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Demodulation and purification of ellagic acid from pomegranate fruit and study antimicrobial activity against the pathogenic bacteria.

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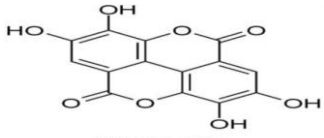
ABSTRACT

The ellagic acid is isolated from pomegranate fruit by liquid–liquid extraction method. This method includes extraction with 20% (v/v) methanol under reflux conditions for purification of acid by preparative TLC method using glass plates (20 × 20 cm) coated with silica gel .This acid was detected by TLC, HPLC, UV methods and functional group of the acidwhich was extracted by FTIR technique. The antibacterial activity of Ellagic acid extract of pomegranate fruit powder was defined by petri Dish technique versus different microbial types, Gram-positive (Staphylococcus aureus), Gram-negative (Escherichia coli, Pseudomonas aeruginosa) and two Yeasts(C. glabrata and C. albicams).

Introduction

A natural Ellagic acid ($C_{14}H_6O_8$) can be obtained from some plants and it is one of the polyphenolic compounds, can be absorbed readily through gastero intestinal system in mammals including human[1,2] . It is found in the plants when tannins hydrolyses to ellagitannis. Ellagitannins are esters of glucose, the last yield ellagic acid [3,4], and it is present in the fruits and vegetables. The acid is a bioactive compound and has important applications in the food, pharmaceutical and cosmetic industryare due to its beneficial biological properties[5]. The nutritional toxicology of tannins and related polyphenols including ellagic acid was investigated, there results showed that acid was considered to be non- toxic, these concentration of it were nontoxic as determined by biochemical and histological methods[6,7]. It has antibacterial and antiviral properties. [8] The latest studies showed that the acid had an influence on liver cancer , esophageal, prostate, and colorectal cancer cell lines.[9] Ellagic acid, which has an important antioxidant potential and plays a great role on human health to protect against reactive oxygen species (ROS)[10], and inhibits lipid peroxidation necrosis of skin flaps, However, in other studies need to confirm the results [11].



Ellagic acid

In the present paper we report the isolation of ellagic acid from the methanolic extract of pomegranates[12], as well as the identification of the compound using high-perference liquid chromatography (HPLC-UV-PDA), thin layer chromatography (TLC), Uv and FTIR spectro. We also evaluated the immunological activities of the isolated compound and compared with standard ellagic acid [13,14].

Experimental

Instrumentation:

Knauer system HPLC with C-18 Phenomenex column (250 × 4.6mm, 5µ) with Uv detector and auto sampler (3455) was employed for chromatographic separation., glass plates (20 × 20 cm) coated with silica gel (60 F254),, with 250 µm layer thickness, using shimadzu UV-VIS Spectrophotometer 1800 with 3 ml capacity quartz cells and FTIR, Digital balance and pH Meter were used in the study.

Materials:

The HPLC grade methanol, hexane, hydrochloric acid hexane, ethanol , acetic acid, THF, acetonitrile, were purchased from local market.

Methods:

The dried powder of pomegranate fruit (200 gm) was extracted with 500 ml of 20% (v/v) methanol under reflux conditions for 1 h at 60°C,The solution was filtered. after 12-24 h by using a Whatmann filter paper No 42, the filtrate was evaporated to a 10 ml and extracted with hexane to remove the lipid, a methanol was added to the extract and this solution was used for purification ellagic acid by TLC technique .

Purification and isolation of ellagic acid by preparative TLC

The dried plates were placed in the oven at 100°C of 30 minutes to activate it and cooled at room temperature. Each extract was antroducat at 1 cm above the edge of the chromatographic plate with 4 mm width using 100 µl sample syringe (Hamilton, Switzerland) Chromatographic chamber which was already saturated with a solvent system,other solvents system such as hexane: ethanol : acetic acid, THF: acetonitrile were also used but 25ml of ethanol : water (80:20) as a mobile phase gave better separation with migration distance 12cm of the compound, the chromatograms were air-dried and visualized under UV light at 254 nm and the fluorescence or the color were noted. The spot of Ellagic acid was scraped separately and dissolved with 20 mL methanol and shaken for 10 min. The obtained extract was filtered and the residue was shaken twice with 20 mL methanol for 10 min, the resulting was identified by comparing with standard.

Table (1) The RF values of one- dimensional TLC for crude Extract for ellagic acid, tannic acid and nicotinic acid standards in different mobile phase.

Mobile phase	Rf Crude extract	Rf Ellagic acid standard	Rf Tannic acid standard	Rf Nicotinic acid standard
Ethanol: water (80:20)v/v	0.29, 0.38.0.78	0.39	0,29	0.23
Hexan:ethanol: acetic acid (70:20:10)	0.13,o.17, o.38	0.29	0.12	0.15
THF:acetonitrile(15:85)	0.15, 0.23, 0.39	0.78	0.38	0.39

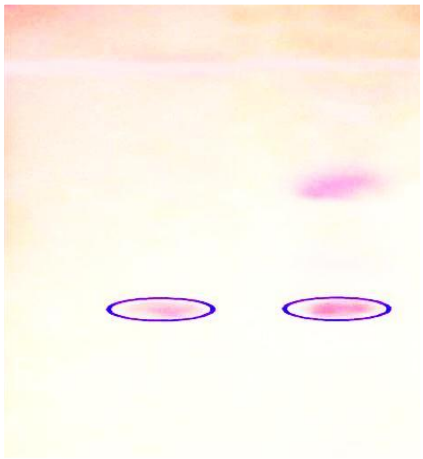


Fig- 1,TLC chromatogram of standard eiiagic acid and extract only by (ethanol:water,80:20) mobile phase

Determination of ellagic acid by HPLC :

The ellagic acid sample was separated and identified by HPLC analysis using the mobile phase consisted of 0.1% orthophospharic acid at a flow rate of 0.5 mL min^{-1}. The UV detection wavelength was set at 272 nm. The extract of ellagic acid was determinted by injection 20 µl of the extract after separation by TLC and it was compared with standard (injection 20 µl under same condition) the Rt value of the ellagic acid extract and standard were shown in table (2).

Table (2) tR value of HPLC

Compound	Retention time (sec)
Ellagic acid extract	6.58
Ellagic acid standard	6.59

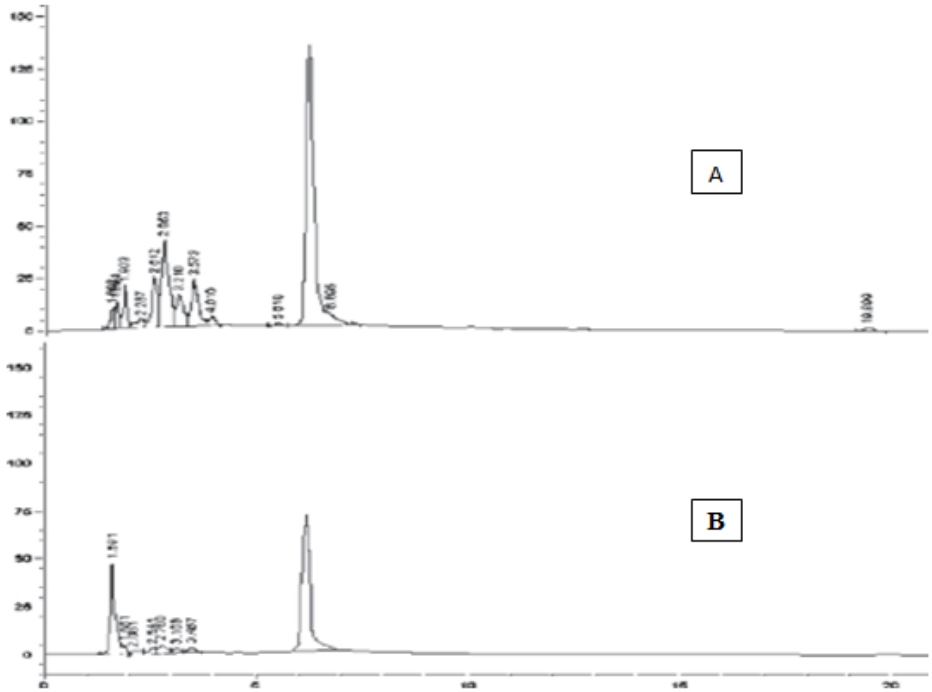


Fig-2, HPLC chromatogram of ellagic acid samples (A) and standard (B)

UV visible and FT-IR Spectroscopic analysis ;
The sample was analyzed using FT-IR and UV spectrophotometer after centrifugation
at 3000 rpm for 10 minutes and filtered through Whatmann No. 41 filter paper using high pressure vacuum pump. The extracts were examined in the UV-VIS wavelength ranging from 200-700 nm after dissolving in analytically pure methanol and the characteristic peaks were detected. FTIR Spectrum recognized the functional group of the chemical compounds depends on the wave number in the infrared technique area(400-4000) , and the peak values were recorded.

Results and discussion

The analysis process leads to the results by using 80% methanol/water, ethanol was distilled out and the aqueous layer was sequentially extracted with hexane, the resdeu dissolved by methanol , It is important to use a 60°C temperature and avoid light during the extraction process with constant shake up, samples should be filtered to eliminate contaminants such as fibers, pigments, and other compounds after 12 or 24 h of extraction process.

TLC: Ellagic acid from plant samples was confirmed by TLC, HPLC hromatogram, IR spectra and UV. When the developed TLC plate was sprayed with 50% sulphuric acid they showed dark coloured spots of the reference and ellagic acid. Rf value of ellagic acid isolated from the samples and standard was equal to (0.39).

HPLC: The identification of the constituentswhich is presented in the chromatographic profile of extract was carried out by comparison with retention times (Rt) of peaks in the standard solution ellagic acid.

FTIR- spectrophotometry ;
The main absorption bands were to the valence vibrations corresponding to OH, C=O, C-O-C groups, C-H bonds and to aromatic rings vibrations. The IR spectrum showed stretching vibration in regions 1612 cm^{-1} for(C=C streach, aromatic ring), OH stretching bond1724 cm^{-1} for (C=O, oxidic carbonyl group) and 3400 cm^{-1} For (H bonded hydroxyl group)The bands observed in the rang 1669-1500 $cm^{-1}$are aromatic ring **(Fig-3**) The results of the FTIR confirmed the existence of ellagic acid.

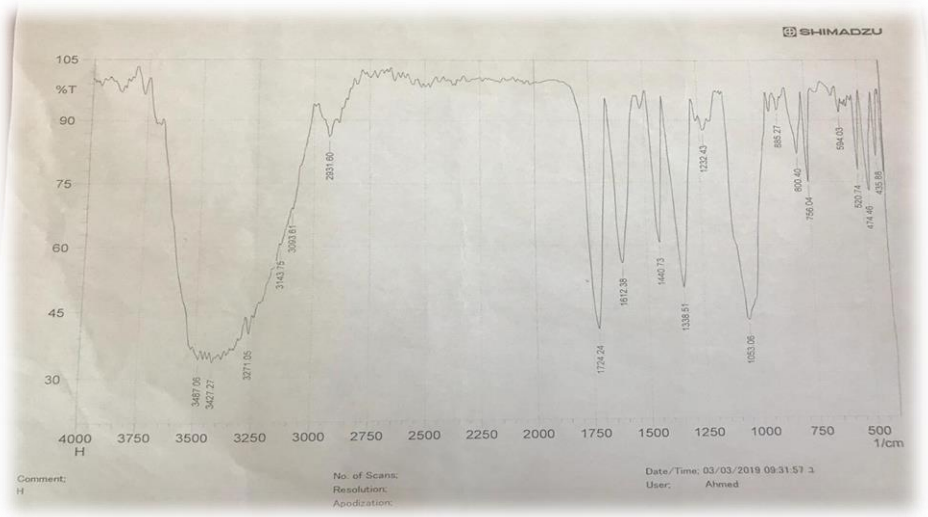


Fig- 3, FT-IR spectrum of ellagic acid extract

UV- spectrophotometry:

The UV-VIS spectra for the extract in methanol was recorded for the range 200-800 nm. The ellagic acid was determined at 273nm and compared with the standard ellagic acid, Fig(4, 5).

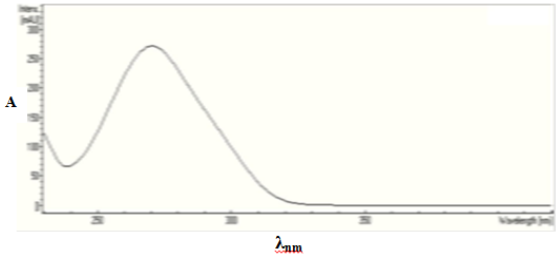


Fig –4, Uv-spectrum of ellagic acid standard

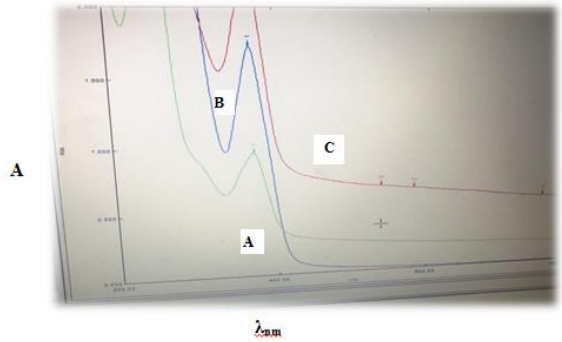


Fig -5, Uv-spectrum different concentration (A,B,C) of ellagic acid extract

A. concentration of elagic acid(75%).
B. concentration of elagic acid (50%).
C. concentration of elagic acid (25%).

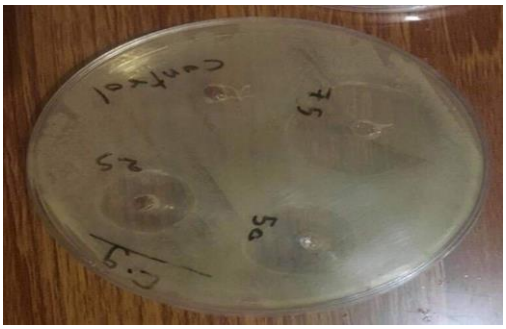
The standard solution was scanned in the range 200-800nm. Wave lengths of maximum absorbance was found to be at 273nm,for determination of extract sample ,an aliquot volume of sample were prepared as in the standard sample and measured at 273nm.

Biological activity study:

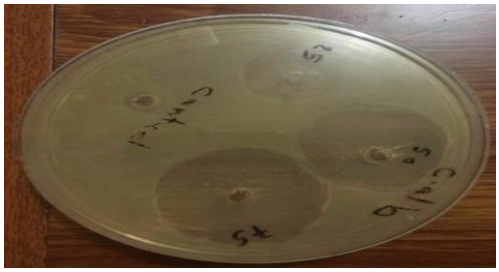
presence of some polyphenols compounds such as ellagic acid the basis of Antibacterial activity of some plant extracts, which are enriching their biological activity against pathogenic bacteria ,three microorganisms representing different microbial classes, Gram-positive (Staphylococcus aureus), Gram-negative (Escherichia coli, Pseudomonas aeruginosa) and two Yeast(**C. glabrata, C. albicams**) , The Petri dishes were incubated at 37 ± 1c° for 24 hrs; the diameters of zone of inhibition (mm) surrounding each of the wells were verified. the inhibitory effect of pomegranate fruits extracts may be reffered to the effect of phenolic compound. As in ellagic acid which may make complexation with enzyme or substrate in bacteria cell. Ellagic acid toxicity due to its effect on the microorganism membranes. The ability of ellagic acid to form comppunds with the main metals in bacteria cell account was used to detect the toxicity[15].

Effect of Elagic acid in bacteria of Ecoli

Effect of Elagic acid on aurens .Staph



Effect of Elagic acid on C.glabrata



Effect of Elagic acid on C.albicams

Table-3, concentration of ellagic acid effected on thebacteria and yest

Type of bacteria	concentration		
	25 mg/ml	50 mg/ml	75 mg/ml
E.coli	11.5 mg	13.6 mg	14.4 mg
Staph.aurens	13 mg	16 mg	18 mg
Ps.acrogenosa	0	o	o
Type of yeast	concentration		
	25 mg/ml	50 mg/ml	75 mg/ml
C.albicams	13 mg	16.2 mg	17.6 mg
C.glabrata	11 mg	13.2 mg	15.3 mg

Conclusion

The research included the extraction and purification of the elagic acid from the pomegranate fruit, where results were obtained means of the measurements by UV radiation shown λmax at 273nm and the technique of infrared radiation, where the packages provided clear to the acid extracted in addition to TLC and HPLC techniques, which have good results .

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استخلاص وتنقية حامض الايلاجيك من فاكهة الرمان ودراسة الفعالية البايولوجية ضد البكتريا المرضية

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الملخص

طريقة بسيطة للاستخلاص باستخدام الاستخلاص (السائل–السائل) لتحضير مستخلص الرمان الغني بحمض الايلاجيك. تضمنت الطريقة الاستخلاص مع الميثانول الحاوي على ماء بنسبة (20٪ ح / ح) تحت ظروف التصعيد الحراري من اجل تنقية الحامض بواسطة TLC المحضر بواسطة صفائح زجاجية (20 × 20 سم) مغلفة بجيل السيليكا، تم تحديد المركب بواسطة HPLC،TLC و UV, تم تشخيص المجاميع الوظيفية للحامض بواسطة تقنية FTIR، وتم تحديد الفعالية المضادة للبكتيريا لمستخلص حمض الايلاجيك من المسحوق المجفف من فاكهة الرمان بواسطة تقنية الطبق البتري (petri dish) ضد اصناف ميكروبية مختلفة، إيجابية الجرام (Staphylococcus aureus)، سالبة الغرام (Escherichia coli, Pseudomonas aeruginosa) ونوعين من الخمائر . (C. glabrata and C. albicams)