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Two-Step Chronoamperometric Determination of Antioxidant Capacity of Water Extracts from Medicinal Plants †

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Abstract: Medicinal plants contain a wide range of bioactive compounds including antioxidants. Thus, the evaluation of the antioxidant capacity of medicinal plant extracts used in phytotherapy is of practical interest. Water extracts from 11 plants obtained by sonication for 30 min were studied by cyclic voltammetry at bare glassy carbon electrode (GCE) and GCE modified with a mixture of 1 mg mL^{-1} CeO_2 and SnO_2 nanoparticles (NPs) dispersed in 0.10 mM cetylpyridinium bromide. A two-step chronoamperometric approach (at 400 and 900 mV for 75 s each one) was developed to estimate the antioxidant capacity of medicinal plant extracts. A strong and very strong correlation level has been obtained between antioxidant capacity and total phenolic contents or antioxidant capacity toward 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$).

Keywords: voltammetry; chronoamperometry; chemically modified electrodes; metal oxide nanoparticles; antioxidants; medicinal plants

1. Introduction

Since ancient times, medicinal plants have been used for the treatment of human diseases. The wide range of bioactive compounds contained in plants stipulates the development of new phytopharmaceuticals [1]. Traditional phytotherapy is also still applied as part of complex treatment [2,3].

Antioxidants are one of the largest groups of bioactive compounds of plant origin [4] that are widely distributed in medicinal plants. Thus, the evaluation of the antioxidant capacity of medicinal plant extracts used in phytotherapy is of practical interest.

Electrochemical methods have been shown to be a highly effective tool for the evaluation of total antioxidant parameters of plant materials [5]. They combine high precision, cost-efficiency, rapid response, simplicity, and in-field applicability. In application to medicinal plants, the electrochemical response usually corresponds to the contents of several antioxidants of similar structure. Depending on the method applied, the impact of major components can be evaluated as it occurs in voltammetry or the total contents of oxidizable components can be obtained if the chrono methods are used. The last are preferable because they allow estimation of a wider range of components including those ones presented at low concentration [6,7].

Thus, the current work is focused on the development of a novel chronoamperometric method for the determination of the antioxidant capacity of water extracts from medicinal plants obtained by sonication using glassy carbon electrode (GCE) modified with a mixture of 1 mg mL^{-1} CeO_2 and SnO_2 nanoparticles (NPs) dispersed in 0.10 mM cetylpyridinium bromide.

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2. Materials and Methods

Commercially available medicinal plants material (11 samples) was studied. The corresponding water extracts were obtained by sonication for 30 min in an ultrasonic bath (WiseClean WUC-A03H) (DAIHAN Scientific Co., Ltd., Wonju-si, Republic of Korea). The plant/water ratio was 1:10, 1:20, or 1:33 depending on the type of plant material, its water absorption and consumption coefficients. The extracts were filtered, their volume was filled to the initial value, and used in a further study.

Gallic acid (99% purity), Folin-Ciocalteu reagent were obtained from Sigma (Steinheim, Germany) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•) was purchased from Aldrich (Steinheim, Germany). Standard solutions of gallic acid (100 mg mL^{-1}) and DPPH• (0.20 mM) were prepared by dissolving the exact weight of the substance in distilled water and methanol (c.p.), respectively. The Folin-Ciocalteu reagent solution was diluted in a 1:10 ratio with distilled water prior to the experiment.

CeO_2 and SnO_2 NPs were used as electrode surface modifier. Their 1 mg mL^{-1} mixture was prepared from commercial reagents (10% CeO_2 NPs water dispersion from Sigma-Aldrich (St. Louis, MO, USA) and SnO_2 NPs powder from Aldrich (Steinheim, Germany)) using 0.10 mM solution of cetylpyridinium bromide as dispersive medium. The standard 1.0 mM solution of cetylpyridinium bromide in water was prepared from a 98% purity reagent (Aldrich, Steinheim, Germany).

Electrode surface modification was performed by drop casting 5 μL of dispersion of CeO_2 and SnO_2 NPs mixture after careful cleaning of the GCE surface at alumina slurry (0.05 μm grain).

Voltammetric and chronoamperometric measurements were carried out at the potentiostat/galvanostat μAutolab Type III (Eco Chemie B.V., Utrecht, The Netherlands) using GPES 4.9 software. GCE (3 mm diameter) from CH Instruments, Inc. (Bee Cave, TX, USA) or modified electrode, reference Ag/AgCl electrode, and auxiliary electrode (platinum wire) were placed in an electrochemical glass cell containing phosphate buffer pH 7.0 and cyclic voltammograms were recorded from 0.0 to 1.2 V with a potential scan rate of 100 mV s^{-1}. Chronoamperometry was performed at +400 and +900 mV in phosphate buffer pH 7.0.

The total phenolic content was measured by standard spectrophotometric method based on the reaction with the Folin-Ciocalteu reagent [8]. Gallic acid was used as a standard. The total phenolic content was recalculated per 100 mL of the extract.

The antioxidant activity toward DPPH• was studied by spectrophotometry [9] and expressed as a relative percentage of the decrease in DPPH• absorption after the reaction with the antioxidants of the extract. The extract volume was 50 μL for all samples under study.

3. Results and Discussion

3.1. Voltammetric Behaviour of Water Extracts from Medicinal Plants

At first, the cyclic voltammetry on bare GCE in phosphate buffer pH 7.0 was applied for the characterization of medicinal plant water extracts. All samples excluding *Urticae folia* show oxidation steps, the shapes and potentials of which depend on the type of medicinal plant (Figure 1). The oxidation potentials vary due to the classes of compounds contained in each sample (Table 1). The contents of oxidizable compounds also affect the oxidation potential value as far as overlap of the oxidation steps occurs in the case of multicomponent samples that leads to the shift of the oxidation potential value. However, a high sample volume should be used for the majority of extracts to obtain a more or less pronounced signal on the voltammograms. Nevertheless, the oxidation currents at nanoampere level are observed for several samples or even full absence of the oxidation steps as for *Urticae folia* extract (Table 1).

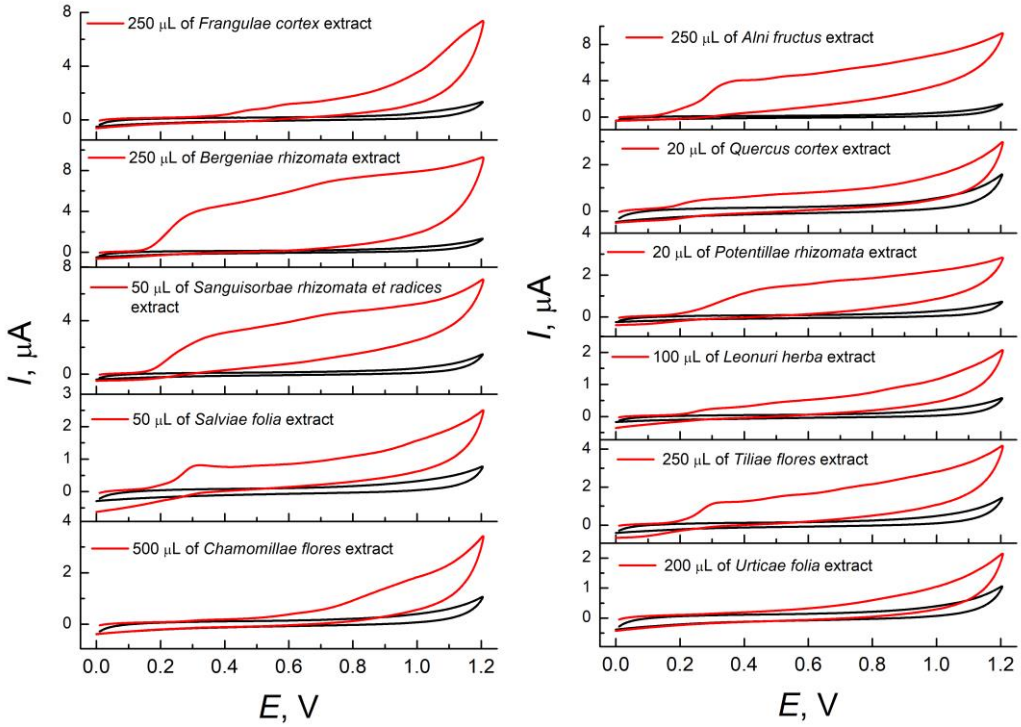

Figure 1. Cyclic voltammograms of medicinal plant water extracts on bare GCE in phosphate buffer pH 7.0. Potential scan rate is 100 mV s^{-1}.

Table 1. Voltammetric characteristics of water extracts from medicinal plants on the bare and modified with a mixture of CeO_2 and SnO_2 NPs GCE in phosphate buffer pH 7.0 (n = 5; P = 0.95).

Medicinal Plant Material	Bare GCE			GCE Modified with Mixed CeO_2 and SnO_2 NPs		
	V_{al}, μL	E_{ox}, V	I, μA	V_{al}, μL	E_{ox}, V	I, μA
Alni fructus	250	0.34; 0.52	0.97 ± 0.08; 0.070 ± 0.005	50	0.33, 0.52	0.26 ± 0.04; 0.025 ± 0.002
Quercus cortex	20	0.25; 0.50	0.11 ± 0.04; 0.014 ± 0.002	20	0.34	0.18 ± 0.02
Frangulae cortex	250	0.47; 0.59	0.060 ± 0.03; 0.076 ± 0.002	500	0.44, 0.61	0.28 ± 0.04; 0.16 ± 0.01
Potentillae rhizomata	20	0.43; 0.68	0.26 ± 0.02; 0.032 ± 0.001	20	0.32	0.84 ± 0.06
Bergeniae rhizomata	250	0.29; 0.76	1.4 ± 0.09; 0.41 ± 0.03	100	0.32, 0.74	1.1 ± 0.1; 0.044 ± 0.008
Sanguisorbae rhizomata et radices	50	0.33; 0.72	0.87 ± 0.07; 0.14 ± 0.02	20	0.35, 0.74	0.60 ± 0.01; 0.017 ± 0.001
Leonuri herba	100	0.29; 0.51; 0.87	0.053 ± 0.001; 0.017 ± 0.002; 0.009 ± 0.002	100	0.31, 0.53	0.011 ± 0.002; 0.018 ± 0.002
Tiliae flores	250	0.30; 0.52; 0.75	0.40 ± 0.05; 0.051 ± 0.005, 0.025 ± 0.003	250	0.39, 0.76	0.45 ± 0.02; 0.017 ± 0.001
Salviae folia	50	0.31	0.45 ± 0.02	50	0.34	0.59 ± 0.04
Urticae folia	250	—	—	500	0.90	0.016 ± 0.003
Chamomillae flores	500	0.32; 0.97	0.017 ± 0.02; 0.015 ± 0.002	500	0.32	0.023 ± 0.002

Phenolic compounds are one of the most widely distributed groups of bioactive compounds in medicinal plants [10–12] that are electroactive at anodic potentials. As shown earlier on typical plant phenolics [13–15], the application of chemically modified electrodes based on CeO_2 or SnO_2 NPs improves voltammetric response parameters. Therefore, GCE modified with a mixture of CeO_2 and SnO_2 NPs dispersed in 0.10 mM cetylpyridinium bromide has been tested for sensing of water extracts from medicinal plants.

There are well-defined oxidation steps on the voltammograms at the modified electrode. The oxidation potentials are close to those observed at the bare GCE (Table 1). The

exclusion is *Potentillae rhizomata* extract, for which a 110 mV shift of oxidation potential to less positive values has been registered. Changes in voltammetric characteristics are also obtained for the *Quercus cortex* extract. Two oxidation steps are shifted close to each other forming one oxidation peak. The oxidation currents of the extracts at the modified electrode are increased vs. bare GCE that is explained by a 4.9-fold increase in the electroactive surface area of the modified electrode (40 ± 2 and 8.2 ± 0.3 mm^2, respectively).

3.2. Chronoamperometry of Water Extracts from Medicinal Plants

As known [5], the voltammetric response of medicinal plant extracts is integral, i.e., each oxidation step is caused by the presence of various antioxidants including structurally related. Moreover, the major contributors to the voltammetric response are components with a high content in the sample. Chronoamperometry allows to cover a wider range of antioxidants independently on their contents providing more information about the sample and improvement in the accuracy of antioxidant capacity assay.

For all extracts under study, the first oxidation step occurs in the range of 310–400 mV and the second one—in the range of 520–900 mV at the modified electrode (Table 1). These values agree well with the classification of the antioxidants by the reducing power [16,17].

Therefore, two-step chronoamperometry was used at 400 and 900 mV for antioxidant capacity evaluation. Components with low concentration also made an impact in the analytical response in this case. The effect of electrolysis time on the chronoamperometric response of medicinal plant extracts was studied. The electrolysis steady state was achieved after 75 s in each step. The corresponding chronoamperograms are presented in Figure 2.

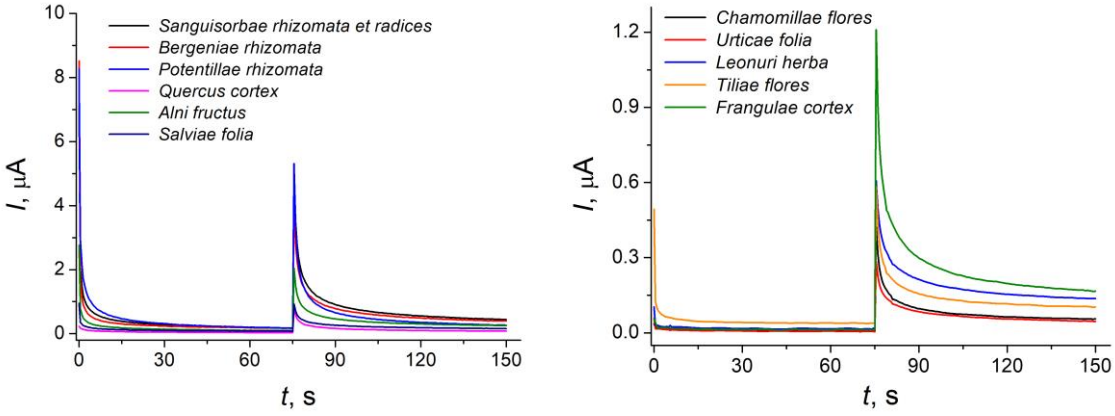

Figure 2. Two-step background subtracted chronoamperograms of 50 μL of medicinal plant extracts at the GCE modified with a mixture of 1 mg mL^{-1} CeO_2 and SnO_2 NPs. Supporting electrolyte is phosphate buffer pH 7.0, E_1 = 400 mV, E_2 = 900 mV.

The antioxidant capacity has been expressed as current at the corresponding potential recalculated per 100 mL of the extract (Table 2).

Table 2. Antioxidant capacity (AOC) of water extracts from medicinal plants based on chronoamperometric measurements (n = 5; p = 0.95).

Medicinal Plant Material	AOC_{400}, μA per 100 mL	RSD, %	AOC_{900}, μA per 100 mL	RSD, %
Alni fructus	747 ± 57	3.1	2053 ± 81	3.2
Quercus cortex	938 ± 51	2.2	1860 ± 86	1.9
Frangulae cortex	80.0 ± 0.9	0.88	1320 ± 99	3.0
Potentillae rhizomata	1404 ± 101	2.9	2204 ± 101	1.8
Bergeniae rhizomata	1360 ± 52	2.4	2840 ± 99	1.4

Sanguisorbae rhizomata et radices	1347 ± 57	1.7	3400 ± 99	1.2
Leonuri herba	112 ± 5	2.9	1073 ± 40	1.5
Tiliae flores	297 ± 18	3.9	832 ± 45	3.4
Salviae folia	518 ± 37	5.7	1104 ± 60	2.2
Urticae folia	33 ± 2	5.5	325 ± 10	1.3
Chamomillae flores	43 ± 3	6.8	403 ± 23	2.3

The antioxidant capacity at 900 mV reflects the total contents of the antioxidants, i. e. components of high, intermediate, and low antioxidant power [16]. The highest antioxidant capacity was observed for high-density types of plant material in particular roots, rhizomes, and bark that agrees well with the literature data [18–20]. The lower antioxidant capacity was obtained for the *Urticae folia* and *Chamomillae flores* being in line with the voltammetric data.

Comparison of the data obtained with the standard antioxidant parameters (total phenolic contents and antioxidant capacity toward DPPH$^{\bullet}$) has shown a strong (r = 0.7–0.9) and very strong (r = 0.9–1.0) correlation level (Table 3) according to the Chaddock scale.

Table 3. Correlation of antioxidant parameters of water extracts from medicinal plants obtained by chronoamperometry and spectrophotometry (n = 11; p = 0.95), r_{crit} = 0.6021.

Antioxidant Parameter	Total Phenolic Content, mg Gallic Acid per 100 mL	Antioxidant Capacity Toward DPPH$^{\bullet}$, %
AOC_{400}, μA per 100 mL	0.8977	0.9311
AOC_{900}, μA per 100 mL	0.9249	0.8803

The results obtained confirm the accuracy of the developed chronoamperometric method. Furthermore, the methos developed is simple and does not require the application of additional specific reagents such as the Folin-Ciocalteu reagent or DPPH$^{\bullet}$ which is unstable and highly affected by the presence of light and water. The use of phosphate buffer pH 7.0 is close to the physiological conditions making possible partial prediction of medicinal plants effect in the biosystems. Another advantage of chronoamperometry is a rapid response due to the absence of an incubation stage that makes the method applicable for fast screening tests.

4. Conclusions

A two-step chronoamperometric method has been developed for the evaluation of the antioxidant capacity of water extracts from medicinal plants for the first time. GCE modified with the mixture of CeO_2 and SnO_2 NPs dispersed in cetylpyridinium bromide provides improvement of the sample antioxidant response and enlarges the number of components contributing to the antioxidant parameters. The use of two potentials for the electrolysis allows discrimination of the antioxidants by power. The chronoamperometric approach is based on the direct response of antioxidants and doesn't need special reagents usually used for the evaluation of the antioxidant properties of the plant samples.

Further development in the field can be focused on the fabrication of portable devices for the *on-site* monitoring of the antioxidants in the samples of plant origin. To solve this problem, the electrodes with a long-term stable and reliable response have to be developed.

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