DILLA UNIVERSITY

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DETERMINATION AND CHARACTERIZATION OF CAFFEINE CONTENT OF SMALL AND LARGE SIZE COFFEE BEANS IN SIDAMA AND KAMBATA TAMBARO ZONES SOUTHERN, ETHIOPIA USING UV-VIS SPECTROPHOTOMETER

Research Thesis

By

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August 2019
Dilla University,
Ethiopia

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A Thesis Submitted to the School of Graduate studies

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By

Tsegaye Bojago

August 2019

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APPROVAL SHEET OF THESIS

SCHOOL OF GRADUATE STUDIES

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As thesis research advisor, I herby certify that I have read and evaluated this thesis research prepared, under my guidance by: Tsegaye Bojago, entitled: Determination and Characterization of caffeine content of small and large size coffee beans in Sidama andKambata Tambaro zones southern, Ethiopia using Uv-vis spectrophotometer andrecommend that it be accepted as fulfilling the thesis research requirement.

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ABBREVIATIONS AND ACCRONYMS

ANOVA	analysis of variance
UV-VIS	ultraviolet visible
CNS	central nervous system
BP	blood pressure
FT-IR	Fourier transforms infrared spectrophotometer
EXC	Ethiopia exchange commodity
PPM	parts per million
CM	centimeter
MM	millimeter
SD	standard deviation
CONC	concentration
VS	versus
DCM	dichloromethane

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ABSTRACT

In this study work using UV/vis spectrophotometer, the caffeine determination and comparison, as well as characterization of caffeine from small and large size roasted coffee by molar decadic absorption coefficient of pure caffeine were studied. The molar decadic absorption coefficient of pure caffeine in water, wavelength at 272.5nm and dichloromethane at275nm were 27.73 and respectively.

The extraction of caffeine from coffee sample in water solution was helped by dichloromethane and the mean value of caffeine in coffee sample calculated one way analysis of variance (ANOVA) and based on the ANOVA result at 0.05significance of confident level. The mean value of Caffeine concentration in small and large size coffee bean were significantly different. the mean concentration of caffeine was significantly high in small coffee beans as compared to large coffee beans (mean 29.75 Vs 22.42 with SD of 4.070 Vs 4.641; t= 4.1; P<0.001).

The mean mass of caffeine in small and large size coffee bean in (g) were (0.07665) and (0.0567 The mean percentagecontent in small and large size coffee bean in (g) were (7.665Respectively.The mean molar decadic absorption coefficient in small and large size roasted coffee sample were (31.65 32.28) respectively, The transition dipole moment of pure caffeine in water at 272.5nm and dichloromethane at 275nm were (Cm) and (.

The mean absorbance of coffee sample in small and large roasted coffee beans were (0.946 0.7225) respectively=3.47145; p=0.01328.

***Keywords**; caffeine content, coffee, extraction, determination, uv-vis spectrophotometer.*

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Coffee is one of the most popular beverages and an important commodity, for many years next to oil, (Ramalakshmi, Raghavan, 1999; Tsegaye, Y; O, Getachew and Z. Tesfaye, 2000) whatever the resources of the woodland flora may be the two main species exploited in the world at the present time are coffee Arabica L. and coffee canephor Pierre (Robusta). Other species of coffee are being cultivated less extensively, although they are still found in certain countries for various reasons.

Coffee canephor has a very wide geographic distribution, extending from the western to the central tropical and subtropical regions of the African continent, from Guinea and Liberia to Sudan and the Uganda forest, with a high concentration of types in the Democratic Republic of Congo (Carencro, 1997). Coffee canephors or Robusta, grows at low altitudes (about 850 m), and accounts for 80% of African coffee production. However, Robusta has also been cultivated in American and Asian countries (Vinod et al., 2006)

The history of coffee has been recorded as far back as the ninth century. During that time, coffee beans were available only in their native habitat, Ethiopia. A popular legend traces its discovery to a goat herder named kaldi, who apparently observed goats that become elated and sleepless at night after grazing on coffee shrubs and, upon trying the berries that the goats had been eating, experienced the same vitality. In 1587, MalayeJaziri compiled a work tracing the history and legal controversies of coffee. In this work, Jaziri recorded that one sheikh, Jamal-al-Coffee is originated in the highlands of Ethiopia, and from there spread into the Arabic world and became known in Europe during the early 17th century, at first as a medicine and then as a social drink in the Arab tradition. Drinking coffee, which in the Amharic language is called ―Buna‖, is an important element of cultural beverage in Ethiopia. Enormous industrial plantations are situated in the South-West of the country, but it is possible to come across single small trees literally everywhere. The peoples of the South who cannot afford genuine coffee prepare a beverage with the use of the husks of coffee grains,(Ethiopian Commodity Exchange Authority, 2008).

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Coffee is a crucial to the economies and politics of many developing countries and the worlds' least developed countries, exports of coffee account for more than 50% of their foreign exchange earnings.Its cultivation, processing, trading, transportation and marketing provide employment for hundreds of millions of people worldwide. In Ethiopia, coffee had been and still contributes to the Lion's share in its national economy being the leading source of foreign exchange earnings. Besides, the livelihood of a quarter (25%) of the Ethiopian population depends directly or indirectly on the different processes of production and marketing along the coffee value-chain, (Grima et al., and 2008). It is a typical global commodity because it is usually produced in developing countries and consumed in developed countries. Therefore, marketing channels extend beyond borders, hand the price of coffee is basically determined at international exchange markets in New York and London (International coffee organization (ICO), 2009; Kodama, 2007). Most of Ethiopian coffee is practically organic, and only a fee for the organic certificate is required.

There are four types of production systems in Ethiopia; forest coffee, semi-forest coffee, garden coffee and plantation coffee. 95% of the coffee production from these systems can be considered as organic, although not yet officially certified. Forest coffee accounts for about 10%, semi- forest coffee accounts for about 35%, garden coffee accounts for about 50%, and plantation coffee accounts for about 5% of total coffee production in Ethiopia. Most of Ethiopian coffee is practically organic, and only a fee for the organic certificate is required.

The export volume of Ethiopian organic coffee is the second largest in the world next to that of Peru (Kodama, 2007; International coffee organization (ICO), 2009. Due to the economic importance of coffee there is an increasing demand for proper quality control for certification of its contents and substances in standardized products. Therefore by using accurate analytical methods for quantitative determinations of chemical substances in coffee are needed. Nowadays, coffee is produced in a large number of countries worldwide.

Coffee grows under diverse environmental conditions ranging from 550m to 2600 m above sea level, with annual rainfall from 1000-2000 mm, temperature (minimum and maximum from 8-150C, and 24-310C, respectively), requires deep, well drained, loamy and slightly acidic soils (Paulos and Tesfaye, 2000). The estimated area of land covered by

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coffee is about 662,000 hectares, whereas the estimated annual national production of clean coffee is about 350,000 tons (Almayehu et al., 2008).

Caffeine is a bitter, white crystalline xanthenes alkaloid that is a psychoactive stimulant drug. Caffeine was discovered by a German chemist, Friedrich Ferdinand Runge, in 1819. He coined the term caffeine, a chemical compound in coffee (the German word for which is kaffee), which in English become caffeine.

Caffeine is an alkaloid of the methylxanthine family which is a naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide (Wanyika et al., 2010 H. N.; Gatebe, E. G.; Gitu, L. M.; Ngumba, E. K.; and Maritim, C. W.). Pure caffeine occurs as odorless, white fleecy masses, glistening needles of powder. Its molecular weight is 194.19 g/mole, melting point 236 ^{o}C, point at which caffeine sublimes is 178 Atmosphericpressure, specific gravity is 1.2, volatility is 0.5%, vapor pressure is 760 mmHg at 178 ^{o}C; solubility in water is 2.17%, vapor density 6.7 and pH values in the range of 6 to 9, (Belay ;2011).

The world's primary source of caffeine is the coffee bean which is actually the seed of the coffee plant, from which coffee is, brewed (Wanyika et al,2010).The highest concentrations of caffeine are found in the leaves and beans of the coffee plant, in tea, yerba mate, guarana berries, the kola nut and cocoa (Meltzer et al,2008). In the literature the amount of caffeine found in these products have been reported as the highest amounts are found in guarana (4–7%), followed by tea leaves (3.5%), mate tea leaves (0.89–1.73%), coffee beans (1.1–2.2%), cola nuts (1.5%), and cocoa beans (0.03%) (Clifford, N, (1990). Ramirez, Martinez, J. R (1990).

The caffeine is degraded relatively slowly and involves demethylation steps to yield theobromine and theophyline. Theophyline is catabolized to xanthenes via 3-methyl xanthenes. However, it is unclear either 3-methyl xanthenes or 7-methyl xanthenes are intermediates in the conversion of theobromine to xanthenes (Kumar et al., 2006).

Caffeine found in many plant species, where it acts as a natural pesticide, with high caffeine levels being observed in seedlings that are still developing foliage, but are lacking mechanical protection. Common sources of caffeine are coffee, tea, and a lesser extent chocolate derived from cocoa beans. Pure caffeine occurs as odorless, white fleecy masses, glistening needles of powder. Its molecular weight is 194.19g, melting point is 236 point at which caffeine sublimes is 178 at atmospheric pressure, pH is 6.9(1% solution), specific

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gravity is 1.2, volatility is 0.5%, vapor pressure is 760mmHg at 178; solubility in water is 2.17%, vapor density 6.7.

1.2. Statement of the Problem

One of the world's primary sources of caffeine is the coffee "bean" (which is the seed of the coffee plant), from which coffee is brewed. Caffeine content in coffee varies widely depending on the type of coffee varieties and the method of preparation used. Caffeine is a central nervous system (CNS) and metabolic stimulant, and is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs (Nehlig, A; Daval, JL; Debry, G (1992).Caffeine stimulates the CNS first the higher levels, resulting in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination, and later at the spinal cord level at higher doses.

Extraction of caffeine from coffee, to produce decaffeinated coffee and caffeine is an important industrial process and can be performed using a number of different solvents. Benzene, chloroform, trichloroethylene and dichloromethane have all been used over the years but for reasons of safety, environmental impact, cost and flavor, they have been superseded by water extraction and supercritical carbon dioxide extraction methods.

Caffeine absorbs strongly in the ultraviolet region (272 nm in water and 276 nm in dichloromethane) and this provides the basis of innumerable spectrophotometer methods, (Clarke and Marroe, 1985; R. J.; Macarae, Z. R. 1985). For caffeine content determination from roasted coffee many analytical methods have been reported. Normally, high-performance liquid chromatography separation and UV/Vis spectrophotometer detection methods are applied.

Spectrophotometer method is relatively simple sensitive, rapid, reproducible valid and the most suitable for on line monitoring, when considerable attention is paid to the removal of interfering compounds using mathematical methods. A method is used for quantitative determination of one or more species in mixtures by Beer-Lambert’s law and for determination of the particular species in a mixture or the identification of certain functional groups in a compound under structural investigation (Thorne, 1988; Rao, 1975; Bauman, 1962).

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In this research, it is intended to determine the quantitative comparison, compositional analysis of Caffeine compound of small and large roasted coffee beans using spectrophotometer via experimental and computational methods.

Caffeine is provided through a number of different sources, most commonly through coffee, tea and soft drinks. Like many other drinks, coffee is a mixture of many macro and micro nutrients within it. Therefore, it is the most popular beverages consumed and worldwide, worth it is considered to nutritional contribution to make our balance diet, in coffee, it is believed to have implication upon human health. Thus it has been very important; to determine caffeine amount in the coffee due to the reason that coffee is used in our day to day consumption with high amount of caffeine. In addition, it is also important to see presence of caffeine amount difference in large and small size coffee beans.

In spite of the fact that coffee is the most economic source in SNNPR region, caffeine amount in coffee was not well documented in the region. Therefore, this study is intended to determine the amount of caffeine and its difference in large and small size as well as to characterize caffeine content in roasted coffee beans by molar decadic absorption and transitional dipole moment in selected Zones (Sidama and KambataTambaro) of SNNPR, Ethiopia.

1.3. Objective of the Study

1.3.1. General Objective

The main objective of this study is to determine the caffeine content of small and large size coffee beans from Sidama and KambataTambaro zones southern, Ethiopia using uv-vis spectrophotometer.

1.3.2. Specific Objectives

- To determine the caffeine content in small and large size roasted coffee beans.
- To compare caffeine content in small and large size coffee beans.
- To characterize caffeine content in coffee bean by molar decadic absorption coefficients and translational dipole moment of pure caffeine.

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1.4. Significance of the Study

The aim of this study is to determine the amount of caffeine in small and large size roasted coffee beans. Currently Regular daily consumption of caffeine containing beverages mainly coffee is widespread throughout the globe. However; excessive caffeine consumption should be avoided by people who are being treated for certain condition such as depression, anxiety and heart problems.

In line with this, the study provide basic information on the amount of caffeine in small and large size roasted coffee beans, in ordered to determine whether roasted coffee beans from selected zones should be decaffeinated before used .in addition to this, the study will provide base line information about the caffeine content in small and large size roasted coffee beans. Moreover, it will give the base line data for further study.

1.5. Scope of the Study

The scope of this study is to determine the amount of caffeine in small and large roasted coffee bean by using uv–vis spectrophotometer and characterization of coffee bean by molar decadic absorption coefficients and transitional dipole moment of pure caffeine.

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CHAPTER TWO

2. LITERATURE REVIEW

The coffee tree belongs to the large Rubiaceae family within which it constitutes the coffee genus. Although more than 80 coffee species have been identified worldwide (Clarke, 2003), only two are economically important. Coffee Arabica, also known as Arabica coffee, is responsible for approximately 70% of the global coffee market, and coffee canephor or Robusta coffee (commercial name of one of the main c. canephor cultivars) accounts for the rest (ICO, 2011). Arabica coffee is the species that has been known for the longest time and is also the widespread throughout the world. It is the only tetraploid in the genus, with 2n=44, (Almayehu ; Wilson).

There is much speculation but only limited evidence of coffee consumption being linked to protective effects on human health. Literatures reveal coffee beans contain efficient water soluble antioxidants, such as chlorogenic acids, caffeic acids, ferulic acids, p-coumarou acids, melanoids and alkaloids (Adam et al., 2006) and their content depends mainly on the coffee species, origin and degree of roasting (Huck et al., 2005; Belay et al., 2008).

The caffeine content of green coffee beans varies according to the species. During roasting there is no significant loss in terms of Caffeine, Caffeine was discovered by a German chemist, Friedrich Ferdinand Runge, in 1819. He coined the term caffeine, a chemical compound in coffee (the German word for which is kaffee), which in English become caffeine (Anonymous, 2009). Caffeine found in many plant species, where it acts as a natural pesticide, with high caffeine levels being observed in seedlings that are still developing foliages, but are lacking mechanical protection (Frischknecht et al., 1986)Experimental studies that investigated the cardiovascular effects of caffeine found an increase in systolic or diastolic blood pressure (BP), increase in blood sugar, increase in gastric acid and pepsin secretion, increased plasma levels of fatty acids and decreased heart beat rate (HR) at doses as low as 1 mg/kg in children and 1.4 mg/kg in adults. Caffeine intakes of ≥1.4 mg/kg increased aortic stiffness, in-creased vascular resistance, decreased cerebral blood flow, increased plasma epinephrine, and rennin activity reported by,(BlenWeldegebreal al,2016) ,(Milanez,S.2011).

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Recently appeared methods (1996–2012) reporting the determination of caffeine in various sample matrices (environmental, biological, plants, food, etc.) cover a broad spectrum of instrumental analysis (Ally, 2013). Many methods exist for determining the methylxanthine contents of food and beverages. Some of these methods include UV-Visible spectrophotometers, electro analytical, the overall land area devoted to coffee production due to new plantings is increasing and estimated to be 662,000 hectares, of which 496,000 hectares are estimated to be productive. The average annual production is amounting to about 350,000 ton and productivity of about 0.71 t/ha (Almayehu, 2008).

There is much speculation but only limited evidence of coffee consumption being linked to protective effects on human health. Literatures reveal coffee beans contain efficient water soluble antioxidants, such as chlorogenic acids, caffeic acids, ferulic acids, p-coumarou acids, melanoids and alkaloids (Adam et al., 2006) and their content depends mainly on the coffee species, origin and degree of roasting (Huck et al., 2005; Belay et al. Common sources of caffeine are coffee, tea, and a lesser extent chocolate derived from cocoa beans (Matissek, 1997). Pure caffeine occurs as odorless, white fleecy masses, glistening needles of powder. Its molecular weight is 194.19g, melting point is 236, point at which caffeine sublimes is 178 at atmospheric pressure, pH is 6.9(1% solution), specific gravity is 1.2, volatility is 0.5%, vapour caffeine (Ramalakshmi and Raghavan, 1999).

Caffeine is one of the most widely used psychoactive substances in the world, its estimated global consumption being 120,000 tons per year. Caffeine is of major importance with respect to the physiological properties of coffee, and also in determining the strength, body and bitterness of brewed coffee. It also increases the effectiveness of certain drugs. Hence, it is used with some over-the-counter drugs for the treatment of conditions such as migraine and cluster headaches (Salihovic et al., 2014).

For most healthy adults, consuming moderate doses of caffeine, or about 200 to 300 mg a day, equal to about two to four cups of brewed coffee, is not harmful. Studies have shown it can help relieve pain, thwart migraine headaches, reduce asthma symptoms, and elevate mood. Although caffeine can contribute to dehydration, recent studies show that it is not dehydrating in moderate amounts, even for athletes (Nicole and Olsen, 2013).

For caffeine content determination from roasted coffee many analytical methods have been reported. Normally, high-performance liquid chromatography separation and UV/Vis spectrophotometer detection methods are applied. Also other methods such as capillary

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electrophoresis, thin layer chromatography and gas chromatography, are used for separation of caffeine in the analysis of mixtures, combined with several other detection methods such as mass spectroscopy and FTIR spectrophotometric measurements. However, very costly instrumentation, highly skilled technicians and complicated and time consuming procedures are required for such methods. Spectrometric method is simple sensitive, rapid, reproducible valid and the most suitable for on line monitoring, when considerable attention is paid to the removal of interfering compounds using mathematical methods.

2.1. Spectroscopy

Spectroscopy is a scientific measurement technique. It measures light that is emitted, absorbed, or scattered by materials and can be used to study, identify and quantify those materials. Spectroscopy is the study of the interaction of light and matter; it plays an important role in the emergence and validation of quantum theory in the early twentieth century. Molecular spectroscopy is the study of the absorption or emission of electromagnetic radiation by molecules. Quantum theory and Einstein's concept of photons converge in our modern microscopic view of spectroscopy.

A typical spectrum consists of the intensity of a certain response, such as absorption of light, as a function of frequency of the light. The intensity is a measure of the rate at which molecules make transitions from one energy level to another, while the frequency is directly related to the difference in the initial and final energies of the molecule (Bauman's. P,1962), Several processes leading to emission or absorption may occur in the atom or molecule, to a good approximation each of these processes is associated with radiation of very different frequency ranges. Several types of spectroscopy may be distinguished on the bases of the frequency range in which measurements are made as well as the type of information sought.(Laqua K. et al, 1988).One can often understand the nature of the molecular charges that are responsible for the emission or absorption of the radiation. In such cases, the experimental spectroscopic data can be used to determine quantitative value for various molecular properties. When the theory of molecular spectra is treated; it is convenient to classify Spectra according to the type of molecular energy that is being altered in the emission and absorption process.

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In this way the principal headings for the material that is to be presented can be arrived at. These are: Rotational spectra due to changes in the rotational energy of the molecule; Vibration spectra due to changes in the vibration energy of the molecule and Electronic spectra due to changes in the electronic energy of the molecule due to different electron arrangements. The quantized internal energy E_{int} of a molecule in its electronic ground or excited state can be approximated with sufficient accuracy for analytical purposes, by (2.1) where E_{el} is the electronic, E_{vib} the vibration and the rotational energy respectively.

Absorption of a photon results in a change of the electronic energy accompanied by changes in the vibration and rotational energies. Each veronica transition, i.e. a particular electronic plus vibration transition, corresponds to an absorption band consisting of the rotational lines in liquids and solids the rotational lines are broad and overlap so that no rotational structure is distinguishable. The experimental data that such studies provide are the frequencies, or wavelengths, of the radiation and the amount of radiation emitted or absorbed by the sample, one can often understand the nature of the molecular charges that are responsible for the emission or absorption of the radiation.

Spectroscopic techniques provide a non destructive, fast and cheap determination of caffeine in green and roasted coffee beans. But UV-Vis spectrophotometer method cannot be used directly for determination of caffeine in coffee beans extracted with water owing to the matrix effect of UV-Vis absorbing substances in the sample matrix by (Belay et al, 2008).In roasted coffee beans it was observed that there is spectral interference from caffeine and chlorogenic acid in the wavelength regions of 200-500 nm. Yet this method requires the extraction of Caffeine from coffee powder in water solution using dichloromethane for the spectroscopic determination. This is necessary since the caffeine spectrum is overlapped with other compounds found in coffee. Hence, the use of dichloromethane limits from wider application of UV-Vis method which is described above

In such cases, the experimental spectroscopic data can be used to determine quantitative value for various molecular properties when the theory of molecular spectra is treated; it is convenient to classify spectra according to the type of molecular energy that is being

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2.2. Interaction of Light with Matter

One of the most important and interesting aspects of spectroscopy are study the propagation of light through matter. In this the interaction of light with matter by classical and semi-classical theory are discussed. The mathematical derivation that can often n used for qualitative and quantitative understanding of how transitions induced in molecular System are presented by semi-classical approach. The time dependent Schrodinger equation has been solved to relate, the theoretical expressions, (the transition dipole moment) with the experimental quantities, (the molar extinction coefficient, and extinction coefficient) that are important in UV-is spectroscopy. Only green light can be transmitted (pass through) this material. Interactions between light and matter determine the appearance of everything around us.

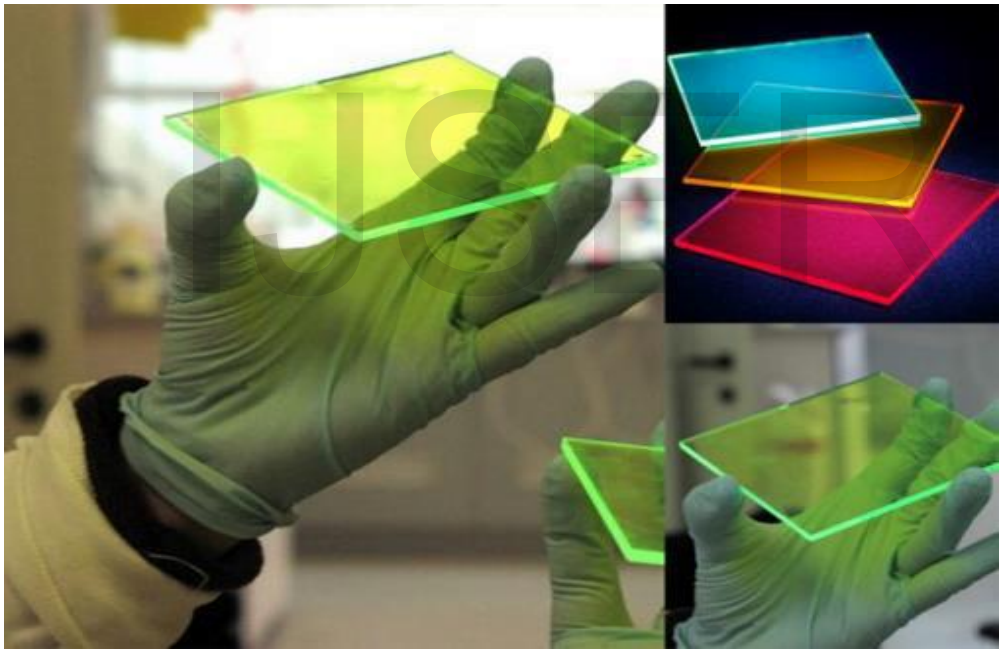

***Figure 9**: Interactions of Light with Matter,*

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2.3. Beer-Lambert's Law and Integrated Absorption Technique

In optics the quantitative spectrophotometers is based on the two principal law of Photometry namely Beer and Lamberts law. The Beer-Lambert's law relates the absorption of light to the properties of the materials through which the light travels. The law states that there is a logarithmic dependence between the transmission, T, of light through substance and the absorption coefficient of substance, α, and the distance the light travel through the material (the path length), *l*.

The absorption coefficient can, in turn be written as a product of either a molar absorption coefficient of the absorber, ε, and the concentration c, of the absorbing species in material or the absorption cross-section, σ, and number density N of the absorbers. For liquids, these relations are usually written as.,

—

Where and are the intensities of the incident and transmitted light respectively, for gases and in particular among physicist and for some spectroscopic techniques (Bauman, 1962; Thorne; 1988; Milonni and Eberly, 1988). The transmission for liquid substances expressed in terms of absorbance as follow,

(—)

The absorption cross-section σ, related to the absorption coefficient α at a single frequency for N number of molecules per unit volume can be expressed by the following relation, (Thorne; 1988; Milonni and Eberly, 1988)

However, in a UV-Vis spectrophotometer, the absorption of molecules in a liquid occurs over a certain range of frequencies rather than at a single frequency reported by, (Abebe et al;Thorne, Ture, K.; Redi, M.; Asfa , 1988; Rao, 1975).Therefore, absorption coefficient measured at any single frequency may not express the true intensity of the molecular transition. An Integrated absorption coefficient which is the sum of absorption coefficients for all frequencies in the band is preferable in such cases; the technique is useful for

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different applications since it is independent of the line function which may vary by parameters like

Pressure, temperature, concentration of the solute and solute-solvent interaction as reported by,(Thorne, 1988; Rao, 1975; Liptay, 1969). In addition to that, the technique is very important in the absence of a high-resolution spectrometer, (Ephraim, GD.; Grima, W.W.; Araya S, A. Ramsey, 1951).Therefore, in liquids and solutions where the above effects are observed, the true integrated absorption intensity of a band should be defined by the following equation. :

$$\alpha_t = \int \alpha \, d\nu \qquad 2.1.4$$

Using Eq (2.1.2) into (2.1.4) yields

$$\alpha_t = \frac{1}{l}\int \log\left(\frac{I_o}{I}\right) d\nu \qquad 2.1.5$$

From the integrated absorption coefficient, the integrated absorption cross-section can be calculated using the following equation (Milonni and Eberly, 1988).

$$\alpha_t = \frac{1}{Nl}\int \log\left(\frac{I_o}{I}\right) d\nu \qquad 2.1.6$$

Where, is the integrated absorption cross-section, and α_t the integrated absorption coefficient, and N, is the number density

2.4. UV-Visible Absorption Spectra

The visible region of the spectrum comprises photon energies of 36 to 72 kcal=mole, and the near ultraviolet region, out to 200 nm, extends this energy range to 14kcal=mole. Ultraviolet radiation having wavelengths less than200nm is difficult to handle.

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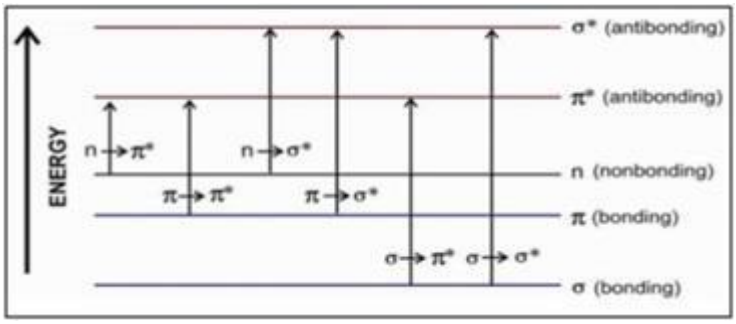

Figure 10: UV-Visible Absorption spectra

Squinty, absorption the six transitions outlined, only the two lowest energy ones are achieved by the energies available in the 200 to 800 nm spectrum. For molecules that possess π bonding energy that is available can promote electrons from a π bonding molecular orbital to a π anti-bonding molecular orbital.This is called a $\pi^* \leftarrow \pi$ transition. The energy difference for such a transition to occur will depend upon the atoms π bonded to each other, other atoms attached as well as the relationship between two or more π bonds within the molecule. π bonds between two carbon atoms will have a different $\pi^* \leftarrow \pi$ transition compared to π bonds between a carbon and an oxygen atom (a carbonyl) or a π bond between a carbon atom and a nitrogen atom (in a nitrite) : This is because there will be a different energy gap between the π bonding and π anti-bonding molecular orbital energy states. The greater the energy of transition the shorter is the wavelength of UV orVisible radiation will have to be for electrons to be promoted from the bonding to the anti-bonding state. Every group of atoms with π bonding will have a different wavelength where maximum absorption will take place. This is called the λ_{max} the wavelength where maximum absorption takes place, and the group of atoms with the π bonding is called a chromospheres' each chromophore will have a different energy of transition between the bonding and anti-bonding molecular orbital for which the electron transition takes place.

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2.5. Comparison the Theoretical Results with Experimental Quantities

Now, let us relate the measurable quantities, the molar decadic absorption coefficient ε to quantum mechanical expression that means the theoretical expression for the rate of transfer of molecules from ground to excited state. The molar decadic absorption coefficient, Eq (2.4.1) related to the absorption coefficient by the following Eq (2.4.2)

(Barrow, 1962; Ban well, 1972).

2.4.7

and where c, is the concentration

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CHAPTER THERE

3. MATERIALS AND METHODS

In this chapter Study area and study period, Samples Collection, laboratory instruments, chemicals and samples experimental procedures were included.

3.1. Study Area and Study Period

The study was carried out in two selected Zones of southern Ethiopia from November 2017 to December 2017. The South Region shares borders with Kenya in the South part, South Sudan in the West part and Oromia Regional State in the East and North parts (Fig.3).Coffee is one of the best cash plant in southern Region. In the region there are 10 and 1 special woredas Zones that are mainly depending on coffee plantings.

Of them the two Zones such as Sidama and KambataTambaro were selected based on their high coffee productivity and geographically feasibility.

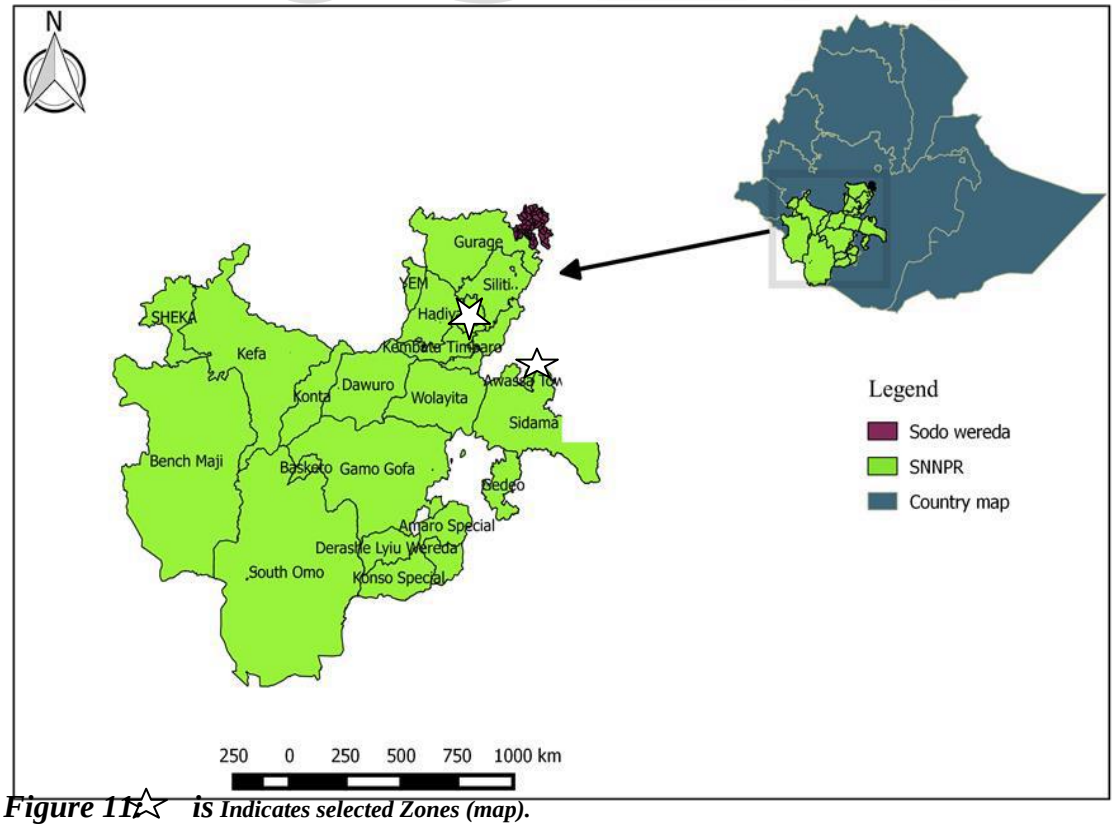

Figure 11:☆ is Indicates selected Zones (map).

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3.2. Samples Collection

The green coffee beans were collected from southern region, Sidama (wondogenet and Boricha) and kambataTambaro (kachabira and hadaro) Zones, SNNPR, Ethiopia. The collected green coffee samples were species of coffee Arabica informed from each woredas farmer. Then collected eight coffee samples were taken to Ethiopia, commodity exchange (ECX) laboratory Woliata at where the roasting and grinding process was occurred.

3.3. Laboratory Instruments

All the laboratory instruments were safely used for their roles and activity,

- Electronic balance and Electronic microbalance were used to measure mass of coffee before roast and pure caffeine respectively.
- The Electric motor grinder (GUATEMALA SB, USA) was used to grinding roasted coffee beans and ProbatWerkeEmmeirch Am Rhein Germany (AC Induction Motor 41K25GN-CF, USA) used to roast green coffee beans,
- Beakers were thoroughly washed and used to measuring volume of conc., de-ionized water and dichloromethane.
- Safely washed Cylinders were used to measuring volume of extracted concentration of caffeine.
- Spatula was used to add the coffee sample powder and pure caffeine in to solvents.
- Quartz cuvette was used to take measuring concentration in the UV/Vis spectrophotometer.
- separator funnels caffeine from were used to extract the coffee water solution,
- The glass filter was used to filtrate coffee water solution from interfering substances, the coffee water solution was stirred with magnetic stirrer with hot plate, and the watch was needed to record the duration of time.
- The UV/Vis spectrophotometer was the instruments which used to measure the absorbance of standard and sample concentration in laboratory. The spectrophotometer was interfaced (linked) with personal computer which is operated by software.

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3.4. Chemicals

In this section, all necessary reagents and chemicals were used for the determination of caffeine content in coffee beans were 854ml of Dichloromethane which is 98- 99% of efficient to extract caffeine from coffee sample as reported by,(A,Abebe et al.),78mg of pure caffeine and 800mlde-ionized water were used for the experiment.

3.5. Method of the Experiment

3.5.1. Coffee sample preparation

Collected green coffee beans were screened with sieving sieve to separate the size of coffee sample as 15mm and above for large and 14mm and below for small size coffee beans, in Woliata Ethiopia exchange commodity (EXC) at where the roasting and grinding process was done ,the figure-4, below illustrates the standard scale of the size separation of sieve

Figure 12; coffee screening process with sieving mesh

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3.5.2. Roasting coffee bean

50 grams of each coffee bean were measured and thoroughly washed with pure water, and then dried at equal time of 30 minutes separately each green coffee bean. The safely dried each green coffee beans were roasted by using Probat Werke Emmeirch Am Rhein Germany at 80 temperature for 8-12 minutes. Figure 3, below illustrates the instrument used to roast coffee sample in the above favorable.

Figure: 13 roasting process of coffee beans

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3.5.3. Grinding coffee beans

The well prepared and safely roasted coffee beans were ground by using GUATEMALA SBGERMANY; the grinding condition used was 250-450µms sieve for powder were the laboratory apparatus used for the experiment at Woliata exc., Coffee sample powdered were sealed and transported to Arbminch University where caffeine analysis and extraction was occurred. Figure 6.illustrates

Figure: 14 grinding process of roasted coffee.

Figure 15 sealed ground coffee powder

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3.6. Instrumental Calibrations

To determine the caffeine content of the coffee samples, the validation was properly done by using the prepared standard solutions for the experiment. The stocks solution was (500ppm) of caffeine was prepared by dissolving 25 mg of the pure standards in 50 mL De-ionized water. This prepared solution where strewed with magnetic stirrer 30 minutes. Then working standard solutions were (0, 10,30,60,80 and 100 ppm)5standards were prepared with concentrations in the range of 1–100 mg L-1.The solutions were measured by using uv-vis spectrophotometer and 0 ppm solution did no peak or absorbance but the rest standard solutions were (10, 30, 60, 80 and 100ppm) gave their responses. Moreover, the peak area was found with increasing of concentration. The absorbances of the standard solutions were listed in table 2 below.

3.6.1. Calibration curve graph for standard solution

A calibration curve for absorbance versus concentration of working caffeine standards was constructed to validate for the quantification of caffeine in case of linearity and calibration purpose. Then curve showed good linear relationship between the absorbance and concentrations of the standard solutions as well as serial and absorbance. The equation formulated was derived as Y=0.5265x+2.1377 and calibration curve of standard (= 0.9978), where Y absorbance, X is concentration of caffeine (mg/L) and R is the linear correlation factor. Therefore the method was taken as cheap rapid and reproducible for the quantitative determination of caffeine extracted by dichloromethane from coffee sample.

Table 1: Absorbance of standard solution

Concentration in(mg/L)	Absorbance in(au)
10	2.635
30	3.211
60	3.751
80	4.232
100	4.757

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3.7. Characterization of Caffeine in Pure Water

The characterization of pure caffeine in de-ionized water and dichloromethane were don independently adding a mass of 25mg pure caffeine powder and dissolved in 30 ml of de-ionized water and 28mg of pure caffeine powder was added to 34ml of dichloromethane solvent.

The solved quantitative value of the concentration of two solution were C=4.29 mol and C=4.23 mol respectively were stirred 50 minutes using magnetic stirrer, then the maximum peak of absorbance were found by wavelength at 243—300nm for in water solution and 243-312nm for dichloromethane solution. Then absorbances of the above solutions were measured by UV/vis spectrophotometer at room temperature, so that from the above conc. each cuvette (cm) was measured at given wavelength and its absorbance recorded. From the absorbance, molar decadic absorption coefficient, integrated area under curve and transitional dipole moment of caffeine in water and dichloromethane were obtained respectively.

3.7.1. Caffeine analysis

Powdered coffee sample was prepared and 1.00g of each was measured by using electronic balance and poured into 250ml volumetric flasks separately for each coffee powder. Then 50ml de-ionized water was added and stirred with magnetic stirrer for 50 minutes and heated gently to 80 to extract caffeine from water solution. The solution was cooled and filtered with filter glass. The filtrates were pipette into safely washed 100ml volumetric flasks and formed mobile phase.

Figure 16 ; water coffee solution

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3.7.2. Caffeine extraction with dichloromethane

Due to the interfering matrices in the water coffee solution, 30ml of Dichloromethane was used to overcome this difficulty. The reagent used to extract caffeine easily from coffee solution. This is a quite similar to those of used by (Blen Weldegebreal et al, 2016;Belay et al, 2008).Then 30ml of dichloromethane was dissolved and stirred with magnetic stirrer for 10 minutes for each of the solution filtered.

After filtration the solution was transferred into the separator funnel, due to the high solubility of dichloromethane Coffee solution was occurred upper part and extracted caffeine with dichloromethane was at lower part of separator funnel, therefore the volume of the extracted caffeine was filtered and recorded (4-times repeated)and fourth round was negligibly zero. Then mean value was taken after extraction of coffee water solution by dichloromethane. The extracting steps illustrated b by figure 7, 8 respectively

Figure: 17, the process of stirring filtrated coffee water solution by DCM.

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The figure10 below illustrates filtaring technques to each of the solution in above figure 9 and measured by the cylinders (three times).

Figure:18 ,The extracting process of caffeine by DCM.

The caffeine concentrations measured in above figure 10 were repeated three times and each of absorbance was measured by uv-vis spectrophotometer in figure11 below.

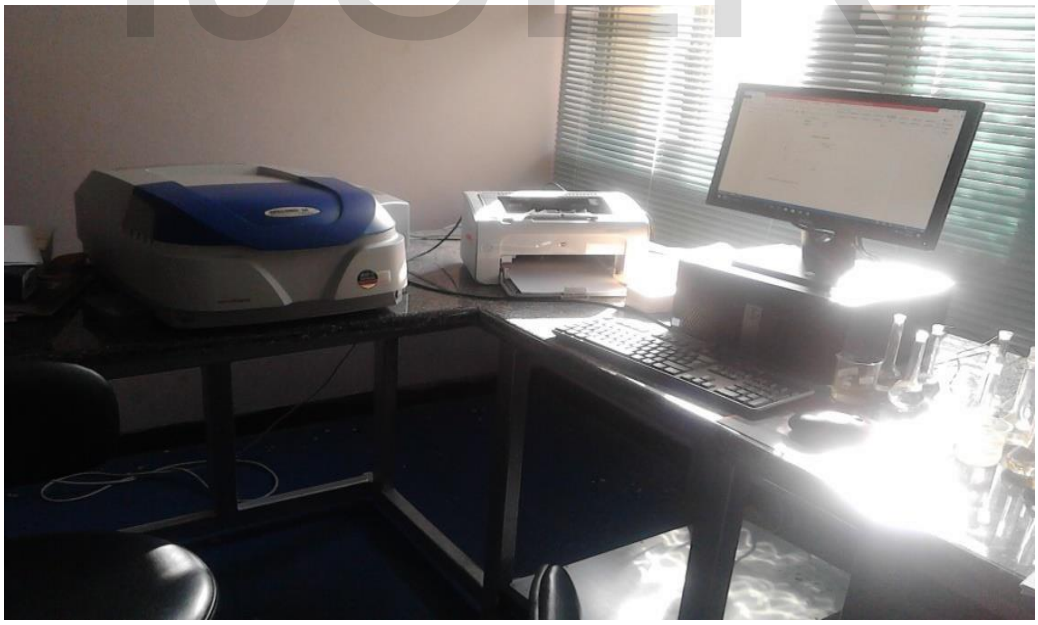

Figure:19Absorption of caffeine concentration measured byuv-vis spectrophotometer

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CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Data Analysis

4.1.1. Linearity of calibration graph

The calibration graph was generated using 10 mm curvet. Five different standard concentrations (10, 30, 60, 80 and, 100) of caffeine from 10 ppm to 100 ppm were analyzed according to experimental conditions. The absorbance of the dilution of serial number above calculated was (0.04, 0.16, 0.22, 0.34, and 1.4) then the calibration curve was established according to the obtained response (peak area) and the concentrations of caffeine in standard solutions. The results show a good linear relationship that was found to be 0.999. The calibration graph is also shown in below the absorbance versus concentration from the table 2.

Table 2 the measured value of absorbance from prepared standards.

Concentration in(ppm)	Absorbance in Concentration
10	2.635
30	3.211
60	3.751
80	4.232
100	4.757

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The calibration graph below in the figure 11 shows the linearity and the regression factor and y=0.5265x+2.1377, were y indicates the maximum absorbance and xindicates concentration.

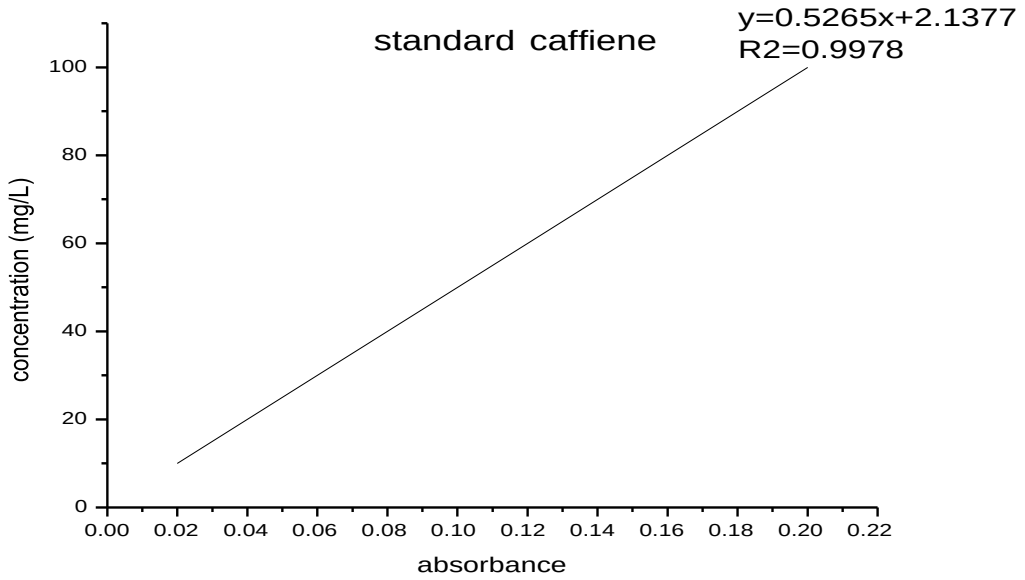

Figure 20 calibration graph of standard solution.

4.1.2. Characterization of pure caffeine

UV–vis absorption of caffeine in de-ionized water and dichloromethane; the UV–vis absorption spectrum of caffeine in de-ionized water was found to be in the region of 243-300nm and its intensity is drops the wavelength greater than this.

The peak absorbance of the solution is found to be IA = 1.190 at the maximum wavelength =272.5nm.this experiment was quite similar to those reported by (A, Abebe et al, Clarke and Macrae (1985).The molar decadic absorption coefficient was measured at a given wavelength and it's the peak absorbance 1.190 taken to calculate value of using Beer–Lambert's e equation (Liptay, 1969).

The molar decadic absorption coefficient of pure caffeine in water is computed and its value 27.73 by using beer lamberts law; equation 2.1.2 in review literature reported by (Bauman,(1962),Thorne;1988;Milonni and Eberly, 1988).Transitional dipole moment was calculated by integrating the coefficient from integral line of frequencies band v1=34000 to v2=412000 and its value was ,μi=15.20 .

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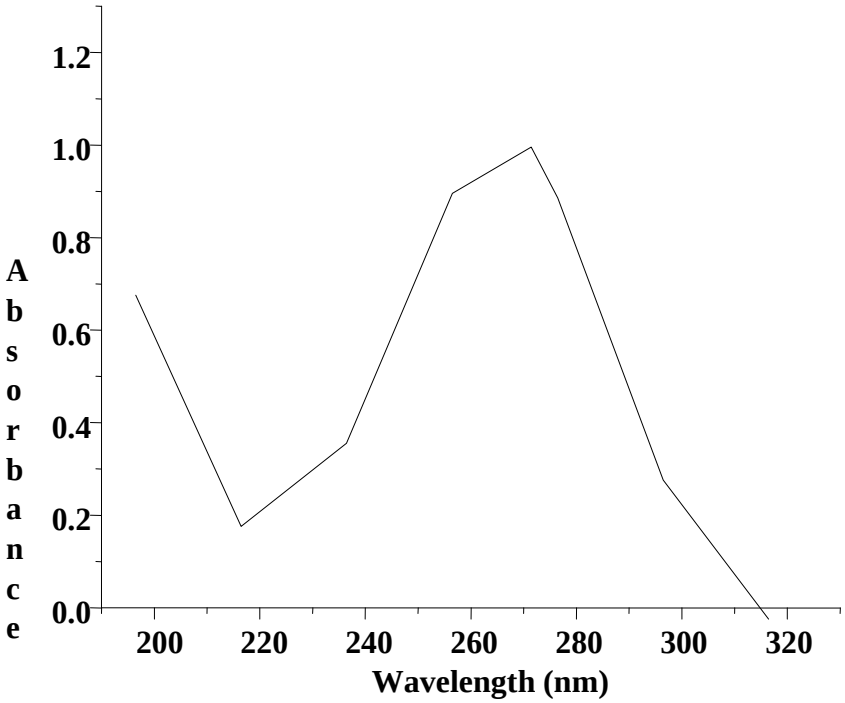

Figure 21 UV–vis absorption spectra of caffeine in water were found to be in region of 2 43-300nm.

The UV/vis absorption spectra of caffeine in dichloromethane were found to be in the region of 246–320 nm. Its peak absorbance A = 1.359 was obtained at maximum wavelength max 275 nm (fig-4).The maximum peak absorbance for caffeine observed in these experiments was almost similar to those reported by (A,Abebe et al., 2017;Clarke and Macrae, 1985).

The molar decadic absorption coefficient measuring the intensity of optical property at a given wavelength was calculated by using Beer–Lambert's equation 2.1.2 given in review literature (Liptay, 1969).the value was .and integrated area under the curve by using the integral line of the frequency bands v1=312000 to v2=406000 then, A=228.64 , again the calculated value of transitional dipole moment, μi=15.293 .

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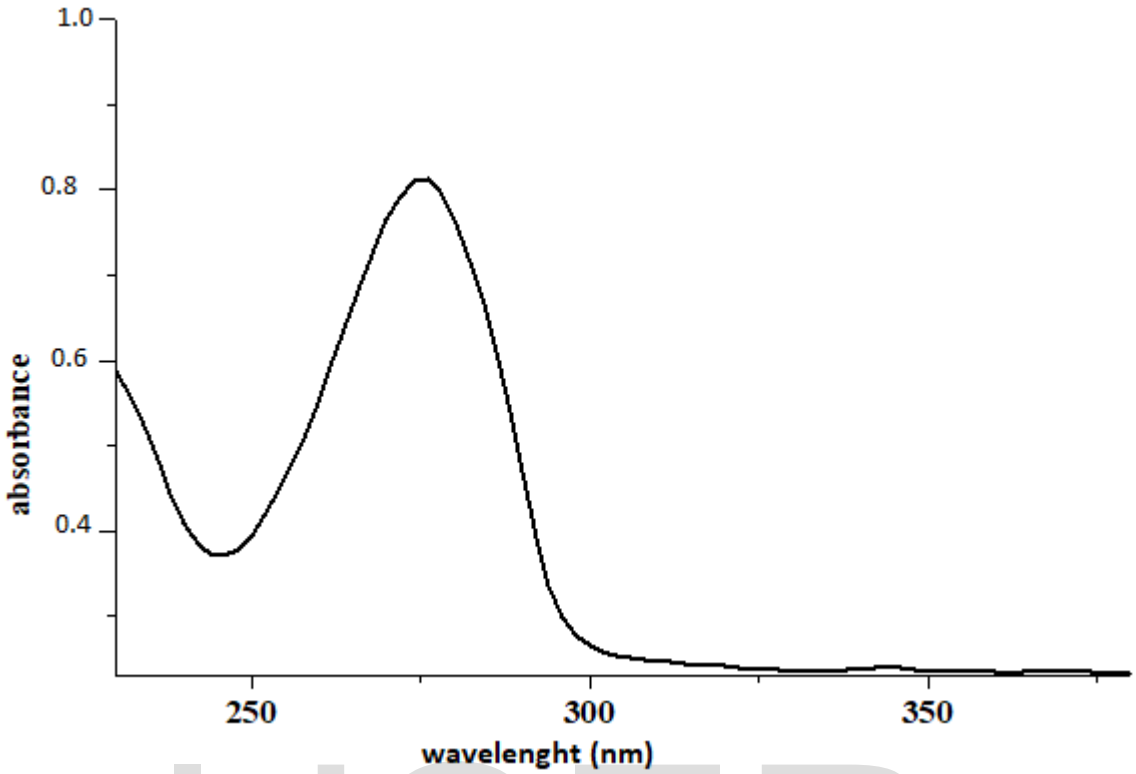

Figure 22 the UV/vis absorption spectra of caffeine in dichloromethane were found to be in the region of 200–320 nm.

4.1.3. Determination of caffeine in small and large size roasted coffee sample

Determination of caffeine in the laboratory were helped by uv-vis spectrophotometer method direct way to owing the matrix effect of UV –Vis spectrophotometer absorbing substances in the simple matrix (A, Belay et al, 2017; Ortega-Burrales., 2002; Zhang et al., 2005).The matrix effect coffee sample in water solution was clearly seen before extracted by dichloromethane. In order to minimize this interfering substance in coffee was first dissolved in water and caffeine extracted from solution Using dichloromethane

Dichloromethane is the most commonly powerful solvent used to the extraction of caffeine from small and large roasted coffee beans (AlmayehuKros, 2008; Rofti, 1971). Many commercial products applied dichloromethane for decaffeinating the coffee roasted coffee Beans for its extraction efficiency 98–99% (A, Belay et al ;Clarke, 1980).Then the extraction was made three times and the extracted concentration was measured by uv-vis

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spectrophotometer. Then the peak absorbance of coffee sample versus wavelength caffeine content and absorbance were obtained.

4.1.4. Coffee sample extraction by dichloromethane

Caffeine concentration in coffee, triplicate measurements and standard deviation After dissolving the coffee powder in water were obtained but there was some interfering matrices, hence to minimize this, the most common extracting solvent dichloromethane was used and the caffeine concentration measured three times by the procedure in figure 9, then Result of triplicate measurements, standard deviation and mean caffeine concentrations were listed in table 3 below.

Table 3 Experimental result and measured value of coffee sample

Origin of Coffee sample	Size of Coffee	Triplicates of Measurements In (ppm)	Mean value of conc. in (ppm)	Std.dev. Value
Boricha	Small	28.5,29 .0, 32.5	30.0	2.345
	Large	18.5 21 22.8	20.16	2.989
Wondogenet	Small	30, 31.5 31.5	31.0	1.224
	Large	22.5, 23.5 23	23.0	0.707
Kachabira	Small	25.5,27,28.5	27.0	2.212
	Large	20,19.5,21.5	20.3	1.473
Hadaro	Small	31.5,31.5,30	31.0	1.224
	Large	25,22.5,26.5	24.6	2.860

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4.1.5. Determination of caffeine mass and its percentage from experimental data

Extraction by dichloromethane in the table below 4; summarizes caffeine concentration, mass of caffeine and their percentage of caffeine in coffee sample. Hence, the result of caffeine content and mean caffeine concentrations are listed in table 4 below.

Table: 4 mass of caffeine (g) and its percentages from experimental data.

Origin of coffee sample	Size of coffee	Mass of coffee powder (g)	Conc. of caffeine in Mean (ppm)	Mass of caffeine in (g)	Percentage of caffeine in coffee (w/w) %
Borcha	Small	1.00	30	0.0773	7.73
	Large	1.00	20.16	0.0519	5.19
Wondogenet	Small	1.00	31	0.0799	7.99
	Large	1.00	23	0.0592	5.92
Kachabira	Small	1.00	27	0.0695	6.95
	Large	1.00	20.3	0.0523	5.23
Hadaro	Small	1.00	31	0.0799	1.050
	Large	1.00	24.16	0.0634	0.790

4.1.6. Caffeine concentration in selected Wordas of South Ethiopia

The Mean concentrations of caffeine in small and large roasted coffee from four selected Woreda's of South Ethiopia were measured at max =275nm and analyzed by one way analysis of variance (ANOVA). The result was shown in table-4. The minimum concentration was observed in Kachabira and maximum concentration was observed in Hadaro (Fig-3). However, there was no significant difference in caffeine concentration among coffee from four woredas (F test= 0.888; P=0.464). Our result suggests that the concentration of caffeine in coffee may not depend on the geographical variation.

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Table 5: Mean concentrations of caffeine in small and large roasted coffee samples from four woredas of South Ethiopia

Woredas	No	Mean Conc. (ppm)	Std. Deviation	95% Confidence Interval		F-test	P-value
				Lower	Upper		
Boricha	6	25.00	6.753	17.91	32.09		
Wondogenet	6	27.00	4.517	22.26	31.74	0.888	0.448
Kachabira	6	23.67	7.421	15.88	31.45		
Hadaro	6	28.67	3.141	25.37	31.96		
Total	24	26.08	5.679	23.69	28.48		

- The mean caffeine concentration of small size coffee sample and large coffee sample were illustrated in figure 5 below, in which the red line indicates mean caffeine concentration of small coffee sample where the black one indicates large

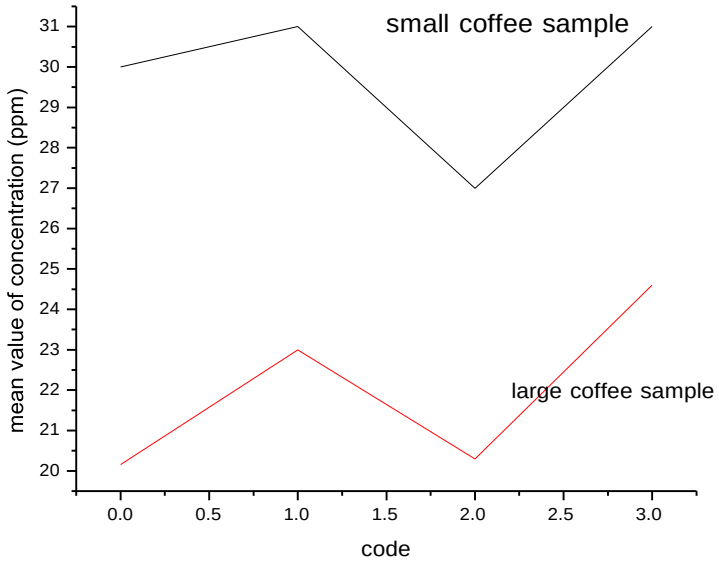

Figure:15 Comparssion of mean caffeine concentration in small and large.

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Where, 0 indicate Boricha, 1 Wondogenet, 2 Kachabira and 3 Hadaro Figure 15; Comparssion of mean caffeine concentration in four Woredas of southern Ethiopia, Where, 0 indicate Boricha, 1 Wondogenet, 2 Kachabira and 3 Hadaro.

4.1.7. Determination of caffeine content in Small and Large Roasted coffee from four selected Woredas of South Ethiopia

The mean concentration differences of caffeine between large and small roasted coffee was analyzed using independent t-test regardless of the area. As illustrated in table-6, the mean concentration of caffeine was significantly high in small coffee beans as compared to large coffee beans (mean 29.75 Vs 22.42 with SD of 4.070 Vs 4.641; t= 4.1; P<0.001). High concentration of caffeine was found in small sized coffee beans while as compared to large. Therefore the hypothesis suggests that the concentration of caffeine was depend on the size of coffee.

Table 6. Determination of caffeine content in Small and Large Roasted coffee from four selected Woredas of South Ethiopia

Coffee size	No	Mean conc. In(ppm)	Std. Deviation	t-test	P-Value	95% Confidence Interval	
						Lower	Upper
Small	12	29.75	4.070	4.115	<001	3.638	11.029
Large	12	22.42	4.641			3.634	11.033

4.1.8. Caffeine content in coffee beans collected from each woredas

Caffeine content was determined in small and large sized roasted coffee from each four selected woredas. The caffeine content was significantly high in small coffee beans collected from Boricha, Wondogenet and Hadaro as compared to large sized coffee beans (P<0.05), however, the caffeine content was not significantly changed between small and large coffee beans from Kachabira (P=0.343).

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Table 1 The mean caffeine concentration in Small and Large Roasted coffee from each selected woredas

Woredas	Coffee Size	Mean Con (ppm)	Std Dev	T-test	P-value	95% CI	
						Lower	Upper
Boricha	Small	30.00	5.696	2.74	0.050	0.001	20.011
	Large	20.00	3.464			0.60	20.623
Wondogenet	Small	31.00	0.00	8.00	0.001	5.224	10.776
	Large	23.00	1.732			3.697	12.303
Kachabira	Small	31.00	6.928	1.074	0.343	-10.040	22.707
	Large	24.67	7.506			-10.081	22.748
Hadaro	Small	31.50	0.866	13.59	<0.001	6.498	9.835
	Large	23.333	0.574			6.396	9.937

4.1.9. Absorbance of mean caffeine concentration extracted from coffee sample

The absorbance of mean caffeine concentration was measured by uv-vis spectrophotometer at 275 wavelength and the experimental recorded values were listed in table 6.the reap tidily measured mean value of the absorbance is high in small and low in large size roasted coffee beans from the selected four Woredas. This suggests that concentration and the absorbance are directly proportional; the literature review in

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equation 2.1.2, Beer-lamberts law, A=cl ,(Bauman, 1962; Thorne; 1988; Milonni and Eberly, 1988).

Table 2; mean absorbance of caffeine of coffee sample by uv-vis spectrophotometer.

Origin of coffee sample	Coffee size	Mean Caffeine conc. in(ppm)	Mean mass of caffeine in (g)	Mean Absorbance in (au
Boricha	Small	30	0.0773	0.870
	Large	20.16	0.0519	0.689
Wondogenet	Small	31	0.0799	1.030
	Large	23	0.0592	0.760
Kachabira	Small	27	0.0695	0.830
	Large	20.3	0.0523	0.651
Hadaro	Small	31	0.0799	1.050
	Large	24.6	0.0634	0.790

4.1.10. Mean absorbance of caffeine concentration in small and large size coffee from selected four woredas

The mean absorbance differences of caffeine between large and small roasted coffee was analyzed using independent t-test regardless of the area. . As illustrated in table-7, the mean absorbance of caffeine was significantly high in small coffee beans as compared to large coffee beans (mean0.945 Vs 0.7225 with SD of 0.01237 Vs 0.00407; t= -3.47145; P<0.01328). High absorbance of caffeine in small sized coffee beans as compared to large

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as hypothesis suggest that the absorbance of caffeine was depend on the concentration of caffeine in coffee size rather geographical difference.

Table 9, mean absorbance of caffeine and its standard deviation from selected four woredas

Size of coffee	N of sample	Mean absorbance(au)	Std.deviation In absorbance	t-value absorbance	P-value absorbance
Small	12	0.945	0.01237	-3.47145	0.01328
Large	12	0.7225	0.00407		

4.2. Decadic molar absorption from coffee sample

The molar decadic absorption of coffee sample were calculated by using the mean conc. of caffeine from coffee sample in(ppm),path length l in(cm) and the absorbance of sample in (au). Hence rearranging the Beer-Lambert equation 2.1.2 into – reported by review which is quite similar to(Bauman, 1962; Thorne; 1988; Milonni and Eberly, 1988),. Molar absorptive, c is the concentration of absorbing species, A, is amount of light absorbed by sample for the given wavelength at 275nm and l, is the distance that light travels through the solution,

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Table: 10 Mean molar decadic absorption of coffee sample from conc. and absorbance

Origin of coffee sample	Size of coffee	Mean conc. of caffeine in(ppm)	Absorbance of sample in(au)	Molar decadic absorption in ( )
Boricha	small	30.0	0.870	29.0
	large	20.16	0.689	31.9
.Wondogenet	small	31.0	1.030	33.22
	large	23.0	0.760	33.04
Kachabira	Small	27.0	0.830	30.74
	Large	20.3	0.651	32.06
Hadaro	Small	31.0	1.050	33.87
	Large	24.6	0.790	32.12

4.2.1. The mean and standard deviation of molar decadic absorption coefficient in coffee

The calculated value was helped by the equation 2.1.2 in review literature, reported by Bauman, 1962; Thorne; 1988; Milonni and Eberly, 1988).And mean molar decadic absorption coefficient with its standard value of molar decadic absorption from small and large size roasted coffee sample, in (were (31.652vs32.28) with SD of (1.998 vs 0.892) respectively.

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Table: 11 the calculated value of mean molar decadic absorption coefficient and standard deviation.

Coffee size	No	Caffeine mean conc. in(ppm)	Molar decadic absorption coefficient, in(	Standard mean deviation SD
Small	12	29.75	31.65	1.998
Large	12	22.42	32.28	0.892

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CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The right methods were developed to determine caffeine content in the small and large size roasted coffee beans from SNNPR, Ethiopia using UV-visible spectrophotometer. In this research, determination of caffeine content, comparison and characterization of caffeine in roasted coffee beans by decadic molar absorption coefficients at optimum temperature were analyzed and the results agreed with the others literature value of analytical methods.

To make the value more accurate, the experiments were measured three times and the mean values were taken. The result of caffeine concentration was extracted by dichloromethane, and the determined value of content in small and large size coffee sample was analyzed by one way analysis of variance (ANOVA). The minimum content of caffeine from selected zones was observed in Kachabira and maximum content was observed in Hadaro. However, there was no significant difference in caffeine content among coffee from four woredas (F test= 0.888; P=0.464). Our result suggests that the content of caffeine in coffee may not depend on the geographical variation.

The mean caffeine content differences of caffeine between large and small roasted coffee was analyzed using independent t-test regardless of the area. The mean content of caffeine was significantly high in small coffee beans as compared to large coffee beans. The mean value of caffeine in small and large size roasted coffee were (7.665 Vs 5.67) with SD of 2.422 Vs 3.118 ; t= 5.3605; P<0.0017).respectively. High concentration of caffeine in small sized coffee beans as compared to large, hypothesis suggest that the concentration of caffeine was merely depend on the size of coffee rather geographical difference. Therefore, determination of caffeine in small and large roasted coffee beans have practical done by using uv-vis spectrophotometer with fast, simple and inexpensive methods. In generally the caffeine concentration and mass of caffeine have significance difference in small and large size coffee beans from selected zones. Moreover,

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the data showed that the caffeine contents of the small and large size coffee samples needed in this study are quietly limited to that of standards for coffee Arabica and its value in range was (5.67 7.665) by uv-vis spectrophotometer**.** In addition to this, the methods have been tested for small and large size green coffee beans as well as green coffee leaves are applied without any restriction.

5.2. Recommendation

In the work of this study findings and the conclusion made above, the following recommendations are forwarded for other studies those not included in this thesis**.**

- By the help of other extrac ants like chloroform, hydrochloric acid, Sulpheric acid should also be checked for the quantification of caffeine content in small and large size roasted coffee samples from different places.
- This thesis work might be repeated with NIR FT-IR, and FT-IR-ATR to determine and compare the caffeine content in small and large size coffee samples from different coffee producing regions.
- Determining and characterizing of the concentration of caffeine in coffee sample of different species should be encouraged**.**

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7. APPENDICES

Appendix I. The UV/vis absorption spectra of caffeine in coffee samples

The UV/vis absorption spectra of caffeine in coffee samples from hadaro (small) found to be in the region of 243–320 nm.

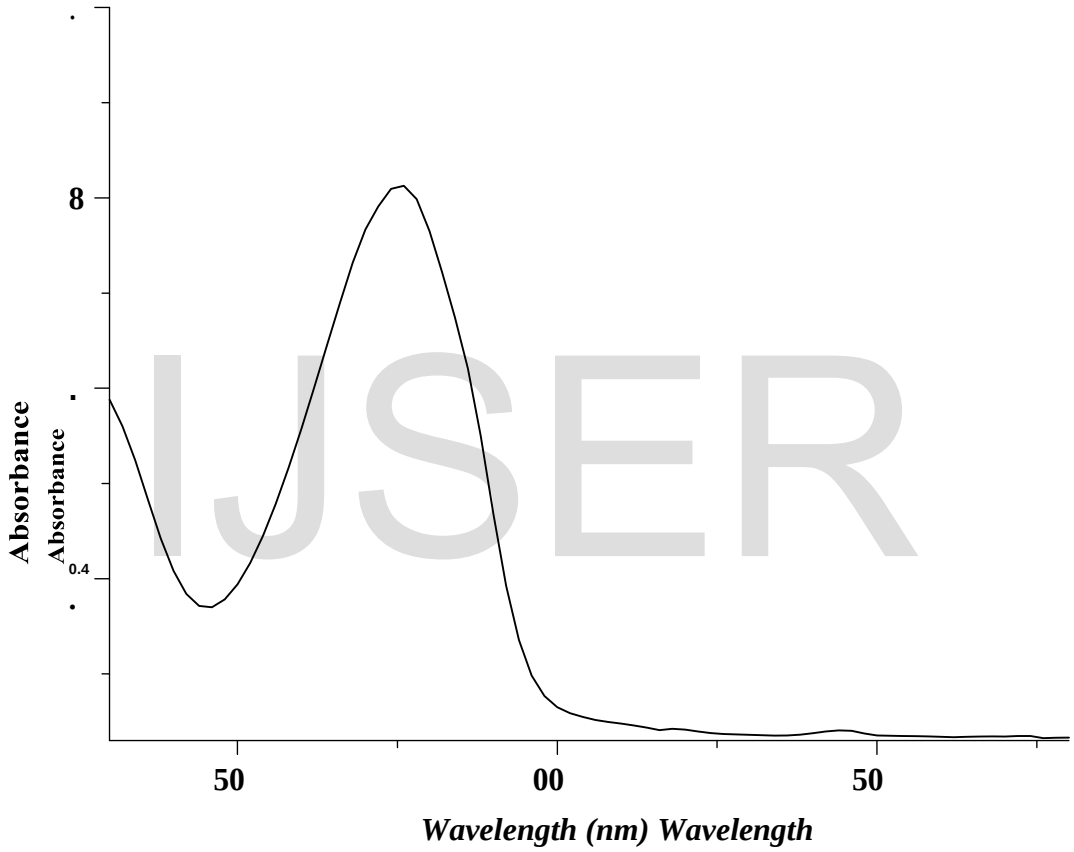

Figure: 23 Uv-vis absorption spectra of Boricha small size coffee sample

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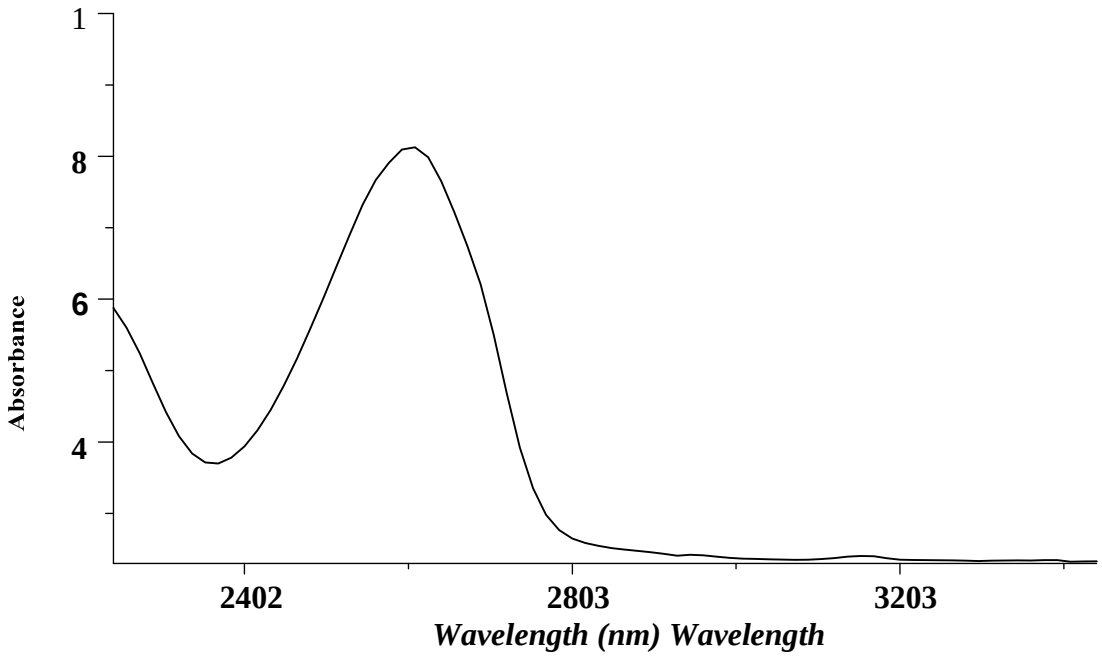

Figure: 24 Uv-vis absorption spectra of Boricha large size coffee sample

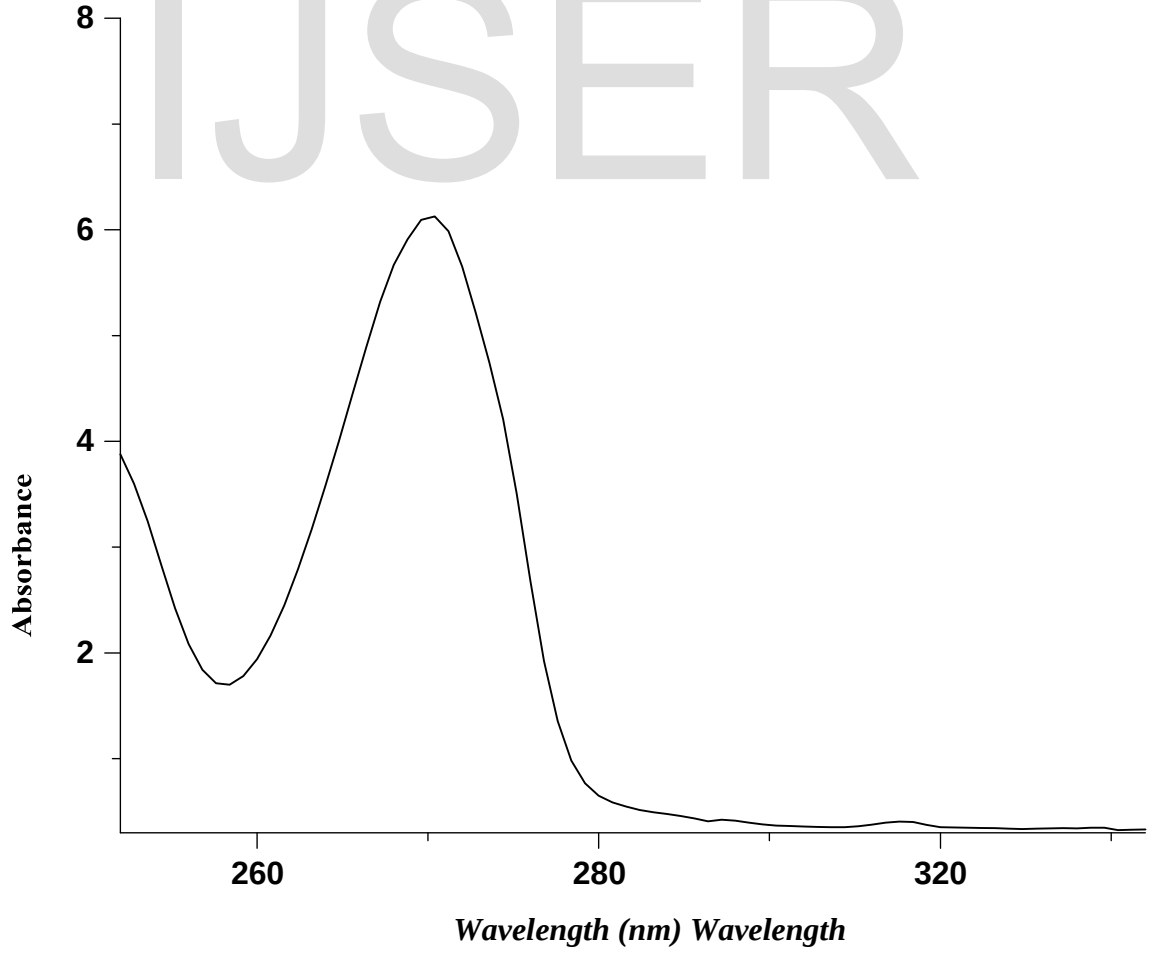

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Figure: 25 Uv-vis absorption spectra of Wondogenet small size coffee sample.

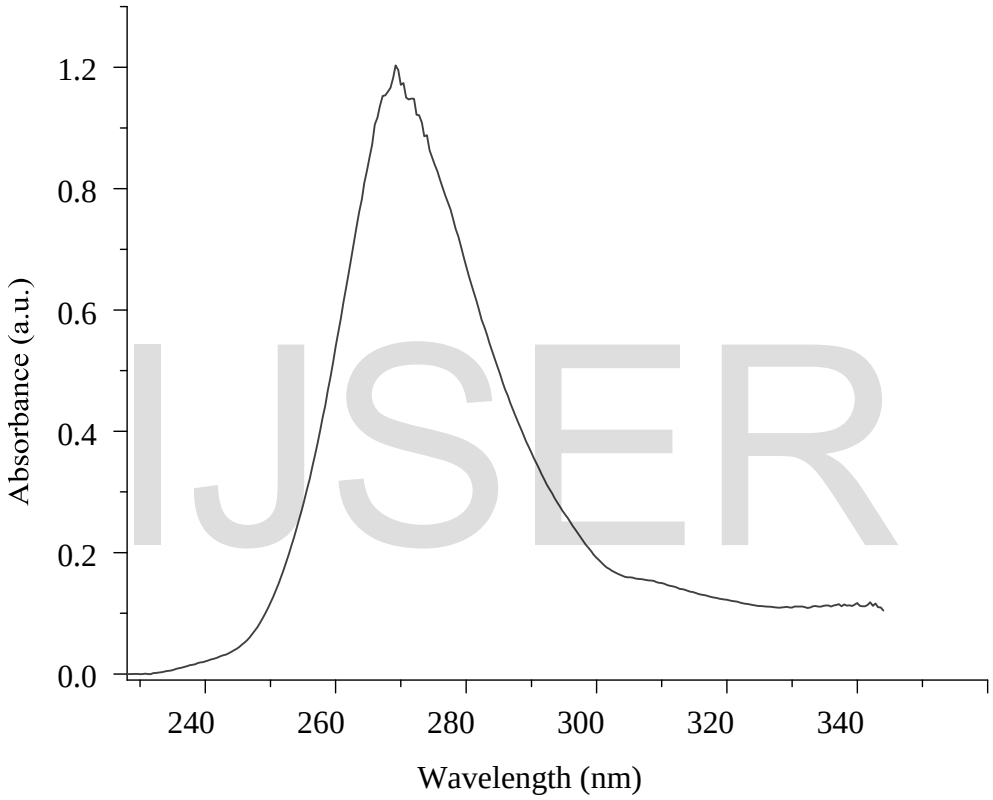

Figure 26: Uv-vis absorption spectra of Wondogenet large size coffee sample.

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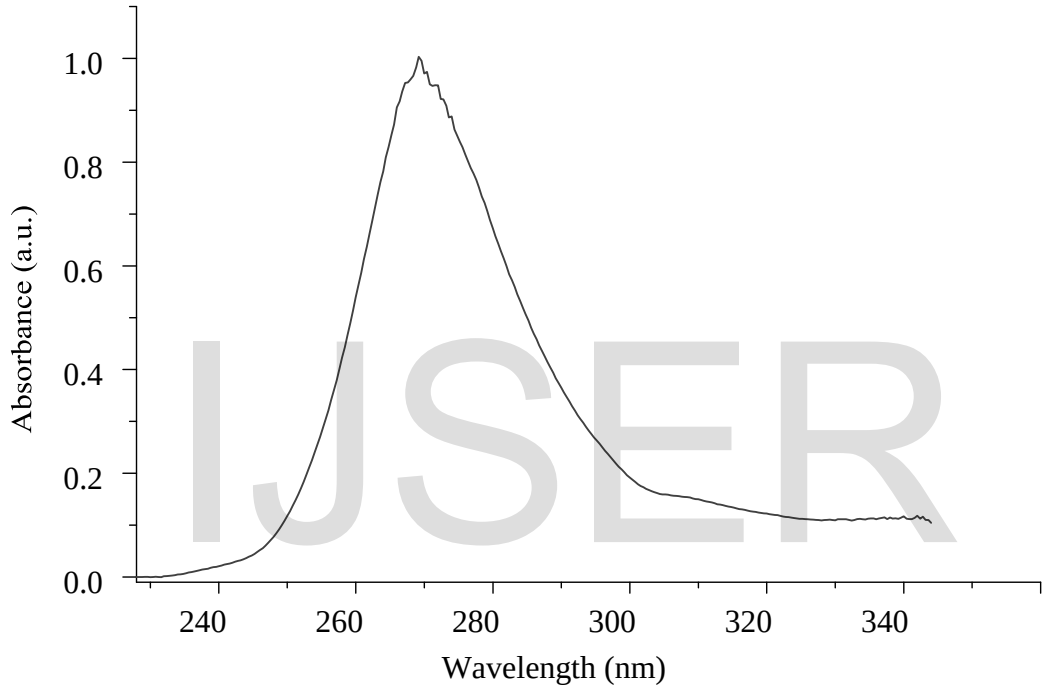

Figure 27 Uv-vis absorption spectra of Kachabira small size coffee sample

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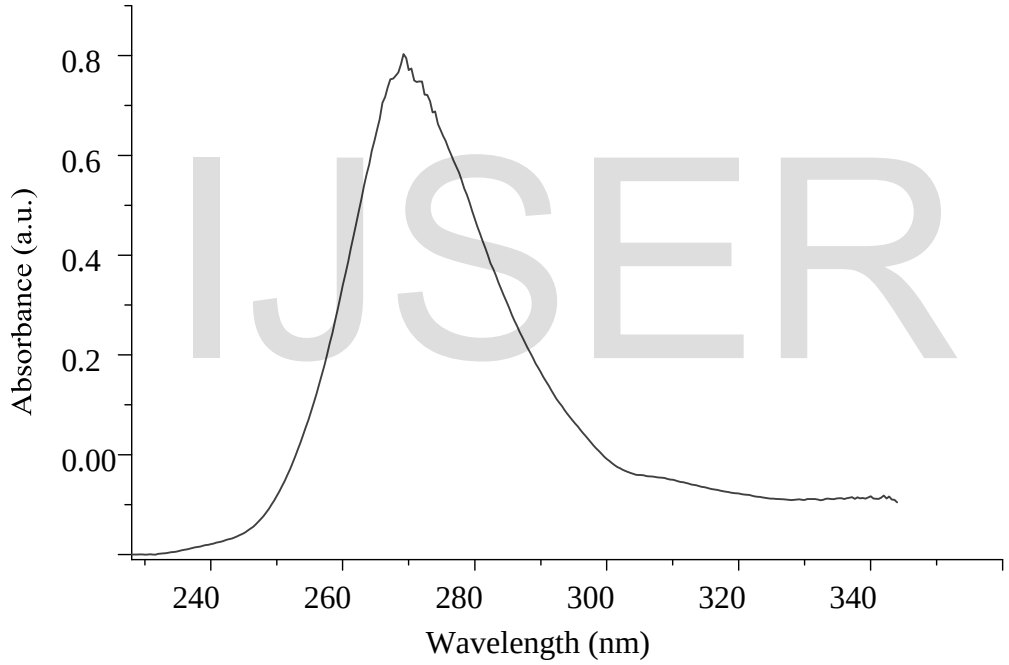

Figure: 28 Uv-vis absorption spectra of Kachabira large size coffee sample

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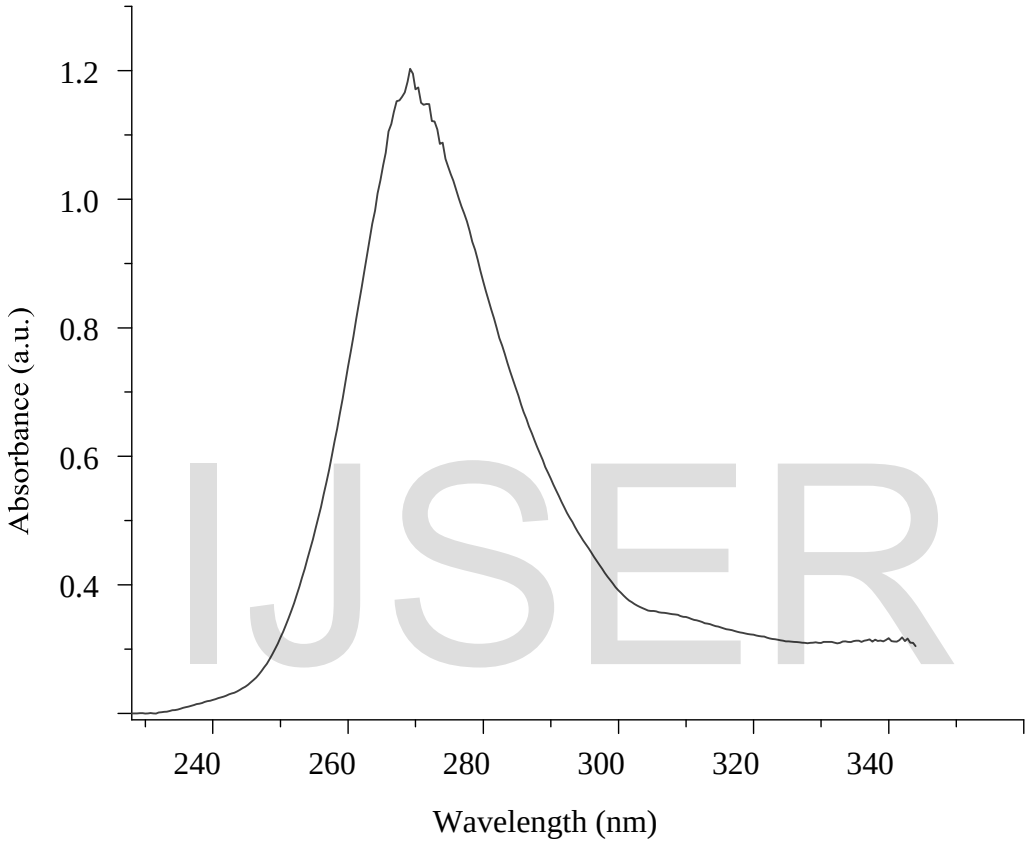

Figure: 29 Uv-vis absorption spectra of Hadaro small size coffee sample.

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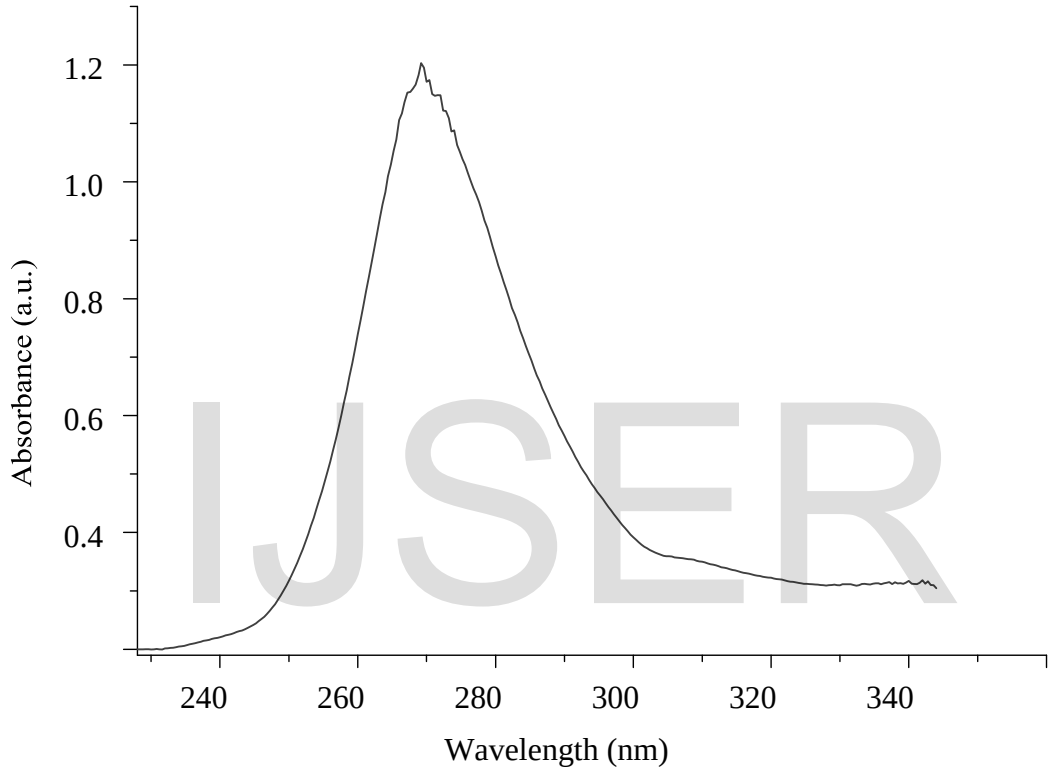

Figure: 30 Uv-vis absorption spectra of Hadaro large size coffee sample.

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