Phytochemical analysis of herbal teas containing caffeic acid

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Abstract

Objectives. The present analysis was to investigate the content of caffeic acid derivatives, antioxidant activity, and the presence of caffeic acid in alcoholic extracts obtained from 16 simple teas from the following medicinal plants: Vaccinium myrtillus, Camellia sinensis, Coffea arabica, Melissa officinalis, Ocimum basilicum, Rosmarinus officinalis, Salvia officinalis, Hyssopus officinalis, Artemisia absinthium, Cynara scolymus, Calendula officinalis, Coriandrum sativum, Foeniculum vulgare, Carum carvi, Rosa canina, Crataegus monogyna.

Materials and methods. The presence of caffeic acid was evaluated using the thin layer chromatography method (TLC). The total content of phenylpropanoids from the medicinal herbs alcoholic extracts was determined using a spectrophotometric method.

Outcomes. Results were in the range of 0.046-2.426% caffeic acid derivatives (CAE%). The antioxidant properties were measured using the DPPH radical scavenging mechanism. The results were found to be in the range of 63.93% and 0.38% inhibition level (I%). The greatest antioxidant activity was measured for Camellia sinensis (I = 45.54%), followed by Vaccinium myrtillus (I = 50.289%).

Conclusions. In this paper it was shown that these medicinal plants are rich in caffeic acid derivatives with antioxidant action capitalized in the prevention of serious diseases.

Keywords: caffeic acid, tea, antioxidant potential

INTRODUCTION

Caffeic acid is an aromatic compound of type C6-C3 (phenylpropane) that has actions: antioxidants, anti-inflammatory, immunomodulators, etc. Caffeic acid has a higher antioxidant activity against human LDL oxidation than p-coumaric acid and ferulic acid [1]. Its main function is to reduce the oxidative stress caused by various free radicals and to protect the body’s cells against their harmful action. Caffeic acid can be found in many herbs, such as green tea, blueberry, sage, basil. Due to its antioxidant activity, caffeic acid is used in the treatment of various medical conditions like AIDS, herpes, cancer, physical effort induced fatigue, and others.

There are various extraction methods, ranging from the most simple and conventional to ultramodern...
technology. The most commonly used method is heated alcohol extraction [2].

Modern techniques of extraction that even manage separation of cis and trans isomers of caffeic acid use, are high chromatography liquid chromatography, ultraviolet rays [3], ultrasound, gas chromatography [4].

The purpose of this study was to evaluate and compare the content of caffeic acid derivatives in various herbal products, conditioned in the form of medicinal tea, on the Romanian pharmaceutical market, as well as to evaluate their antioxidant activity.

MATERIALS AND METHODS

For the pharmacognostic study, 16 herbal teas from the Romanian market were analyzed. Based on literature data, Table 1 presents the content of caffeic acid derivatives expressed in caffeic acid equivalents (CAE) for each of the studied plant species: Vaccinium myrtillus, Camellia sinensis, Coffea arabica, Melissa officinalis, Ocimum basilicum, Rosmarinus officinalis, Salvia officinalis, Hyssopus officinalis, Artemisia absinthium, Cynara scolymus, Calendula officinalis, Carum carvi, Rosa canina, Crataegus monogyna.

Their chemical composition includes aromatic compounds of type C6-C3, phenylpropane derivatives, caffeic acid, chlorogenic acid, and others. They are used in phytotherapy due to their various pharmacological properties: antioxidant, cardioprotective, coronary dilator, anti-inflammatory, antimicrobial, cytoprotective [5].

Our study included three stages:
1. Qualitative determination of caffeic acid derivatives in the alcoholic extracts of the 16 plant products, using the thin layer chromatography (TLC) method.
2. Quantitative determination of caffeic acid derivatives in the alcoholic extracts, by spectrophotometric methods.
3. Evaluation of antioxidant activity using the DPPH method.

**Qualitative determination of caffeic acid derivatives by TLC**

Chromatography is a physico-chemical method used for separating substances from a mixture which is dissolved in a fluid called the mobile phase, which carries it through a system on which is fixed a material called the stationary phase. The method is based on the differentiated migration of the substances in the mixture, due to differences in adsorption, distribution, solubility, molecule size, etc. In the TLC method the stationary phase is represented by a fine and uniform adsorbent powder of chromatographic purity (silica gel, microcrystalline cellulose, aluminum oxide, polyamide, etc. with or without a binder) which is applied in a thin and uniform layer (~ 0.25 mm) on chromatographic plates [7]. The mobile phase may consist of a single solvent or, more frequently, of a mixture of solvents (methanol, chloroform, butanol, ethyl ether, toluene, ethyl acetate). The separation of the components from the mixture is based on the different distribution between the two phases: stationary and mobile. The detection of spots can be done in daylight and UV light as such or after the plate is treated with appropriate reagents [8,9].

The materials needed for the application of the samples and the subsequent processing of the data are: a Silicagel plate, the capillaries for manual sample application. After application the plate was placed in a tank containing the mobile phase which consists of: ethyl acetate, formic acid, acetic acid and water in a ratio of 100: 11: 11: 26. The plate was kept in contact...
with the mobile phase until it migrated to the front line. Once the front line was reached, the plate was removed from the tank and sprayed with the NEU-PEG detection reagent. After revelation, the plate was analyzed in the darkroom under UV light (365 nm), and the pictures were taken with the help of the Samsung Galaxy S5 mobile phone with a 16 megapixel camera.

The alcoholic plant extracts were prepared as follows: 2500 g of vegetal product mixed with 25 ml of 70° methyl alcohol were put to boil on the water bath for 30 minutes. The standard solution is a 1% caffeic acid solution in concentrated methyl alcohol [8,9].

Standard solutions, as well as the samples are applied as bands on the Silicagel plate. The size of the plates used is 20 * 10 cm. In order to be able to apply all 16 extracts, it was needed one plate, but for a more accurate determination, for the second analysis of the samples, two plates were used, applying 8 extracts on each plate. On both plates the first eight strips correspond to the vegetal extract, the first application being that of the caffeic acid standard (1% in methanol).

For the first plate (A) table 2 summarizes the method of applying the samples on a single plate and for the second application the plates (B, C) table 3 respectively table 4.

**Quantitative determination of of caffeic acid derivatives**

The principle of the method is based on the property of phenols to form nitro compounds with the Arnow reagent, which are then isomerized to oximes, which due to their weakly acidic characteristic, dissolve in alkaline agents, resulting in a red coloured solution [10].

2500 g of vegetal product powder is extracted in 25 ml of 70% methyl alcohol in a round-bottomed flask, which is heated at reflux on the water bath, where it is kept for 30 minutes from when it starts to boil. The hot solution is filtered through a paper filter, in a graded cylinder and after it has cooled to make up to 25 ml, the residue is washed with the same solvent, solution A, and poured in the cylinder in order to reach 25 ml. 5 ml of this extractive solution is diluted with methanol to 10 ml in a flask rated solution B.

To continue, 1 ml of this solution was poured in a volumetric flask, and then, the following were added: 1 ml of 0.5 N hydrochloric acid, 1 ml of Arnow reagent (R), 1 ml of 1N sodium hydroxide (in this precise order)

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**TABLE 2. Sample application on plate A**

| Band  | Sample                  | Applications (µl) |
|-------|-------------------------|-------------------|
| 1     | 0- Standard of acid cafeic | 3x0,5             |
| 2     | 1- Vaccinium myrtillus   | 3x0,5             |
| 3     | 2- Camellia sinensis     | 3x0,5             |
| 4     | 3- Coffea arabica        | 3x0,5             |
| 5     | 4- Mellisa officinalis   | 3x0,5             |
| 6     | 5- Ocimum basilicicum    | 3x0,5             |
| 7     | 6- Rosmarinus officinalis| 3x0,5             |
| 8     | 7- Salvia officinalis    | 3x0,5             |
| 9     | 8- Hyssopus officinalis  | 3x0,5             |
| 10    | 9- Artemisia absinthium  | 3x0,5             |
| 11    | 10- Cynara scolymus      | 3x0,5             |
| 12    | 11- Calendula officinalis| 3x0,5             |
| 13    | 12- Coriandrum sativum   | 3x0,5             |
| 14    | 13- Foeniculum vulgare   | 3x0,5             |
| 15    | 14- Curum carvi          | 3x0,5             |
| 16    | 15- Rosa canina          | 3x0,5             |
| 17    | 16- Crataegus monogyna   | 3x0,5             |

**TABLE 3. Sample application on plate B**

| Band  | Sample                  | Applications (µl) |
|-------|-------------------------|-------------------|
| 1     | 0- Standard of acid cafeic | 2x0,5             |
| 2     | 1- Vaccinium myrtillus   | 3x0,5             |
| 3     | 2- Camellia sinensis     | 3x0,5             |
| 4     | 3- Coffea arabica        | 3x0,5             |
| 5     | 4- Mellisa officinalis   | 3x0,5             |
| 6     | 5- Ocimum basilicicum    | 3x0,5             |
| 7     | 6- Rosmarinus officinalis| 3x0,5             |
| 8     | 7- Salvia officinalis    | 3x0,5             |
| 9     | 8- Hyssopus officinalis  | 3x0,5             |

**TABLE 4. Sample application on plate C**

| Band  | Sample                  | Applications (µl) |
|-------|-------------------------|-------------------|
| 1     | 0- Standard of acid cafeic | 2x0,5             |
| 2     | 9- Artemisia absinthium  | 3x0,5             |
| 3     | 10- Cynara scolymus      | 3x0,5             |
| 4     | 11- Calendula officinalis| 3x0,5             |
| 5     | 12- Coriandrum sativum   | 3x0,5             |
| 6     | 13- Foeniculum vulgare   | 3x0,5             |
| 7     | 14- Curum carvi          | 3x0,5             |
| 8     | 15- Rosa canina          | 3x0,5             |
| 9     | 16- Crataegus monogyna   | 3x0,5             |
Water was added in small portions and under continued stirring, to get to a volume of 10 ml. The mixture turned to a rather red solution. After it was allowed to sit for 10 minutes, the absorbance of the solution was determined at a wavelength of 500 nm, using as a control a mixture obtained from 1 ml of solution A, 1 ml of 0,5N hydrochloric acid, 1 ml of Arnow reagent (R), 1 ml 1N sodium hydroxide and water up to 10 ml.

If the extinction of the sample is lower than 0.15 or higher than 0.30, appropriate dilutions shall be made to obtain values within this range, which shall comply with the Lambert-Beer law.

The concentration of phenylpropane compounds, namely caffeic acid, is calculated using a standard curve. In a series of 10 ml volumetric flasks, 0.0125% caffeic acid solution is added with the pipette in volumes of 0.1; 0.2; 0.3; 0.4; 0.5 ml. To this is added as follows: 1 ml of 0.5N hydrochloric acid, 1 ml of Arnow reagent (R) and gently shake to mix. Then 1 ml of sodium hydroxide 1N is added. A red coloration appears. The absorbance of each sample is measured (λ = 500 nm) after dilution and homogenization of the sample, up to mark with 70% methanol and homogenizing.

With the standard caffeic acid absorbance values in relation to their corresponding concentrations, the standard curve is drawn using the equation: $y = 0,0790 + 1,4752x$; where: $y =$ absorbance of the sample at 500 nm, $x =$ concentration of caffeic acid corresponding to the measured absorbance expressed in mg per 100 g of plant product.

The concentration of phenylpropane compounds can be calculated using the formula: $C% = x \cdot \frac{10/5 \cdot 25}{a \cdot 100/1000} = \frac{5 \cdot x}{a}$; where $a =$ the amount of plant product used and $x =$ the concentration of caffeic acid present in the measured absorbance expressed in mg per 100 g of plant product.

Evaluation of antioxidant activity by DPPH method

Antioxidants are compounds that have the ability to inhibit the oxidation of other substances. Oxidation processes usually result in free radicals (reactive oxygen species = ROS), which are indispensable for the normal functioning of cells when their level is within physiologic limits, but, when exceeding, they can affect the structure and normal function of cells.

Natural products are one of the main sources of antioxidants, through the active compounds with polyphenolic structure (flavonoids, polyphenolcarboxylic acids, tannins etc.), triterpene compounds, carotenoids, etc. which can be used for therapeutic or prophylactic purposes.

The evaluation of antioxidant activity of the compounds in a plant product can be done through various methods that have as principle the neutralization of oxygen radical, hydrogen peroxide or DPPH free radical. The DPPH method is a spectrophotometric method, widely used to test the ability of substances to neutralize free radicals or their ability to donate hydrogen. The method is based on the reaction of sample solutions (plant extracts) with the stable radical 2,2-diphenyl-pycrylhydrazil (DPPH) in an alcoholic solution. For the evaluation of the antioxidant activity (AOA) DPPH is used as a radical source, using a spectrophotometric method for monitorization. The purple DPPH radical is reduced in the presence of an antioxidant to a pale yellow compound; the DPPH absorbance variation is measured at 517 nm. [10]. 1 ml of methanolic extract of plant products (10%) diluted (200-300 times); 0.25, 0.5, 1.0, 1.25, 1.5, 1.75, 2.0 ml were brought to 2.0 ml with methanol and mixed with 2 ml methanolic solution DPPH (0.1 g/L-1). Place on the water bath at 40°C for 30 minutes, then read the absorbance at 517 nm against a DPPH solution consisting of 2 ml of 10% DPPH solution and 2 ml of methanol. Calculate the percentage of inhibition, $I\%$, using the following formula: $I(\%)=\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$; where: $A_{\text{control}}$ is the absorbance of the DPPH radical + methanol (the witness solution contains all reagents except for the tested sample), $A_{\text{sample}}$ is the absorption of the radical DPPH + sample [11-14].

RESULTS AND DISCUSSIONS

Qualitative determination of caffeic acid derivatives

The identification of caffeic acid was based on the fluorescence of this phenolic compound. Being only a qualitative analysis of methanolic extracts of plant products and not being coupled with another method of quantitative dosing, the assessment of the presence of caffeic acid was made visually after exposure of the plates in UV light (365 nm), then calculating RF values for the spots that presented interest (Fig. 1,2 3). Thus, from the visual analysis of the plates, especially of plates B and C which have only 9 bands each, one of
which is that of the 1% caffeic acid standard, we can say that in extracts 3, 4, 5, 6, 7, 8 (plate B) caffeic acid was identified, with the mention that spots 3, 6, 7 have a higher intensity. From the analysis of plate C, it can be stated that we have a weak presence of caffeic acid in samples 11, 12, 13, 16.

Following the visual analysis in UV spectre of the chromatographic plates and the calculation of the Rf for each spot, the reporting being made to the Rf of the caffeic acid standard (~ 0.9) the presence of caffeic acid could be qualitatively determined.
Thus, it can be stated that caffeic acid was identified in the following extracts of: *Coffea arabica*, *Mellisa officinalis*, *Ocimum bassilicum*, *Rosmarinus officinalis*, *Salvia officinalis*, *Hyssopus officinalis*, *Calendula officinalis*, *Coriandrum sativum*, *Foeniculum vulgare*, *Crataegus monogyna*.

**Quantitative determination of caffeic acid derivatives**

The results obtained from the quantitative determination of phenylpropane compounds by spectrophotometric method are presented in Table 5, which are expressed in equivalents of caffeic acid (%).

As can be seen, the highest amount of phenylpropane compounds was determined in the extract of *Mellisa officinalis* (2.426%) followed by the extract of *Rosmarinus officinalis* (2.234%), these being the only ones that exceed the value of 2%. A concentration of over 1.5% phenylpropane compounds is found in *Vaccinium myrtillus* (1.658%) and between 1.5% and 1% we have several plant products (presented in descending order): *Coffea arabica* (1.390%), *Rosa canina* (1.388%), *Crataegus monogyna* (1.292%), *Ocimum bassilicum* (1.152%), *Hyssopus officinalis* (1.054%). Next, concentrations between 1%-0.5% were determined in *Artemisia absinthium* (0.936%), *Salvia officinalis* (0.854%), *Camelia sinensis* (0.746%), *Calendula officinalis* (0.524%). Below 0.5% phenylpropane compounds were determined in *Foeniculum vulgare* (0.420%), and low concentrations in *Carum carvi* (0.162), *Cynara scolimus* (0.112%), the
lowest being in Coriandrum sativum (0.046%). Some concentrations obtained in this study do not correspond to the data obtained by other researchers, this may be due to several reasons such as: different methods of determining the concentration of caffeic acid, different pedo-climatic conditions, the origin of the plant product studied (from different medicinal teas) the freshness of the product at the time of making the determinations, etc.

Antioxidant activity assessment using DPPH method

The results obtained after evaluating the antioxidant activity of the extracts obtained from the studied vegetal products are presented in table 6, the inhibition being presented as a percentage (I%).

Before interpreting the results, an important mention would be the fact that in the case of extracts “1” and “2” the determinations were performed on dilute solutions compared to the rest of the extracts, namely: for extract “1” a dilution of 1: 2.5 was performed and for extract “2”, a dilution of 1: 5. Thus, in the case of extract “1” instead of 50 µl solution, the determination was performed on 20 µl solution, and in the case of extract “2” the determination was performed on 10 µl solution.

The highest percentage of inhibition, given the effective dilutions, is in extract no. 2 (Camellia sinensis) - 45.540% (1: 5 dilution), green tea being the best antioxidant among the products studied. Vaccinium myrtillus also has a high antioxidant action, the inhibition percentage being 50.289% (1: 2.5 dilution).

Increased antioxidant activity also shows the following plant products: Mellisa officinalis (63.939%)> Rosmarinus officinalis (60.625%)> Rosa canina (58.944%)> Coffea arabica (55.788%), all of which have a percentage of inhibition greater than 50. Of the products with less than 50% inhibition, Crataegus monogyna (44.047%) and Ocimum basilicum (33.120%) stand out, the rest having a low antioxidant activity, the lowest being Coriandrum sativum (0.386%), Cynara scolymus (1.627%) and Carum carvi (3.730%).

The results obtained from the determination of antioxidant activity largely correspond to the results obtained from the quantitative dosing of phenylpropane compounds by the spectrophotometric method, in the sense that a high

| No. | Sample Absorbance | Inhibition percentage (%) |
|-----|-------------------|---------------------------|
| 0.  | Soluție DPPH 1,7077 | -                         |
| 1.  | Vaccinium myrtillus 0,8489 | 50,289*                  |
| 2.  | Camellia sinensis 0,9300 | 45,540**                 |
| 3.  | Coffea arabica 0,7550 | 55,788                    |
| 4.  | Mellisa officinalis 0,6158 | 63,939                   |
| 5.  | Ocimum basilicum 1,1421 | 33,120                    |
| 6.  | Rosmarinus officinalis 0,6724 | 60,625                   |
| 7.  | Salvia officinalis 1,2899 | 24,465                    |
| 8.  | Hyssopus officinalis 1,4168 | 17,034                    |
| 9.  | Artemisia absinthium 1,5186 | 11,073                    |
| 10. | Cynara scolymus 1,6799 | 1,627                     |
| 11. | Calendula officinalis 1,5834 | 7,278                     |
| 12. | Coriandrum sativum 1,7011 | 0,386                     |
| 13. | Foeniculum vulgare 1,6091 | 5,773                     |
| 14. | Carum carvi 1,6440 | 3,730                     |
| 15. | Rosa canina 0,7011 | 58,944                    |
| 16. | Crataegus monogyna 0,9555 | 44,047                    |

| No. | Samples | Caffeic acid derivatives (CAE%) | % |
|-----|---------|---------------------------------|---|
| 0.  | Soluție DPPH | -                              | - |
| 1.  | Vaccinium myrtillus 1,658 | 50,289                      |
| 2.  | Camellia sinensis 0,746 | 45,540                      |
| 3.  | Coffea arabica 1,390 | 55,788                      |
| 4.  | Mellisa officinalis 2,426 | 63,939                      |
| 5.  | Ocimum basilicum 1,152 | 33,120                      |
| 6.  | Rosmarinus officinalis 2,234 | 60,625                     |
| 7.  | Salvia officinalis 0,854 | 24,465                      |
| 8.  | Hyssopus officinalis 1,054 | 17,034                      |
| 9.  | Artemisia absinthium 0,936 | 11,073                      |
| 10. | Cynara scolymus 0,112 | 1,627                       |
| 11. | Calendula officinalis 0,524 | 7,278                       |
| 12. | Coriandrum sativum 0,046 | 0,386                       |
| 13. | Foeniculum vulgare 0,420 | 5,773                       |
| 14. | Carum carvi 0,162 | 3,730                       |
| 15. | Rosa canina 1,388 | 58,944                      |
| 16. | Crataegus monogyna 1,292 | 44,047                      |
concentration of phenylpropane compounds determines an increased percentage of inhibition.

As can be seen in Table 7, Melissa officinalis, which has the highest amount of phenylpropane compounds 2,426% has a high antioxidant activity of 63.939%, and Rosmarinus officinalis with 2.234% phenylpropane compounds has an inhibition rate of 60.625%. In the category of products with low concentration of phenylpropane compounds are Coriandrum sativum with a quantity of caffeic acid of 0.046% but which has a low antioxidant activity (I% = 0.386) and Cynara scolymus with (0.112%) phenylpropane compounds => (1.627%) percentage of inhibition.

The biggest differences between the results of spectrophotometry and those from the evaluation of antioxidant activity are recorded in Camellia sinensis and Vaccinium myrtillus. Table 7 shows written in green the plant products which have a direct proportionality between the amount of phenylpropane compounds expressed in caffeic acid and the percentage of inhibition and written in red the extracts which show an indirect proportionality.

CONCLUSIONS

In this study 16 teas from the Romanian pharmaceutical market were analyzed, their choice being made randomly, without taking into account the manufacturing company.

These vegetable products contain aromatic compounds type C6-C3, caffeic acid, chlorogenic acid and isomers, cyanin and isomers, coumaric acid, cinnamic acid, etc. and are used in therapy for properties like: antioxidant, anti-inflammatory, antibacterial, diuretic, cholagogue-choleretic, expectorant etc.

Quantitative analysis of phenylpropane compounds in the plant products studied was performed by a spectrophotometric method, the highest concentrations being obtained in the species: Melissa officinalis, Rosmarinus officinalis, Vaccinium myrtillus, Coffea arabica, Rosa canina and Crataegus monogyna, and the identification of caffeic acid was performed with the help of thin layer chromatographic method.

The antioxidant activity of plant extracts was evaluated by the DPPH method, obtaining a high antioxidant effect for Camellia sinensis and Vaccinium myrtillus species.

The results obtained from performing these determinations can confirm the therapeutic indications of some dietary supplements on the Romanian pharmaceutical market, in order to use them safely and efficiently.

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