/N=3) as low as 15 nM. Table 1 summarizes the comparison between the electrochemical studies for RA sensors in the current study and other reported RA sensors in the literature, demonstrating the relatively low detection limit value and broad linear range of IL/CNTs-PE between the RA electrochemical sensors. It is associated with the benefit of IL's synergistic effect on the surface of CNTs in IL/CNTs composite [63], including numerous nucleation sites originated from the functional groups on IL molecules on the large surface area derived from CNTs' porous network structure, which effectively promotes CNT dispersion and improves sensing performances of IL/CNTs-PE [64-66].



- **Figure 5.** DPV curves and calibration graph of IL/CNTs-PE at applied potentials between 0.00 V to 0.42 V in potential scanning rate of 50 mVs⁻¹ under following repeated injections of 40 μ M RA solutions in an electrochemical cell containing 0.1 M PBS (pH 7.4).
- **Table 1.** Comparison among the electrochemical studies for RA sensors in current study and other reported RA sensor in literatures.

Electrode	Techniqu	Linear	Detection	Ref.
	e	range	limit (nM)	
		(µM)		
IL/CNTs-PE	DPV	0–680	15	This
				work
Pt NPs/poly(orthophenylenediamine)/GCE	DPV	1–55	700	[19]
Fe ₃ O ₄ /phthalocyanine/carbonylated	DPV	0.2–400	182	[20]
MWCNTs				
DNA/chitosan/CNTs	SWSV	0.040-1.5	14	[21]
Magnetic functionalized molecularly	DPV	100-500	85	[22]
imprinted polymer				

Disposable pencil graphite electrode	DPV	0.01_10	7.93	[23]
Disposable periori grapinte electrode		0.01-10	1.75	$\lfloor 2 \rfloor$
Peptide/Graphene Oxide Screen-Printed	CV	0.1-3.20	96.6	[24]
Carbon Electrode				
Au/Laccase/chitosan/MWCNTs	CA	0.910-12.1	233	[25]

SWSV: Square Wave Stripping Voltammetry; CA: Chronoamperometry

In the presence of several organic and inorganic chemicals, the selectivity of IL/CNTs-PE as an RA electrochemical sensor was evaluated. Table 2 shows the results of electrocatalytic peak current measurements of DPV using IL/CNTs-PE at applied potentials ranging from 0.00 V to 0.42 V at a scan rate of 50 mVs⁻¹ after repeated injections of 5 M RA and 30 M interfering chemical compound solutions in an electrochemical cell containing 0.1 M PBS (pH 7.4). The results show that the proposed method has an excellent electrocatalytic response to the addition of RA in an electrochemical cell, and that the addition of interfering chemical compound solutions has no significant effect on the electrocatalytic response of IL/CNTs-PE. These findings are consistent with the fact that IL/CNTs-PE increases the ionic strength of the membrane, making selective transport of target molecules to the membrane phase possible [67, 68].

Table 2. The outcomes of obtained electrocatalytic peak current of DPV measurements using IL/CNTs-PE at applied potentials between 0.00 V to 0.42 V in potential scanning rate of 50 mVs⁻¹ under following repeated injections of 5 μ M RA and 30 μ M interfering chemical compounds solutions in an electrochemical cell containing 0.1 M PBS (pH 7.4).

Substance	Added	Electrocatalytic peak	RSD
	(µM)	current (µA)	
RA	5	0.4755	±0.0022
Gallic acid	30	0.0204	±0.0015
Caffeic acid	30	0.0150	±0.0012
Epigallocatechin-3 gallate	30	0.0127	±0.0017
Glucose	30	0.0292	±0.0010
Vanillic acid	30	0.0201	±0.0012
Theaflavin	30	0.0162	±0.0016
Quercetin	30	0.0142	±0.0011
Epicatechin	30	0.0122	±0.0018
Catechin	30	0.0241	±0.0017
Epigallocatechin	30	0.0142	±0.0010
Rutin	30	0.0150	±0.0012
Vitamin C	30	0.0225	±0.0014

In prepared real samples of Lavandula angustifolia, the accuracy of IL/CNTs-PE for determining RA was tested. Figure 6 shows DPV measurements using IL/CNTs-PE at applied potentials ranging from 0.00 V to 0.42 V at a scanning rate of 50 mVs⁻¹ after repeated injections of 40 μ M RA solution in an electrochemical cell containing 0.1 M PBS (pH 7.4) prepared from Lavandula angustifolia extracted

liquid. The calibration plot obtained in inset Figure 6 shows that the RA level in the processed sample is 3.20μ M. Table 3 summarizes the analytical results, which show low RSD values (4.33 to 5.14%) and high recovery values (98.75 to 99.50%), indicating appropriate, accurate, and valid IL/CNTs-PE results for the determination of the RA level in the prepared sample from the extracted liquid of flowers.



- **Figure 6.** DPV measurements and obtained calibration plot using IL/CNTs-PE at applied potentials between 0.00 V to 0.42 V in potential scanning rate of 50 mVs⁻¹ under following repeated injections of 40 μ M RA solution in an electrochemical cell containing 0.1 M PBS (pH 7.4) which prepared from extracted liquid of Lavandula angustifolia.
- **Table 3.** The analytical results using DPV measurements for determination RA in prepared real samples from extracted liquid of Lavandula angustifolia.

Spiked (µM)	Detected (µM)	Recovery (%)	RSD (%)
40.0	39.8	99.50	5.14
80.0	79.0	98.75	4.77
120.0	119.1	99.25	4.33
160.0	159.2	99.50	4.95

4. CONCULUSION

The current study's goal was to create an IL/CNTs-PE nanocomposite that could be used as a precise and sensitive electrochemical sensor to quantify RA as phenolic acid in a prepared sample of Lavandula angustifolia. Structural analyses revealed that IL was successfully incorporated on the surface of CNTs in the IL/CNTs composite. Electrochemical studies revealed that combining ILs and carbon CNTs improves the electrocatalytic activity, selectivity, and sensitivity of IL/CNTs-PE to determine RA by simple ion exchange reactions because ILs on the CNTs surface can act as a suitable charge transfer

bridge to facilitate electron transfer efficiency by lowering charge transfer resistance. The results showed that a stable linear range of 0 to 680 μ M and a sensitivity of 0.0951 μ A/ μ M were achieved, with detection limits as low as 15 nM. The current study's electrochemical studies for RA sensors and other reported RA sensors revealed a relatively low detection limit value and a broad linear range of IL/CNTs-PE between the RA electrochemical sensors. The results of determining RA in prepared real samples from Lavandula angustifolia showed low RSD values and high recovery values, indicating that the IL/CNTs-PE results were accurate and valid for determining RA level in the prepared sample from the extracted liquid of flowers.

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