



EXTRACTING AND IDENTIFYING SOME ACTIVE COMPOUNDS FROM POMEGRANATE SEEDS AND BANANA PEELS AND THEIR INHIBITORY EFFECT ON SOME MICROORGANISMS

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ABSTRACT

The study aimed to extract phenolic and flavonoid compounds from pomegranate seeds and banana peels by microwave technology, where the percentages of phenolic compounds in pomegranate seeds and banana peels were 40.60 and 32.27 mg GAE/g dry weight, respectively, while the flavonoid compounds were in pomegranate seeds and banana peels 2.48 and 17.8 mg catechins/g dry weight, and they were diagnosed using a High-performance liquid chromatography(HPLC) Technique device, and it was found that there is a number of phenolic compounds included (galic acid, caffeic acid, chlorogenic and ellagic acid), while flavonoid compounds included (catechins, quercetin, coumarin, and myricetin). and the inhibitory activity of the plant extracts was tested against a number of bacteria *Escherichia coli* (*E.coli*), *Bacillus cereus*(*B.cereus*), *Staphylococcus aureus*(*S.aureus* and *Pseudomonas aeruginosa* (*P.aeruginosa*), at three concentrations 3, 7and 10% which reached the highest inhibitory effectiveness of pomegranate seed extract and according to the order of its effect on bacteria as follows *P.aeruginosa*, *E. coli*, and finally *B. cereus*, as the diameter of the inhibition halo at a concentration of 10% reached 21 mm against *P.aeruginosa* bacteria, and pomegranate seed extract didn't show any inhibitory activity against *S.aureus* bacteria. Banana peel extract showed lower inhibitory efficacy than pomegranate seed extract, as the diameter of the inhibition halo against *P.aeruginosa* bacteria at a concentration of 10% to 11 mm, while the extract didn't show any inhibitory activity against *B. cereus* bacteria at any concentration. Pomegranate seed extract exceeded the highest concentration of phenolic substances in addition to containing a higher concentration of phenolic compounds and flavonoids diagnosed and had the highest inhibitory effectiveness against bacteria and molds.

Keywords: Phenol, flavonoid. Microwave. Mold. Bacteria

*The article is taken from the master's thesis of the first researcher.



استخلاص وتشخيص بعض المركبات الفعالة من بذور الرمان وقشور الموز وتأثيرها التثبيطي على بعض الاحياء المجهرية

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الخلاصة

هدفت الدراسة الى استخلاص المركبات الفينولية والفلافونويدية من بذور الرمان وقشور الموز بتقنية المايكروويف حيث بلغت نسب المركبات الفينولية في بذور الرمان وقشور الموز 32.27, 40.60 ملغم/GAE و 2.48 و 17.8 ملغم كاتيكين/ غرام جاف على التوالي، اما المركبات الفلافونويدية فكانت في بذور الرمان وقشور الموز (2.48 و 17.8 ملغم كاتيكين/ غرام وزن جاف، وشخصت باستخدام جهاز الكروماتوغرافي السائل عالي الأداء وتبين وجود عدد من المركبات الفينولية شملت (حامض الكاليك حامض الكافيك، الكلوروجينك وحامض الايلاجيك) اما المركبات الفلافونويدية شملت (الكاتيكين، الكورستين، الكومارين والماييستين)، كما تم اختبار الفعالية التثبيطية للمستخلصات النباتية تجاه عدد من البكتريا *E. coli*, *S. aureus*, *B. cereus*, *P. aeruginosa* وبثلاث تراكيز 3, 7, 10 % اذ بلغت اعلى فعالية تثبيطية لمستخلص بذور الرمان وبحسب ترتيب تأثيرها على البكتريا كالتالي: *E. coli* , *p. aeruginosa* , وأخيرا *B. cereus* اذ وصل قطر هالة التثبيط عند تركيز 10% الى 21 ملم تجاه بكتريا *pseudomonas* ، ولم يبدي مستخلص بذور الرمان أي فعالية تثبيطية تجاه بكتريا *S. aureus* . اظهر مستخلص قشور الموز فعالية تثبيطية ادنى من مستخلص بذور الرمان اذ وصل قطر هالة تثبيط مستخلص قشور الموز تجاه بكتريا *P.aeruginosa* عند تركيز 10% لـ 11 ملم في حين لم يظهر المستخلص أي فعالية تثبيطية تجاه بكتريا *B.cereus* عند أي تركيز. اذ تفوق مستخلص بذور الرمان بأعلى تركيز للمواد الفينولية بالإضافة الى احتوائه على تركيز اعلى من المركبات الفينولية والفلافونويدية المشخصة وكان لها اعلى فعالية تثبيطية تجاه البكتريا والاعفان.

الكلمات المفتاحية: فينول، فلافونويد، مساعدة المايكروويف، غفن، بكتريا.

INTRODUCTION

The plant residues resulting from fruit juice, such as peels and seeds, are among the important sources of antioxidant compounds and microorganisms they contain, as they are free radical inhibitors and useful at the same time as agents against cancer, heart disease, and aging factors (Al-janabi *et al.*, 2015). In addition, modern methods have focused on creating effective packaging made from biodegradable materials from plant waste to reduce waste in the environment (Chalob& Abdul-Rahman, 2018). Pomegranate trees have been known since ancient times, as they were planted in the hanging gardens in Babylon and drawn on the walls of the Pharaonic tombs, and their flowers were called Gulnar. Pomegranate follows (*Punica granatum* L.) Pomegranate family (*Punicaceae*) . The number of Pomegranate trees in Iraq is 11,997,000 trees, and their production of fruits is (304,300) thousand tons, and the average production of one seed is (25.4) kg. More than 23 varieties of pomegranate are grown in Iraq, the most common of which is the Salimi variety (Karume & Jumaa, 2005). More than 23 varieties of pomegranates are grown in Iraq. The Slimy variety is considered the most cultivated and produced in the central region. Pomegranate fruits ripen 135-170 days after flowering, as the color of the peel becomes yellow, and when the peel is tapped or struck, it gives a metallic sound. Pomegranates are considered one of the climacteric fruits (Tahish & Jumaa, 2005). Pomegranate fruits consist of three parts: juice, seeds, and peel. Pomegranate fruits contain sugars, acids, water, protein materials, fibers, tannins, mineral elements, vitamins (A, B, C), and small amounts of iron, phosphorus, sulfur, calcium, potassium, and manganese. Pomegranate peel constitutes about 40%. From the pomegranate fruit, pomegranate peel contains quantities of phenolic compounds such as (flavonoids, anthocyanins, catechins and



other complex flavonoids). Pomegranate peels are rich in tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). Pomegranate is antimicrobial, anticancer, antioxidant, antibacterial, and antifungal (Al-Qazzaz, 2014). Pomegranate is very rich in antioxidants, which are very important in reducing oxidation processes, and the most important active substances in it are polyphenols. Phenolic compounds are among the most important antioxidants in pomegranate fruit to combat free radicals (Banana, 2020). Pomegranate peels showed effectiveness in inhibiting fungi and some other microorganisms. Phenolic compounds were used in pomegranate peels, which proved highly effective in inhibiting fungi, which are the causes of many diseases, including the fungus *Pythium* sp (Al-Juboory & Saleh, 2013). The common banana, scientifically known as *Musa sapientum*, is a popular fruit that has a number of beneficial pharmacological effects and can be distributed throughout the world. It can be grown in lowland wetlands to tropical highlands. Banana peels are an unused part of the banana fruit. There are many compounds in the banana peel such as enzymes such as polyphenoloxidase and pectin as the gelling agent. Banana peels contain a high percentage of antioxidants, both phenols and minerals. This peel is biodegradable and causes an environmental problem due to the amount of nitrogen and phosphorus. As a result, the best solution to protect humans, get some profits and stop the waste of wealth is to extract banana peels. Banana peel contains more antioxidants than the pulp. Banana peel can also be marketed due to its antioxidant quality and quantity. You will not deal with banana pulp in the production of finished products especially in the food industry. Previous research confirms that bananas can consistently serve as a natural source of antioxidants. Banana peel also contains dietary fiber, polyunsaturated fatty acids, essential amino acids, proteins, and potassium (Farhan *et al.*, 2016). Banana peel extract is used alone or with a cream or ointment, and the medical benefits of the extract include reducing pain, swelling, and itching (Hikal *et al.*, 2022). Bananas are considered prebiotics because they contain fructo oligosaccharides and resistant starch in varying quantities, as well as possessing anti-microbial, anti-diarrheal, anti-tumor and anti-mutagenic properties, as well as anti-diabetic and other properties (Al-Zobaie & Sherida, 2013). The Cell Quest preparation, which contains banana extract and a high percentage of tannin, has shown an anti-cancer effect on some cancer cell lines outside of the body in vitro. The reason for this is the effectiveness of tannic acid in inhibiting proteasomal activity and inducing cell death. It was found that the aqueous extract of the shell has an anti-inflammatory effect. antioxidants, bacteria and algae (Al-Ansari *et al.*, 2010).

The current study aims to:

The exploitation of plant waste to be a source of active compounds instead of disposal to preserve the environment.

MATERIALS AND METHODS

Sample preparation

Ripe pomegranate and banana fruit samples were selected from the local markets of Anbar governorate to use certain parts left over from them as raw materials and sources of active compounds. A general cleaning and washing of the models was carried out to get rid of dust and impurities, they were cut and then dried in the convection oven at a temperature of 40 °C, stirring occasionally until the drying process was completed, after which the models were crushed with an electric grinder finely and then sieved with a fine sieve to obtain dry powders



that were placed in polyethylene bags. It is stored in the refrigerator at a temperature of 5°C until use.

Extraction using microwave

The extraction was carried out with this technique by mixing the extract at a rate of 10% using solvents (distilled water and 60% ethanol) and using a home microwave oven. The mixtures were exposed to radiation in a Microwave oven (with a power of 700 watts) according to (Proestos & Komaitis, 2008) with some modifications as follows :45 sec of operation to reach the required temperature of about 85-90 °C in order to ensure that the solution does not boil, and 30 sec of shutdown, then 3 sec of operation for heating, and 10 sec of shutdown for cooling to room temperature for 3 min. After the extraction process, the liquid is separated by filtration under vacuum using filter paper. (What man No. 1) The filtrate was then concentrated by evaporation in a rotary evaporator at a temperature of 40°C, after which the concentrated liquid was placed in glass dishes in an electric oven at 45 °C until it dried completely. After that, the samples were scraped off and placed in tightly sealed containers until used.

Determination of Total Phenols

The total phenolic content of pomegranate seed and banana peel extracts was estimated according to the method reported by (Vu *et al.*, 2019). 0.5 ml of dried plant extract (prepared by dissolving 0.5 g of dry powder in 10 ml distilled water) or distilled water as a Blank control sample was taken and placed in test tubes with 2.5 ml. Of Folen's reagent prepared simultaneously at a concentration of 10% v/v, the tubes were shaken well with a Vortex mixer and then left in the dark for 8 minutes. Then 2 ml of 7.5% sodium carbonate solution, Na₂CO₃, was added, shaking again, and the tubes were kept in the dark at room temperature. Then the optical absorption was measured with a spectrophotometer at a wavelength of 760 nm. The total content of the phenols under study was estimated using the standard curve for gallic acid. The total phenolic substances were expressed as gallic acid equivalents (GAE) in mg/g dry weight figure (1).

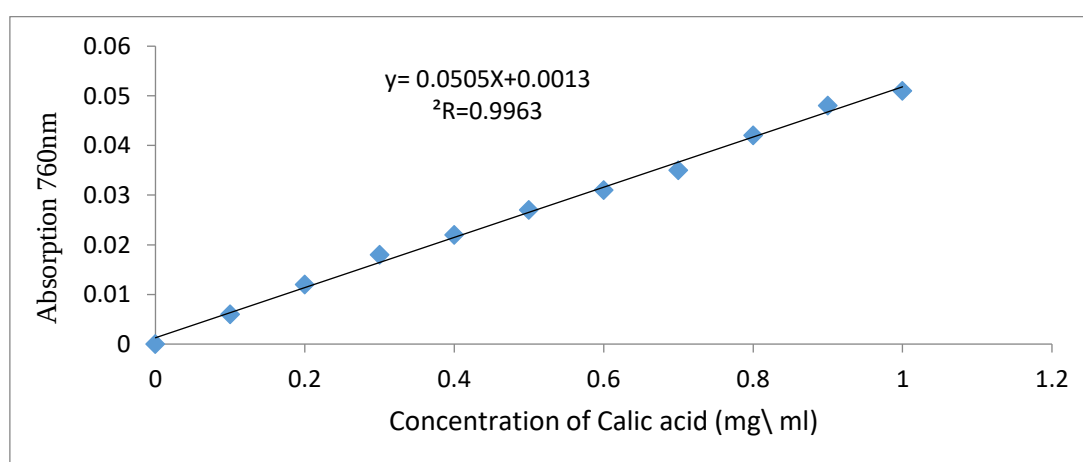


figure (1): The standard curve of Gallic acid for estimating total phenolic substances.

Total Flavonoids

The aluminum chloride method AlCl_3 mentioned by (Srinivasa *et al.*, 2012) was followed to estimate the total content of flavonoids in plant extracts, by mixing 1 ml of dried extract (1 Mg/ml) in a 10 ml volumetric flask with 5 ml distilled water and adding 0.3 ml of 5% NaNO_2 solution. After 5 minutes, 0.6 ml of 5% AlCl_3 solution was added to it, and after 5 minutes, 2 ml of 1 M sodium hydroxide solution, NaOH , was added and the volume was completed to the mark. Then the absorbance was measured at a wavelength of 510 nm based on the standard curve for catechins figure (2).

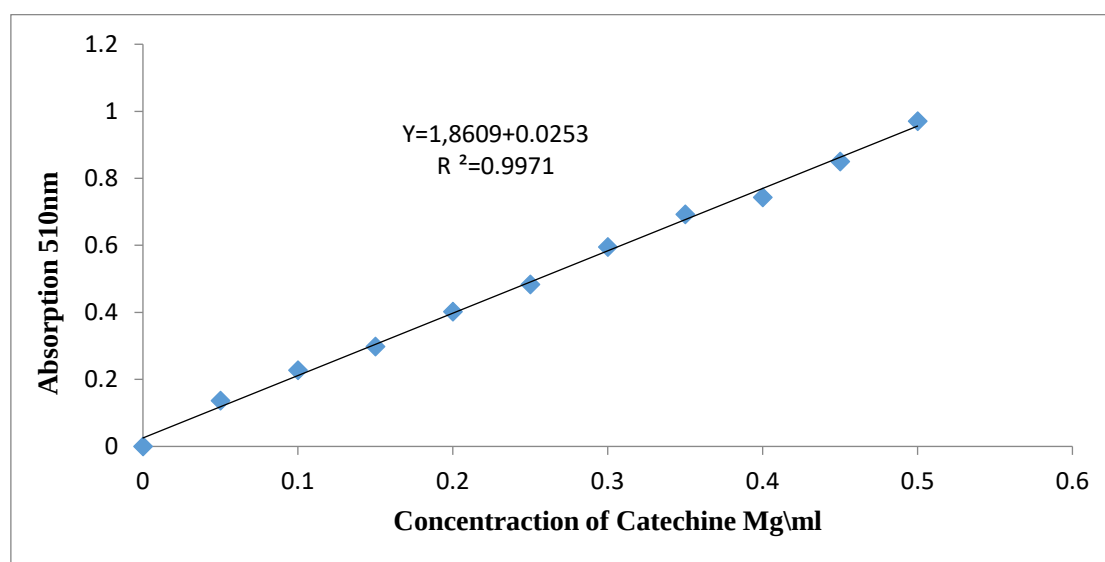


figure (2): The standard curve of Catechin for estimating total Flavonoids substances.

Diagnosis of active compounds using HPLC technology

Some active compounds of pomegranate seed and banana peel extracts were identified using a reverse-phase HPLC with an ultraviolet system in the Ministry of Science and Technology/Materials Science Laboratories based on the method developed before (Al-Qazzaz, 2014). Chromatographic separation was performed on a VertiSepTM pHendure C18 column. Programmed to keep the column temperature constant at 25°C using an Agilent G1316A column oven. The mobile phase was composed of a solution of acetonitrile 99.9% v/v and formic acid 0.1% v/v in a ratio 15:85 with pH 2.5, prepared immediately. Set the flow rate at 1 ml/min. The sample/injected volume was 20 μL and all standards and sample solutions were injected in three replicates. Use a UV detector with a wavelength of 275 nm. The compounds were identified based on the retention time matching the standard compounds.

Model concentration = $\frac{\text{area of the model curve}}{\text{area of the standard sample curve}} \times \text{standard sample concentration} \times \text{dilution factor}$

Measuring the inhibitory activity of pomegranate seed extract and banana peels against some types of bacteria



Activation of bacterial isolates and the effectiveness of the extract in inhibiting them: Four isolates of Gram-positive (*P. aeruginosa* and *B. cereus*) and Gram-negative (*S. aureus* and *E. coli*) From the Ministry of Science and Technology/Food Research Laboratory and was used for the purpose of qualitative detection of the inhibitory effectiveness of pomegranate seed and banana peel extracts. In order to measure the antibacterial effectiveness, the method described by (Saleem & Saeed, 2020), was adopted with some modifications, where the bacterial isolates were activated by transferring 0.5 mL of cultures preserved in nutrient broth medium to 10 mL of sterilized nutrient broth medium, and the tubes were incubated at a temperature of 37°C for 24 days. hour . The growth of the bacterial suspension was then compared with the turbidity of McFarland's standard solution and was adjusted to give a cell count of approximately 10^8 CFU/mL. Then, the method of diffusion by etching was used to detect the inhibitory effectiveness of the extract used in the study, by spreading 0.1 mL of the bacterial suspension containing 10^8 CFU/mL of previously activated bacteria on the surface of sterilized nutrient agar medium placed in sterilized petri dishes using L.shape. I made 4 holes and placed (50) microliters of the extract, each separately at different concentrations, where a 10% concentration of the extract was placed in the first hole. In the second hole, a concentration of 7% was placed, and in the third hole, a concentration of 3%. As for the fourth hole, there was no extract, as only water was used and it was taken as a control treatment. The dishes were incubated at a temperature of 37°C for 24 h. After the incubation period, the inhibition zones were measured, as they represent the diameter of the surrounding areola. With holes and growth-free Clear Zone(CZ) inhibition.

Testing the inhibitory effectiveness of plant extracts against some molds

The effect of alcoholic extracts of pomegranate seeds and banana peels was tested on the growth of two identified mold isolates (*P. digitatum* and *A. niger*), which were obtained from the Ministry of Science and Technology/Food Research Laboratories. The method mentioned by (Niamah, 2014) was followed with some modification, where 1 mL of the plant extract at a concentration of 10% was mixed with the nutrient medium Potato Dextrose Agar (PDA), and the extract was added to the sterile nutrient medium cooled to (45) degrees Celsius under conditions Sterilized, it was mixed thoroughly, and 12 mL of the mixture was poured into Petri dishes. After solidifying the medium, the dishes were inoculated by placing a piece of mold colony with a diameter of approximately 6 mm from an activated culture on PDA medium at 7 d old in the center of each dish using sterile forceps. The dishes were incubated at temperature 25°C with the control dish containing mold only without the extract for 2-3 d. To express the anti-mold effectiveness, the diameter of the mold was measured by calculating the average of two perpendicular diameters of the growth of each colony, and the inhibition percentage was calculated according to the equation (Farhan *et al.*, 2016):

Growth inhibition percentage =

$$\frac{\text{Dimeter of mold growth in the control sample} - \text{Dimeter of mold growth treated with the extract}}{\text{Dimeter of mold growth in control sample}} \times 100$$



RESULT AND DISCUSSION

Total phenolic content of plant extracts

Phenolic compounds were extracted from pomegranate seeds and banana peels with the help of a microwave and using a 60% ethanol solvent, where the amount of phenols was (40.60, 32.27 mg GAE/g dry weight). In a study conducted on pomegranate peels, the percentage of total phenols in the alcoholic extract was estimated at about 23-25. 3 mg (Yasoubi *et al.*, 2007). It was noted that the result of aqueous extraction of banana peels is less than what was recorded by (Vu *et al.*, 2019), as they were able to significantly improve the efficiency of extracting phenols from banana peels by using water as a solvent and with the help of the microwave by increasing its power to reach 960 watts and the time (6 min) and they were able to obtain (50.55 mg) of phenols. The reason for obtaining the highest yield of extraction of phenolic materials from Portuguese cherries of the Saco type using microwave technology compared to the traditional one could be the result of homogeneous heating of the microwave irradiation mechanism, which leads to an increase in temperature from the inside of the system to the outside, which leads to cell rupture and the consequent transfer of compounds phenolic to solvent. Unlike the traditional method, where the heat increases from the outside to the inside, so it is inefficient (Vilas-Boas *et al.*, 2020).

Total flavonoid content of plant extracts

The aqueous extract of banana peels had the highest amount of total flavonoids 17.8 mg/g. This result is consistent with what was indicated by (Ishak *et al.*, 2019), where two types of banana peels were used, and based on the standard curve of the rutin compound, the percentage for one of the types ranged from (1.94-17.19 mg/g).), and the amount of flavonoids in pomegranate seed extract was (2.48 mg/g), and the amount of flavonoids mentioned by (Vladić *et al.*, 2020) when he studied pomegranate peels was (3.34 mg CE/100 g) using an alcoholic solvent (ethanol 50%) and with a power of 800 watts, where it was found that the content of flavonoids It was higher when the energy was increased, as the increase in energy leads to heating of the solution and enhances the transfer of polyphenols from the cell to the extraction system. It must be noted that exposure to energy for a long time leads to plants losing their sensitive components, so it is necessary to choose the appropriate energy and time to avoid these effects. (Sood & Gupta, 2015) indicated that the total amount of flavonoids in pomegranate peels, based on the standard compound quercetin, ranged between (15.8 - 17.8 mg/g). The reason for this discrepancy in the content of flavonoids may be due to the difference in the efficiency of the solvents used in extraction or to the type of flavonoids whose solubility varies in these compounds.

Identification of active compounds in plant extracts using a high-performance chromatography (HPLC)

Figures (3) (4) (5) show the Retention Time (RT) values of the compounds identified in the extracts. It is clear from these curves that there are a number of phenolic and flavonoid compounds in the extract of banana peels and the extract of pomegranate seeds, which included (calic acid, caffeic acid, catechins, quercetin, chlorogenic, ellagic acid, Coumarin and myristin), where it was observed that the retention time of the standard compounds and the identified compounds matched, and the concentrations of these compounds varied in each extract, where the concentration of gallic acid in banana peels was 0.124 Parts per million (ppm), while in pomegranate seeds its concentration was 0.40 ppm, while the concentration of

gallic acid was 0.40 ppm. Caffeic acid in banana peels, it was 0.50 ppm, and its concentration in pomegranate seeds was 0.99 ppm. The concentration of catechins in banana peels was 0.54 ppm, and its concentration in pomegranate seeds was 1.13 ppm. The concentration of quercetin in banana peels reached 0.44 ppm, while its concentration in pomegranate seeds was 0.99 ppm. Per million, the chlorogenic concentration in banana peels was 0.43 ppm, and its concentration in pomegranate seeds was 0.97 ppm, and the ellagic concentration in banana peels was 0.45 ppm, and its concentration in pomegranate seeds was 0.99 ppm, while the coumarin concentration in banana peels was 0.42 ppm. Pomegranate seeds contain 0.98 parts per million, while the last compound, myricetin, was found in banana peels only at a concentration of 0.37 parts per million. Use (Russo *et al.*, 2018) HPLC device to conduct a quantitative and qualitative analysis of the main phenolic compounds in different parts of pomegranate fruits. There was a difference in the concentrations of phenolic compounds, which allows distinguishing between them. The most prominent compounds that were detected in pomegranate juice included (catechin, quercetin, ellagic acid, cyanidin-3-glucoside, punicalagin α , punicalagin β , gallic acid, caffeic acid and p-coumaric). acid). And that the content of phenolic substances and flavonoids depends strongly on the type of solvent and its polarity. Ethanol and methanol appeared to be more effective than water as a solvent. (Aboul-Enein *et al.*, 2016) was able to extract the following compounds from banana peels and stated that the separation process by HPLC was affected by various factors such as the degree of purity of the sample, the types of columns and the reagents used (quercetin, catechin, cyanamic, caffeic, coumarin, gallic, vanillic, chrysin). And that the content of phenolic substances and flavonoids depends strongly on the type of solvent and its polarity, and it was shown that ethanol and methanol are more effective than water as a solvent.

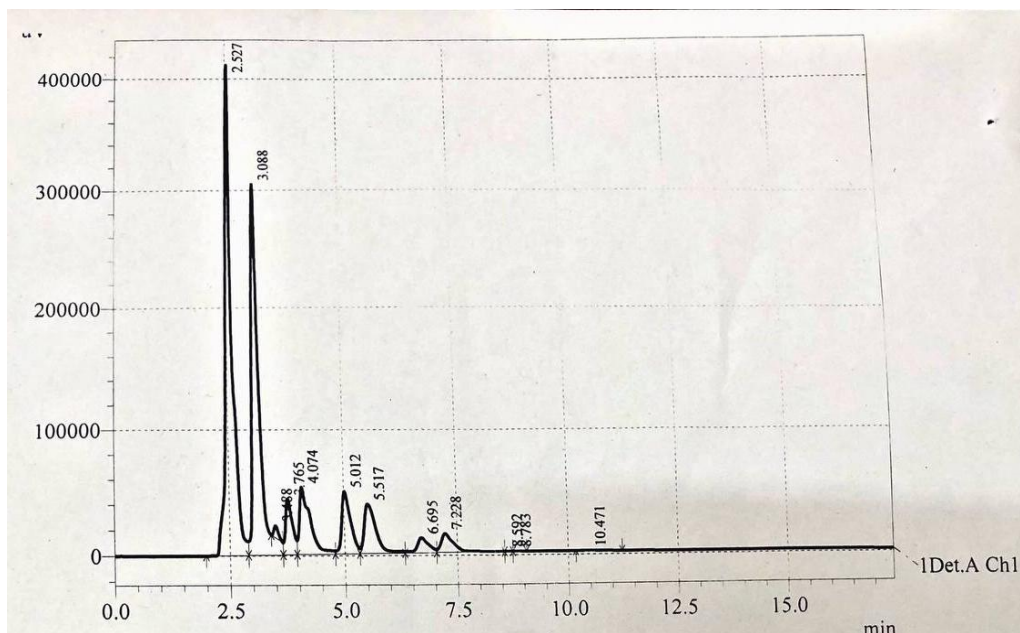


Figure (3): Detention time for standard compounds in the HPLC device.

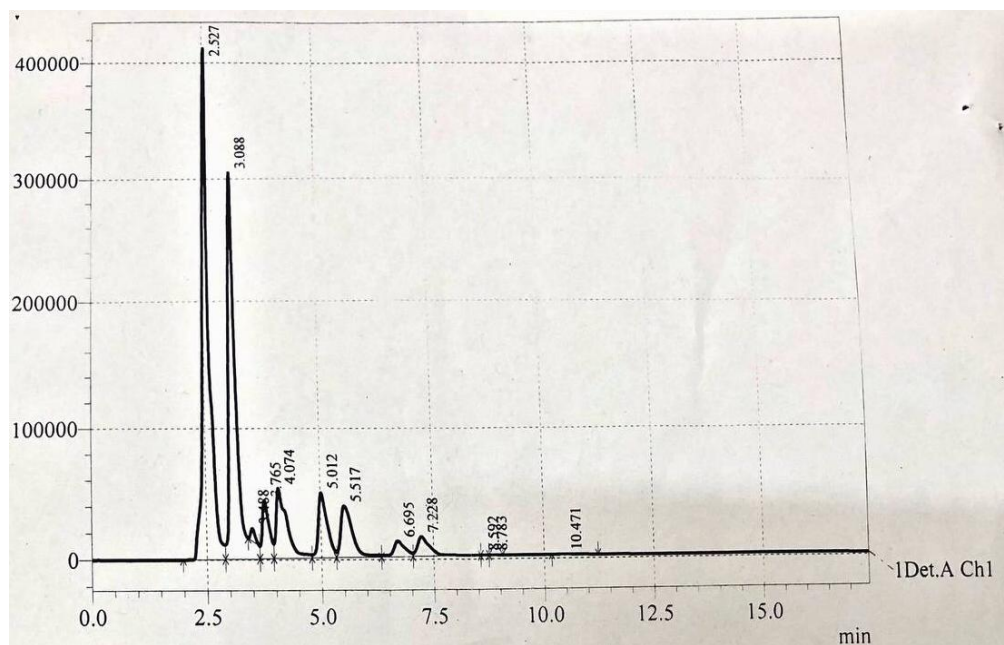


Figure (4): the retention time of the active compounds in the extract of pomegranate seeds diagnosed by the HPLC.

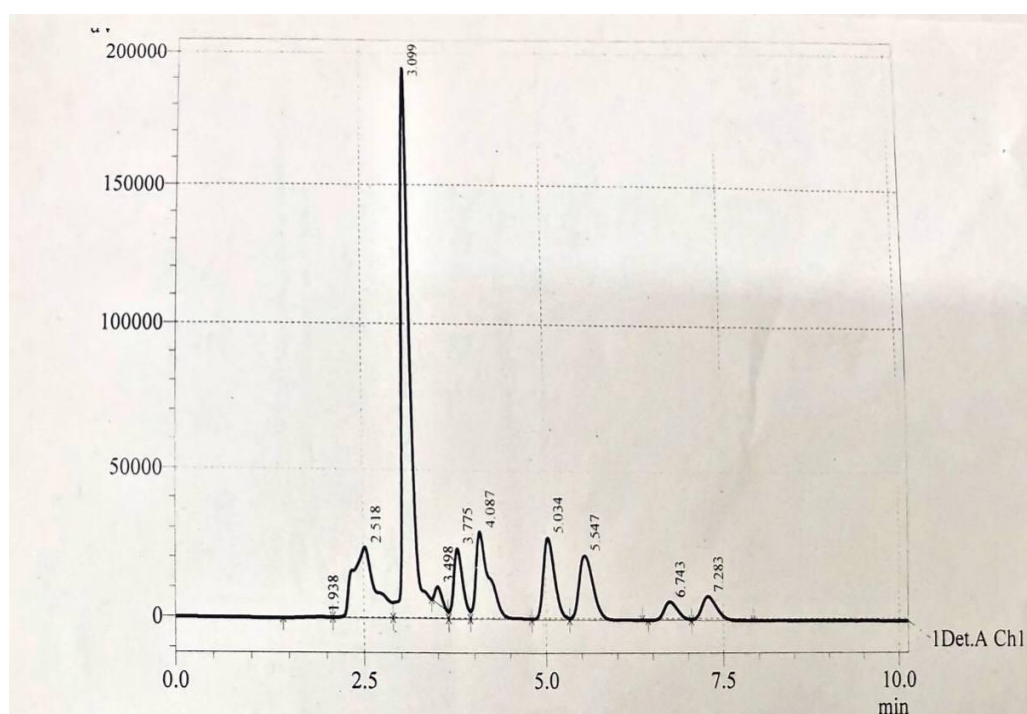


Figure (5): Retention time of active compounds in banana peel extract identified by HPLC device.



Measuring the inhibitory effectiveness of plant extracts against some types of bacteria

It is clear from the results shown in Table (1) that there are significant differences in the ratio between the types of pathological bacteria and the concentration of pomegranate seed extract, as follows :*P. aeruginosa*, *E. coli*, and finally *B. cereus*, as the diameter of the inhibition halo at a concentration of 10% reached 21 mm against *P. aeruginosa* bacteria. At 7% and 3% concentrations, the average diameters of the inhibition haloes for the same bacteria reached (16.14 mm), respectively, in While the effect of the extract on *E. coli* and *B. cereus* bacteria was less, the diameter of the corona at a concentration of 10% for the two types of bacteria reached 19 mm and 17 mm, respectively. It is noted that the lower the concentrations of the extracts, the smaller the diameters of the transparent coronas formed. That is, with increasing concentration, growth inhibition increases. Bacteria. Pomegranate seed extract did not show significant differences inhibitory activity against *S. aureus* bacteria.

Table (1): The effect of adding different concentrations of pomegranate seed and banana peel extracts on inhibiting the number of bacterial growth.

Abstract	Extract concentration %	Pathological isolates				
		<i>B.cereus</i>	<i>p.aeruginosa</i>	<i>S. aureus</i>	<i>E.coli</i>	
		Diameter of the inhibition zone (mm)				
Pomegranate seeds	3	10	14	0	12	4.094 ** (0.0001)
	7	13	16	0	14	4.137 ** (0.0001)
	10	17	21	0	19	5.024 ** (0.0001)
	LSD (P-value)	4.891 * (0.0317)	5.023 * (0.0395)	0.00 NS (0.000)	4.883 * (0.0296)	---
Banana peels	3	0	9	7	8	4.052 ** (0.0001)
	7	0	10	9	10	5.021 ** (0.0001)
	10	0	11	10	12	4.172 ** (0.0001)
	LSD (P-value)	0.00 NS (0.000)	3.071 NS (0.288)	3.184 NS (0.164)	3.759 * (0.0447)	---
* (P≤0.05), ** (P≤0.01) (P≤0.05) * Moral , (P≤0.01) ** High Morale , NS:Not Moral						



In a study conducted on different parts of pomegranate, including peel extract, red and white seed extract, juice and whole fruit, and using two solvents (water and methanol) as anti-growth of a number of food-contaminated bacteria, including (*B.cereus*, *E.coli*, *S. aureus*, *p.aeruginosa*), the superiority of the methanolic peel extract appeared with the highest inhibition halo diameters (25, 20, 22, 25 mm), respectively, followed by the red seeds with halo diameters (8, 11, 10, 19 mm), respectively, while the white seeds had The inhibition diameters were (10, 8, 10, and 10 mm), respectively, and it appeared that the alcoholic extracts were more effective than the aqueous extracts, and that the good activity of the peel as an antibacterial was due to Because it contains the compound (Punicalagin), which has antimicrobial activity, pomegranate is also considered a source of bioactive substances such as phenols, flavonoids, anthocyanins, and tannins (**Dahham et al., 2010**). Banana peel extract showed there are significant differences in the ratio between the types of pathological bacteria and the concentration inhibition activity against the tested bacteria, but it was lower than the effectiveness of pomegranate seed extract, where the diameter of the inhibition corona of banana peel extract against *P.aeruginosa* bacteria at a concentration of 10% reached 11 mm, while the diameter of the inhibition corona for the other concentrations was 7.3%. It is (10.9 mm), while for *E. coli* bacteria, the diameter of the inhibition halo for the three concentrations (10%, 3%, 7%) was (12, 10.8 mm), respectively. While the extract did not show significant differences against *Bacillus* bacteria at any concentration, while it had inhibitory activity against *S. aureus* bacteria, where the diameters of the inhibition circles for the three concentrations reached (10.9 and 7 mm), respectively. (**Niamah, 2014**) also tested the effectiveness of methanolic yellow banana peel extract against types of bacteria, including (ATCC25922 *E. coli*, ATCC25923 *S. aureus*, SP, *Bacillus p.aeruginosa*) at three concentrations of 300, 200, and 100 mg/ml of the extract. The diameters of the inhibition halos ranged at a concentration of 300 mg of (13-24 mm), as it was noted that the rate of inhibition diameters increased with increasing concentration of the extract, and the Gram-positive bacteria were more affected than the negative bacteria. Active compounds cause disruption of the cell membrane and dissipate cellular energy in the form of ATP, as well as organic acids found in plant species inhibit the oxidation of NADH and thereby eliminate it. Flavonoids are considered to be microbiocenes due to their ability to penetrate the cell membrane and change its acidity (**Gyawali et al., 2015**).

Testing the inhibitory effectiveness of plant extracts against some molds

The results in Figures (6)(7) showed that the pomegranate seed extract had an inhibitory effectiveness estimated at 63.6% against the mold (*P. digitatum*). It also showed anti-growth activity against the fungus (*Aspergillus niger*), causing its growth to be inhibited by 56%. While the effectiveness of the banana peel extract was less against The two isolates reached (40% and 57%), respectively (**Hanaf et al., 2021**) showed that pomegranate peel extract has an inhibitory activity against (*A. niger*) when compared with extracts of orange peels and banana peels, using two solvents: ethanol and methanol, where the percentage of antioxidant activity in the ethanolic extract was 20% and in the methanolic extract 21%, as for the peel extract. Bananas did not show any effectiveness. The antimicrobial effect of fruit peel extracts is attributed to the presence of antimicrobial compounds in plants, such as antioxidants, phenols, flavonoids, tannins, as well as secondary metabolites. Therefore, antimicrobial activities vary from one plant extract to another due to the difference in the

extraction method in addition to its chemical composition. Banana peels are effective against mold (*A. niger*), as (Prakash *et al.*, 2017) indicated when studying the extracts of three varieties of banana peels to inhibit three types of rot (*Aspergillus niger*, *Aspergillus flavus*, *Penicillium* spp), that the extract of peels of *Musa parasiadica* (kadali) has effective. Banana peels are known to contain anti-fungal properties and are rich in vitamins and fibre. Medicinal plants containing flavonoids are recognized as safe and endowed with numerous biological functions. Various flavonoids have been extracted and investigated in association with their anti-fungal activities and can be promising, efficient, and cost-effective agents for the inhibition of fungal infections. They often inhibit fungal growth in various underlying mechanisms by enhancing the disruption of the plasma membrane and mitochondrial dysfunction; and inhibiting cell wall formation, cell division, protein synthesis, and the efflux-mediated pumping system. These flavonoids are capable and efficient in synergetic combination therapy with conventional drugs, which can be more appropriate and supportive for finding novel drug therapies against fungal pathogens (Al Aboody & Mickymaray, 2020).

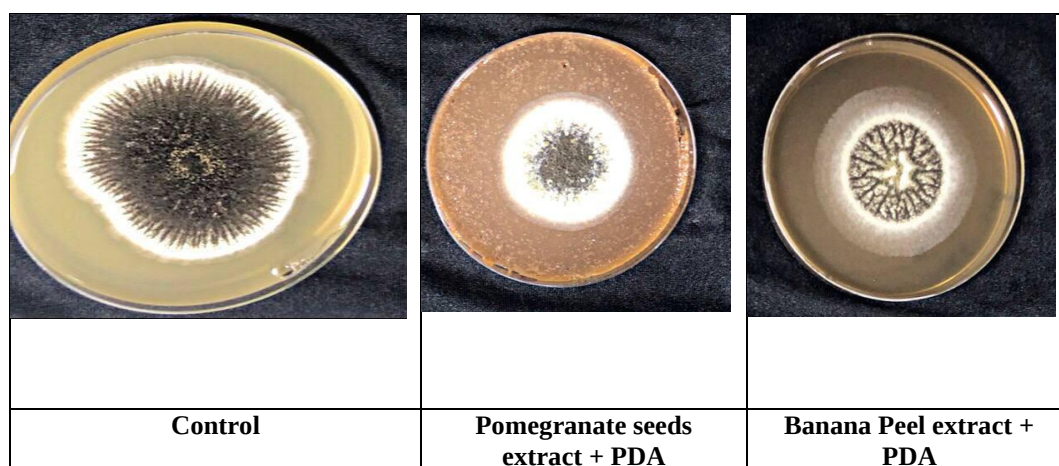


Figure (6): Effect of pomegranate seed extract and banana peel extract on the growth of *Aspergillus niger* fungus.



Figure (7): The effect of pomegranate seed extract and banana peel extract on the growth of the *Penicillium digitatum* fungus.



CONCLUSION

various plant waste are important sources of effective compounds that play an important role as antibiotics, pomegranate seed extract showed inhibitory effectiveness against *pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* and showed no effect on *staphylococcus aureus* bacteria, while banana peel extract showed inhibitory effectiveness against *pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* are effective against *Bacillus cereus* bacteria. pomegranate seed extract is superior in its inhibitory effectiveness against *Aspergillus niger* and *Penicillium digitatum* rot compared to banana peel extract.

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