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DETERMINATION OF CAFFEINE IN THE LEAVES AND FLOWERS OF *CAMELLIA CHRYSANTHA* BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DAD DETECTION

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Abstract. Caffeine (1,3,7-trimethylxanthine) is naturally found in the leaves, seeds or fruits of over 63 plants species worldwide. It is contained highly in coffee, tea, cacao beverage products. Caffeine showed good effects in enhancing performance and perception of exercise, reducing fatigue and drowsiness, and improving memory and learning effects. Although caffeine intoxications are rare, they showed its unhealthy potential factor, resulting in tachycardia, atrial arrhythmias, convulsions or even coma. Thus, the national standard technical committee has limited the caffeine content in beverage products. In this paper, we present an HPLC method for determination of caffeine. The results show optimal conditions for the rapid analysis of caffeine with high precision and accuracy which are suitable for its determination in the plant. The obtained results revealed that no caffeine is detected in the leaves and flowers of golden camellia (*Camellia chrysantha* (Hu) Tuyama). Thus, we may suggest that using golden camellia leaves and flowers as natural alternatives to current decaffeinated tea would avoid some unwanted side effects of caffeine.

Keywords: caffeine, *Camellia chrysantha*, Golden Camellia, HPLC method.

Classification numbers: 1.1.1, 1.1.3.

1. INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is the active alkaloid component which is naturally found in the leaves, seeds or fruits of over 63 plants species worldwide. It was found in the samples of green teas, oolong teas, black teas, and pu-erh teas as well as in fresh leaves. Many studies have demonstrated the biological effects of caffeine in enhancing performance and perception of exercise in young and elderly people [1]. It stimulates the central nervous system to reduce fatigue and drowsiness, and enhance learning and memory effects [2]. Caffeine has also used in prevention and treatment of bronchopulmonary dysplasia in premature infants, as

well as language and cognitive delay [3, 4]. It appeared that caffeine improved airway function in people with asthma, increasing forced expiratory volume up to 18 % [5]. When combined with paracetamol or ibuprofen, caffeine improved pain rapidly in 10 % of people [6].

Beside of good maner, caffeine has claimed for its many side effects on human health such as cardiovascular disease [7, 8], heart rate variability [9], ischemic stroke as a result of arrhythmia [10], aerial stiffness, further elevating the arterial pressure [11], and miscarriages [12]. Thus, caffeine has been limited in cacaos and beverage products by the national standard technical committee with a TCVN 11037:2015 number.

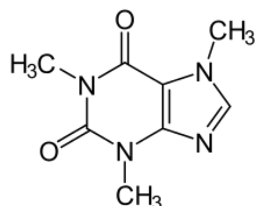

Figure 1. Chemical structure of caffeine.

Today, caffeine is determined by HPLC method, an easy and accurate procedure published by Directorate for Standards, Metrology and Quality (STAMEQ). It appears as high content in coffee and green tea, and the quantity could be influenced by brewing process [13]. However, researchers have found the absence of caffeine in yellow tea by a quantitative analysis of six different yellow tea leave samples which collected at Yellow Camellia Garden in Guangxi, China [14].

Camellia chrysantha (Theaceae), from a group of yellow-flowering species called golden camellia which is native to Viet Nam, is grown as an ornamental plant worldwide [15]. *C. chrysantha* leaves's extracts have shown antioxidant activities in DPPH radicals, superoxide anions, and hydroxyl free radicals scavenging assays [16]. Many evidences have suggested the important roles of oxygen free radicals in the expansion of tumor clones, acquisition of malignant properties [17], and development of hyperlipidemia [15]. Golden camellia has shown special benefits of reducing the risk of atherosclerosis caused by blood lipids, regulation of blood pressure and blood sugar, treatment of dysentery and hematochezia [18]. In this study, we described the caffeine content in the fresh leave and flower materials of *C. chrysantha* which collected from Ba Che (Quang Ninh), Viet Nam.

2. MATERIALS AND METHODS

2.1. Materials

Plant materials: The leaves and flowers of *Camellia chrysantha* (Hu) Tuyama were collected in Ba Che commune, Quang Ninh province, Vietnam in February, 2015, and identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, VAST, Viet Nam. A voucher specimen (THV08.2015) was deposited at the Herbarium of Institute of Natural Products Chemistry (VAST).

Standard: The reference standard of caffeine (> 98 % purity) was purchased from Chengdu Biopurity Phytochemicals, China.

Chemicals: HPLC-grade methanol and acetonitrile were purchased from Merck, Germany. Other reagents were of analytical grade and distilled water was used throughout the experiment.

Equipments: The analysis was performed on a liquid chromatography Agilent 1260 system (USA) composed of LC-10Advp pump, an SIL-20AC autosampler, a Zorbax Eclipse XDB-C18 (4.6 × 150 mm I.D, 5 µm particle size) column and an UV-Vis/DAD detector. The data were aquired by an Agilent LC solution software.

2.2. Methods

Preparation of samples: After collection, the plant samples were separated into different parts of leaves and flowers, and then they were pulverized and dried at 50 °C. The dried materials were extracted using sonication with methanol for 2 hours at 50 °C, then filtered. The process was repeated three times. The extracts were combined and evaporated to dryness in vacuum to give dark solid extracts which were stored in a refrigerator prior to use.

Sample solutions were prepared in a similar manner to the standard solution: 1 mg of extract was dissolved in 1 mL of methanol using sonication for 10 min at 30 °C.

Chromatography: The mobile phase was composed of a gradient of methanol in water (15:85 v/v) in 60 min. After preparation, the mobile phase was filtered through a 0.22 µm nylon membrane and subsequently ultrasonically degassed before being used. The chromatographic separation was conducted on a Zorbax Eclipse XDB-C18 (4.6 × 150 mm I.D, 5 µm particle size) column. The column temperature was kept steady at 30 °C. The DAD wavelengths were scanned from 190 to 400 nm.

3. RESULTS AND DISCUSSION

3.1. Calibration and linearity

The UV spectrum (Figure 2a) of the analytes was obtained from the peak raised in the HPLC spectrum (Figure 2b) to determine the optimal wavelengths as shown in Table 1. Caffeine was detected by DAD detector at 280 and 365 nm wavelengths which were selected through scanning the wavelength range of caffeine and combining it with the reference [17]. Peak signal indicates the presence of caffeine at 32.529 min in Figure 2.

Table 1. HPLC programme and parameters for determination of caffeine.

HPLC programme and parameters
Injection volume: 10 µL
Column temperature: 30 °C
Column: XDB-C18 (4.6 × 150 mm; 5 µm particle size)
Mobile phase: methanol (Channels A)/ H_2O + 0.1 % formic acid (Channels B) = 15:85
Flow rate: 0.5 mL/min
Data channels: 280 and 365 nm

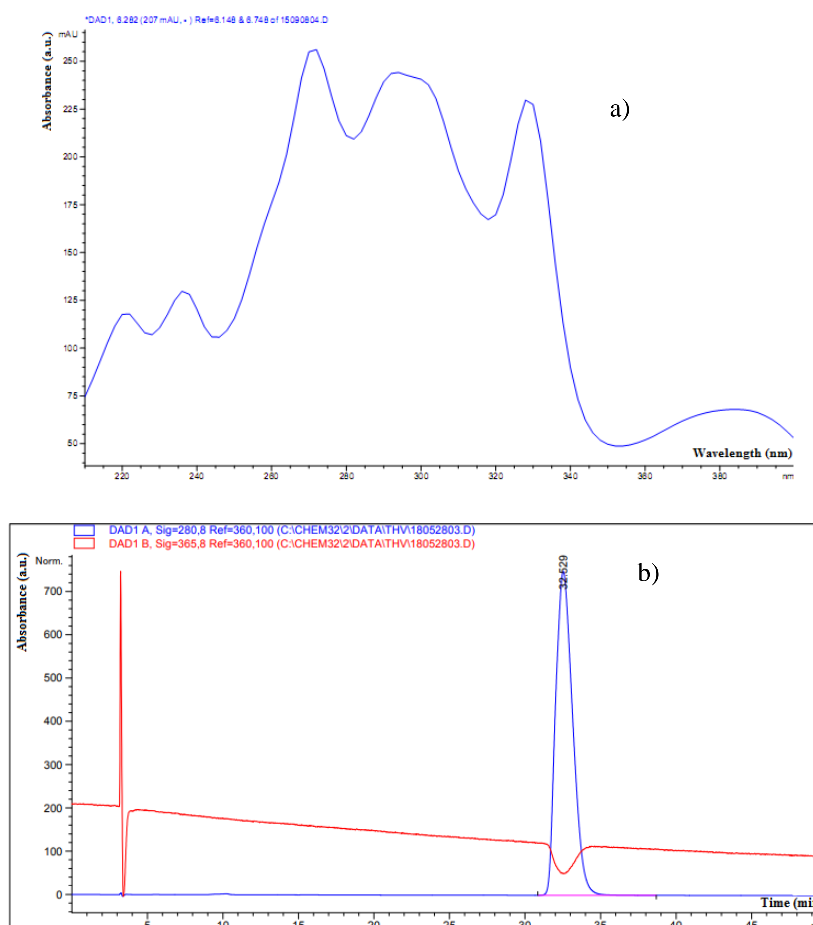

Figure 2. (a) UV-spectrum of caffeine showing maximal absorptions at 275, 295 and 330 nm; (b) HPLC chromatogram of caffeine showing retention time at 32.529 min.

Table 2. Calibration curve, regression coeffecient and linear relationship of caffeine.

Calibration curve	**Concentration**	**Peak area**
r^2: 0.99951	50 ppm	1009.00
a: 20.63650	100 ppm	2035.70
b: 26.14389	250 ppm	5358.70
y = area	400 ppm	8414.60
x = concentration	500 ppm	10166.00

The linearity of the method was evaluated by analyzing a series of standard caffeine. Ten microliters of each of the five working standard solutions containing 50 – 500 ppm of standard caffeine was injected into the HPLC. The elution was carried out as the above described method, and the standard calibration curve was obtained by plotting the concentration of standard caffeine versus peak area. Good linear relationship of caffeine was obtained within the

concentration range of 50 – 500 ppm and the regression coefficient (r^2) and the slope of the calibration curve were determined to be 0.99951 and 20.63650, respectively (Table 2).

3.2. Precision of method

Precision of the method was investigated by measuring the peak areas of the same standard solution at a concentration of 10 ppm on the six consecutive assays and was evaluated by the values of SD (Standard Deviation) and RSD (Relative Standard Deviation) of the measurements (Table 3). The obtained results revealed that this method has good precision with standard deviation and relative standard deviation values of 0.1470 and 0.0136 ppm, respectively.

Table 3. SD, RSD, LOD, and LOQ values of caffeine.

No.	Initial Concentration	Detected concentration	Mean value (ppm)	SD (ppm)	RSD (ppm)	LOD (ppm)	LOQ (ppm)
1	10 ppm	10.7889 ppm	10.7820	0.1470	0.0136	0.0235	0.0712
2	10 ppm	10.8428 ppm					
3	10 ppm	10.8581 ppm					
4	10 ppm	10.9095 ppm					
5	10 ppm	10.7885 ppm					
6	10 ppm	10.4958 ppm					

3.3. Detemination of LOD and LOQ of caffeine

The limit of detection (LOD) and limit of quantitation (LOQ) were determined from the calibration curve of each standard. LOD was calculated according to the expression **3.3*SD/a** where **SD** is the standard deviation of the response and **a** is the slope of the calibration curve. LOQ was established by using the expression **10*SD/a**.

According to the data obtained in Table 3, the LOD and LOQ of caffeine were 2.350 and 7.122 %, respectively.

3.4. Application to the quantitation of caffeine in *Camellia chrysantha*

The contents of caffeine in the leaves and flowers of *C. chrysantha* were calculated on the basis of linear calibration function and with regard to the dilution factor. The sample volumes of the leaf and flower extracts of *Camellia chrysantha* which were injected and obtained chromatographies are shown in Figure 3. According to the result, no peak was observed around the retention time of 32.529 min in both the leave and flower extracts, indicating that these materials may not contain any caffeine.

Caffeine is well known for its stimulant properties, being constituent of drinks, foods and plenty of drugs available on the market. In our study, no caffeine was detected in the leaves and flowers of golden camellia *C. chrysantha*. This feature makes a difference between golden camellia and other camellia species.

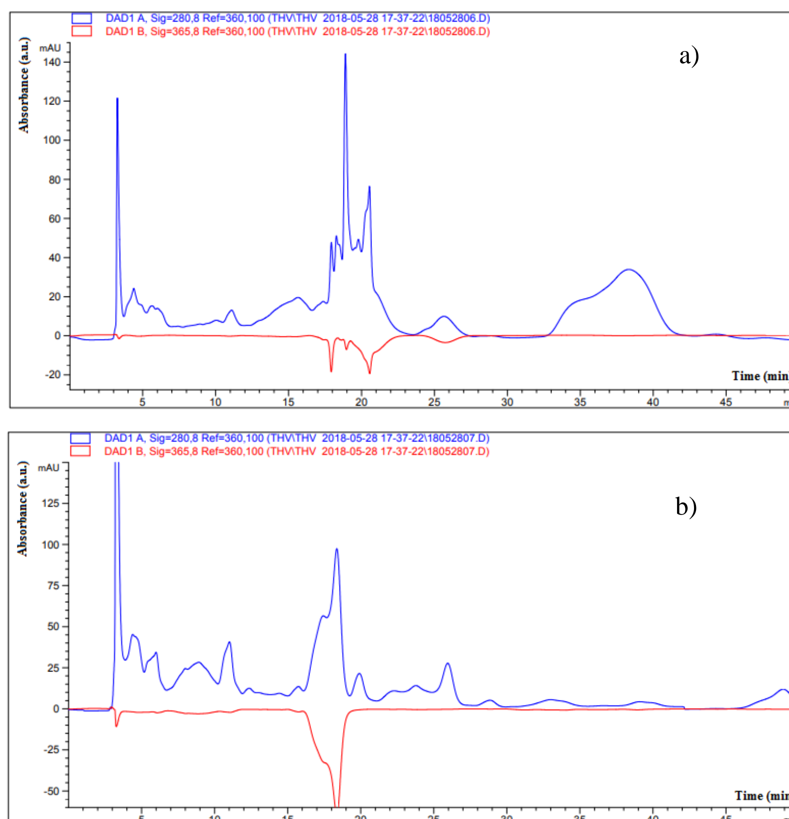

Figure 3. HPLC chromatograms: (a) Leaf extract; (b) Flower extract of *Camellia chrysantha.*

4. CONCLUSION

A reliable HPLC method with DAD detection was developed for analysis of caffeine content in different parts of *Camellia chrysantha* (Hu) Tuyama. This method was successfully employed and applicable for routine quality assurance of caffeine in *C. chrysantha*. The results showed that no caffeine was detected in the leaves and flowers of *C. chrysantha*. Thus, it is suggested that taking golden camellia tea for a long period may not be suffered from caffeine-induced side effects and using it as a natural alternative to current caffeine-free tea.

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