Isolation and characterization of ellagicacid from Iraqi white flesh pomegranate

Jasim M. Salman

Al Kunooze university college, Basrah, Iraq

Corresponding author: +9647832742860, E-mail: jasim.s@kunoozu.edu.iq

Abstract

The main aim of this work was to prepare specific antioxidant material as ellagicacid which was extracted from white flesh Iraqi pomegranate. We designed a locally pioneer plant for production a cheap ellagicacid from Iraqi pomegranates which collected from pomegranates agriculture fields south of Baghdad.

The production process was evaluated based on the plant quantity scale used in the production of the ellagicacid. It was found that each 1 kg of pomegranate contains 400 g of white leaf. It was found that 1 ton of pomegranate will produce about 5.8 to 6.2 kg of elagicacid.

Key Words: Ellagic acid; White flesh pomegranate; Isolation; Characterization.

عزل وتوصيف الإيلاجيكاسيد من رمان الشحم الأبيض العراقي

الخلاصة

ان الهدف الرئيسي من هذا العمل هو تحضير مواد مضادة للأكسدة معينة مثل الإيلاجيكاسيد الذي تم استخلاصه من رمان عراقي شحم الأبيض. لقد صممنا مصنعًا رائدًا محليًا لإنتاج الإيلاجيكاسيد الرخيص من رمان عراقي تم جمعه من الحقول الزراعية لرمان في جنوب بغداد.

تم تقييم عملية الإنتاج بناءً على مقياس كمية النبات المستخدم في إنتاج الإيلاجيكاسيد. وقد وجد أن كل 1 كجم من الرمان يحتوي على 400 غرام من ورقة بيضاء. وقد وجد أن 1 طن من الرمان ينتج حوالي 5.8 إلى 6.2 كجم من الإيلاجيكاسيد.

الكلمات المفتاحية: حمض الإيلاجيك الشحم الرمان الأبيض. عزل؛ التوصيف.

1. Introduction:

1.1. Chemistry of ellagicacid

The structure of ellagicacid as shown in Fig.1 has two substituted benzene rings in a form of dilactone. The dilactone form is representing of the keto form for the enolic form of hexahydroxydiphenic acid (HHDP). Ellagic acid is moderately soluble in dimethylsulphoxide, slightly soluble in other organic solvents.

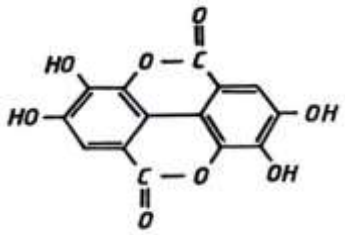


Fig. 1: Ellagicacid Chemical Composition

1.2. Ellagicacid's Anti-carcinogenic Properties

Ellagic Acid is a phenolic compound that has become a known as a potent anti-carcinogenic/anti-mutagenic compound. It also has anti-bacterial and anti-viral properties. Ellagic acid itself is not thought to be naturally present in plants. Instead, polymers of ellagicacid and hexahydroxydipenoyl (HHDP) are linked to glucose centers to form class of compounds known as ellagitannins. When two ellagicacid groups become linked side-by-side within a tannin molecule and HHDP group is formed.

Ellagic acid is the result when the HHDP group is cleaved from the tannin molecule and spontaneously rearranges. It is the ellagitannins that are present in red raspberries. Some articles in which ellagitannins are quantified refer to ellagicacid because quantitation of ellagitannins is done by breaking them down into ellagicacid subunits and quantifying the subunits.

The availability to the body of ellagicacid from dietary sources has only been confirmed with red raspberries. Other foods such as strawberries, pomegranates, and walnuts contain far lesser amounts ellagicacid yet the bioavailability has not been confirmed. Recently Dr. Eiichi Furusawa and Anne Hirazumi, of the Department of

Pharmacology at the University of Hawaii, have performed a series of studies concerning the potential health benefits of this fruit. In three successive studies (in-vitro or in vivo with mice), the researchers showed that the juice enhances the immune system and thereby attacks tumor cells. The active portion of the fruit (the ethanol-insoluble fraction) contains gum arabicheteropolysaccharide, which is composed mainly of the sugars glucuronic acid, galactose, arabinose, and rhamnose. Dr. Furusawa treated tumor-bearing mice with a combination of these polysaccharides and conventional chemotherapeutic agents and found that polysaccharides of this fruit had a significantly beneficial effect. He concluded, the possibility of combining [the active component of extract] with sub-optimal doses of chemotherapy may not only reduce the toxic side effects of chemotherapy by lowering its dose, but may improve the curative property. Another group of researchers in the Department of Bacteriology at the University of Hawaii found that the fruit is moderately effective against bacteria.

2. Methods

2.1. Preparation of ellagicacid

Ellagic acid can be prepared by oxidation of tannin solution at acidic medium or by oxidation of ellagicacid solution at alkali medium with suitable oxidizing agents. In this work, high purity tannic acid was used as the following method.

2.2. Preparation of tannic acid solution

Fifteen grams of tannic acid was dissolved in 100 ml of distilled water .The solution was filtered and adjusted to pH 8.00 by 20% Sodium hydroxide solution, 8.4 g of Sodium bicarbonate were added (1 M sodium bicarbonate). The mixture was stirred gently by magnetic stirrer to obtain a homogenized solution.

2.3. Oxidation reaction

The oxidation reaction was performed via a suitable reactor, which is depending on the scale of the reaction solution and the type of the oxidizing agent. In this work, two types of oxidizing agents were used, the hydrogen peroxide solution and the oxygen gas. 100 ml of the prepared solution (above step) was transferred to 250 ml conical flask connected to the dropping funnel containing 20 ml of 28% (W/V) hydrogen peroxide solution, which is added dropwise to the solution at a constant rate with

continuous stirring .The reaction was conducted at 25ºC for 5 hour. After completion of the reaction, the mixture was allowed to stand for 20 hours at 25Cº. The resulted slurry was filtered to separate the formed ellagic acid.

In the case of the oxygen gas, 1 Liter of the prepared solution (above step) was placed in a 2-liter flask connected to quick fit two-way adapters for allowing oxygen gas to pass through the solution in constant rate using oxygen gas flow. The rate of oxygen gas was adjusted to be 0.1 V/V /min.(gas volume / solution volume/minute) by oxygen gas flow meter. The reaction was started when the oxygen gas passed through solution with continuous stirring. It is performed at 25ºC for 20 hours. After completion of the reaction, the mixture was allowed to stand for 24 hours and the formed slurry was collected by filtration.

2.4. Analysis of the prepared ellagicacid by HPLC technique

The chemical analysis was conducted for the purpose of measuring the concentration of the ellagicacid using liquid chromatography model LC-10AV equipped with UV-visible spectrometer (Shimadzu, Kyoto, Japan SPD 10 Avp). The mobile phase consists of 1.5% acetic acid in the solvent water (A) with 1.5% hydrochloric acid in methanol solvent (B) and a flow velocity of 1 ml per minute. The chromatography of the alveolar acid was determined on the 360 nm wavelength. The qualitative and quantitative analysis of the prepared ellagicacid were performed on ODS –C18 (250 × 4.6mm I.D.) column, mobile phase, 0.03M phosphate buffer pH (7): methanol 70:30(V/V), flow rate 1ml /min., temp. 35ºC and UV-vis detector at 256 nm. 1 gm of the prepared ellagicacid was dissolved in 100 ml of methanol; the solution was completed with same solvent up to 250 ml in a volumetric flask. 20 µl of the prepared sample was injected into column and the eluted ellagicacid peak was monitored via UV-vis. detector set at 256 nm.

The concentration of ellagicacid was calculated according to the following relation:

Conc. of sample (µg /ml) =A sampl/A standard× Conc. Standard. (µg /ml) (1)

Where,

A sample = Area of sample.

A standard = Area of standard.

3. Results

Ellagic acid extracted and separated from the Iraqi pomegranate white flesh with high purity for the purpose of preparation of a pharmaceutical combination of ellagicacid with some additives. Fig. 2 shows the calibration curve of ellagicacid.

Table 1 show the analytical specifications and results of ellagicacid which extracted from pomegranate white flesh.

When evaluating the economic feasibility in the case of the use of an integrated experimental system must take into account the cost and method of collection of raw materials. In order to reduce the cost, the possibility of producing the acid of the pomegranate was studied. The production process was evaluated based on the plant quantity scale used in the production of the ellagicacid. It was found that each 1 kg of pomegranate contains 400 g of white fat. Of the soft or frozen matter of pomegranate will produce 6 g of lactic acid in addition to impurities. This means that 170 kg of pomegranate will produce about 1 kg of lactic acid and that 1 ton of pomegranate will produce about 5.8 to 6.2 kg of lactic acid.

Table 1: The specifications of prepared and standard ellagicacid

Result	Specification	Item
Dark brown powder	Dark brown powder	Appearance
Typical	Typical	smell
Bitterness	Bitterness	taste
8.1	max 10%	Ash
Compatible	100% from 60 mesh	Particle size
white flesh of pomegranate	Strawberry	Type of plant
75,2%	65% min	HPLC

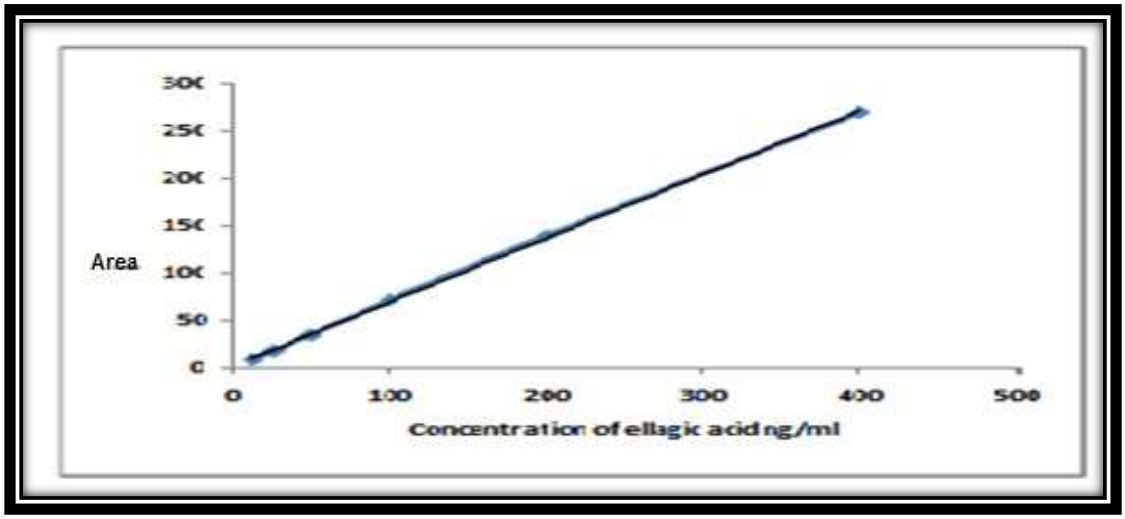
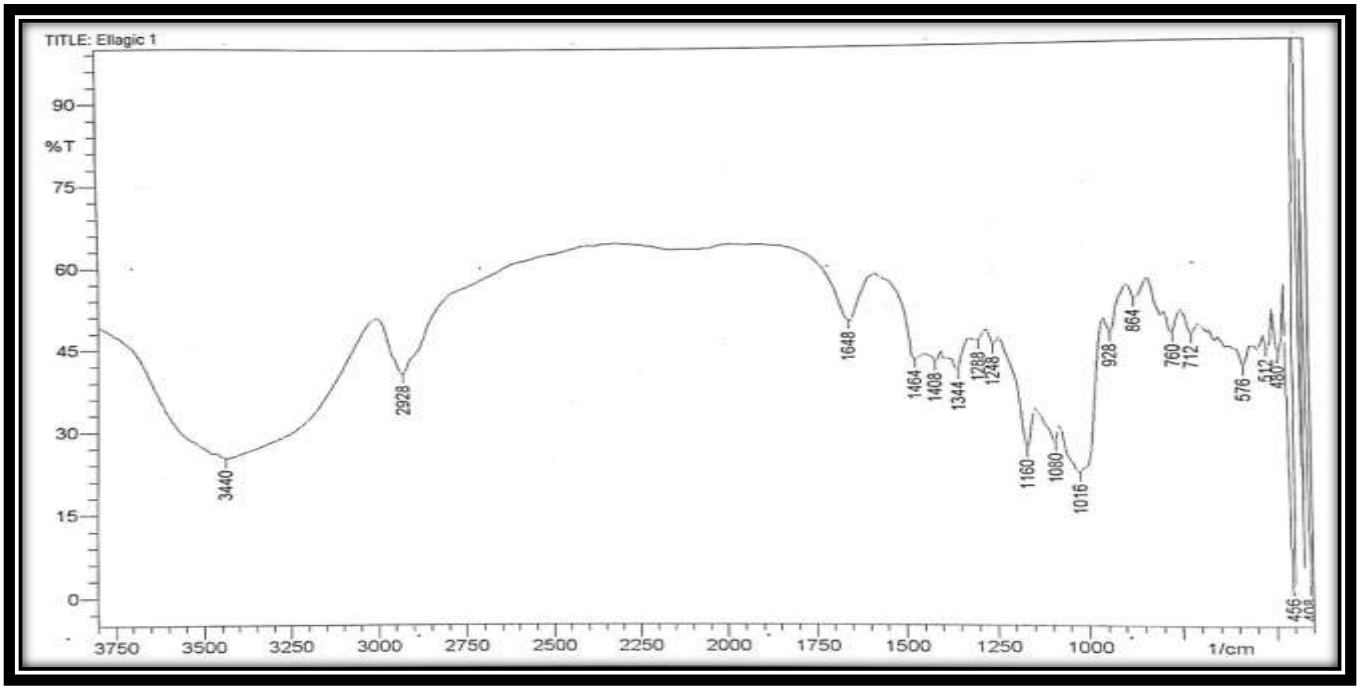


Fig. 2: The linear function of the standard ellagicacid concentrations under the optimum conditions for separation using HPLC technique.

Figures 3 and 4 shows the Infrared spectrum of ellagicacid extracted from white flesh Iraqi pomegranate and the standard one we purchased from the suppliers for the purpose of compare between the prepared and the standard, we can see the matching of the functional groups of the two materials.



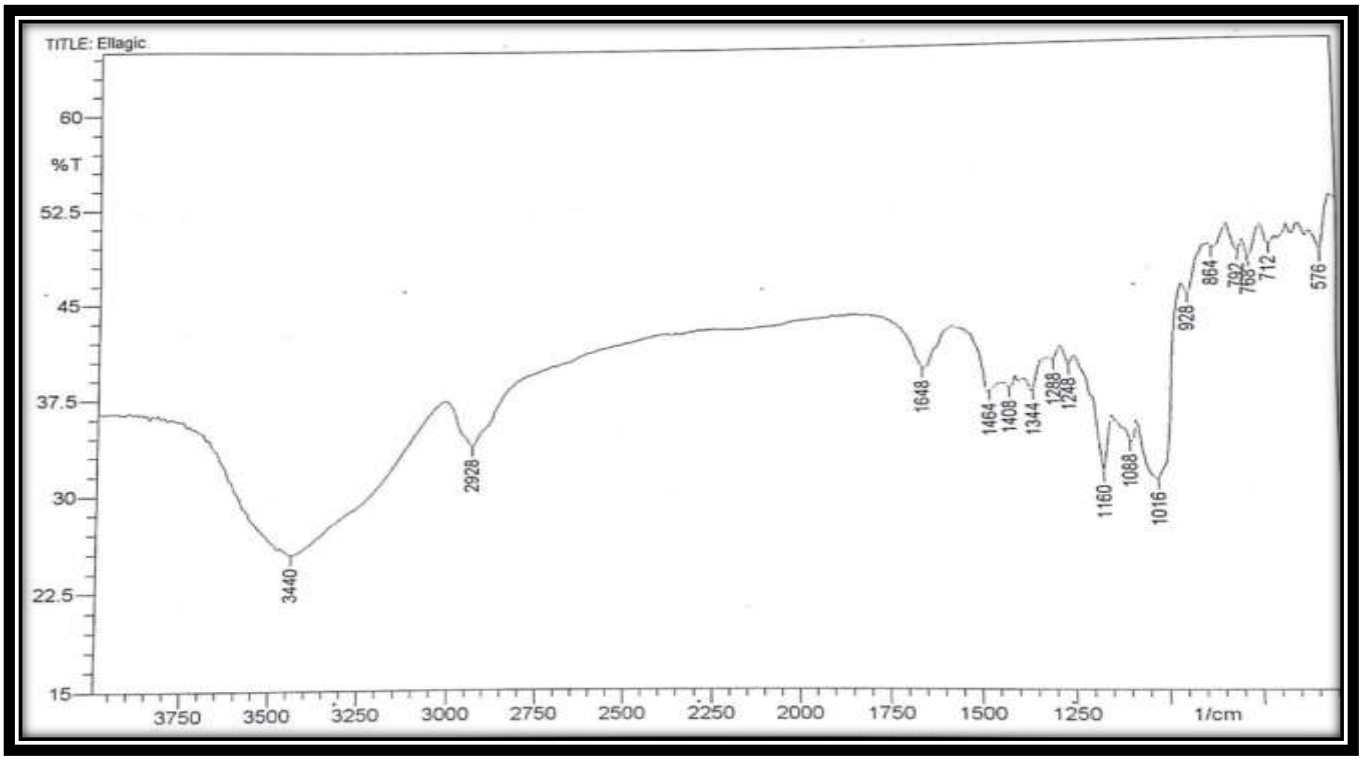


Fig. 4: Infrared spectrum of ellagicacid extracted from white flesh pomegranate.

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