

Short Communication

Determination of γ -Terpinenes in *Bunium Persicum* by Voltammetric Method and GC/Mass Analysis

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A new method using a glassy carbon electrode modified with gold nanoparticles (GNP_S/GCE) was developed and validated for determination of γ -terpinene in *Bunium Persicum* (*B. Persicum*) oil. Voltammetric methods including cyclic voltammetry and differential pulse voltammetry were used. Cyclic voltammetry was used to investigate the redox properties of the GNP_S/GCE. The peak current increased linearly with the concentration of γ -terpinene. The GNP_S/GCE was then successfully used to determine the concentration of γ -terpinene in *B. Persicum* oil. Also *B. Persicum* oil constituent are identified by gas chromatography coupled with the mass detector (GC/Mass).

Keywords: γ -Terpinene; Gold Nanoparticles; *Bunium Persicum*; Voltammetry Methods; GC/Mass

1. INTRODUCTION

B. Persicum (Boiss.) B. Fedtsch. (Carum Persicum, Zire Kuhi) (Fig. 1) is an herbaceous and perennial plant belonging to the Apiaceae family [1]. The flowers are hermaphrodite and are pollinated by insects. It is also one of the most valuable indigenous medicinal plants in Iran, which is found locally in many parts of the country. Also *B. Persicum* is one of the most important indigenous spices of Iran, India, Tajikistan, Afghanistan, Pakistan, Kashmir and Central Asia [2].

B. Persicum is one of the most important and economical medicinal plants. The essential oil of the seed has expectorant, stimulant, antispasmodic and diuretic properties [3]. Antifungal effect [4],

sample preparation. Gold nanoparticles (GNPs) modified glassy carbon electrode (GCE) have been successfully applied for the determination of γ -terpinene.

2. EXPERIMENTAL SECTION

2.1. Reagents

Acetonitrile (HPLC, 99%), lithium perchlorate (98%), γ -terpinene and Chloroauric acid (HAuCl_4) were obtained from Merck and used without any further purification. The glassy carbon electrode (0.0314 cm², Azar Electrode Co., Urmia, I. R. Iran) was used for assembly process. *B. Persicum* seeds were purchased from pakan bazr co. in Isfahan (Iran) which was harvested from Jandagh Mountains in July 2012. The electrochemical study was carried out in acetonitrile (0.1 M LiClO_4). The measurements were conducted in solutions made free from oxygen by the use of bubbling nitrogen.

2.2. Isolation Procedure

The oils were obtained from 25.0 g of freshly ground seeds of *B. Persicum* by hydrodistillation for 4 h in 250 ml water, according to the determination of oil content in vegetable drugs, using the apparatus described in the Iranian herbal pharmacopeia [26]. The oils were separated and stored below 8°C in a refrigerator until voltammetry and GC/mass analysis was performed.

2.3. Electrochemical experiments

All electrochemical experiments were carried out using an Autolab potentiostat-galvanostat PGSTAT 35 (Eco chemie Utrecht, Netherlands) equipped with GPES 4.9 software. The electrochemical cell was equipped with a GNP_s/GCE as the working electrode, a platinum electrode as the counter electrode, and a silver/silver chloride (Ag/AgCl) electrode as the reference electrode. A personal computer (pentium IV) was used for data storage and processing.

The bare GCE was polished into a mirror-like 100 surface with 0.5 and 0.05 μm alpha Al_2O_3 , then rinsed ultrasonically with water / chloroform / water baths, each for 5 minutes, so that any physically adsorbed species be removed. The bare GCE electrode was immersed in gold nanoparticle (GNPs) solution for 10 h at room temperature (25 °C). An ultrasound bath (Bandelin Sonorex, Germany) was used at a constant frequency of 35 kHz during experimentation.

2.4. Preparation of colloidal gold

The GNPs were prepared according the following procedure, by adding 0.5 ml of 1 % sodium citrate solution to 50 ml boiling solution of 0.01 % HAuCl_4 . The mixture was maintained at boiling point for 15 min and stirred for another 15 min after removing the heating source to produce 24 nm-

diameter colloidal gold nanoparticles. Color of external solution for GNPs (24 nm) was pinkish red. The λ_{\max} of the GNPs by UV-vis spectra was observed to be 528 nm. The solution was stored in a refrigerator in a dark-colored glass bottle.

2.5. GC/mass analysis

The components of the *B. persicum* were identified by GC–MS analyses. The GC–MS apparatus was a Hewlett Packard 5972A spectrometer consisting equipped with a fused-silica column HP-5MS (30 m - 0.25 mm i.d., film thickness 0.25 μm) The operating conditions were as follows: oven temperature 60–240 °C with a rate of 4 °C/min, injector temperature 250 °C, injector mode: split injection, with carrier gas, He, flow rate 2 ml/min, mass spectra: electronic impact, ionisation potential 70 eV, ion source temperature 200 °C.

2.6. Identification of components

The oil components were identified from their retention indices obtained with reference to the n-alkane series on the DB-5 column, mass spectra with those of authentic samples, composition of their mass spectra and fragmentation patterns reported in the literature, and computer matching with MS-data bank. Quantification of the relative amount of the individual components was performed according to the area percentage method.

3. RESULTS AND DISCUSSION

3.1. Voltammetric behavior of γ -terpinene

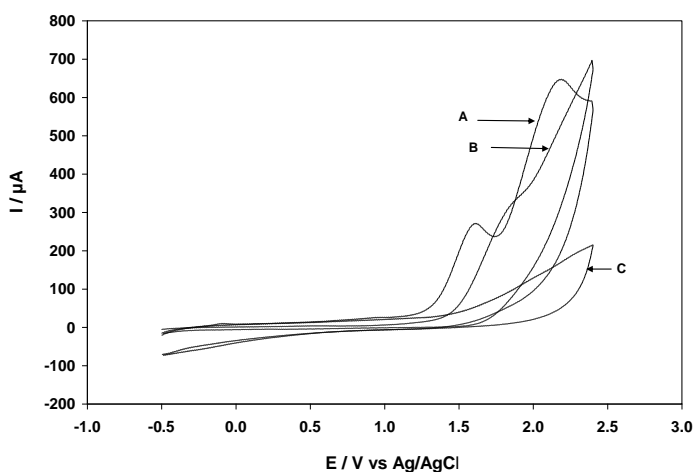


Figure 2. Cyclic voltammetry of (A) $9 \times 10^{-3} \text{ M}$ γ -terpinene on the surface of GNPs/GCE in acetonitrile (0.1 M LiClO_4) (B) $9 \times 10^{-3} \text{ M}$ γ -terpinene on the surface of bare CPE in acetonitrile (0.1 M LiClO_4) (C) bare GCE in acetonitrile (0.1 M LiClO_4).

The voltammograms of γ -terpinene solution with bare CPE and GNPs/CPE electrodes were investigated. Fig. 2 shows the cyclic voltammogram responses from $9 \times 10^{-3} \text{ M}$ γ -terpinene on the surface of bare GCE and GNPs/GCE in acetonitrile (0.1 M LiClO₄). At the bare electrode, γ -terpinene shows a relatively broad and two weak oxidation peaks at 1800 and 2200 mV. Nevertheless, with the GNPs/GCE, the oxidation peak becomes well-defined and sharp, with a magnification current of 1500 and 2200 mV. The lower over potential and the increase in current response are clear evidence of the catalytic effect of the modified electrode on the oxidation of γ -terpinene. From these voltammograms, it can be seen that the peak potential of γ -terpinene shifts to less positive potentials and the peak currents increase.

3.2. Effect of scan rate

The effect of potential scan rate on the electrocatalytic oxidation of γ -terpinene at the GNPs/GCE was investigated using cyclic voltammetry. By increasing the scan rate, the oxidation peak potential shifted towards a more positive potential, confirming the kinetic limitation of the electrochemical reaction. This result suggests that, at sufficiently positive potentials, the reaction is controlled by γ -terpinene diffusion. The linear regression equation is shown in Equation [27].

$$I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C v^{1/2}$$

Where C is the bulk concentration, D is the diffusion coefficient, A is the electrode surface area (0.029 cm²), α is the transfer coefficient, n is the number of electrons transferred in the reaction and v is the scan rate.

The effect of scan rate (v) on the electrochemical behavior of γ -terpinene in the GNPs/GCE in acetonitrile (0.1 M LiClO₄) containing 1 mM γ -terpinene is shown in Fig. 3A.

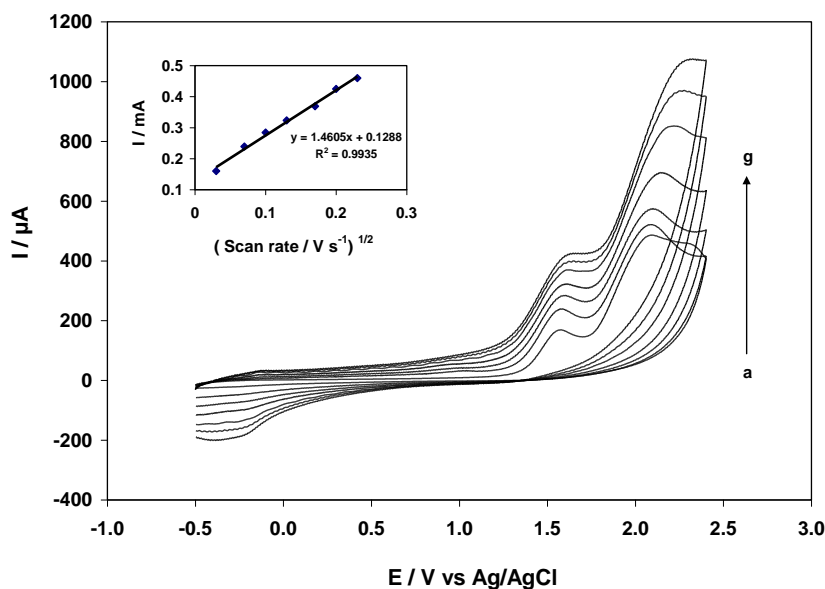


Figure 3. Cyclic voltammogram of a GNPs/GCE in 1mM BR at scan rates of 30 - 230 m V s⁻¹ Inset: (A) Plot of peak current vs. E/mV.

For scan rates between 30 and 230 m V s^{-1} , the γ -terpinene oxidation peak current also increases linearly with the square root of the scan rate suggesting that the reaction is diffusion-limited (Fig. 3B). The linear regression equation for γ -terpinene was found to be:

$$I_{\text{Pa, } \gamma\text{-terpinene}} = 1.46 V^{1/2} + 0.128, R^2 = 0.993$$

3.3. Differential pulse voltammetry investigations for the measurement of γ -terpinene

Given that differential pulse voltammetry (DPV) has a much higher current sensitivity and better resolution than cyclic voltammetry (CV), it was used to estimate the detection limits for γ -terpinene. Moreover, the charging current contribution to the background current, which is a limiting factor in the analytical measurements, is negligible in DPV. Fig. 4 shows the DPV data obtained for the oxidation of different concentrations of γ -terpinene on the GNPs/CPE in acetonitrile (0.1 M LiClO_4) at a scan rate of 25 mV s^{-1} .

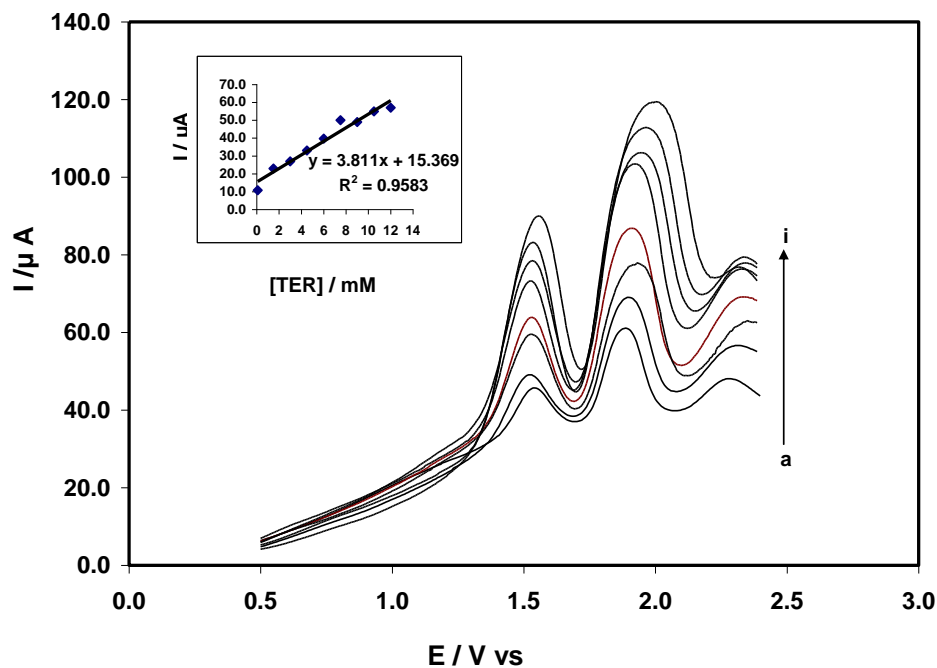


Figure 4. Differential pulse voltammograms of γ -terpinene on the GNPs/GCE in acetonitrile (0.1 M LiClO_4) at a scan rate of 25 mV s^{-1} (A) Plots of peak current as a function of γ -terpinene concentration in the range of $1.2 \times 10^{-2} \text{M}$ - $1 \times 10^{-4} \text{M}$.

Inset A Fig. 4 shows the dependencies of the peak current on the γ -terpinene concentration. This figure shows that the plot of peak current versus γ -terpinene concentration is linear in the range of $1.2 \times 10^{-2} \text{M}$ - $1 \times 10^{-4} \text{M}$. From the analysis of these data, we can estimate that the detection limit of γ -terpinene is $5 \times 10^{-5} \text{M}$. The relative standard deviation (RSD) of 3.2% in the oxidation peak current and 0.95% in the peak potential for five repeated detections of $1 \times 10^{-4} \text{M}$ of γ -terpinene suggests that there is excellent reproducibility of results using GNPs/GCE.

We report the oxidation of γ -terpinene by a GNP_s/GCE in DPV method and compare a voltammetric and GC/Mass method and validated it for determination of γ -terpinene in essential oil of *B. Persicum* seed. Determination of γ -terpinene in essential oil was achieved by the standard addition method, yielding a plot of peak height versus γ -terpinene concentration. A linear equation ($i_p = 0.011C + 0.0296$) was obtained with a correlation coefficient of 0.999. Under these conditions, quantity of γ -terpinene in *B. Persicum* oil was obtained as 36.7g. The amount of γ -terpinene obtained by voltammetry method has good agreement with the value obtained by GC/Mass method. The results are in good agreement with each other and acceptable and the proposed method for determination of γ -terpinene can be used.

3.4. GC/mass results

Table 1. GC/mass results for *B. persicum* oil

NO.	Name	Retention time (min)	RI	%
1	α -thujene	3.39	928	t
2	α -pinene	3.55	938	1.6
3	apiole	24.50	1682	0.2
4	β -pinene	4.36	981	5.5
5	myrcene	4.58	993	t
6	dill apiole	22.92	1626	0.3
7	p -cymene	5.47	1029	17.5
8	limonene	5.56	1034	5.3
9	Cis- β -ocimene	5.67	1043	0.1
10	γ -terpinene	6.36	1066	31.2
11	terpinolene	6.95	1091	0.3
12	terpinene-4-ol	9.48	1180	t
13	cuminal	11.82	1246	28.7
14	spathulenol	21.65	1581	0.3
15	bornyl acetate	12.89	1290	2.9
16	elimicin	21.20	1566	2.4
17	p -cymen- 7-ol	13.15	1293	1.5
18	α -copaene	15.46	1374	t
19	E -caryophyllene	16.78	1416	0.1
20	myristicin	20.08	1527	0.9
21	Ar -curcumene	18.77	1483	0.2
22	β -selinene	19.02	1491	t
23	β -bisabolene	19.56	1509	0.3

The GC/mass analysis of *B. persicum* oil was performed and results shown in table 1. The total numbers of chemical constituents identified in the oil were measured to be 23. Volatile oils have different chemical structure components, so different behavior for each of them is predictable. The main components were found to contain γ -terpinene (31.2%), cuminal (28.7%), p -cymene (17.5%), β -pinene (5.5%), limonene (5.3%), bornyl acetate (2.9%), α -pinene (1.6%), p -cymen- 7-ol (1.5%). As

shown in Table 1, γ -terpinene in the *B. persicum* oil was the main monoterpene hydrocarbon with 31.2% and shows that the GC/mass results are similar to the DPV results. In order to address the qualitative determination of composition of *B. persicum* oil by GC-MS, it is necessary to utilize retention indices identities [28]. The retention time (RT) and retention index (RI) for each compound are also presented in Table 1.

4. CONCLUSION

The GC/mass analysis of *B. Persicum* oil was performed and results showed that γ -terpinene is the main component of *B. Persicum* oil. A new method using a glassy carbon electrode modified with gold nanoparticles (GNP_s/GCE) was developed and validated for determination of γ -terpinene. The GNP_s/GCE was then successfully used to determine the concentration of γ -terpinene in *B. Persicum* oil. The concentration of γ -terpinene obtained by this electrode was on the same order of value as the concentration obtained by GC/mass analysis. In addition, high selectivity, good sensitivity, low detection limits and simple, fast and low cost make this method suitable for accurate analysis in pharmaceutical and herbal medicine.

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