

Full Length Research Paper

Characterization of caffeine and determination of caffeine in tea leaves using uv-visible spectrometer

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In this research, characterization of pure caffeine and the method for determination of caffeine content in twelve commercial tea leaves have been reported using UV-visible spectrometer. The optical transition properties of caffeine were measured in different solvents (dichloromethane, water, chloroform and ethyl acetate). The results show that, caffeine has highest optical transitions in dichloromethane than the other solvents. After characterization of the electron transition, the content of caffeine in 6 Ethiopian black and green tea leaves and 5 different black tea leaves from South Africa, Kenya, 2 India, Zimbabwe, and Sri Lanka green teas, were analyzed at 30°C. Four Ethiopian tea samples were also analyzed at the boiling temperature using smaller extraction time. The result of the experiment at both temperatures indicates that, the caffeine content of Ethiopian green tea leaves is greatest of all the Ethiopian teas while black lion tea has the least. Of all the samples tested, Kenyan black tea had the highest caffeine content and Sri Lankan green teas had the least caffeine. The result also indicates that, caffeine can be extracted more at the boiling temperature than at 30°C. Furthermore, the absorption range of cinnamon tea was greater than the others by 16 nm.

Key words: Tea leaves, caffeine, extraction, UV-visible spectrometer, optical transition properties.

INTRODUCTION

Tea refers to the agricultural products of the leaves, leaf buds and internodes of the *Camellia sinensis* plant. It has been consumed as a beverage for almost 2,000 years starting in China. It is the most widely consumed beverage after water (Alan and Iris, 2004).

Teas from many areas may be blended. The aim is to obtain better taste, higher price or both, as a more expensive, better tasting tea may cover the inferior taste of cheaper varieties. There are two major kinds of tea, black tea and green tea. Both contain caffeine (1 to 5) % of its dry weight (Amro et al., 2006) depending on type, brand (Bennett and Bonnie, 2001) and brewing method (Hicks et al., 1996). This is why the reported values in the literature are so variable.

Caffeine belongs to a family of naturally occurring components known as xanthines. The xanthines which come from plants are possibly the oldest known stimulants. Caffeine is the most powerful xanthine, in its ability to increase alertness, put off sleep and to improve

attention in study (Boltonad, 1981), caffeine is a vasodilator (relaxes the blood vessels) as well as a diuretic (increase urination). On the other hand, severe restlessness and excitement, leading to mild delirium, muscular tension and twisting and cardiovascular disturbances such as tachycardia, are negative effects of caffeine at large doses (Boltonad, 1981). The spinal cord is stimulated at higher doses, convulsions and death may result (Bolton and Null, 1981).

Many consumers prefer to avoid caffeine partially or altogether, due to its stimulant effects and others, still on health concern. This makes decaffeination of tea an important industrial process. In addition, caffeine has a slightly bitter flavor. As a result, decaffeinating coffee beans and tea leaves will leave the flavor slightly changed, even if no other components are lost. It should be noted that, decaffeinated coffee and tea are not caffeine free.

Decaffeination is done by the use of solvent, which extract caffeine. For this purpose, the currently available solvents are chloroform, dichloromethane, ethyl acetate, super critical carbondioxide, etc. Dichloromethane is used to decaffeinate a high proportion of conventional

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teas. It is also a relatively non-toxic solvent and is often used in place of chloroform. Ethyl acetate also removes caffeine from tea leaves effectively; it can also extract other chemical components as well (Abdul Mumin et al., 2006). Studies on green tea, decaffeinated with ethyl acetate, have shown a potential of up to 30% of epigallocatechin gallate (EGCG, considered to be the beneficial component in green tea) and other beneficial antioxidant compounds, to be extracted along with caffeine (Kirmer, 1988; Johnson, 1988; Jefferson, 1998).

Due to the wide spread consumption of caffeine, it is important to collect precise information of their content in foods. Most research activities have been focused on chromatographic methods; however, spectrophotometric determination is preferred because of its rapidity, high accuracy and reproducibility (Komes et al., 2009). Furthermore, UV-visible spectrophotometer is cheap and it is found in many laboratories. But caffeine content in tea leaves cannot be determined directly using UV visible spectrometer, due to the matrix effect of UV absorbing substances (Guzin, 2002).

In this study, we have developed a method to use UV-visible spectrometer for the determination of caffeine in tea leaves. That is, dissolving tea leaves first in distilled water and then extracting caffeine by dichloromethane and analyzing it using UV-visible spectrophotometer, to define the caffeine content of different Ethiopian tea leaves and to examine the extraction efficiency of caffeine by hot water, with respect to extraction time and temperature. For the comparison purpose, caffeine content of Sri Lankan green tea, Kenyan, Indian, South African, and Zimbabwean black tea leaves were also analyzed. The method includes characterizing pure caffeine in water, dichloromethane, chloroform and ethyl acetate.

MATERIALS AND METHODS

Samples

A set of twelve commercial tea samples made up of, five Ethiopian black teas (Addis, Black lion, Cinnamon, Tosign and Ginger), one Ethiopian green tea, one Sri Lankan green tea, two Indian (Tatagold, Taj Mahal), one South African, one Kenyan and one Zimbabwean (Taganda) black tea. All Ethiopian tea samples and Sri Lankan green tea were from supermarkets in Addis Ababa. The other tea leaves were purchased from their respective countries. These samples were from tea bags except black lion and Indian Tatagold black tea. Cinnamon, tosign, and ginger tea leaves are blended teas with cinnamon, tosign and ginger respectively.

Materials and chemicals

UV-visible spectrometer (Perkin-Elmer Lambda 19, wavelength range 170 nm to 3200 nm, double monochromator), microbalance (Mettler At250, 0.1/0.01 mg) for measuring mass of caffeine, balance (BP 210S, $d = 0.1$ mg, Max 210 g) for measuring mass of tea leaves, measuring cylinder, magnetic stirrer, glass filter (2

'Pyrex', England), beakers, thermometer, 1 cm quartz cuvette, separatory funnel, funnel, ice bath, distilled water, dichloromethane (assay: 99.6%, Aldrich Germany), chloroform (assay: 99.8%, European Union), ethyl acetate (assay: 99%, Indian), caffeine (M.W. 194.19 g/mol, Aldrich Germany).

Preparation of samples and standard solution

Standard solution preparation

To characterize caffeine in different solvents and to determine caffeine content in different samples of tea, solutions of caffeine in different solvents were prepared. 0.46 mg, 1.40 mg, 0.85 mg and 0.80 mg of pure caffeine was immersed into 25 ml, 50 ml, 50 ml and 40 ml of dichloromethane, water, chloroform and ethyl acetate respectively and stirred by magnetic stirrer for 30 min. The absorbance versus wavelength of the solution was measured using UV-visible spectrometer. These procedures were repeated ten times for different concentrations. Finally, the molar decadic absorption coefficient, transitional dipole moment and integrated absorption coefficient were calculated.

Tea sample preparation

For 12 tea samples (all the tea samples listed in the samples above) when distilled water for dissolving tea leaves was at 30°C: 30 ml of distilled water was heated to 30°C. 50 mg of tea leaves were immersed into the hot water and stirred for 30 min with a magnetic stirrer. It was then filtered through a glass filter and cooled to room temperature (21°C). 40 ml of dichloromethane was poured into the tea infusion and stirred for 10 min with a magnetic stirrer. Since caffeine is more soluble in dichloromethane (140 mg/ml) than it is in water (22 mg/ml), it readily dissolves in dichloromethane. The water phase and the dichloromethane phase were then separated by a separatory funnel. The volume of dichloromethane phase was measured. This solution was poured into 1 cm quartz cuvette and placed into the sample holder of the spectrometer and the spectrum was then taken. These procedures were repeated four times. The total content of caffeine in tea samples were found from the spectrum of combinations of the four extractions. The contents of caffeine in the 5th extract were found negligible.

For four tea samples of only Ethiopian origin (Addis, black lion and cinnamon black teas and green tea) at the boiling temperature (94°C): 50 mg tea leaves were immersed into 30 ml boiled distilled water and stirred for 3 min and filtered through a glass filter and cooled to room temperature using ice bath. 40 ml of dichloromethane was poured into the tea infusion and stirred for 5 min by a magnetic stirrer. The dichloromethane phase and water phase were separated by a separatory funnel. Volume of dichloromethane phase was measured and analyzed by UV-visible spectrometer. These procedures were repeated up to the fourth extraction, similar to that of 1 and the percentage of caffeine was determined.

Note that, back ground correction using dichloromethane was taken before taking spectrum of the above measurements and the cuvette was rinsed four times and dried before taking the next measurement. To make the result more reliable, three independent measurements were taken for each sample and the average values were taken.

RESULTS AND DISCUSSION

The absorption spectra of pure caffeine and caffeine in tea leaves were measured using UV-visible spectrometer

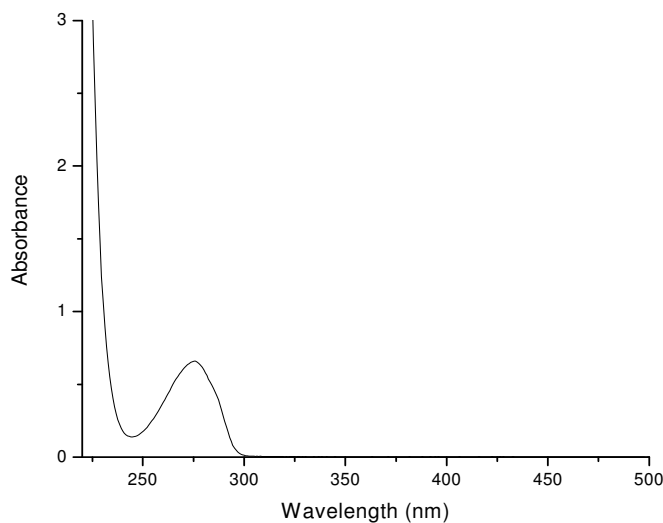


Figure 1. Absorption spectrum of caffeine in dichloromethane.

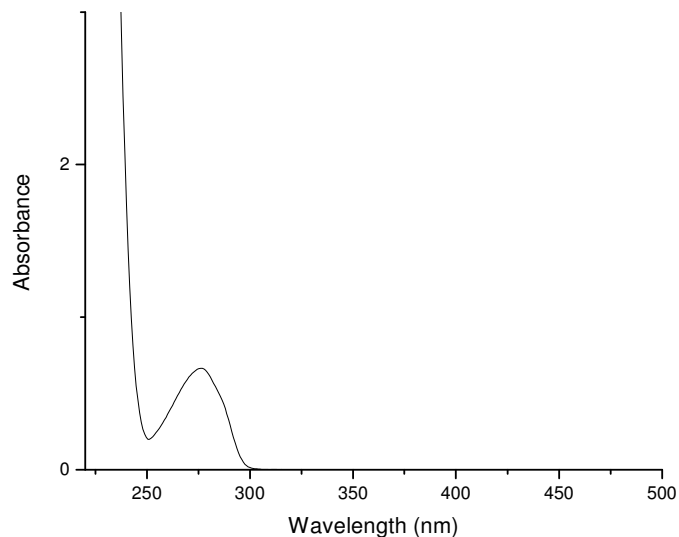


Figure 3. Absorption spectrum of caffeine in chloroform.

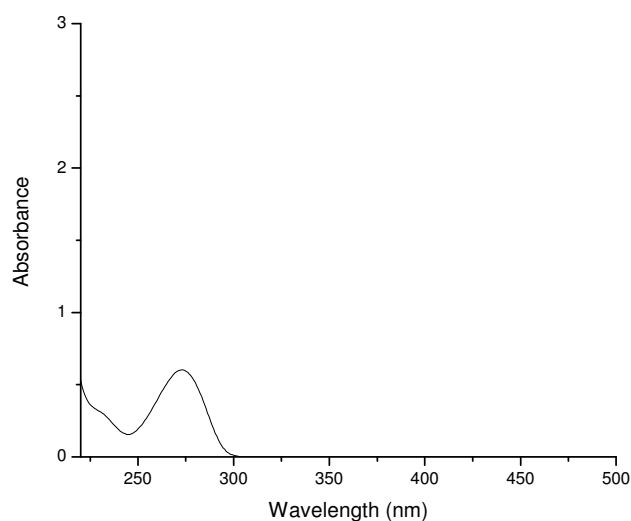


Figure 2. Absorption spectrum of caffeine in water.

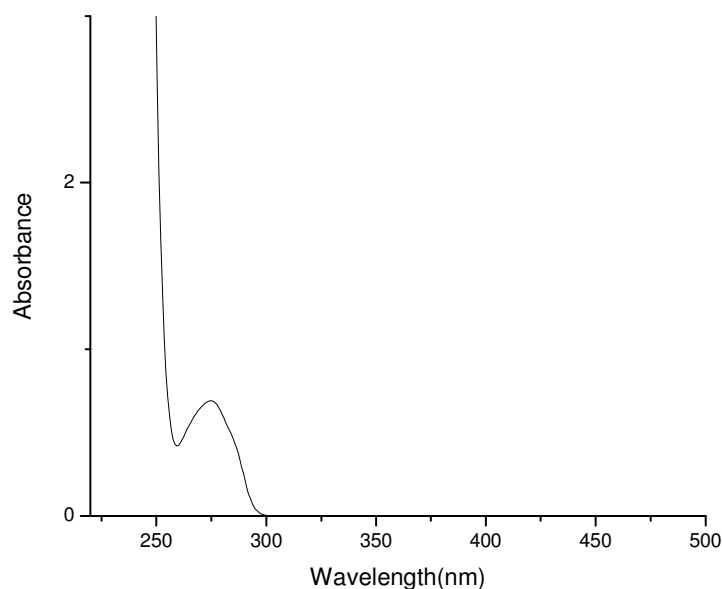


Figure 4. Absorption spectrum of caffeine in ethyl acetate.

in the spectral range of 200 to 500 nm. The spectra were recorded at a spectral bandwidth of 2 nm and scan speed of 480 nm per min. Each data in these experimental activities was found from computerized recorder interfaced with the spectrometer. It was then analyzed using origin 6.1 software.

UV-visible absorbance of caffeine in dichloromethane, water, chloroform, and ethyl acetate

To determine the molar decadic absorption coefficient, integrated absorption cross-section and transitional dipole moments of caffeine in dichloromethane, water, chloroform and ethyl acetate, a series of solutions each

with a known concentration was prepared. The absorbance versus wavelength was measured using UV-visible spectrometer and the results were presented in Figures 1,2,3,4.

From the spectra, it can be observed that caffeine absorbs in the spectral range between 244 nm to 300 nm in dichloromethane and water with λ_{\max} at 276 nm and 272.8 nm respectively, 250 nm to 300 nm at λ_{\max} of 276 nm in chloroform and 258 nm to 300 nm at λ_{\max} of 274.4 nm in ethyl acetate.

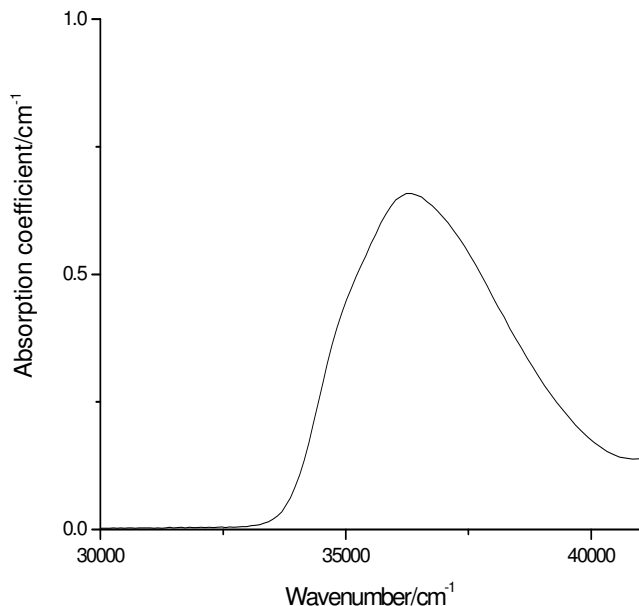


Figure 5. a_ν versus ν of caffeine in dichloromethane.

Optical transition properties of caffeine in dichloromethane, water, chloroform and ethyl acetate

From UV-visible absorption spectra, the optical transition properties of caffeine were calculated in solvents to compare the strength of transition. For incident light intensity I_0 , propagating a distance l in the absorbing medium, the transmitted light intensity I can be described as:

$$I = I_0 e^{-a_\lambda l}, \quad (1)$$

where a_λ is the absorption coefficient.

The Beer-Lambert law results directly from Equation (1) (Gunter and Tuan, 2003).

$$I = I_0 e^{-\varepsilon(\nu)cl}, \quad (2)$$

where $\varepsilon(\nu)$ is the molar decadic absorption coefficient and c is the concentration of the absorbing compound. The Beer-Lambert law is usually expressed in its logarithmic form:

$$\ln\left(\frac{I_0}{I}\right) = A = \varepsilon(\nu)cl, \quad (3)$$

where A is the dimensionless quantity called absorbance, and I/I_0 transmittance (T). The molar decadic absorption

coefficient measures the intensity of optical absorption at a given wavelength (Angew, 1969) was calculated from Equation (3) at λ_{\max} for caffeine in the four solvents. From Equation (1), the absorption coefficient is given by

$$a_\lambda = \frac{1}{l} \ln \frac{I_0}{I} \quad (4)$$

The integrated absorption coefficient at which is the sum of absorption coefficient for all frequencies in the band is expressed as:

$$a_i = \int a_\lambda d\nu, \quad (5)$$

where ν is the frequency.

The integrated absorption coefficient is independent of line function, which may vary with parameters like pressure, temperature and solute solvent interaction. It thus provides a measure of the inherent absorbing strength of atoms. The integrated absorption cross-section δ_i , can be calculated by the following equation:

$$\delta_i = \frac{1}{N} \int a_\lambda d\nu, \quad (6)$$

where N is the number density of the molecules.

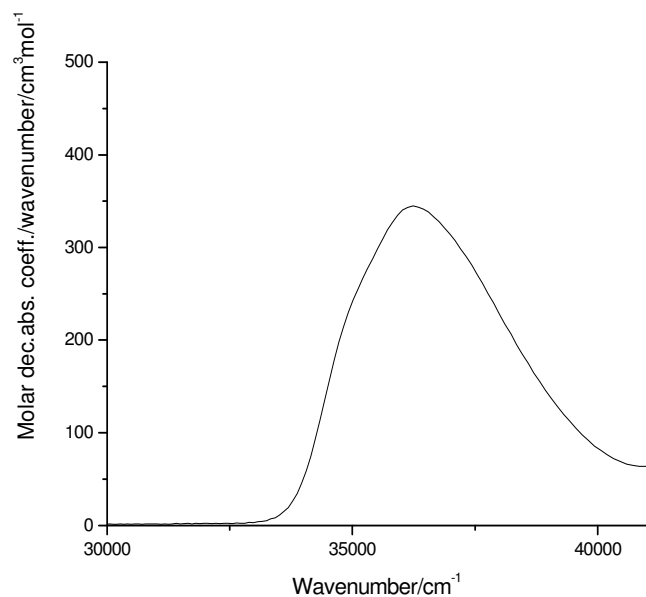
The integrated absorption cross-section of caffeine can be found by recalculating the absorbance versus wave number using origin 6.1 software. The integrated area under the curve was obtained by integrating from 32,981.5303 to 40,849.6732 cm^{-1} . Figure 5 shows the spectra of absorption coefficient versus wavenumber of caffeine in dichloromethane. The transitional dipole moment of the dissolved molecule, which is related to the molar decadic absorption coefficient by the integral absorption coefficient, is calculated by the equation (Liptay, 1969 and Michale, 1999):

$$|A| = \int_{\text{band}} \frac{\varepsilon(\nu)}{\bar{\nu}} d\bar{\nu} = \frac{1}{3} \frac{2h\pi^2 Na}{\ln(10)c_0 \varepsilon_0} |\mu_{fi}|^2 = \frac{1}{3} S |\mu_{fi}|^2, \quad (7)$$

where $S = 2.9352 \times 1060 \text{ c-2mol}^{-1}$. The transitional dipole moment of caffeine was found by recalculating the absorbance versus wavelength into $\frac{\varepsilon(\nu)}{\bar{\nu}}$ versus ν using origin 6.1 software along with Equation 7. Figure 6 shows the spectra of molar decadic absorption coefficient over wavenumber versus wave number $\left(\frac{\varepsilon(\nu)}{\bar{\nu}} \text{ versus } \nu\right)$ of caffeine in dichloromethane.

Table 1. Optical transition properties of caffeine in dichloromethane, water, chloroform and ethyl acetate.

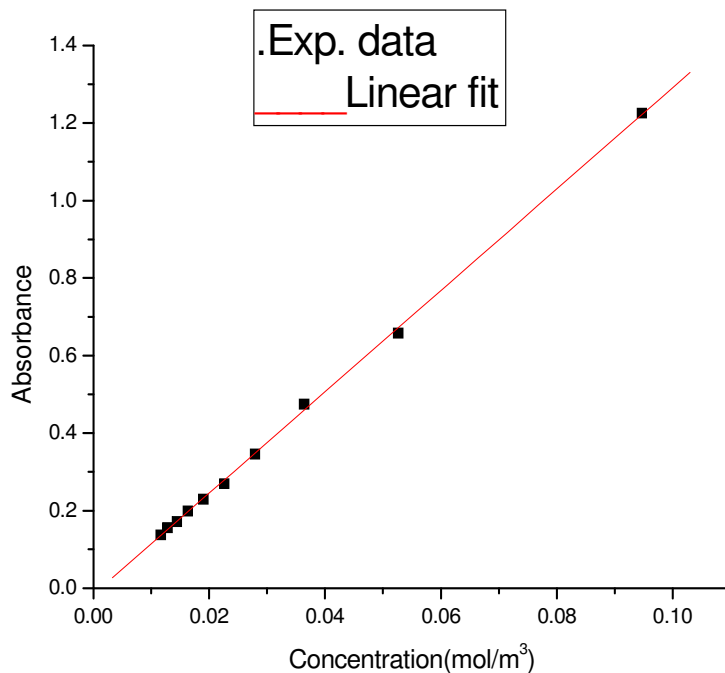
Solvent	Molar decadic absorption coefficient (ϵ_{\max}) in $\text{m}^2\text{mol}^{-1}$	Integrated absorption cross section in $\text{cm}^2\text{mol}^{-1}$	Transitional dipole moment in C m
Dichloromethane	1224±0.689	(86.65±0.47)10 ⁻¹⁵	(12.07±0.06)10 ⁻³⁰
Water	920±0.85	(65.04±0.11)10 ⁻¹⁵	(10.39±0.01)10 ⁻³⁰
Chloroform	658±1.70	(43.91±0.10)10 ⁻¹⁵	(8.58±0.01)10 ⁻³⁰
Ethyl acetate	1008±2.72	(56.81±0.61)10 ⁻¹⁵	(9.74±0.03)10 ⁻³⁰

**Figure 6.** $\frac{\epsilon(\nu)}{\nu}$ versus ν of caffeine in dichloromethane.

In electronic spectroscopy especially in organic molecules, the transition observed in UV-visible region is $\pi^* \leftarrow \pi$. Thus, for pure caffeine the electronic type transition is $\pi^* \leftarrow \pi$ and this transition is the cause for absorption. Using the above equations, the optical transition properties of caffeine in different solvents are presented in Table 1.

Validation of the method

The calibration graph correlating to the absorbance and concentration of pure caffeine in dichloromethane was constructed at the highest peak for concentration range of (1.16 to 9.47)10⁻⁸ molcm⁻³ (Figure 7). The standard deviation is 0.0084 and the linear regression coefficient is 0.99972. From Figure 7, good linear relationships were observed for a wide concentration range. This indicates that absorbance is directly proportional to concentration

**Figure 7.** Absorbance versus concentration graph for caffeine in dichloromethane.

or in other words Beer-Lambert law is valid.

Determination of caffeine in tea leaves

Caffeine content in tea leaves cannot be determined directly using UV-visible spectrometer, due to the matrix effect of UV absorbing substances (Guzin, 2002). This effect can be observed in the spectral bands of tea leaves in water as shown in Figure 8; as seen from the Figure, caffeine spectra interface with other compounds in tea leaves. Therefore, it is necessary to develop a method to overcome this difficulty. The method developed in these experimental activities is to first dissolve caffeine in water and then extract it, using dichloromethane as mentioned in the procedure part.

In Figure 9, the absorption spectra of pure caffeine and caffeine extracted by dichloromethane exactly overlaps.

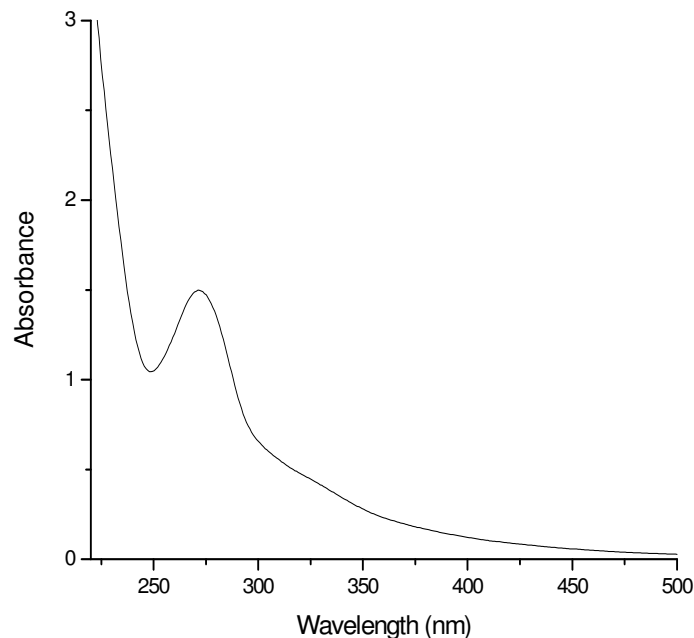


Figure 8. Absorption spectrum of tea leaves in water.

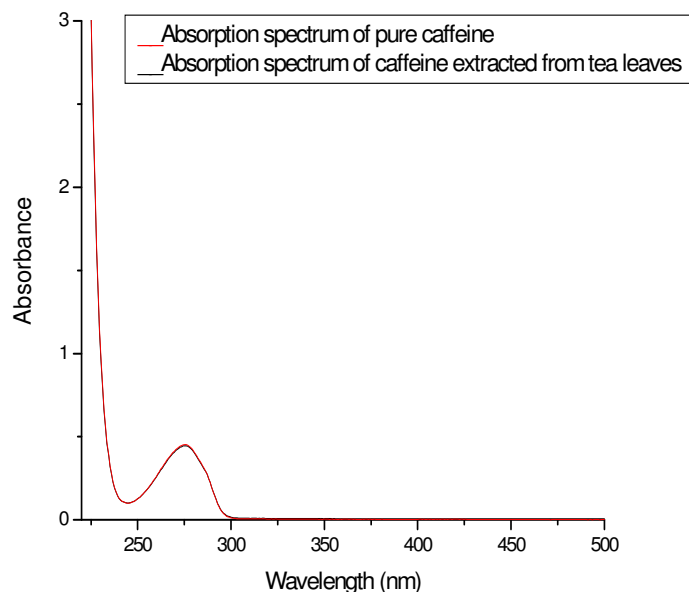


Figure 9. Absorption spectra of pure caffeine and caffeine extracted from tea leaves.

This indicates that dichloromethane was a good solvent used to extract caffeine from tea leaves.

To determine caffeine from tea leaves, the extractions were repeated four times until the spectrum of caffeine becomes flat, when seen under UV-visible spectrometer. In the fifth extraction, the content of caffeine was found negligible (Figure 10).

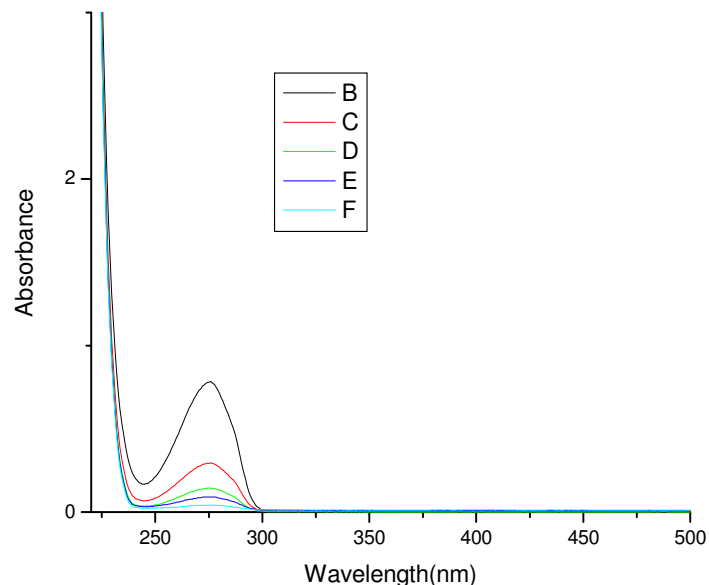


Figure 10. The over lapped spectra of caffeine for different round of extraction (B) for first round, (C) for second round, (D) for third round, (E) for fourth round, and (F) for fifth round.

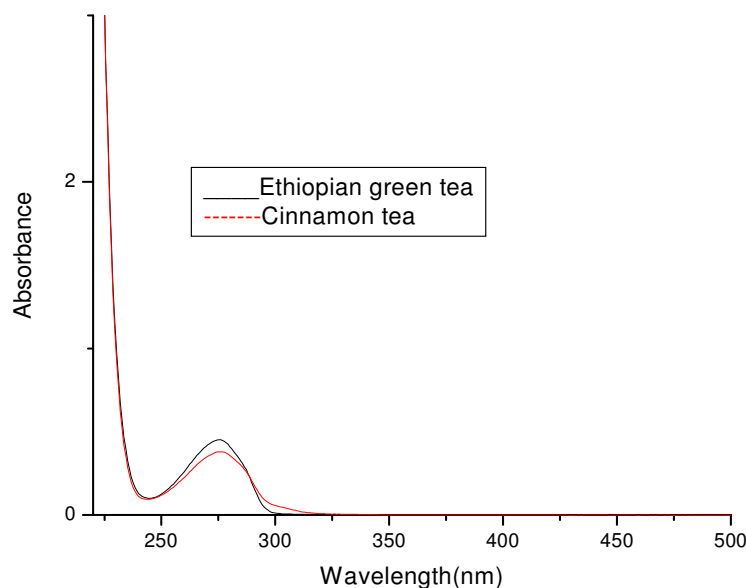
Table 2 presents the experimental result for the determination of caffeine at two different temperatures (30°C and 94°C or boiling temperature). For convenience, we used to represent tea samples by symbols in Table 2. The representations are as follows, Addis black tea (A), Ethiopian green tea (B), black lion tea (C), cinnamon black tea (D), tosign black tea (E), ginger black tea (F), Sri Lankan green tea (G), Indian Tatagold black tea (H), Indian Taj Mahal tea (I), Kenyan black tea (J), South African black tea (K), and Zimbabwean Taganda black tea (L).

The result of the experiment indicates that (not indicated in the Table 2), most caffeine was extracted during the first extraction (about 85% for 30°C and 65% for 94°C) of the total caffeine. The result in Table 2 indicates that at 30°C, the percentage of caffeine in Ethiopian green tea leaves is the greatest, of all the other Ethiopian tea leaves studied. On the other hand, Ethiopian black lion tea has the least caffeine content compared to the other Ethiopian tea leaves. As can be seen from the Table 2, caffeine content of Kenyan black tea leaves is the greatest of all the other tea leaves. Next to Kenyan, Indian Taj Mahal tea has the next higher content of caffeine. To the other end, Sri Lankan green tea, which is purchased in Ethiopian super markets, has the least caffeine content of all the above teas.

From the tea leaves tested at the boiling temperature (94°C), similar to the result at 30°C, the content of caffeine in Ethiopian green tea leaves is the greatest of the other tea leaves and Ethiopian black lion tea has the least caffeine content. When we compare the content of

Table 2. Experimental result of determination of caffeine in tea leaves at two different temperatures (30°C and 94°C) (n = 3).

Sample	30°C			94°C		
	λ_{\max} (nm)	Amax	Percentage of caffeine	λ_{\max} (nm)	Amax	Percentage of caffeine
A	274.80±0.28	0.468±0.011	2.01±0.04	275.5±0.28	0.486±0.010	2.11±0.04
B	275.2±0.00	0.478±0.004	2.08±0.01	275.2±0.00	0.494±0.028	2.15±0.01
C	274.4±0.00	0.346±0.004	1.50±0.00	275.5±0.21	0.366±0.005	1.60±0.01
D	276.8±0.00	0.462±0.025	1.98±0.09	276.0±0.00	0.422±0.019	1.87±0.08
E	275.2±0.00	0.458±0.009	1.94±0.04			
F	275.2±0.00	0.382±0.006	1.63±0.03			
G	275.6±0.28	0.317±0.013	1.34±0.04			
H	275.6±0.28	0.430±0.040	1.87±0.09			
I	275.2±0.00	0.538±0.001	2.30±0.04			
J	275.6±0.28	0.547±0.006	2.36±0.00			
K	275.2±0.56	0.401±0.011	1.70±0.05			
L	275.6±0.28	0.357±0.005	1.52±0.01			

**Figure 11.** Overlapped spectra of caffeine extracted from Ethiopian green tea and cinnamon tea.

caffeine at 30°C and boiling temperature, the percentage of caffeine at the boiling temperature is greater.

Caffeine content in tea leaves reported in this research is in the range of values reported (1 to 5%). As it can be seen in this research, the maximum content of caffeine is 2.36% which is almost similar to the results obtained for the caffeine contents of marketed tea by Misra et al. (2009), using HPLC in which the maximum concentration of caffeine in tea samples of marketed tea was 2.145%. Abdul et al. (2006) also reported using HPLC that, the caffeine content for black tea was 3.34% and green tea was 2.44%.

Precision of the method was determined by three replications of each sample. The precision (%RSD) of the

replications was found to be less than two which is indicative of a precise method. The UV-visible absorbance range of caffeine is from 243 nm to 302 nm. But for cinnamon tea leaves the range is from 243 nm to 318 nm (Figure 11). This indicates that the addition of cinnamon to tea leaves increases the wavelength range by 16 nm.

Conclusion

Alternative methods were developed to determine caffeine content in real tea leaves using UV visible spectrometer. In this research, optical transition

properties of pure caffeine and caffeine content of real tea leaves at two different temperatures were analyzed and the result agrees with the literature values of other analytical methods. To make the result more reliable, the experiments were repeated three times and the average values were taken. The results at two different temperatures reveal that, caffeine is better extracted at the boiling temperature than that of 30°C and more extraction time. But the differences were very small. Therefore, it can be said that, by taking more extraction time and at higher temperatures, more caffeine can be extracted than at boiling temperature and a less extraction time.

The result of the experiment indicates that for cinnamon tea, the wavelength range increases to the longer wavelength. This indicates that, addition of cinnamon to tea leaves increases the wavelength range. The broadening of absorption spectrum when tea is blended with cinnamon can be explained by the fact that in this tea, caffeine and compounds in cinnamon will have different natural frequencies.

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