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Detection and Estimation of Tannic Acid in Rubus-Idaeus (Red Raspberry) as Natural Chelating Agent for Iron Using HPLC.

Hazim Ali Hussein*[1], Mufeed. J Ewadh[2], and Moaed E AL gazally[2].

[1]Pharmacist/Ministry of health/AL Furat Al awset hospital/Al Najaf Al ashraf /Iraq.
[2]College of Medicine / University of Babylon, Hilla-Iraq.

ABSTRACT

Raspberries(*Rubus-Ideaus*)are among the fruits containing the highest antioxidant levels,in addition to vitamin C,the antioxidant activity of raspberries is primarily constituted by two classes of compounds: anthocyanins and ellagitannins.Tannins are water-soluble poly phenolic compounds which are important classes of secondary metabolites and have molecular weight ranged to 500-20000 Daltons. The present study aimed to qualitativeand quantitative estimation of (Tannic Acid) as iron chelating agentin Rubus-Ideaus fruit. SHIMADZU Reverse Phase-High Performance liquid Chromatography (RP-HPLC) and other molecular spectrum instruments were used to conduct the experience. Detection was performed by injecting standard material of pure tannic acid it's concentration was(2µg/ml) under special and specific conditions to detect and determine the true Retention Time (RT),then perform the analysis of the aqueous and alcoholic rubus-idaeus extract under the same conditions.Intensive examinations were performed on aqueous, extract and dry powder of rubus-idaeus fruit using modern and sophisticated analysis instruments for more confirmation and prove the existence of tannins in this plant. Obtained results show that Rubus Idaeus fruit contain **(853 µg/gm)** of Iron chelating agent (tannic acid)**.**We conclude that Rubus Idaeus plant contain good acceptable concentration of tannic acid as natural reducing agent forIron chelating agent.

Keywords: RP-HPLC, Tannins,Tannic acid,RubusIdaeus, Iron overload.

Corresponding author

INTRODUCTION

In 1893, Alfred Werner, a French-Swiss chemist, developed the theory of coordination of compounds .This theory identified a process where metals bind with organic molecules, making them inactive. He won the Nobel Prize in 1913 for his process .This process of binding metals to organic molecules is the basis for chelation therapy. The original application of Werner's theory was within the field of industrial production, to help eliminate heavy metal contamination brought on by the industrialization. Heavy metal toxicity can occur rapidly or be a progressive process, and its symptoms, although similar to other disorders, require treatment to minimize adverse effects. In the 1920s, new paint materials were introduced that contained heavy metals including lead, and the elimination of these heavy metals was critical .The most common binding product of the time, called ethylene-diamine-tetraacetic acid (EDTA), was made in Germany and sold globally for industrial use**[1]**.

Chelating agents are molecules that form stable bonds with metallic atoms, making them more stable, soluble and resistant to disassociation is generally well tolerated; however, the most common side effect is a burning sensation at the site of administration. More serious and potentially fatal side effects occur rarely but include heart failure, hypotension, hypocalcemia, kidney damage, and bone marrow suppression, hypocalcemia and death may occur particularly if the chelation therapy is infused too rapidly **[2]**.

Raspberries are an aggregate fruit commonly grown and consumed throughout the Northern Hemisphere and, as members of the rose family, are closely related to blackberries and other brambles or cane berries, although many species and types of raspberries exist, red and black are the most common. Raspberries belong to the rose family (Rosaceae) and the genus (Rubus), which also includes other brambles or cane berries, such as blackberries, dewberries, and boysenberries,the raspberry fruit and raspberry leaves and blossoms have been used for medicinal purposes for many centuries**[3]**.

Figure (1): Red raspberry (Rubusidaeus) in nature

Red raspberry is a plant which stands about 6 feet tall. It is often found growing wild in hedges and it may be vining or shrubby but tends to grown in thickets**[4]**as it shown in figure (1)**.** Raspberries (*Rubusidaeus*) are among the fruits containing the highest antioxidant levels. In addition to vitamin C, the antioxidant activity of raspberries is primarily constituted by two classes of compounds: anthocyanins and ellagitannins. Anthocyanins, which are red pigment polyphenols, are mainly found in berry fruits and grapes. They have been implicated in protection against coronary heart disease and certain types of cancer**[5].** Anthocyanins, which demonstrate a wide spectrum of colors from pale yellow to deep blue, and the colorless polymerized compounds which areclassified as proanthocyanidins(also known as condensed tannins) are the two common flavonoid metabolites. They are both end-products of the well-known flavonoid pathway. The health-promoting properties of both have been implicated in a wide range.

Anthocyanins have been reported to be involved in enhancing immune function, protecting against age-related neurological disorders and exhibiting anti-cancer properties. Proanthocyanidin concentrations are believed to be an essential factor affecting astringency and bitterness tastes of persimmon **[6].**

Tannins are water-soluble poly phenolic compounds which are important classes of secondary metabolites and have molecular weight ranged to 500-20000 Daltons. Since ancient time tannins are able to

tan animal skins from leather. Tannins are a type of polyphenols extracted from different parts of trees such as in barks, fruits, leaves and woods. Tannins are water soluble compounds and their modified forms are used as adsorbent for metals from waste water. Tannins are divided into four major groups; Gallotannins, ellagitannins, complex, and condensed tannins. Tannic acid is a specific commercial form of gallotannin which are esters of glucose and gallic acid **[7].**

Mechanism action of tannic acid for chelating iron:

A possible mechanism of ion exchange could be considered as a trivalent metal ion (Fe^{3+}) attaching itself to adjacent hydroxyl groups and oxyl groups, which could donate two pairs of electrons to metal ions, forming chelated compounds and releasing two hydrogen ions into solution. The catechol or pyrogallol groups of poylphenols offer special opportunities for the formation of metal complexes. Metals form complexes with the phenolics by two adjacent hydroxyls (catechols), and the presence of a third adjacent hydroxyl (pyrogallols) increases the stability of the complexes. Third-OH group of pyrogallol molecule does not participate the metal-complex formation due to the steric hindrance**[8].**

MATERIALS AND METHODS

Materials

Plant:*Rubus-Idaeus (Red raspberry)*fruit dried, samples were collected freshly from local markets of Sulaimanya city(North of IRAQ). After that samples were stored in clean P.V.C bags until time of extractionSamples grinded by mortar and pestle to produce powdered sample. After that samples were stored at temp below (25ºC) in pvc bags until time of analysis**[9].**

Chemicals: Chemicals that have been used in the present study obtained from thoughtful international companies were high purified as follows;methanol, and Ethanol for HPLC**.** Other chemicals were used in this study as follows; standard tannic acidTartaric Acid, Ethyl Acetate, Gallic acid, and Ortho-Phosphoric acid.

Instrument:- Instrument that have been used in the present study were modern and sophisticated analysis instruments as follows; Preparative and Revers Phase-High Performance Liquid Chromatography (HPLC), FTIR – BRUKER tensor 27, Freeze Dryer ALPHA 1-4 LD plus, Lovibond pH meter 200, Magnetic Stirrer with Hot plate, Sensitive Balance, Centrifuge Hettich EBA 20, and Water Bath (Grant company).

Methods:-

First:- HPLC Technique:

Detection and Estimation of tannic acid in Rubus Idaeus Plant Using Reversed- Phase High-Performance Liquid Chromatography

Reversed phase high performance liquid chromatography was employed for the identification and quantification of phenolic compoundsaccording to**[10],** as itshown in table (1) **[11].**

Table (1): Separation conditions of high performance liquid chromatography

Parameter	Characteristic for tannic acid identification
Mobile phase	Methanol:H_2O (60: 40 ml)
Column Type	C18 – ODS (25cm x 4.6 mm X 5µm)
Volume injection sample	20 µl
Detector	UV Spectrophotometer at 254 nm
Flow Rate	1 ml / min
Temperature	30ºC

Second:-Examination and analysis using (ATR-FTIR) instrument

Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy

FTIR Spectroscopy is a technique based on the determination of the interaction between an IR radiation and a sample that can be solid, liquid or gaseous. It measures the frequencies at which the sample absorbs, and also the intensities of these absorptions. The frequencies are helpful for the identification of the sample's chemical make-up due to the fact that chemical functional groups are responsible for the absorption of radiation at different frequencies. The concentration of component can be determined based on the intensity of the absorption. The spectrum is a two-dimensional plot in which the axes are represented by intensity and frequency of sample absorption **[12].**

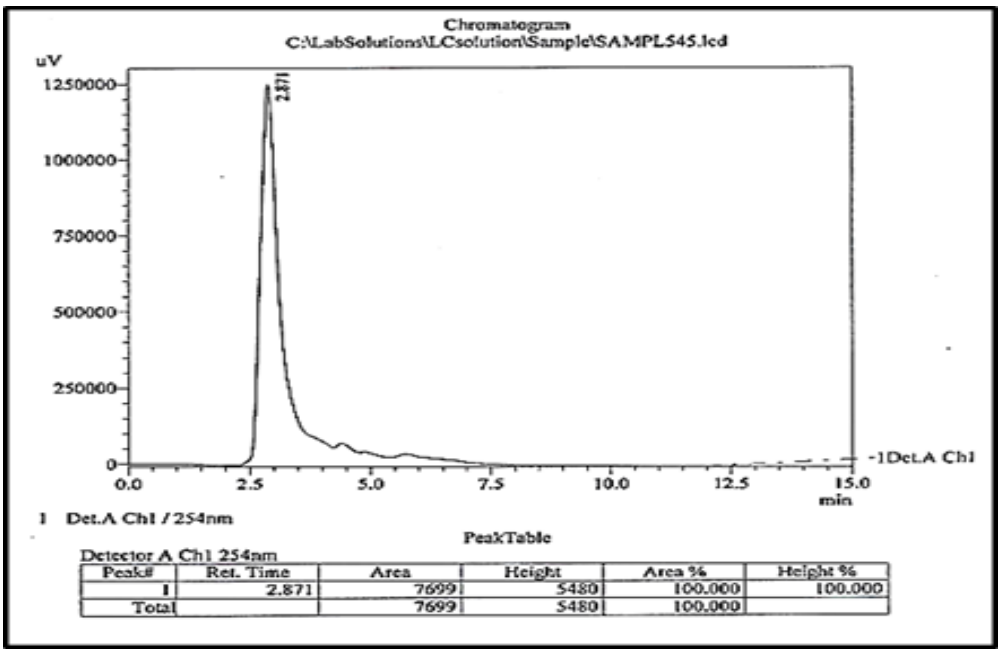
ATR-FTIR spectroscopy

The use of attenuated total reflection (ATR) accessories in conjunction with Fourier transform infrared (FTIR) spectrometers is now commonplace. This accessory provides for the non-destructive measurement of samples with little or no preparation. Most samples can be directly applied to the internal reflection element (IRE) of an ATR without time-consuming dilution with matrices such as Nujol or KBr. The ATR accessory also allows for easy analysis of liquid samples with just a single drop required, applied directly to the IRE crystal. However, by the nature of their design, ATR accessories absorb infrared radiation and consequently reduce the amount of energy that reaches the infrared detector. The attenuation caused by these accessories typically varies from 70–90%**,** Samples placed directly on the accessory crystal for examine and analysis in which intensity graphic of permeability appear within seconds **[13],** as it shown in figures(5,6).

RESULTS

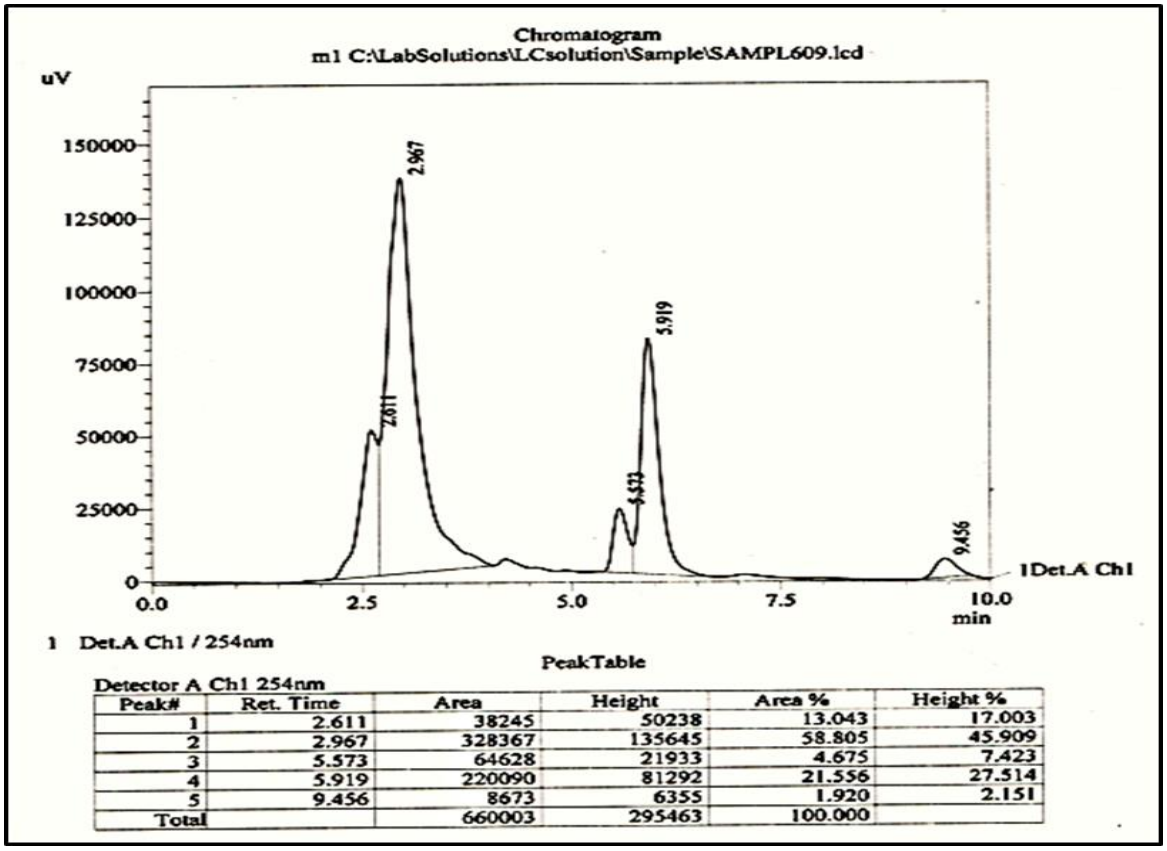
HPLC Analysis:-

In the present study, it has been developed thorough examination to determination of **tannic acid** in the aqueous extract of ***Rubus Idaeus*** **plant**. **Figures (2,3)** show that the complete baseline of separation has been obtained within standard tannic acid and sample by **RP-HPLC.**



Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.871	7699	5480	100.000	100.000
Total		7699	5480	100.000	

Figure (2): HPLC Chromatogram of Standard Tannic Acid, its retention time (RT) is 2.871



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.611	38245	50238	13.043	17.003
2	2.967	328367	135645	58.805	45.909
3	5.573	64628	21933	4.675	7.423
4	5.919	220090	81292	21.556	27.514
5	9.456	8673	6355	1.920	2.151
Total		660003	295463	100.000	

Figure (3): HPLC Chromatogram of aqueous extraction of RubusIdaeus plant, its retention time (RT) is 2.967

Calculations:

Concentration calculations resulting from HPLC [10].

$$C_{sample} = \frac{C_{standard} \ X \ A_{sample}}{A_{standard}} \ x \ \frac{D.F}{Wt. \ or \ V}$$

Using area under the peak to calculate the aqueous extract concentration by the following equation:-

$C_{standard}$: Concentration of standard (µg/ml).

A_{sample}: Area under the peak of sample.

$A_{standard}$: Area under the peak of standard.

D.F: Dilution Factor.

Wt. or V: Weight or Volume of taken sample.

C_{sample} = [(2 µg/ml X 328367) / 7699] X [500ml/ 50gm]

= 853 µg/gm ≈ 853 ppm tannic acid in original plant sample

ATR-FTIR Analysis:

Qualitative analysis obtained from ATR-FTIR shows match spectra of standard material and sample at closely wavenumber as it shown in figures (4,5). These spectra indicate that these analyst compounds have the same effective groups of tannic acid.

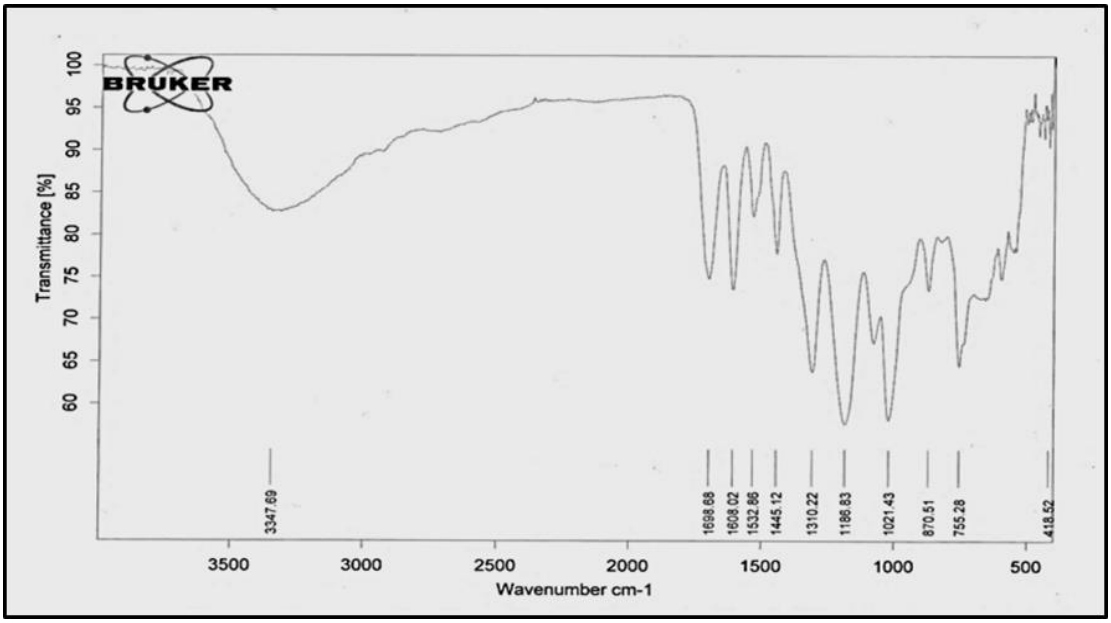
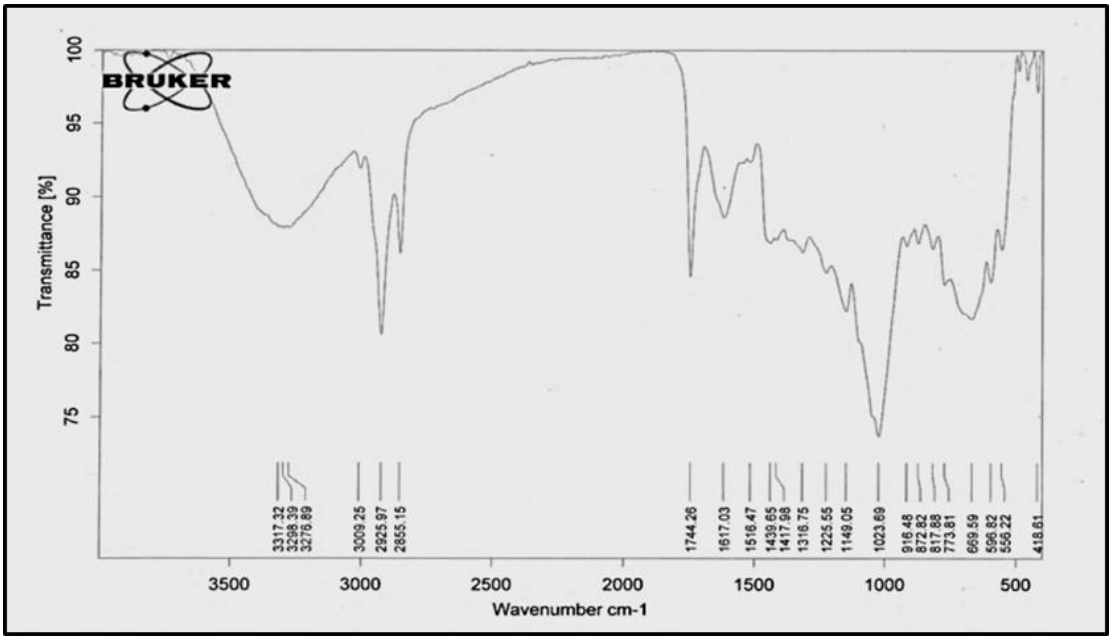


Figure (4) : Molecular spectra (transmittance T%) of effective groups for Standard tannins using ATR-FTIR instrument



Figure(5): Molecular spectra(transmittance T%) of effective groups for dry grinded rubus idaeus plant using ATR-FTIR instrument

CONCLUSIONS

1. The present study concludes that results of detection and analysis confirmed the existence and containing high concentration of tannic acid in Rubus Idaeus fruit.
2. Since fruit of this plant contains high quantity of tannic acid and since tannic acid has a mechanism to chelate iron, so it can be given to those patient having hereditary or transfusional iron overload but must be given in sufficient quantity, and using it in industrial scale after extracted, isolated and purified for use in medical, pharmaceutical fields and alternative medicine, or what is called herbal medicine.

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