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Characterization and determination of catechins in green tea leaves using UV-visible spectrometer

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In this paper, characterizations of pure major catechins and method for determination of total catechins in green tea leaves have been reported using UV-visible spectrometer. Most research activities have been focused on chromatographic methods, however spectrophotometirc method is preferred because of its rapidity, high accuracy, and reproducibility. The optical transition properties of Epigallocatechin Gallate (EGCG) and Epicatechin Gallate (ECG) in water, methanol, ethanol, acetonitrile and Epigallocatechin (EGC) and Epicatechin (EC) in water were measured. The results show that, EGCG has highest molar decadic absorption coefficient in methanol than in the other solvents. ECG has the highest absorption cross-section, transitional dipole moment, and oscillator strength of all the catechins in water. On the other hand, EGC has the least optical transition properties of all the catechins in water. Limits of detection (LOD) were comprised in the range $3.1\times10^{-2}gmL^{-1}$ to $1.6\times10^{-1}gmL^{-1}$ and reproducibilities with RSD lower than 2%. After characterization of the electron transition, a method was developed for UV-Visible determination of total catechins. Using the developed method, the content of total catechins in Ethiopian and Sri Lanka green tea leaves at room temperature was determined. The result of the experiment indicates that, Ethiopian green tea leaves has the greater total catechins (17±0.01%) than Sri Lanka green tea leaves (7.17±0.12%).

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

INTRODUCTION

Tea is one of the most widely consumed beverages in the world after water (Castro et al., 2010). It is used not only as fresh drink but also as traditional herb which has many benefits for human health. Tea has attracted scientific attention for its anticancer and antioxidant activities (Hirota et al., 2002; Karori et al., 2007). There are two major kinds of tea, black tea and green tea, and they both contain caffeine (1 to 5%) with small amounts of other xanthine alkaloids (Amra et al., 2006). The main components of tea are polyphenolic compounds, the main quality parameters for teas, commonly known as catechins, which represent a group of compounds belonging to the flavonoid family. Catechins, may be contained in (5 to 27%) of the dried tea leaf (Leung and

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Foster, 1996), which are divided into four primary compounds epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), epicatechin (EC) (Amra et al., 2006; Salova et al., 2013). EGCG constituting (10 to 50%) of catechins and being the most potent due to its degree of gallation and hydroxylation (Pellilo et al., 2002). It is also the main subject of scientific study with regard to its potential health effects (Kanwar et al., 2012). Polyphenols in green tea are believed as excellent free radical scavengers. Several clinical studies have proved polyphenols to be active in cancer prevention in several ways. Polyphenols have also been recently recognized as functionally active molecules possessing antioxidant, anticancer, anti-mutagenic properties, as well as exerting protective effects against cardiovascular and other diseases (Michael, 1999; John, 2008). With regard to the toxicity of catechins, studies have shown no toxic effects in animals (Chengelis et al., 2008; Takami et al., 2008).

To determine these important tea components in tea, most research activities have been focused on chromatographic methods (Collier and Mallows, 1971; Khokhar and Magnusdottir, 2002; Wang et al., 2003). The methods give good separation of the catechins but they are time consuming and labor intensive (Guanqun et al., 2003). On the other hand, the UV-visible spectromerer method has been simple, fast and inexpensive and normally available in most laboratories. Furthermore, UV-visible spectrometric method is the best method to obtain total catehins in tea leaves. In the present method it is difficult to separate individual catechins in tea leaves.

In this study, we report a method for the determination of total catechins from green tea leaves. The method includes extracting them by water from green tea leaves and removing non-polar components by chloroform followed by determining its content by UV-visible spectrometer. The tea leaves studied in this research were Ethiopian and Sri Lanka green tea leaves. We have studied these two samples of tea leaves because they are the green tea leaves currently available in the Ethiopian supermarkets. In addition, the only green tea that has been produced in Ethiopia is Ethiopian green tea leaves and mostly the imported tea leaves and purchased in the Ethiopian supermarket is the Sri Lanka green tea leaves. The four major catechins were also characterized in water. Furthermore, EGCG and ECG were characterized in methanol, ethanol, and acetonitrile.

MATERIALS AND METHODS

Samples

Ethiopian and Sri Lanka green tea leaves were purchased from supermarkets in Addis Ababa. Sri Lanka green tea leaves (imported and distributed by: Kafco pvt. Ltd. Co., packed by: Qualitea Ceylon pvt. Ltd. Colombo, Sri Lanka). Ethiopian green tea (grown in high lands of Ethiopia, packed by Ethio Agri-ceft). Both of these samples were from tea bags.

Instrumentation

The absorption spectra were recorded on a Perkin-Elmer Lambda 19 spectrometer with wavelength range of 170 to 3200 nm. It consists of radiation source, monochormator, sample area, photometer, and detection area. There are two types of radiation sources. For the UV region, the radiation source is deuterium discharge lamp that emits polychromatic UV radiation which can then be filtered into monochromatic UV radiation. For the visible region, the radiation source is tungsten filament. The monochromator or wavelength selector disperses the light from radiation source into its separate wavelength. The radiation of only a particular wavelength leaves the monochromator through an exit slit. It has double monochromator which offers the advantage of low levels of stray light that is significant to measure a high value of absorbance using 1 cm cuvette. The monochromatic light that emerges from exit slit is pulsed by a chopper and split into sample and reference beam by the beam splitter. A reference beam passes through a sample holder or a quartz cuvette that contains only a solvent. The sample beam passes through a quartz cuvette that contains a sample solution. The radiation beams that passes through the detectors is amplified by difference amplifier and finally reaches the recorder, where the results are recoded digitally in a personal computer attached to a spectrometer. The investigated compound was measured in the UV-visible spectrum range of 200 to 500 nm with a spectral band width of 2 nm and scan speed of 240 nm min^{-1}.

Chemicals

Chloroform (assay: 99.8%, European Union), methanol (assay: 99.8%, Indian), ethanol (assay: 96%, Indian), acetonitrile (assay: 99.99%, European Union), EGCG (M.W. 458.4 g/mol, Aldrich Germany), ECG(M.W. 442.4 g/mol, Aldrich Germany), EGC (M.W. 306.3 g/mol, Aldrich Germany), EC (M.W. 290.27 g/mol, Aldrich Germany).

Preparation of samples and standard solution

Standard solution preparation

For the determination of catechins in green tea leaves and for their characterization in distilled water, 1.06 mg of EGCG was immersed into 15 ml of distilled water and 0.46 mg of ECG, 0.87 mg of EGC and 0.38 mg of EC each immersed into 10 ml of distilled water and stirred for 1hr by magnetic stirrer. Absorbance versus wave length of each solution was taken by the UV-Visible spectrometer from which transitional properties of these compounds were calculated. EGCG and ECG were also characterized in methanol, ethanol and acetonitrile. For this 0.80, 0.55 and 0.88 mg of EGCG and 0.88, 0.55 and 0.61 mg of ECG each were immersed into 15 ml of methanol, ethanol, and acetonitrile respectively and stirred for 30min by magnetic stirrer and analyzed. These procedures were repeated for different concentrations. Finally, the molar decadic absorption coefficient, transitional dipole moment, oscillator strength, and integrated absorption coefficient were calculated.

Tea sample preparation

50mg of Ethiopian and Sri Lanka green tea leaves were added to 40 ml of distilled water at room temperature (to protect the epimerization of primary catechins). It was then stirred by magnetic stirrer for 1 h and filtered through glass filtrate. The tea infusion was washed by 40 ml of chloroform in a separatory funnel to remove caffeine, pigments, and other non-polar impurities. This step was

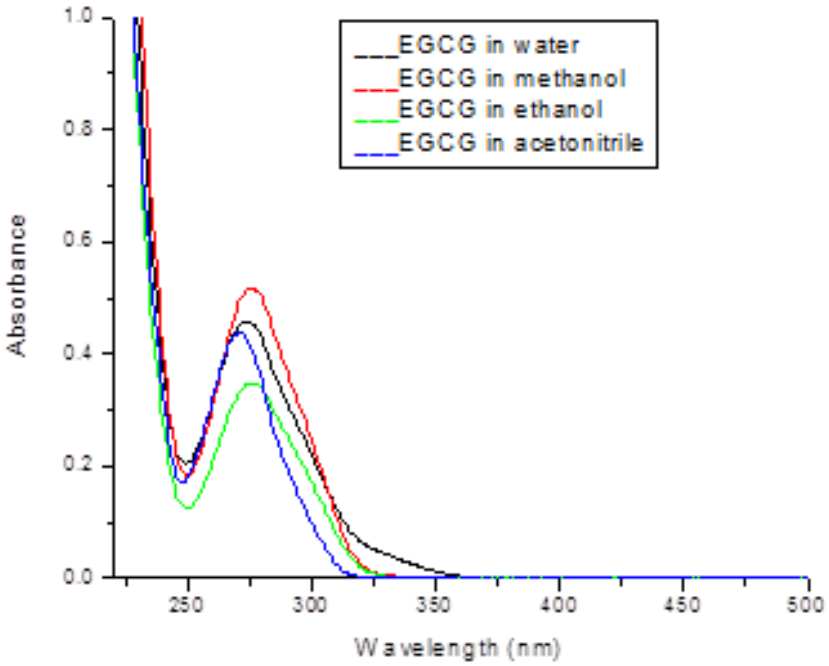

Figure 1. EGCG in water, methanol, ethanol, and acetonitrile.

repeated four times and negligible catechin compounds were found in the chloroform phase owing to their low solubility in chloroform. This was checked by taking the spectrum of chloroform phase until the absorption versus wavelength spectrum was flat when seen by the UV-Visible spectrometer. Then, the volume of water 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 taken. To make the measurement more reliable three independent measurements were taken for each sample (n=3).

RESULTS AND DISCUSSION

The absorption spectra of pure catechins and catechins in tea leaves were measured using UV-visible spectrometer in the spectral range of 200 to 500 nm. The spectra were recorded at a spectral bandwidth of 2 nm and scan speed of 240 nm per minute. Each data in these experimental activities was found from computerized recorder interfaced with the spectrometer. It was then analyzed using origin 6.1 soft ware.

UV-Visible absorbance of catechins in water, methanol, ethanol, and acetonitrile

The absorbance versus wavelength was measured by UV-visible spectrometer for each solution of known concentrations of catechins in water, methanol, ethanol, and acetonitrile. The absorption spectrums of catechins in water, methanol, ethanol, and acetonitrile are shown in Figures 1, 2, 3, and 4 respectively. From the spectra (Figures 1, 2, 3 and 4), it can be observed that EGCG absorbs in the spectral range between 248 to 361 nm in water with λ_{max} at 273.6 nm and in the other solvents, the spectral range is between 246 to 323 nm with λ_{max} 276.0 nm in methanol and ethanol and 271.2 nm in acetonitrile. This indicates that, peak absorbance of EGCG in methanol and ethanol is the same but it is shifted to the shorter wave length (blue shifted) in the acetonitrile. The spectral range of ECG in water is from 246 to 363 nm with λ_{max} 276.8 nm and from 246 to 325 nm in the other solvents with λ_{max} 279.2 nm in methanol and ethanol and 273.6 nm in acetonitrile. Similar to that of EGCG, the maximum absorbance of ECG in methanol and ethanol is the same, and for acetonitrile λ_{max} is shifted to the shorter wave length (blue shifted). The spectral range of EGC in water is between 254 to 378 nm and λ_{max} at 269.6 nm and EC between 252 to 328 nm and λ_{max} at 278.4 nm in water. In the region below the minimum wave length of the band gap shows the region of solvent absorption.

Optical transition properties of catechins in water, methanol, ethanol, and acetonitrile

The optical transition properties of catechins were calculated in solvents from their UV-visible spectra to

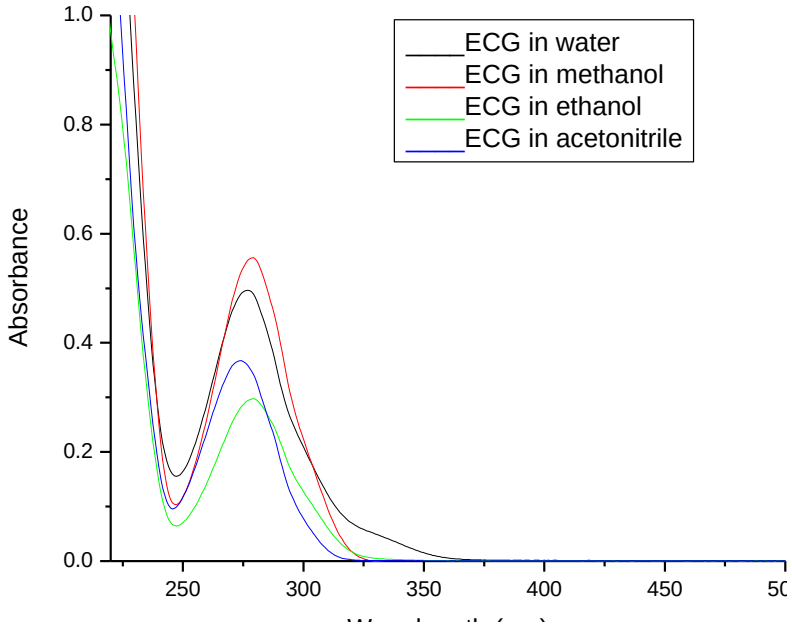

Figure 2. ECG in water, methanol, ethanol, and acetonitrile.

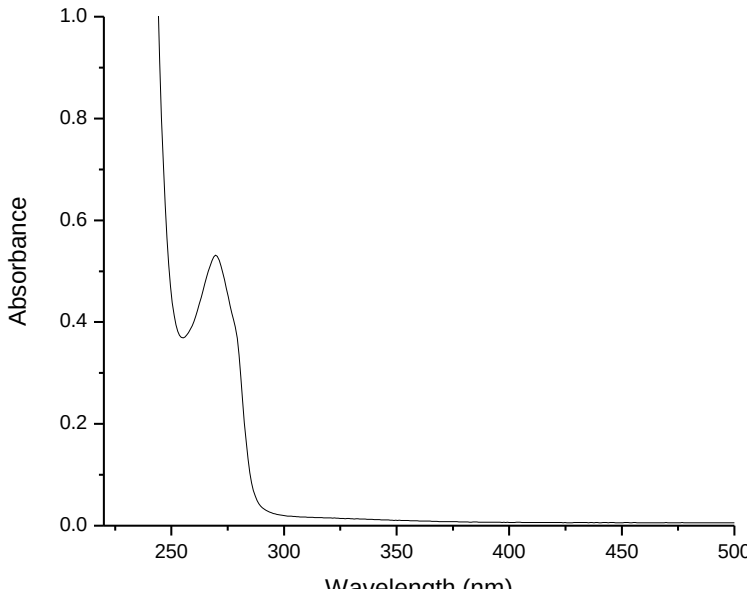

Figure 3. EGC in water.

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).

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

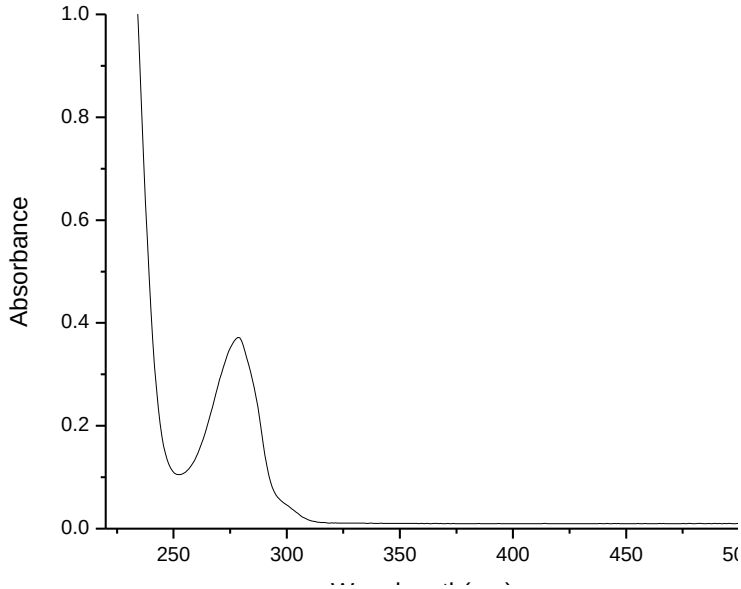

Figure 4. EC in water.

Where $\varepsilon(\nu)$ is the molar decadic absorption coefficient and c is the concentration of the absorbing compound. The molar decadic absorption coefficient $\varepsilon(\nu)$ represents the ability of a molecule to absorb light in a given solvent. 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).

For n-multicomponent case, the relation becomes (Clark et al., 1993).

$$A_{total} = A_1 + A_2 + \ldots + A_n. \quad (4)$$

The molar decadic absorption coefficient was calculated from Equation (3) at λ_{max} for EGCG and ECG in the four solvents and in water for EGC and EC. From Equation (1), the absorption coefficient is given by:

$$a_\lambda = \frac{1}{l}\ln\frac{I_0}{I} \quad (5)$$

An absorption band for a given transition usually extends over a range of frequencies, the integrated absorption coefficient. Thus, the integrated absorption coefficient a_t which is the sum of absorption coefficient for all frequencies in the band is expressed as:

$$a_t = \int a_\lambda d\nu, \quad (6)$$

Where ν is the frequency.

The integrated absorption coefficient is independent of line function, which varies due to pressure, temperature, and solute solvent interaction. The integrated absorption cross-section δ_t which characterize the photon-capture area of a molecule can be calculated by the following equation:

$$\delta_t = \frac{1}{N}\int a_\lambda d\nu, \quad (7)$$

Where N is the number density of the molecules.

The integrated absorption cross-section of catechins can be found by recalculating the absorbance versus wave number using origin 6.1 soft ware. The integrated area under the curve was obtained by integrating in the band gap for the catechins in each solvent. Figures 5, 6, 7, and 8 shows the spectra of absorption coefficient versus wavenumber of catechins in different solvents. 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):

$$I_A = \int_{band} \frac{\varepsilon(\nu)}{\bar{\nu}} d\bar{\nu} = \frac{1}{3}\frac{2h\pi^2 Na}{3\ln(10)c_0\varepsilon_0}\left|\mu_{fi}\right|^2 = \frac{1}{3}S\left|\mu_{fi}\right|^2, \quad (8)$$

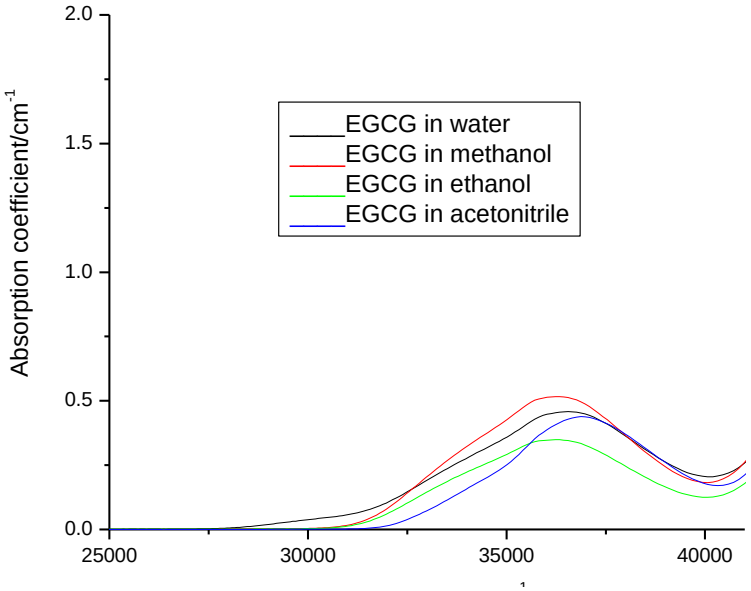

Figure 5. a_ν versus ν of EGCG in water, methanol, ethanol, and acetonitrile.

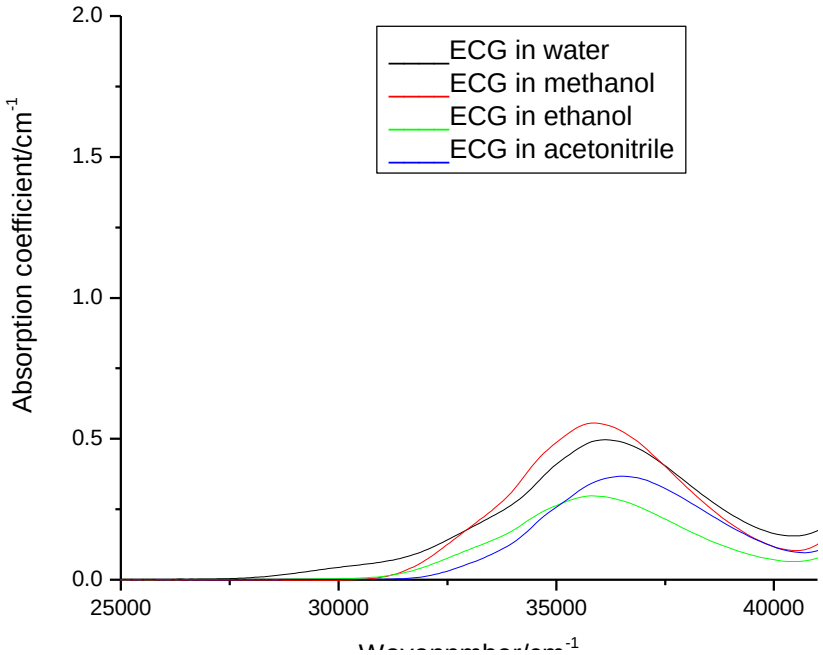

Figure 6. a_ν versus ν of ECG in water, methanol, ethanol, and acetonitrile.

Where S = 2.9352×10^{60} $c^{-2}mol^{-1}$. The transitional dipole moments of catechins were found by recalculating the absorbance versus wave length into $\frac{\varepsilon(\nu)}{\nu}$ versus ν using origin 6.1 soft ware along with Equation (8).

The other important parameter, which provides the relative strength of electron transition, is the oscillator strength (f). It is the average number of elections per atom that can be excited by the incident radiation. Oscillator strength related to molar decadic absorption

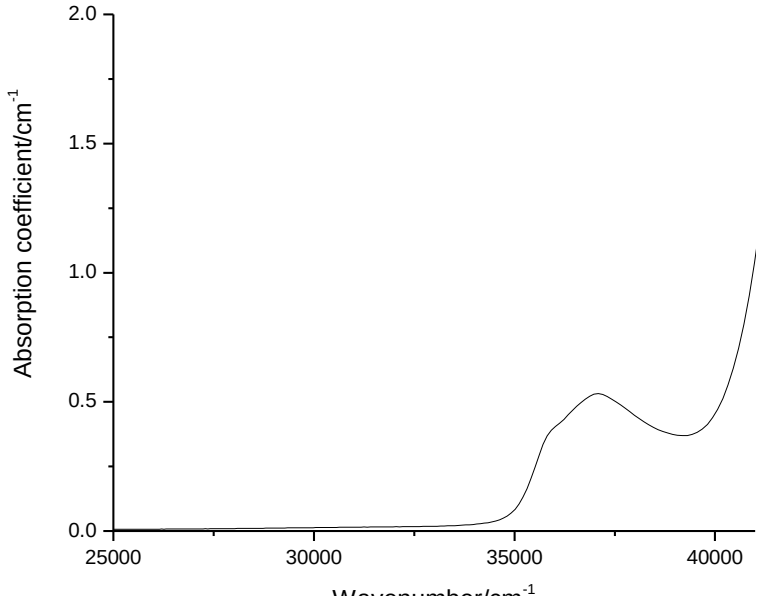

Figure 7. a_ν versus ν of ECG in water.

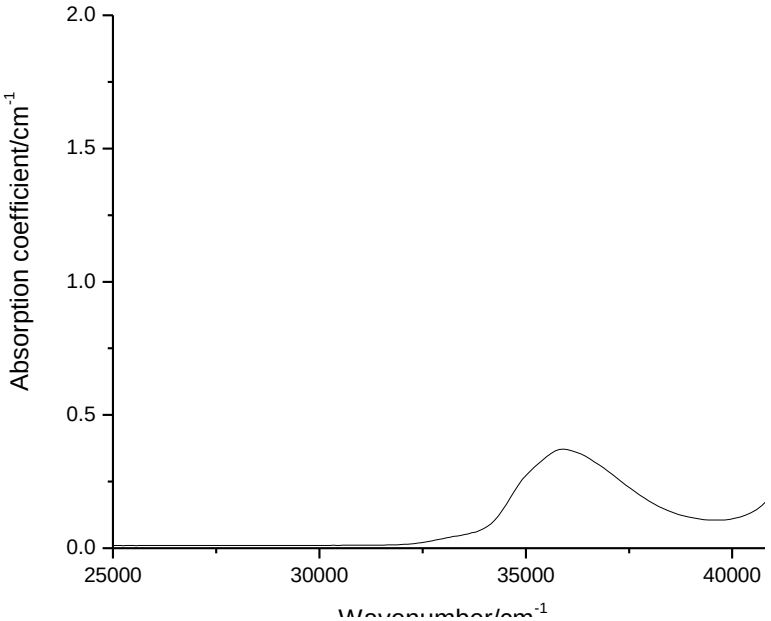

Figure 8. a_ν versus ν of EC in water.

coefficient by the following equation (Georgakopoulous et al., 2004; Radwan, 2007).

$$f = 4.32 \times 10^{-9} \frac{molcm^2}{L} \int \varepsilon(\nu) d\nu . \quad (9)$$

In electronic spectroscopy especially in organic molecules, the transition observed in UV-visible region is $\pi^* \leftarrow \pi$. Thus, for catechins the electronic type transition is $\pi^* \leftarrow \pi$ and this transition is the cause for absorption.

Table 1. Optical transition properties of catechins in water, methanol, ethanol and acetonitrile.

Catechins	Solvent	Molar decadic absorption coefficient(ε_{max}) in m^2mol^{-1}	Integrated absorption cross section in cm mol^{-1}	Transitional dipole moment in cm	Oscillator strength
EGCG	Water	1084±3.92	(106.74±0.34)10^{-15}	(13.54±0.02)10^{-30}	0.278±0.001
	Methanol	1342±7.60	(120.24±0.56)10^{-15}	(14.36±0.03)10^{-30}	0.314±0.001
	Ethanol	1313±1.41	(118.66±0.22)10^{-15}	(14.24±0.02)10^{-30}	0.309±0.001
	Acetonitrile	1255±2.60	(99.90±0.31)10^{-15}	(12.93±0.02)10^{-30}	0.260±0.001
ECG	Water	1430±3.01	(133.61±0.25)10^{-15}	(15.16±0.01)10^{-30}	0.347±0.001
	Methanol	1464±34.50	(117.60±3.40)10^{-15}	(14.16±0.21)10^{-30}	0.306±0.009
	Ethanol	1329±11.45	(112.27±0.89)10^{-15}	(13.86±0.05)10^{-30}	0.292±0.002
	Acetonitrile	1470±10.55	(117.06±0.89)10^{-15}	(14.01±0.05)10^{-30}	0.304±0.002
EGC	Water	184.13±1.18	(10.94±0.02) 10^{-15}	(4.28±0.004)10^{-30}	0.028±0.001
EC	Water	311±10.39	(20.21±0.50) 10^{-15}	(5.94±0.007)10^{-30}	0.053±0.001

Using the above equations, the optical transition properties of catechins in different solvents are summarized in Table 1. From the results of Table 1, it can be seen that, EGCG has highest and lowest molar decadic absorption coefficient in methanol and water respectively. ECG has the highest absorption cross-section, transitional dipole moment, and oscillator strength of all the catechins in water. On the other hand, EGC has the least optical transition properties of all the catechins in water.

Validation of the method

The calibration graph correlating the absorbance and concentration of pure catechins in water was constructed at the highest peak for concentration range of (1.85-15.46)10-8mol cm-3 for EGCG, (1.30-10.40)10-8mol cm-3 for ECG, (9.47-28.40)10-8mol cm-3 for EGC, and (4.36-13.09)10-8mol cm-3 for EC. The standard deviation was determined from linear fit of data points is 0.016, 0.0037, 0.0017, and 0.0070 for EGCG, ECG, EGC, and EC respectively and the linear regression coefficient is 0.99935, 0.99997, 0.99998, and 0.99912 for EGCG, ECG, EGC, and EC respectively. Therefore, good linear relationships were observed for a wide concentration range. This indicates that absorbance is directly proportional to concentration or in other wards Beer-Lambert law is valid. The limits of detection (LOD) were calculated from the peak to noise ratios for the four catechins in water and found out to be $3.1\times10^{-2}gmL^{-1}$, $4.2\times10^{-2}gmL^{-1}$, $1.6\times10^{-1}gmL^{-1}$ and $9.5\times10^{-2}gmL^{-1}$ for ECG, EGCG, EGC, and EC respectively. The low LOD indicates that UV-Visible spectrometer is reliable for analyzing catechins in tea samples.

In Figure 9, the absorption spectra of the combination of pure catechins and catechins extracted by chloroform are similar. This indicates that, chloroform can be used to extract catechins from tea leaves.

Determination of catechins in tea leaves

A method was developed to determine catechins in tea leaves using UV-visible spectrometer. The method includes, first dissolving tea leaves in distilled water and removing caffeine, pigments, and other non- polar impurities using chloroform as mentioned in the procedure part. In this experimental activity, total catechin was determined using Beer's law. First, molar decadic absorption coefficient (ε_{max}) at the maximum peak of the extracted real tea leaves was calculated from pure substances of the four catechins, EGCG, ECG, EGC, and EC, and total molar decadic absorption coefficient ε was then calculated. Total concentration c was then found from Beer's law (c = A/ε *l*), where A is maximum absorbance of real tea leaves and *l* length of the cuvette which is 1 cm. Total mass was then calculated using this concentration value (m = cMV), where M is the molecular weight and V is the volume. Finally, percentage of total catechins was determined. To make the result more reliable, three independent experiments were carried out for each sample (n=3). Table 2 presents the experimental result for the determination of catechins for the green tea leaves of Ethiopian and Sri Lanka origin.

The result of the experiment indicates that, the content of total catechins in Ethiopian green tea leaves is greater than Sri Lanka green tea leaves. This result agrees with the range of result reported by Leung and Foster (1996). Specifically, the result obtained in this research for

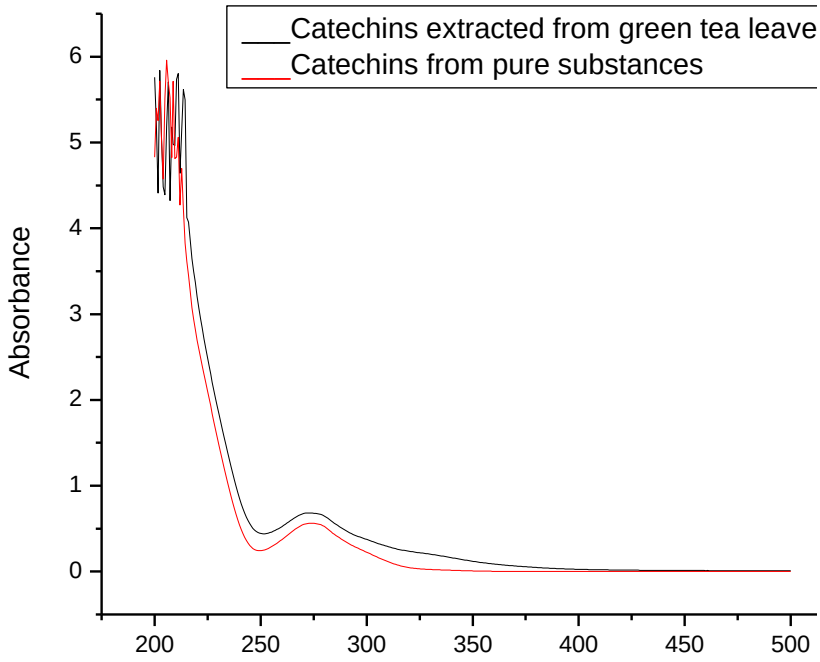

Figure 9. Absorption spectrum of catechins extracted from green tea leaves and catechins from pure substances.

Table 2. Experimental result of determination of catechins in green tea leaves (n = 3).

Sample	λ_{max} (nm)	A_{max}	Percentage of catechins
Ethiopian green tea leaves	272.8±0.0	0.6969±0.03	17.14±0.01
Sri Lanka green tea leaves	270.9±0.2	0.4311±0.02	7.17±0.12

Ethiopian tea leaves was almost similar to the result obtained using HPLC for different samples of tea leaves reported by Quan et al. (2006) in which percentage dried weight of total catechins of maximum 20.49 and minimum 14.32. The results found for the green tea extract followed this same sequence in which the total catechin content was 4 to 45% on average (Dalluge et al., 1998; Khokhar, Magnusdottir, 2002; Mizukami, Sawai, Yamaguchi, 2007). Studies suggest the consumption of 4 to 7 cups of green tea per day in order to obtain the expected health benefits of catechins. 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 (0.06% for Ethiopian green tea leaves and 1.7% for Sri Lanka green tea leaves) which is indicative of a precise method.

Conclusions

A new method was presented to determine total catechins in tea leaves and could be successfully applied for the analysis of real tea samples. Therefore, in this research content of total catechins in Ethiopian and Sri Lanka green tea leaves were determined by the developed method using UV-visible spectrometer. The range of results found in the literature was compared with the results found in this method which came to be noticeably similar. The result of the experiment indicates that, Ethiopian green tea leaves have greater catechins content than Sri Lanka green tea leaves purchased in Ethiopia. So, we can say that one can get more catechins from Ethiopian green tea leaves than Sri Lanka green tea leaves purchased in the same country.

Absorbance versus wavelength of the four pure major catechins (EGCG, ECG, EGC and EC) were taken in water and in addition, EGCG and ECG in methanol, ethanol, and acetonitrile. From the spectra it was found that, peak absorbance of EGCG and ECG in methanol and ethanol is the same but it is shifted to the shorter wavelength (blue shifted) in acetonitrile. As it is expected, the absorption maximum of acetonitrile, that cannot form

hydrogen bond with solute is blue shifted. Optical transition properties of pure major catechins were also calculated in water. In addition, EGCG and ECG were calculated in methanol, ethanol, and acetonitrile, which can be used as reference for further studies.

Conflict of Interest

The authors have not declared any conflict of interest.

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