

**A preliminary Study of the Effect of Drinking Black Tea on the Absorption of Iron in the Blood**

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**Received: 8 April 2017**

**Accepted: 14 November 2017**

**Abstract**

The objective of this work is primary focus on iron-polyphenol complex were synthesized using the extracts obtained from black tea leaves interaction in aqueous solution at different concentrations of iron solutions and the resulting chelators iron-polyphenol complexes was characterized, using infra-red spectroscopy (IR) and ultraviolet-visible spectroscopy (UV). Determination of the iron concentration in blood serum was carried out on nine youth health volunteer's female student from Iraqi population (Diyala University-College of science) who are drinking tea particularly tannin rich dally with the meals, and then re-measuring their iron levels after stop to take it for five days, sensitive atomic absorption spectroscopy has been used for iron determination in serum samples. The results show that the reaction between iron ions and polyphenol can form complex. Based on data from atomic absorption during tea consumption and after stop drinking found that no significant inhibition in the iron level in younger students was noted, however, the iron values that measured in the serum samples by atomic absorption method are agreement with the natural value of iron level in the body.

**Keywords:** Iron chloride, Serum, Black tea, Atomic Absorption Spectroscopy, UV spectroscopy, IR spectroscopy

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### دراسة أولية لتأثير شرب الشاي الاسود على امتصاص الحديد في الدم

لمى سلمان عبد<sup>1</sup> ، محمد علوان فرحان<sup>2</sup> و نضال متعب خميس

قسم الكيمياء- كلية العلوم - جامعة ديالى

#### الخلاصة

تضمنت هذه الدراسة تحضير معقد الحديد- بولي فينول والناتج من تفاعل الحديد على هيئة كلوريد الحديد الثلاثي المائي مع مستخلص أوراق الشاي الاسود عند تراكيز مختلفة من محلول الحديد. شخّصت معقدات الحديد- بولي فينول بتقنية مطياف الأشعة تحت الحمراء ومطياف الأشعة فوق البنفسجية. قياس تركيز الحديد في مصل الدم باستخدام مطيافية الامتصاص الذري. تم اختيار تسع طالبات متطوعات يتمتعن بالصحة من المجتمع العراقي من كلية العلوم جامعة ديالى المرحلة الرابعة يتناولن الشاي الأسود الغني بمادة التانين بصورة يومية مع الوجبات ومن ثم قيست نسبة الحديد بعد ترك شرب الشاي لمدة خمسة ايام. أظهرت مطيافية الأشعة تحت الحمراء وتقنية مطيافية الأشعة فوق البنفسجية إمكانية تكوين معقد بين ايون الحديد ومركب البولي فينول المستخلص من أوراق الشاي ومن خلال مقارنة النتائج التي تم الحصول عليها بواسطة جهاز قياس الامتصاصية أثناء تناول الشاي وبعد ترك شرب الشاي مع النسبة الطبيعية لتركيز الحديد في الجسم أظهرت النتائج أن هناك ستة أشخاص من مجموع تسعة أزداد تركيز الحديد لديهم عند ترك شرب الشاي أما الأشخاص الثلاثة المتبقين من نفس المجموعة لم يلاحظ ارتفاع في تركيز الحديد لديهم لكن جميع النسب التي تم الحصول عليها كانت ضمن الحدود الطبيعية لتركيز الحديد في الدم.

**الكلمات المفتاحية:** كلوريد الحديد المائي، مصل الدم، الشاي الاسود، تقنية الامتصاص الذري، الأشعة تحت الحمراء، الأشعة فوق البنفسجية

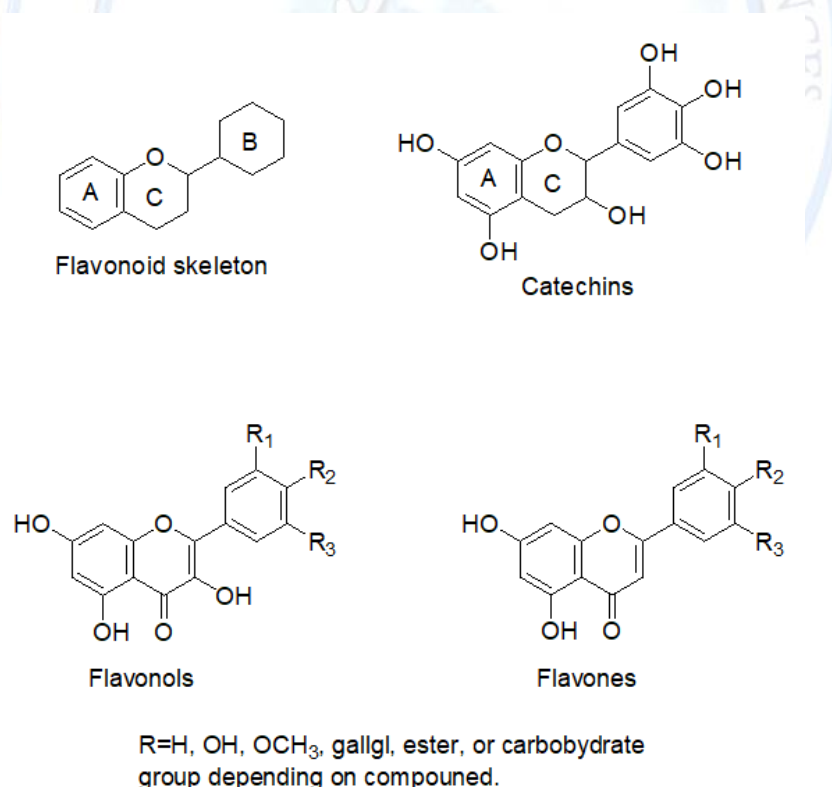
#### Introduction

Due to abundance of antioxidant group and moderate level of caffeine tea consider to be the most commonly consumed beverage in the world however, increase the consumption of tea to inhibit the body absorption of dietary iron, causing a deficiency. Therefore, it was necessary examine the effect of black tea drinking on the natural hemoglobin value in the human body typically (50-170 mg/dl). Iron is one of the essential trace chemical elements for a living human [1], [2]. Iron play an important role in biology, forming complexes with molecular oxygen in hemoglobin and myoglobine also, it has pro-oxidation properties used by the immune system

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to destroy bacterial, iron also required for DNA synthesis [3]. As a result, iron deficient is one of the most common nutritional disorders in the world caused by poor iron absorption from the diet. Tea contains huge of bioactive compounds, including amino acids, caffeine, lignins, proteins, xanthines, fibers, minerals and flavonoids [4]. Black tea is a kind of tea and its flavonoids include up to 40% of the dry weight of it, the interactions of flavonoids with iron metal lead to form chelate complex [5] and the health benefits of tea are most often at tribute to its polyphenol compounds, particularly flavanols and flavonols [4] Polyphenols can be standing into different classes according to the number of phenolic rings in their structure [6]. They contain at least one aromatic ring with one or more hydroxyl group in addition to other substituents. According to Perron *et al* [6] flavonoids can behave as anti-oxidant because of their chelating properties, they also have many other biological activities, such as anti-histamines, anti-inflammatory, antibacterial Figure 1. [6]

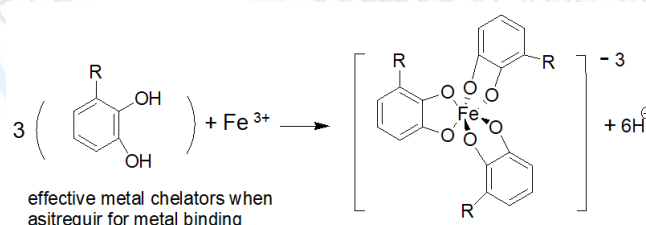


**Figure 1:** Showing general structures of flavan

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Among these compound Catechins was found abundant in the group of polyphenol and contained in (5 to 27%) of the dried tea leaf [7] and many of beneficial of tea are related to its catechins. Although the polyphenols in tea act as antioxidants, but inhibit the non-heme iron absorption. This effect is ascribed to the formation of ligand such as phytates, tannates, phosphates, oxalates and carbonates. Tannins are viewed as the most significant compound inhibitor the non-haem iron absorption by formation of insoluble iron tannate complexes [1, 8]. Researchers reported on the biosynthesis of iron II, III- polyphenol complex using green and black tea extracts however, the reaction between polyphenol compound and ferric iron was study in detail for low and high molecular weight polyphenols [9] In more recent years, Wang [10] have also suggest the mechanism of the reaction between plants extract and iron ion. Moreover, Perron *et al*, [6] have also reviews, the effected of both PH and stability constant on the binding interaction between polyphenol compound and iron. Metal ions that prefer octahedral geometry such as  $Fe^{+2}$  and  $Fe^{+3}$ , can coordination up to three catecholate or gallate groups. Because of this it might be expected that polyphenols with catechol or gallol group would always bind iron in ratio of 3:1 Scheme 1. [6] [9] [10].



**Scheme 1:** General Coordination of Iron-polyphenol Complexes  
Gallols R=OH, Catechols R=H

On the other hand, more sensitive methods and specific AAS \* Atomic Absorption Spectrophotometric\* was used by Abu Mohsen *et al* [11] and Najim [12] to determine iron concentration in blood serum. According to Abu Mohsen *et al* [11] comparative study between two different analytical methods (flame atomic absorption and Atomic Absorption Spectrophotometric were used. In more recent year, estimate the iron concentration for both male and female of Thalassemia patients and normal group was done by Najim [12] and then

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comparison with standard spectrophotometric method. Because of many people have thought about whether or not polyphenols affect nutrient absorption therefore this work seek to answer this question. So accurate method was used to determined the level of iron in human blood serum and comparison with the practical result that obtained by atomic absorption spectroscopy (A.A.S.) Because of differences in views about the effected of tea polyphenols on iron absorption therefore, this research was focused firstly, on iron-binding polyphenols and characterize of polyphenol- iron complex at different concentration of iron solution. Secondly, investigate the effect of tea drinking with the meals on the level of iron in the body.

### Result and discussions

Tea leaves contain mainly Gallic acid, tannins, flavonol, with plenty of OH groups worked as effective metal chelators to form iron-polyphenol complex. These polyphenols interfere with iron absorption reducing the bioavailability of iron and can lead to an iron deficiency. The IR spectra revealing several peaks in the spectra range of (1000 - 3400 $\text{cm}^{-1}$ ) the strong and broad band peak at 3375 $\text{cm}^{-1}$  are attributed to the OH group of of Fe-polyphenol. The peak about 1622  $\text{cm}^{-1}$  can relate to the C=C group of ring stretching in polyphenols, and the weak peak at 1033  $\text{cm}^{-1}$  could be attributed to the phenolic C-O all of these indicate that the tea polyphenols was formed complexes with iron in the solution, this agree with the proposed molecular structure Figure 2,3. It is worth mentioning that, when the  $\text{FeCl}_3$  concentration increased 1:2 volume ratio of plant extract to  $\text{FeCl}_3$ , OH group' borders became sharper Figures 4, 5, 6, 7.

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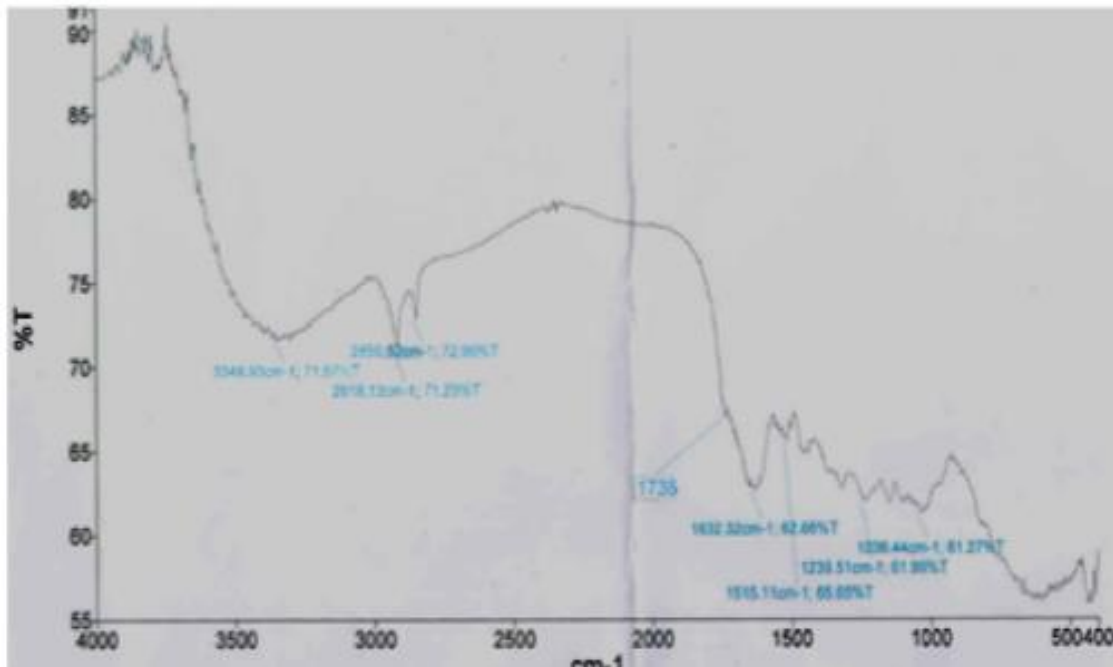


Figure 2: IR spectra of tea

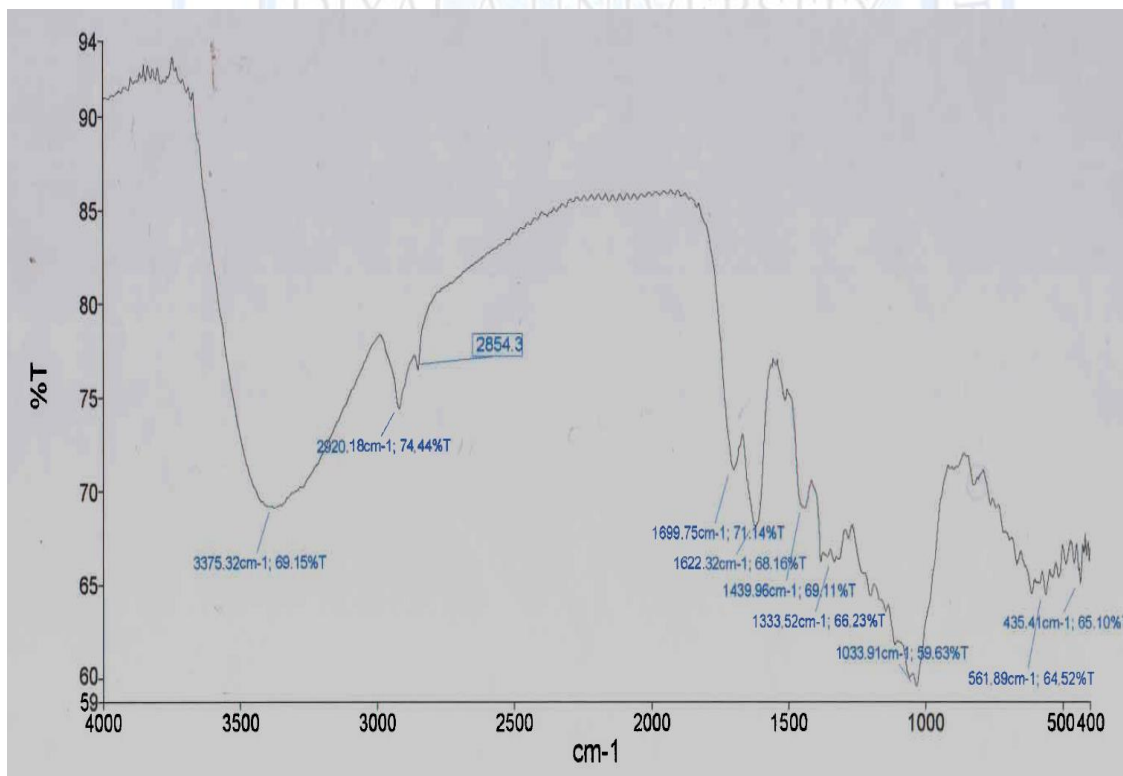


Figure 3: IR spectra of tea after adsorption

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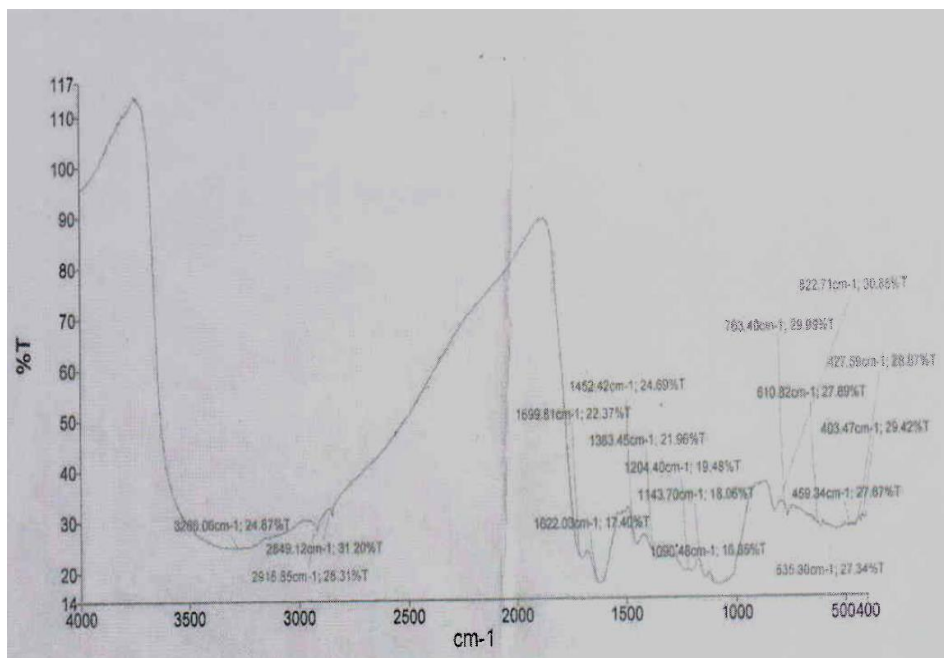


Figure 4: IR spectra of 0.5% of iron concentration

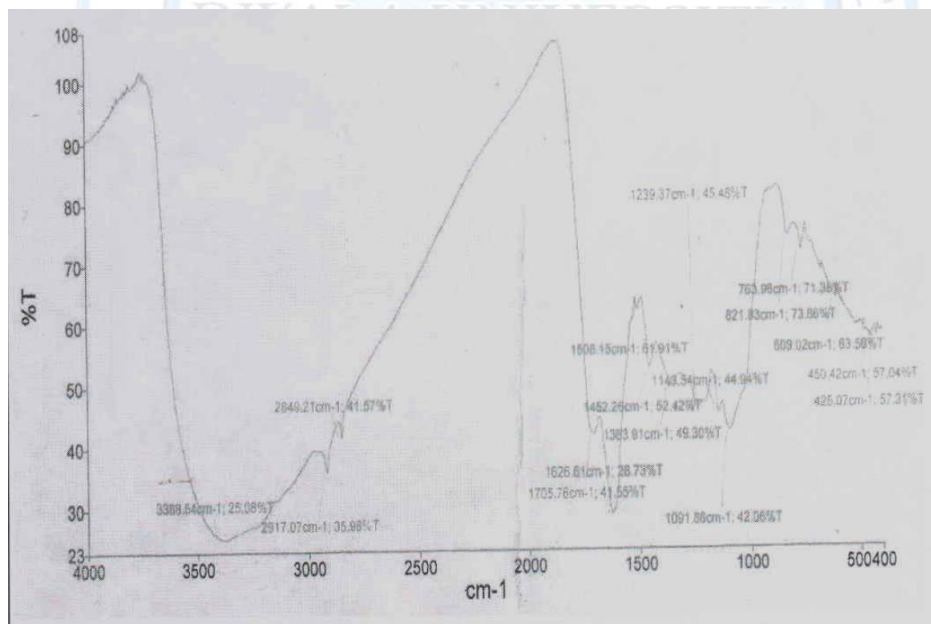


Figure 5: IR spectra of 5% of iron concentration

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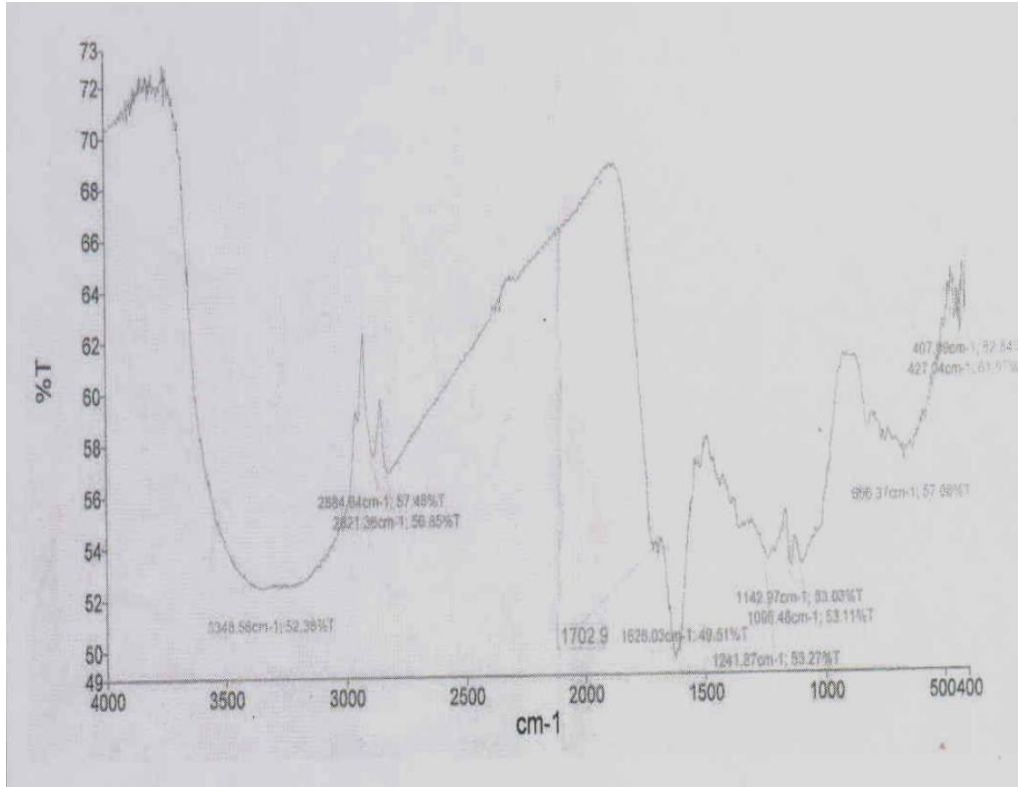


Figure 6: IR spectra of 0.12 of iron concentration

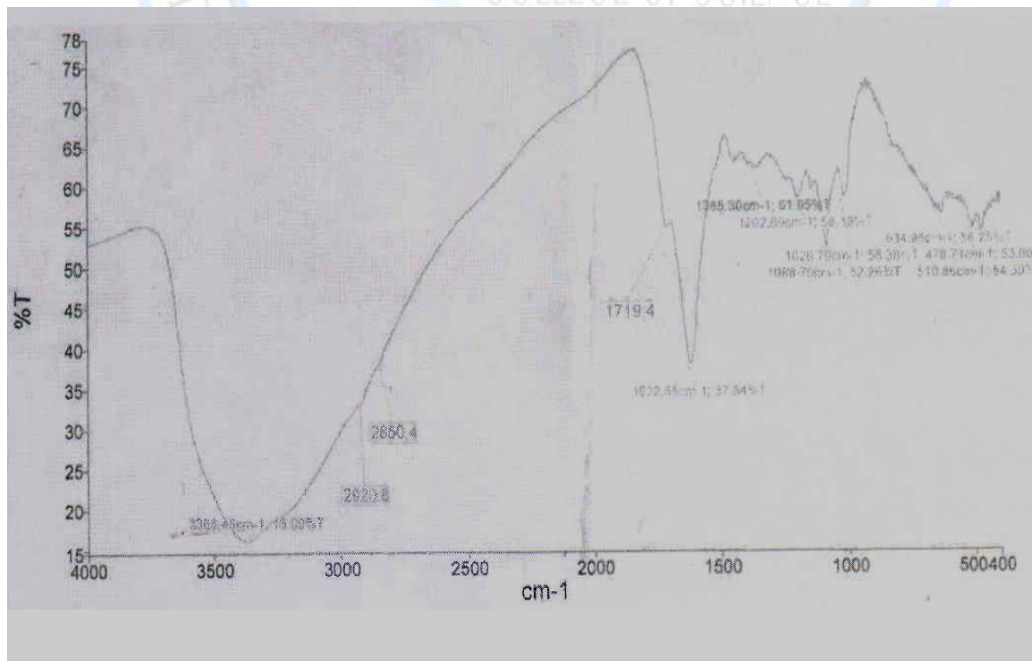


Figure 7: IR spectra 0.24 of iron concentration



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Since the absorption of ultraviolet by molecule lead to electron transition between energy levels of the molecule it is supposed that different compound of polyphenols have different chelating ability to combine iron ions. According to the Perron [6] and Dominguez-Vera [13] polyphenols that contain catechols are the main reductants agent and iron plays an important role in oxidation of polyphenols subsequent the polyphenols can reduced the ferric ion  $Fe^{+3}$  which is relatively biologically inactive to active  $Fe^{+2}$  and in the same time polyphenols catechols is oxidized to quinone depending on the condition particularly PH. At viruses PH value Catechols give three kinds of complex with iron (III) for instant at PH (5- 6.5) iron is bounded by two catecholate for one ion of metal with  $\lambda_{max}$  561-586 nm, at PH less than (<4) mono catecolate iron complex will form in 1:1 ratio with  $\lambda_{max}$ -670 nm. With PH > 9 iron complex of catecolate can be formed with  $\lambda_{max}$ - 483 nm. Because of the catechols produce strongly coloured complexes with iron ion [13] the Fe-Tea complex demonstrate a pure dark green. The UV-VIS absorption spectra of the dark green Fe- polyphenol synthesized is found to be in the region of (402) nm. From the available data it is assume that the iron tea complex being form at PH > 9 although the value obtained is slightly low however in good agreement with data found in the literature. Figure 8, 9.

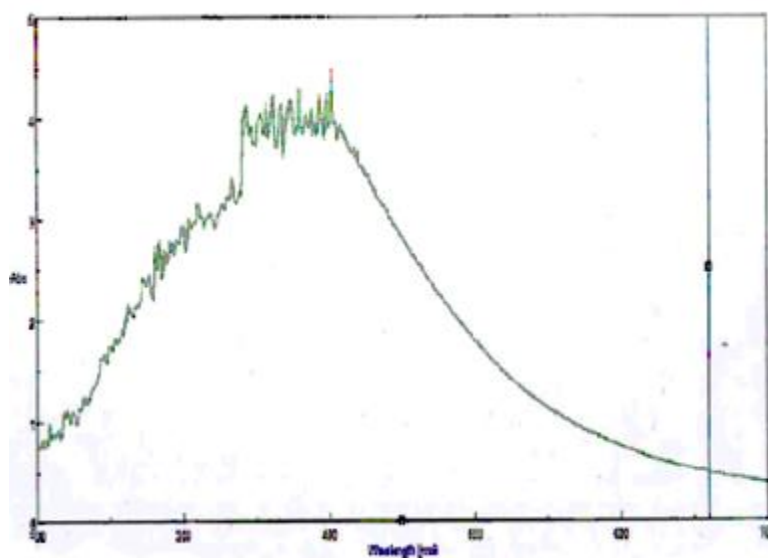
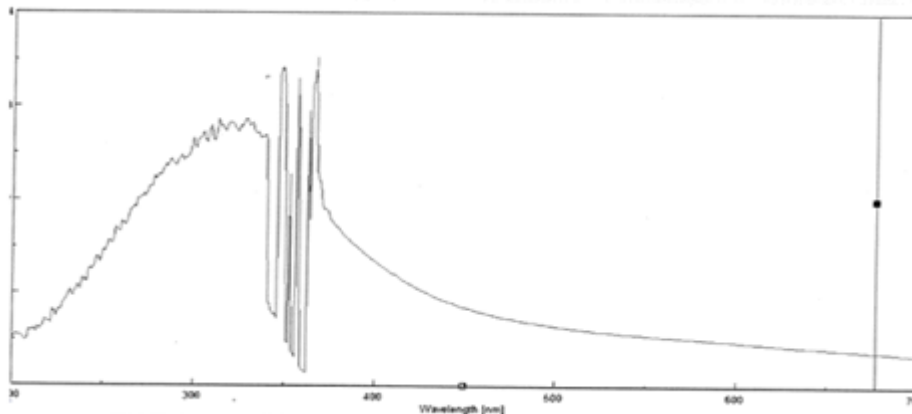


Figure 8: UV spectra of tea

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**Figure 9:** UV spectra of iron –polyphenol complex

As attempt to investigate the effected compounds of tea on iron concentration practically before measure the iron content in blood serum. Tea extracted react with 100 ppm  $\text{FeCl}_3$  solution at room temperature in different composition ranging from 2 mL tea extract +10 mL 100 ppm  $\text{FeCl}_3$  to 10 mL tea extract +10 mL 100 ppm  $\text{FeCl}_3$  as detailed in Table 1. The method below is used to determination iron concentration practically before measure the iron content in blood serum and shows that tea inhibits iron absorption to significant extent.

**Table 1:** Conditions and results of iron concentration practically

Sample	Composition Description	Concentration of iron after absorption by A.A.S.
1	10 mL/100 ppm $\text{FeCl}_3$ solution +2 mL tea extracted	70.1825
2	10 mL/100 ppm $\text{FeCl}_3$ solution +4 mL tea extracted	59.2375
3	10 mL/100 ppm $\text{FeCl}_3$ solution +6 mL tea extracted	45.2350
4	10 mL/100 ppm $\text{FeCl}_3$ solution +8 mL tea extracted	43.2350
5	10 mL/100 ppm $\text{FeCl}_3$ solution +10 mL tea extracted	41.4450

In a clinical study for nine samples of serum were obtained from healthy volunteers and their Iron concentration in the samples are summarized in Table 2

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**Table 2:** Iron concentration in serum samples of volunteer students

Sample	Iron con. mg/dl in serum During tea consuming by A.A.S.	Iron concentration. mg/dl after stop taken for five days by A.A.S.
1	131	147.5
2	138.5	149
3	134.4	229.5
4	179.5	173.8
5	117.2	131
6	135.2	157.5
7	146.7	98
8	111.5	115.8
9	102.5	76.2

According to available results showed in Table 2 indicated that iron quantity in serum after stop having tea has no significantly differences than the natural value of iron in human body which is typically (50-170 mg/dI). Further analysis was carried on with **T-test** as can see in Table 3, 4, 5 the number of significance is 0.510 which is larger than the confidence rate of 0.05 indicated that no significant difference was found in iron concentration after stop taking tea.

**Table 3:** Shows the arithmetic mean of the iron during and after stop taking the tea and the values of standard deviation, standard error

**Statistics 1**

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Iron con. mg/dl during tea consuming by A.A.S. serum	142.0333	9	44.80522	14.93507
	Iron con. mg/dl after stop taken for 5 days by A.A.S	132.9444	9	22.45301	7.48434

**Table 4:** Shows a relationship between iron concentration during and after stop tea drink, and the amount of 0.473 but, the relationship is statistically no significant

**Correlations 2**

		N	Correlation	Sig.
Pair 1	Iron con. mg/dl during tea consuming by A.A.S. serum & Iron con. mg/dl after stop taken for 5 days by A.A.S	9	0.473	0.199

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**Table 5:** Shows no significant difference in the concentration of the iron during and after at the 0.05 level where the value is 0.510 by using *T*- test approved for a couple of variables

### Paired Samples Test 3

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Iron con. mg/dl during tea consuming by A.A.S. serum - Iron con. mg/dl after stop taken for 5 days by A.A.S	9.08889	39.50495	13.16832	-21.27730-	39.45508	0.690	8	0.510

According to the available results there is no restriction on tea drinking in healthy people who are consuming a varied diet with no risk of iron deficiency however, people who have poor iron status should avoid drinking tea with meals or having a tea at least one hour after the meal.

### Materials and methods (First part)

Tea was collected from local market.

Iron was administered as pure ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ).

#### **Preparation of stock solution**

The iron solution was prepared by dissolving 5 g of ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) in 95 mL of distilled water. The plant leaf extract was prepared by heating 2 g of tea leaves with 100 mL of distilled water at  $100^\circ\text{C}$  for 5 min. After settling for 30 min. the extract was filtered and the tea solution was used without further purification. Tea extracted of 50 mL was reacted with 100 mL of 5g/100 mL, 0.5g/100 mL, 0.24g/100 mL, 0.12g/100 mL  $\text{Fe}(\text{III})$  solution. The mixture was left for 15 min at room temperature. Before filtration through filter paper and the dark green precipitate was left to dry at room temperature.

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### Experimental (clinical studies)

Determination iron level in human serum was carry out by atomic absorption spectroscopy (A.A.S).

Reagents and solutions were prepared according to the instruction of Biolabo reagents-Iron recipe kit (France).

**Principle:** At acidic PH transferrin bound iron dissociates into free ferric ions and ferrous then ascorbic acid is used to reduce the ferric iron to the ferrous state. The ferrous iron react with the chromogen ferene to from a dark green complex which absorbs at 600 nm.



Reagents and solution

R1: Ascorbic acid	30 mmol/L
Citric acid	150 mmol/L
Thiourea	27 mmol/L
R2: Ferene	600 $\mu$ mol/L
R3: Standard as FeCl <sub>3</sub> .6H <sub>2</sub> O	200 mg/dl or 35.8 $\mu$ mol/L

#### The working reagent

Assay procedure

Wave length 600 nm

Temperature 25-30 °C

Measurement against blank

50 mL R1+ 1 mL R2

Blank 1 mL R1+ 200  $\mu$ L Distilled water

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### Four sets of solution were prepared as follows:

1. R3 (200  $\mu$ L) mix with R1 (1 mL) read absorbance after 3 min against the blank solution (A1 standard).
2. R3 (200  $\mu$ L) mix with working reagent (1 mL) read absorbance after 5 min against the blank solution (A2 standard).
3. Sample (200  $\mu$ L) mix with R1 (1 mL) read absorbance after 3 min against the blank solution (A1 sample).
4. Sample (200  $\mu$ L) mix with working reagent (1 mL) read absorbance after 5 min against the blank solution (A2 sample).

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