

The Extraction and Purification Of Gallic Acid from the Pomegranate Rind

Entessar H.A. Al-Mosawe and Iman I. Al- Saadi
Biotechnology Division, Applied Sciences Department/ University of Technology,Iraq-Baghdad.

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الخلاصة

هدفت هذه الدراسة الى استخلاص وتنقية حامض الكاليك من قشور الرمان المزروع في العراق. واستعمل كروموتوغرافي الطبقة الرقيقة لعزل المركب من مستخلص الكحول الايثيلي (٤٠%) من قشور الرمان بأستخدام نظام متكون من التولوين: اثيل استنيت: حامض الفورميك بحجم ٣: ٣.٥: ٠.٥ حجم/حجم. وقد اجريت الفحوصات للتأكد من نقاوة حامض الكاليك والتي شملت تقدير الحركة النسبية بواسطة TLC واختبار الاشعة الحمراء والكشف بالمطياف الضوئي لمعرفة منحنى اقصى امتصاص وطريقة تحليل كروموتوغرافيا السائل عالي الكفاءة مع المقارنة بالمادة القياسية لجميع الاختبارات.

ABSTRACT

The study aimed to extract and purify of gallic acid from rind of pomegranate cultivated in Iraq and to investigate the chemical composition of the pomegranate peel this study the original procedure is described for the extraction and purification of gallic acid.

Gallic acid was extracted by ethanol / water (40:60) and identified in the chromatography method (GA in the FT-IR ,UV spectra and HPLC) and then compared the result with the standard test to determin the isolated compound .

Keywords: *Punica granatum*, rind , Gallic Acid.

INTRODUCTION

Pomegranate belongs to punicaceae family. *Punica granatum* (Punicaceae), commonly called pomegranate, recently described as nature's power fruit , is a plant used in folkloric medicine for the treatment of various diseases [1,2]. The Babylonians regarded the seeds of Pomegranate as an agent resurrection, the Persians as conferring invincibility on the battlefield and ancient Chinese it used the bright red juice was mythopoetically regarded as a“soul concentrate”, The rind of Pomegranate homologous to human blood and capable of conferring on a person longevity or even bearing multiple hydroxyl groups that predominate in immortality [3]. Fruits are one of the oldest forms of food known to man.. According to Qur'an, the fruits like grapes, olive and pomegranate are heavenly fruits of God. The people in ancient times regarded fruits to be endowed with magic or divine properties. The pomegranate is an ancient fruit that has not changed much throughout the history of man. It was found in the Indus Valley so early that there is a word in Sanskrit for pomegranate. The pomegranate is also significant in Jewish, Christian and Muslim traditions [4].The

pomegranate is native of Iran and Afghanistan, known in ancient Egypt [5].

Pomegranate has strong antioxidant and anti-inflammatory properties, Recent studies have demonstrated its anti-cancer activity in several human cancers [6]. In addition, pomegranate peel extract with an abundance of flavonoids and tannins has been shown to have a high antioxidant activity [7]. Antimicrobial drug resistance in human bacterial pathogens is a worldwide issue and as a consequence, effective treatment and control of such organisms remain an important challenge. Bacterial resistance has appeared for every major class of antibiotic [8]. The major class of pomegranate phytochemicals is the polyphenols (phenolic rings bearing multiple hydroxyl groups) that predominate (flavonols, flavanols and anthocyanins), condensed in the fruit. Pomegranate polyphenols include flavonoidstannins (proanthocyanidins) and hydrolysable tannins (ellagitannins and gallotannins). Hydrolyzable tannins (HTs) are found in the peels (rind, husk, or pericarp), membranes and piths of the fruit [9]. HTs are predominant polyphenols found in pomegranate juice and account for 92% of its antioxidant activity . Constituents Pomegranate pericarp (Peel, rind) Phenolic punicalagins; gallic acid and other fatty acids; catechin, EGCG; quercetin, rutin and other flavonols; flavones, flavonones; anthocyanidins [10].

Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid, a type of organic acid, also known as 3,4,5-trihydroxybenzoic acid, found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants [11] . The chemical formula is $C_6H_2(OH)_3COOH$ (fig.1). Gallic acid is found both free and as part of tannins. Salts and esters of gallic acid are termed 'gallates'. Despite its name, it does not contain gallium.

Gallic acid is commonly used in the pharmaceutical industry [12] . Gallic acid can also be used as a starting material in the synthesis of the psychedelic alkaloid mescaline [14] .

Gallic acid seems to have anti-fungal and anti-viral properties. Gallic acid acts as an antioxidant and helps to protect human cells against oxidative damage. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells. Gallic acid is used as a remote astringent in cases of internal haemorrhage. Gallic acid is also used to treat albuminuria and diabetes [15] .

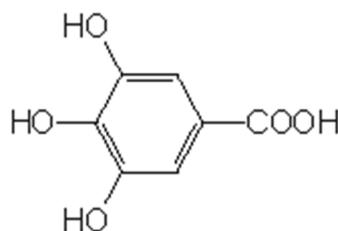


Figure-1: Chemical structure of gallic acid

This study we aimed to extract and purification Gallic acid from rind of pomegranate to use it to biological and chemical test as standard compound.

MATERIAL AND METHODS

Plant materials

The rind of pomegranate collected and washed with water to remove any impurities and then dried at room temperature 25°C without light, the samples put in grinder to transfer it to powder.

Extracting solvents:

We weighed 100g of powder and put it in a round bottom with solution content ethanol : water with ratio 40:60 . The flask was heated in a water bath at 60°C for 60 min . The solution was then filtered to obtain crude (stock solution) [15].

Purification of crude:

The crude distilled to get 1/3rd of the solution using the soxhlet apparatus at 70°C for 3hrs ethanol was recovered to concentrate crude extraction and then the solution is kept overnight at room temperature 25°C to precipitate. The solution and the obtained particles are dried in the oven overnight at 60°C [15]. Water was added in the soxhlet apparatus, and allowed to evaporate at lower temperature.

GALLIC ACID TEST:

To detect Gallic acid we take extract (1 ml of ethanol extract) mixed with 10 ml of distilled water and filtered. Ferric chloride reagent (3drops) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic.

Identification procedure

Thin layered chromatography (TLC): the dried methanol extraction redissolved in 1ml methanol then applied by micropipette on silica gel plate as continuous straight line with a width range (2-4) mm wide. Plates were inserted into a saturated TLC chamber containing Toluene: ethyl

acetate : formic acid system 3:3.5:0.5 as a mobile phase put two spots sample and standard (fig 2)[16].

HPLC: *The yield of extracted from pomegranate peel was gallic acid can be improved by using:*

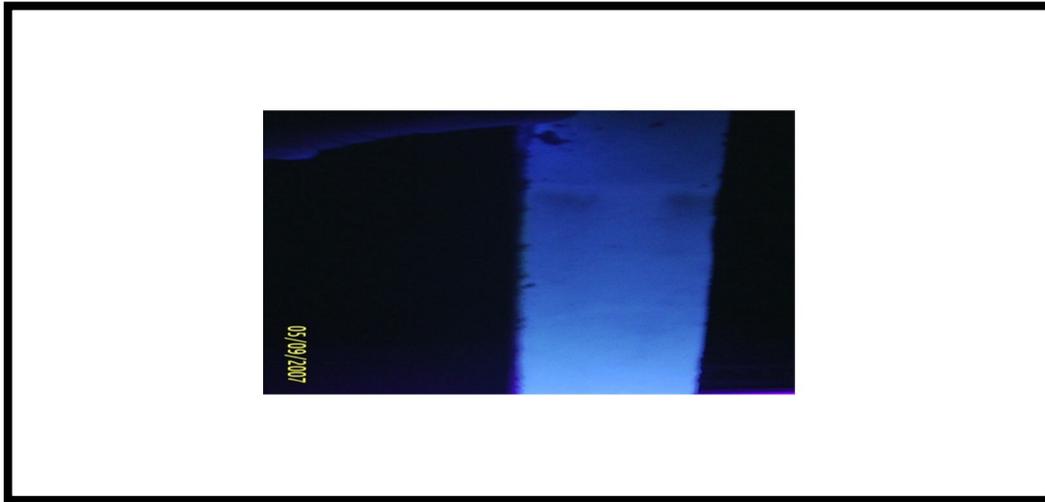
The separation and identification of gallic acid was performed by HPLC using Jasco's Binary Type High Performance Liquid Chromatography; comprising two PU-1550 pumps with 20 μ l loop and a Jasco multi wavelength detector MD-1510. A Cosmosil C₁₈ column (150mm x 4.6mm, 5 μ m particle) was used for the analysis. The mobile phase was a mixture of ethyl acetate: ethanol: water (1:5:4, v/v/v) delivered at a flow rate of 1.0 mL min⁻¹. Peak of Gallic Acid in sample was identified by comparison with retention time of standard Gallic Acid [16]. Separation and identification of Gallic Acid in sample extract was performed by HPLC using Cosmosil C₁₈ column (150mm x 4.6mm, 5 μ m particle) and mobile phase a mixture of ethyl acetate: ethanol: water (1:5:4, v/v/v), a well resolved separate peak of Gallic Acid was identified at retention time of 3.496 min. which was found to be comparable with the retention time of standard (**Figs. 3,4**).

FT-IR : to detect functional groups in resveratrol structure and to be compared with the standard chart. Resveratrol was reported to contain many functional benzene ring, hydroxyl groups and the C=C double bond compared with standard (**figures. 5,6**)[16].

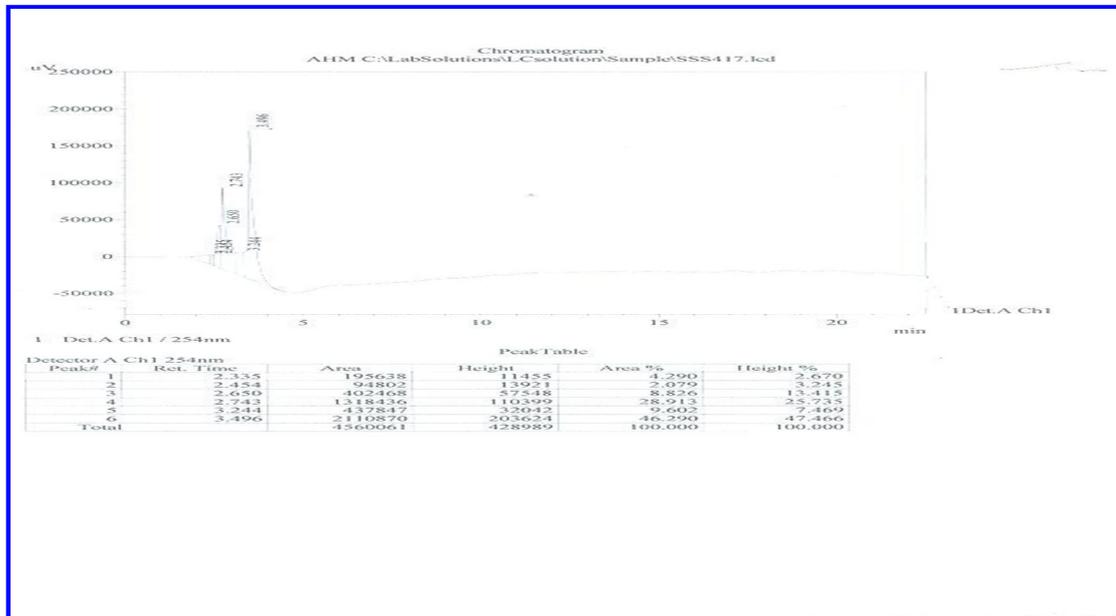
UV absorption : show intense purple fluorescence in UV light that was appear in the λ max at 366 with an inflection at 500 that compared with standard (**figures. 7,8**).

RESULTS AND DISCUSSION

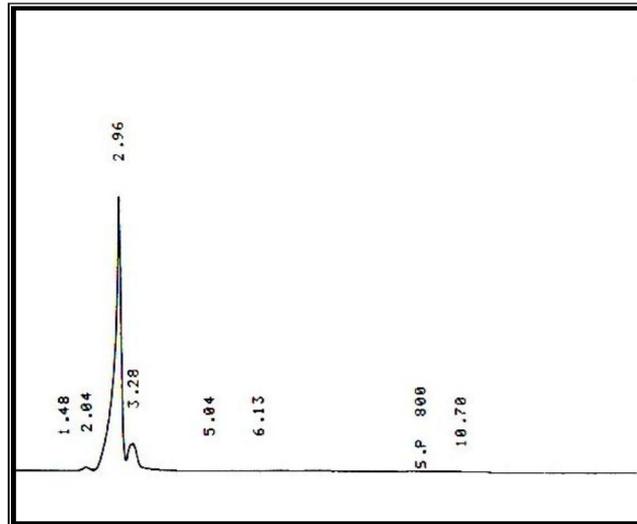
In the TLC obtained one zone were clearly visible: R_f - 0.9 which compared with R_f of Gallic acid standred (fig. 2,3).Some R_f values in the TBA system were identical to luteolin mentioned in literature, and even more in Toluene-atheyl acetate- formic acid system 3:3.5:0.5 due to polarity difference. Bearing in mind the limitations of the identification of substances by the chromatographic method.



Figures-2: TLC of gallic acid A-standard B-sample



Figuer-3: HPLC spectra of gallic acid sample



Figuer-4: HPLC spectra of gallic acid standard

FT-IR Spectral Analysis: The FT-IR analysis fig (5,6) of the were further tested by UV fig(7,8) and and IR spectroscopy methods for identification samples was done and the functional groups associated were determined (table 2).the FT –IR spectrum presence of

Table-2: functional groups in FT –IR spectrum

Major absorption bands	ν : 3414, 3377, 1716, 1616, 1512, 1454, 1226 cm^{-1} (KBr)
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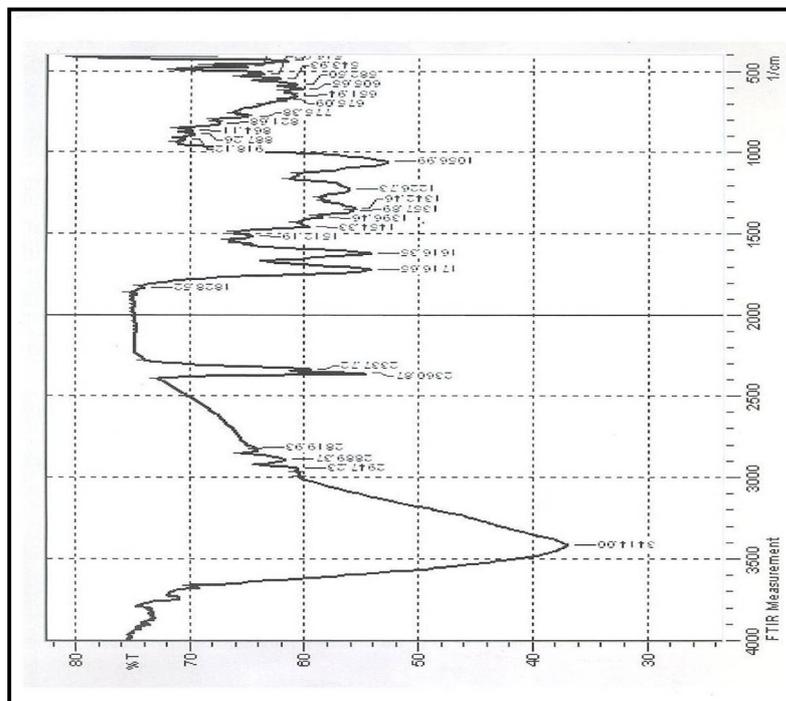


Figure-5: FT-IR spectra of gallic acid sample

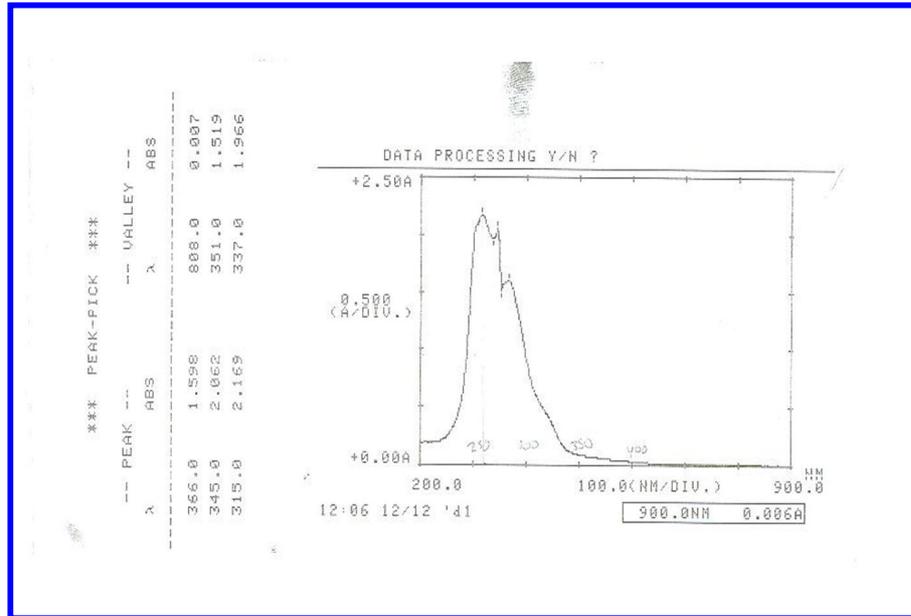


Figure-6: UV. spectra of gallic acid sample

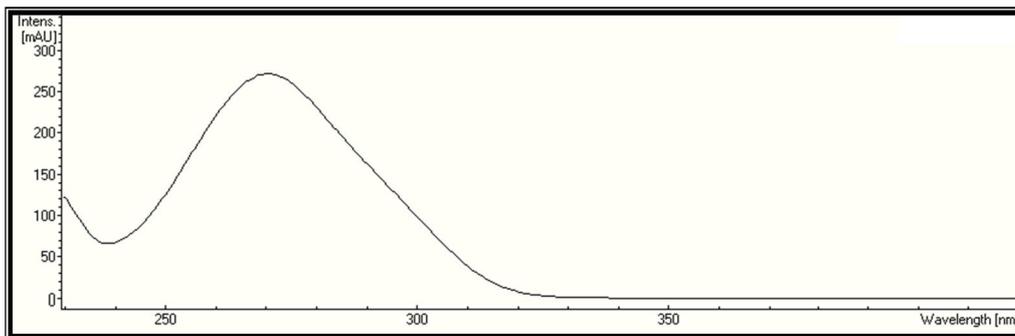


Figure-7: UV. spectra of Gallic acid standard

Peaks of GALLIC ACID in sample was identified by comparison with IR-standar GALLIC ACID .Determination of gallic acid Uv analysis was performed on a uv – visible spectrophotometer 220, 271 nm (ethanol).

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