Digest Journal of Nanomaterials and Biostructures Vol. 7, No. 2, April - June 2012, p. 729 - 736

ANTIOXIDANT CAPACITY OF *CAMELLIA SINENSIS* EXTRACTS

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The term "tea" refers to the flavored beverage obtained by infusion of dried leaves, and the popular name of the plant is Camellia sinensis. A series of tea from Camellia sinensis plant have been analyzed for caffeine content. In recent years, caffeine received increasing attention in food and pharmaceutical industries, due to its pharmacological properties which comprise stimulation of the central nervous system, peripheral vasoconstriction, relaxation of the smooth muscle and myocardial stimulation. The aim of this study was to determine the content of caffeine in different varieties of Camellia sinensis extract in aqueous, acid and alcoholic media. The content of caffeine was determined by using two methods: cyclic voltammetry and high-performance liquid chromatography (HPLC).

(Recieved February 24, 2012; Accepted May 28, 2012)

Keywords: antioxidant activity, caffeine, cyclic voltammetry, HPLC, tea

1. Introduction

Camellia sinensis is a perennial plant of the *Theaceae* family [5]. It is cultivated in tropical and subtropical regions. The product can be green, black or white, fermented or not, smoked or not, knowing a very wide range of types. Tea can be obtained from buds or leaves.The number of leaves from the harvested branches (two or three), as well as the harvest season, have their own importance when determining the tea's quality.

The content of caffeine varies depending on tea type, which is directly attributed to their processing and leaf maturity. Caffeine is the major alkaloid present (about 2-5%) in dry leaves of *Camellia sinensis* and *Camellia assamica*, which also contains small amounts of theobromine and theophylline [4]. It belongs to a class of organic compounds called xanthines. Other common members of this class include theophyline and theobromine. It is found naturally in foods such as coffee, tea, cola nuts, yerbamate, guarana berries, and (in small amounts) cacao beans. For the plant, caffeine acts as a natural pesticide since it paralyzes and kills some of the insects that attempt to feed on the plant.

The main objectives of this study were: to determine the effects of different extraction conditions (solvents influence) and *Camelia sinensis* species for the determination of the qualitative content of caffeine in tea extracts; to compare the caffeine content as well as the antioxidant capacity of *Camellia sinensis* (white, black and green tea).

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2. Experimental part

2.1. Materials and methods

All solutions were prepared with analytical or high-performance liquid chromatography grade reagents (caffeine standard (Dionex), methanol(Aldrich), ultrapure water, ethanol 96% (Aldrich), phosphate buffer with 65% w/v 50 mM disodium hydrogen phosphate and 35% w/v 50 mM sodium dihydrogen phosphate at pH 7.0. For high-performance liquid chromatography, as mobile phase was used 80% methanol: 20% aqueous solution.

2.2 Standard solution

Caffeine solution produced by Dionex was used as standard solution.

2.3 Preparation of tea extracts

2g of tea samples (green tea, white tea and black tea) were extracted in different medium: in 200 ml of: (a) distilled water (80^0C), (b) distilled water (80^0C) with 5 ml of freshly squeezed lemon juice, and (c) aqueous ethanol (70^0C) (20%). Extraction with aqueous ethanol was carried out by pouring the required amount of water over the tea samples and then adding absolute ethanol at room temperature up to 200 ml to obtain the required concentration of ethanol. Tea samples were extracted (5, 15 or 30 min) and then the infusions were filtered through a tea strainer.

2.4 Hydrolysis of tea extracts

A mixture of 1 mL of filtered tea extract and 4 mL of hydrochloric acid (2M) was boiled in a water bath for 30 min. After cooling, the mixture was extracted three times with diethyl ether (4+4+3 mL). The ethereal phases were collected and evaporated. Residue was dissolved in 1 mL of 96% ethanol, filtered through the nylon filter (0.22 μm) and stored at -20^0C.

2.5 Cyclic voltammetry

Samples were prepared for cyclic voltammetry analysis by diluting 2.00 ml of the extracts, typically 50 times, in a phosphate buffer with 65% w/v 50 mM disodium hydrogen phosphate and 35% w/v 50 mM sodium dihydrogen phosphate at pH 7.0. The volumetric flask of tea used in each determination was 75 mL.

Cyclic voltammograms for electrochemical oxidation of tea in aqueous, acid and alcoholic medium, using glassy carbon electrode, were performed using an PGz-402 Universal Dynamic Pulse Voltammetry Eis Radiometer, Copenhagen, Denmark.

Cyclic voltammetry studies were made within -250÷1200 mV, at ambient temperature, at a scan rate of 100mV/s with a sensitivity of 100mA.

The electrochemical cell was equipped with three electrodes: the working electrode was the glassy carbon electrode with 0.07 cm^2 active surface; the platinum counter electrode had 1 cm^2 active surface and the reference electrode was the Ag/AgCl electrode.

HPLC chromatograms were recorded on an Ultimate 3000 – DAD with Acclaim 120 C18 column (5 μm, 4.3 x 10 mm). Separation was achieved using an isocratic elution of 80% methanol and 20% water.

This mobile phase was selected due to separation properties of the column. The UV chromatographic signal was collected at λ=270 nm. The wavelength of 270 nm was determined as the most adequate for caffeine quantification because of the maximum absorbance at this wavelength. Data was collected using Chromeleon software. The flow rate used for separation was 0.500 ml/min. The column temperature was held at 30°C and a 20μl injection volume was used in all analyses.

3. Results and discussions

Most research activities on *Camellia sinensis* have been focused on chromatographic, spectrophotometric or electrochemical methods. Therefore, it is important to develop more

reliable, simpler and faster methods for the determination of caffeine from different sources in order to find a more precise relationship between the amounts of consumed caffeine and its physiological effects [6,7]

Thus, the nature influence of extraction conditions (solvent influence) on oxidation activity was followed, by comparison with the electrochemical methods described in the literature in which the phenols content from different varieties of *Camellia sinensis* was determined [8].

Earlier studies revealed that caffeine content is associated to origin, genetic and environmental variability, harvest time and processing manner of plant material, and can range from 24% to 40%. The displayed results confirmed that caffeine content depends on the age of tea leaves and processes involved in the production of tea [1,2].

The efficiencies of different solvents (water, acid and alcohol) in the extraction of caffeine from leaves of white, black, green after different extraction times (5, 15, 30 min) and the antioxidant capacity of the obtained extracts were investigated. The HPLC analyses of the caffeine content in these extracts were also performed.

Table 1. Extraction conditions for the four types of tea (white, black, green)

Retention time [min]	Temperature [^{0}C]	Solvent
5	80	water
15	80	acid
30	70	alcohol

The determination of the antioxidant character of *Camellia sinensis* species depending on caffeine content was studied by cyclic voltammetry and HPLC chromatography.

3.1. Electrochemical studies

Antioxidant activity of tea has been studied extensively especially on the polyphenols content from different species of *Camellia*.[9]

The study of the antioxidant activity by caffeine content in *Camellia sinensis* species was carried out by cyclic voltammograms.

Figure 1 shows cyclic voltammograms (CVs) recorded for *Camellia sinensis* on glassy carbon electrode in aqueous medium; the sweep rate used for this measurement was 100mV/s.

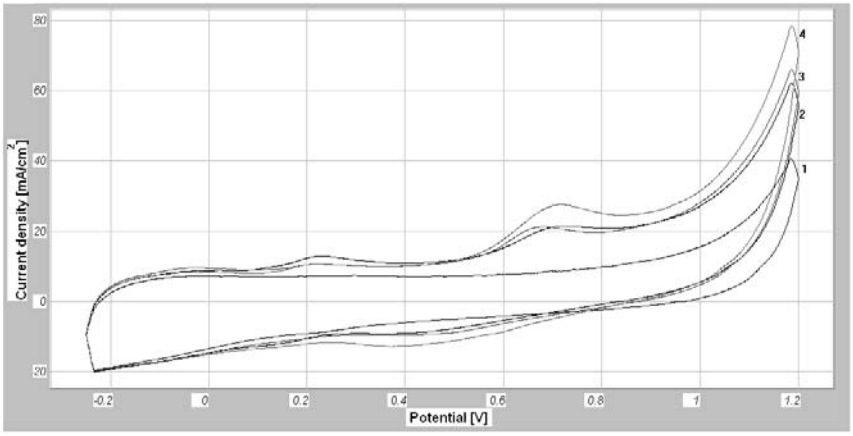

Fig. 1. Cyclic voltammogram of Camellia sinensis in aquous medium; glassy carbon electrode; scan rate 100mV/s;1- baseline, 2- white tea; 3- black tea; 4- green tea

In case of the aqueous reaction medium, the presence of two oxidation peaks can be observed on the anodic branch for the three types of tea (white, black and green): one around the 0.25 V and the other around 0.7 V. It can also be observed that the anodic current peak around the potential value of 0.25 V is followed by the better outlined current peak around 0.7 V. The peak around 0.25 V indicates the presence of caffeine content in the three types of *Camellia sinensis* tea.

Thus, in the aqueous reaction medium green tea has the highest antioxidant character.

Figure 2 shows cyclic voltammograms (CVs) recorded for *Camellia sinensis* on glassy carbon electrode in acid medium; the sweep rate used for this measurement was 100mV/s.

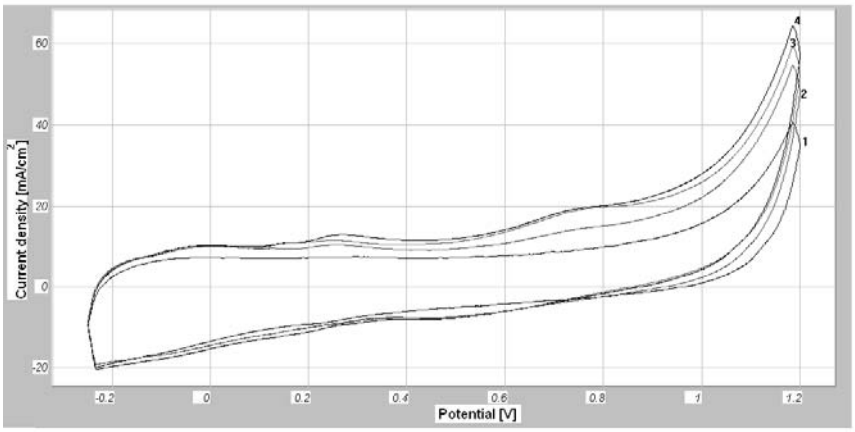

Fig. 2. Cyclic voltammogram of Camellia sinensis in acid medium; glassy carbon electrode; scan rate 100mV/s; 1- baseline, 2- white tea; 3- green tea; 4- black tea

In the case of the acid reaction medium can be observed on the anodic branch for the three types of tea (white, black, green) the appearance of two oxidation peaks poorly pronounced: one around 0.25 V and the other in around 0.7 V. It can also be observed that the anodic current peak around the potential value of 0.25 V is followed by the slightly outlined current peak around 0.7 V. In this case, just like in the previous, the caffeine content is characterized by the peak corresponding to the 0.25V value. In the acid reaction medium, the white tea exhibited the highest antioxidant capacities.

Figure 3 shows cyclic voltammograms (CVs) recorded for *Camellia sinensis* on glassy carbon electrode in alcoholic medium; the sweep rate used for this measurement was 100mV/s.

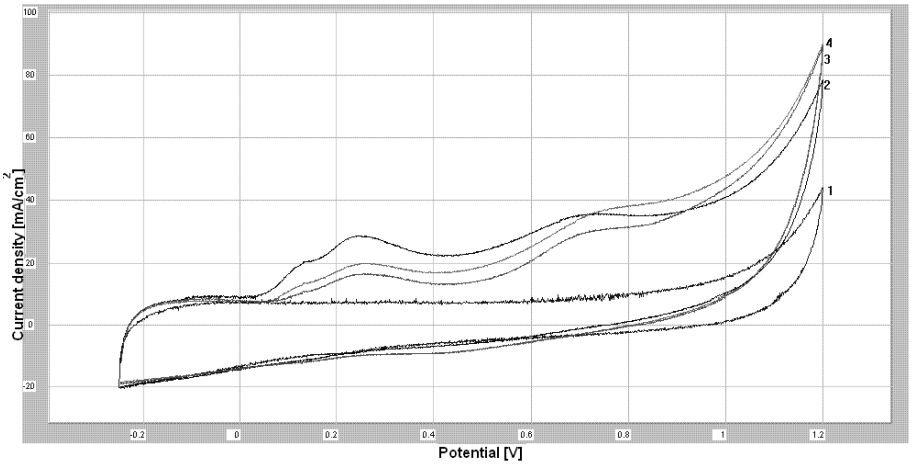

Fig. 3. Cyclic voltammogram of Camellia sinensis in alcoholic medium; glassy carbon electrode; scan rate 100mV/s; 1- baseline, 2- white tea; 3- black tea; 4- green tea

In case of the alcoholic reaction medium, the presence of three oxidation peaks can be observed on the anodic branch for the three types of tea (white, black and green): one around the 0.18 V, the other around 0.25V which indicates the presence of caffeine and one around 0.7V. The oxidation peak around 0.18V potential is followed by a pronounced increase in the peak around 0.25V. Highest antioxidant character in this reaction medium it presents white tea.

3.2. Chromatographic studies

In the literature, the HPLC results show that the major phenolic compound in the *Camellia sinensis* species was epigallocatechin gallate (EGCG), with epicatechin gallate (ECG) [3].

The following HPLC chromatograms were recorded for determining the caffeine peak from the various studied tea extracts: black, green, white. HPLC chromatograms of extracts were carried out in three different mediums: acid (Figure 7), alcoholic (Figure 8) and aqueous (Figure 9).

The caffeine was identified by comparing it's retention times with the caffeine standard retention time.

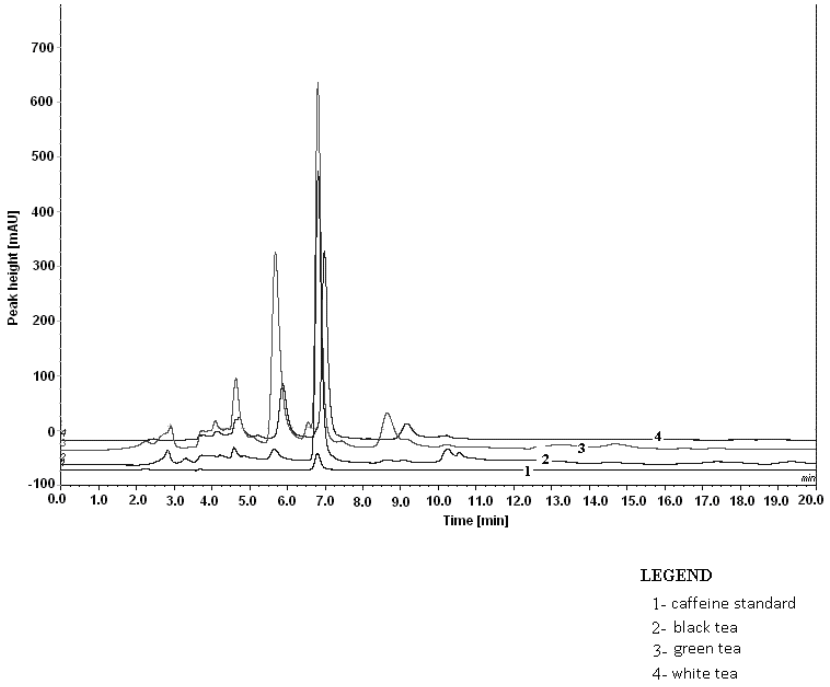

Fig. 7. HPLC chromatogram for tea extracts in acid medium

In Tables 2-4 are presented the chromatographic characteristics of caffeine standard and tea extracts in the three media: acid, alcoholic and aqueous.

Table 2. Chromatographic characteristics of caffeine standard and tea extracts in acid medium

sample	t_R [min]	Peak area [mAu·min]	Peak height [mAu]
Caffeine standard	6.79	5.2830	29.362
Black tea	6.813	95.7957	528.518
Green tea	6.808	109.6364	637.520
White tea	6.981	64.3253	340.689

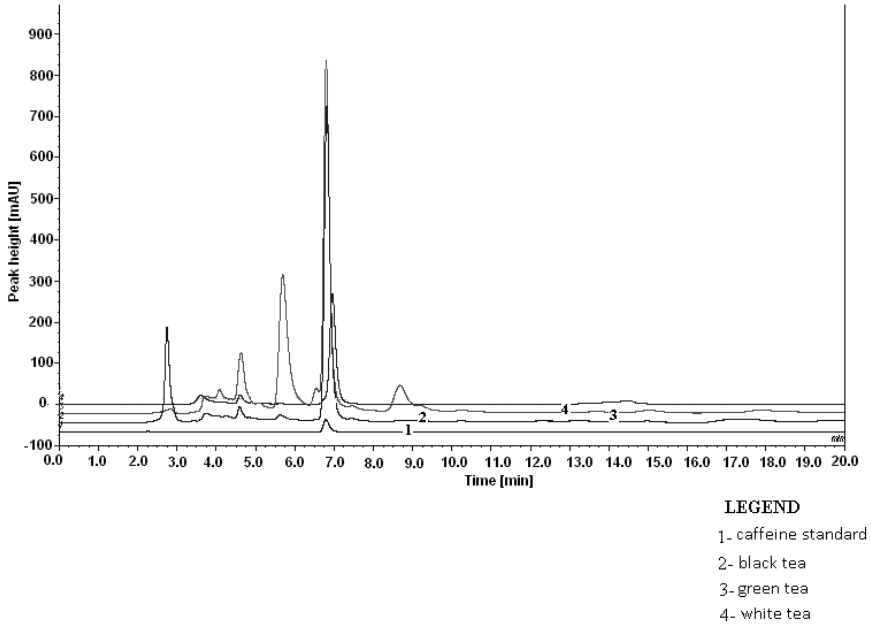

Fig. 8. HPLC chromatogram for tea extracts in alcoholic medium

Table 3. Chromatographic characteristics of caffeine standard and tea extracts in alcoholic medium

sample	t_R [min]	Peak area [mAu·min]	Peak height [mAu]
Caffeine standard	6.79	5.2830	29.362
Black tea	6.797	344.9006	1853.501
Green tea	6.774	255.0475	1335.420
White tea	6.943	45.4064	223.347

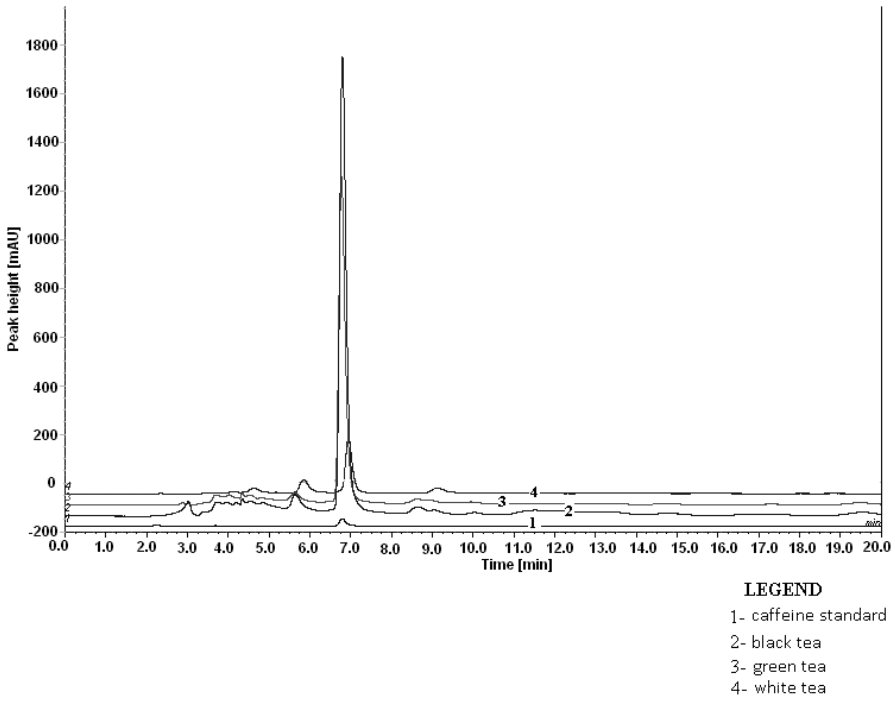

Fig. 9. HPLC chromatogram for tea extracts in aqueous medium

Table 4. Chromatographic characteristics of caffeine standard and tea extracts in aqueous medium

sample	t_R [min]	Peak area [mAu·min]	Peak height [mAu]
Caffeine standard	6.79	5.2830	29.362
Black tea	6.814	132.7592	757.992
Green tea	6.798	139.9885	813.569
White tea	6.958	51.8297	267.579

Analysis performed on different varieties of *Camellia sinensis* extracts in three different solvents leads to the identification of caffeine in tea extracts: white, black, green tea. The retention time of the standard caffeine and that of the caffeine from tea extracts were almost similar, which confirms the identity of caffeine.

4. Conclusions

In this paper, rapid and simple chromatographic and electrochemical procedures were developed for simultaneous determination of caffeine standard and caffeine from white, black, green tea in water, acid and alcohol extracts.

HPLC chromatography

By using HPLC method, the retention time and the relative peak area of extracted purified caffeine was determined. The retention time of the purified caffeine and that of the standard caffeine were almost similar, which confirms the presence of caffeine.

Overlapping the chromatograms for caffeine standard and caffeine from white, black, green tea extracts, it can be observed that the green tea presents highest caffeine contents, followed by black and white tea in aqueous and acid medium. In contrast, in alcoholic medium, caffeine content is highest in black tea extract.

This simple and easy technique can be applied satisfactory to any caffeine quantification in tea extracts.

The HPLC chromatographic studies results demonstrate that the solvent nature affect the caffeine content.

Cyclic voltammetry

This study of the electrochemical cyclic voltammetry behavior of caffeine content on glassy carbon electrodes in three different solutions leads to the following conclusions:

The influence of the solvent's nature

From cyclic voltammetry it can be observed that in the scanning potential domain, the base solution for the *Camellia sinensis species* is electrochemically inert.

The voltammograms presented in this paper showed that in acid and alcoholic medium, white tea has the most powerful antioxidant character. By contrast, in the aqueous medium, the most powerful antioxidant character is that of the green tea.

The electrochemical reactions of caffeine on all types of electrodes are irreversible and the oxidation peaks is much bigger than the reduction ones.

It was found that the electrochemical activity of caffeine depends on the solvent extract. The highest caffeine content was found in two types of teas: green and white tea.

The results of chromatographic studies for the determination of caffeine in different species of Camellia sinensis were almost similar to those obtained by Mumin et al in Determination and characterisation of caffeine in tea, coffe and soft drinks by solid phase extraction and high performance liquid chromatography (SPE-HPLC) journal, 2006 [10].

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