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# Pomegranate peel (Punica granatum L.) versus Juice: Properties and Benefits

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**M.Sc. Thesis**

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# Pomegranate peel (Punica granatum L.) versus Juice: Properties and Benefits

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## Thesis Approval

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## Dedication

This thesis is heartily and proudly dedicated to my parents who gave everything they have to ensure I would have the opportunity to be the best version of myself. Their encouragement, endless love, and support allowed me to pursue my goals

To my brothers and family for being my source of inspiration and motivation. I am forever grateful

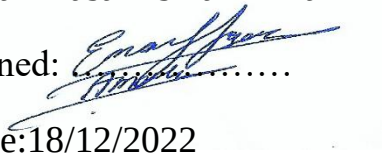
To faculty of Prof. Ibrahim Kayali, Dr. Jawad Shoqeir, classmates, and friends who extended their help in the midst of problems while doing this work

To my company Al-Reef For Investment And Agricultural Marketing for supporting me in every step when I work on my thesis

## Declaration

I certify that this thesis submitted for the degree of a master's is my research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Eman Hasan Ismail Amar

Signed: 

Date:18/12/2022

بسم الله الرحمن الرحيم

قال تعالى {وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنْ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ انْظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَلِكُمْ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ} (الأنعام، 99)

وقال تعالى : {فِيهِمَا فَاكِهَةٌ وَنَخْلٌ وَرُمَّانٌ} (الرحمن، 68)

## Table of content

## Chapter one: Introduction



## Chapter two: literature review



## Chapter three: materials & methods

## Chapter four: Result and Discussion

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In the name of Allah, the most gracious the most merciful. Words cannot express my gratitude to my professor Ibrahim Kayali for his invaluable patience and feedback, who generously provided knowledge and expertise, I could not have undertaken this journey without his guidance. Additionally, this endeavor would not have been possible without the generous support from Dr. Fuad Rimawi (College of science and technology, Al-Quds University), Dr. Ibrahim Abbasi, biology Department, Dr . Kifaiah Azmi (Faculty of medicine ), Mr. Sameh Nusybeh, Soil & Hydrology Laboratory For giving me the permission to use their devices, Mrs. Reem Yaghmour, the teacher in biology Department, for helping me in this work.

Eman Hasan Ismail Amar

## Abstract

The presence of antioxidants and polyphenols in pomegranate peels and juice has been linked to several medical benefits. In the juice, you'll find vitamins and minerals. It is possible to understand the pomegranate's features and benefits, which are beneficial for treating a variety of disorders, by using various extracts (alcoholic extract and ethyl acetate). To develop treatments for diverse ailments, this study is crucial.

In this study, the pomegranate peel was extracted using the Soxhlet extraction apparatus, which was able to essentially remove all of the active compounds from this plant.

The Folin-Ciocalteu test was used to measure the total phenolic content (TPC) of pomegranate peel extracts and juice. All tests were performed using a UV-Visible spectrophotometer. Pomegranate juice and peel were shown to have antioxidant capabilities and high phenolic content. While the total phenolic content (TPC) of pomegranate peel extracted with ethanol 99% produced a result of (78.81mg/g) higher than that of pomegranate peel extracted with, ethanol 35% which is (74.94mg/g) and ethyl acetate (56.61 mg/g) and juice(23.88 mg/g), where the total flavonoids content (TFC) ended with a result of pomegranate extract with ethanol 35% (18.09 mg/g) higher than the extracted with ethanol 99% (7.95 mg/g) and pomegranate juice (0.0547 mg/g).

HPLC analysis of polyphenolic standards compounds and flavonoids was detected and identified by comparing the peaks retention times in the sample chromatogram of pomegranate peel (extracted using ethanol) with that of the standard. After conducting the HPLC analysis, the results showed, Rutin and Gallic acid

By scavenging free radicals with the DPPH method, the antioxidant activity (AA) of pomegranate peel extracts was identified. Pomegranate peel was extracted using ethanol 99.9% and ethanol 35% as a solvent, yielding results of (51.9 mg/g), (9.86 mg/g), and juice (16.79 mg/g), as opposed to (76.8 mg/g) when ethyl acetate was used as the solvent.

The extracts from peel pomegranate (extracted using ethanol 99.9%) antibacterial activity were determined in vitro using the agar disc diffusion method  The existence

of positive control. the result showed antibacterial activities against *Pseudomonas aeruginosa*, *Escherichia coli*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Staphylococcus aureus* and the juice does not have a direct effect on the bacteria, as the bacteria appeared diluted.

The anti-glycation formation of the end product was assessed using an *in vitro* glucose-bovine serum albumin (BSA) test. The results of the study showed that pomegranate peel extract with ethanol has an anti-glycation effect, whereas pomegranate juice has less of it.

The cream was made based on pomegranate peel extracted using ethanol and had several features, including moisturizing, and smoothing, has a pleasant scent, absorption rapidly (without leaving a film), does not irritate the skin (pH= 5.5-6), has excellent color, easily distributed on the skin

The enzyme HMG-CoA(3-hydroxy-3-methylglutaryl-coenzyme A) reductase is essential for the mevalonate pathway that produces cholesterol. The liver produces less cholesterol when HMG-CoA reductase is present. Pomegranate peel (extracted with 99.9% ethanol) had a 45% inhibitory effect, and pomegranate juice had a 74% inhibitory effect.

## List of Tables:

## List of Figures

## List of Abbreviations, Symbols, and Terminology:

| Abbreviation | Definition |
|---|---|
| HMG-CoA | 3-hydroxy -3-methyl-coenzyme A |
| EtOH | Ethanol |
| TFC | Total Flavonoid Content |
| TPC | Total Phenolic Content |
| SDG | Secoisolariciresinol diglucoside |
| PJ | Pomegranate juice |
| MRSA | *Methicillin-resistant Staphylococcus aureus* |
| PPE | pomegranate peel extract |
| FA | Fatty acids |
| LDL | Low-density lipoprotein |
| *S.aureus* | *Staphylococcus aureus* |
| *E.coli* | *Escherichia coil* |
| LPS | Lipopolysaccharides |
| PP | Pomegranate peel |
| (HaCaT) | keratinocytes |
| LDL-C | Low-density lipoprotein cholesterol |
| BSA | Bovine Serum Albumin |

| | |
|---|---|
| DPPH | 2,2-Diphenyl-1-picrylhydrazyl propane hydrochloride |
| HPLC-DAD | High-performance liquid chromatography |
| DW | Distilled water |
| (T2D) | type 2 diabetes |
| (PSO) | Pomegranate seed oil |
| (ROS) | reactive oxygen species |
| (TNF-α) | as tumor necrosis factor-alpha |
| (PC) | phenolic content |
| (AGEs) | glycated end products |
| (AA) | Antioxidant activity |
| (Ats) | anthocyanins |
| (HTs) | Hydrolysable tannins |
| (UAE) | Ultrasound-assisted extraction method |
| (BJ) | normal human fibroblast |
| NMR | Nuclear magnetic resonance spectrometers |
| MS | Mass spectrometry |
| ABTS | ethylbenzothiazolin-6-sulfonoic acid |
| DMPD | N-N-dimethyl-p-phenylene-diamine |
| FRAP | fluorescence recovery after photobleaching |
| TEAC | Trolox equivalents antioxidant capacity |
| *Pa* | *Pseudomonas aeruginosa* |

# Chapter One:
# Introduction

## 1.1 pomegranate

Pomegranate (Punica granatum L.) is categorized as a grain yet really has a place with the Punicaceae group of phytophagous plants. Most of them are filled in Mediterranean countries including Turkey, Egypt, Tunisia, Spain, Morocco, Iran, and India. Pomegranate, on the other hand, is a desiccation and long-lasting plant. Pomegranate trees frequently flourish in dry and semiarid regions. Punica is the sole species, and P. granatum, a generic type, and its extracts are employed as botanical components in herbal treatments and dietary supplements. (Walid Elfalleh, et al,.2011; Aida Zarfeshany, et al,.2014)

Pomegranate (Punica granatum, Punicaceae) has been used extensively in many cultures' hordes medicine. On cell lines, in preclinical models, and in a few human studies, various parts of the fruit have been shown to exert antioxidant, anti-inflammatory, anticarcinogenic, antiatherosclerosis, hypolipidemic, antidiabetic, antiviral, antibacterial, and antifungal activities. The main properties of the pomegranate that have been identified thus far are associated with its antioxidant capacity, which is three times higher than extracts of red wine and tea. Also, its structure includes anthocyanins and tannins. (Mélanie SpilmontZahin, et al,.2015)

The pomegranate peel performs nearly 26%–30% of the pomegranate fruit. The pomegranate peel has high antioxidant efficiency (92% of the total antioxidant activity) on account of its major content of flavonoids (anthocyanins, catechins), hydrolyzable tannins (punicalagin, penicillin, gallic, ellagic acid, and pedunculagin), and polyphenols like punicalagin. Pomegranate peel tannins have treated various common diseases such as intestinal worms, diarrhea, cough, infertility, and inflammation and have been cured by using pomegranate peel extract, have been known traditionally for its medical properties. ( Mélanie SpilmontZahin, et al,.2015)

Fructose, sucrose, and glucose can all be found in abundance in pomegranate juice. Additionally, it contains ascorbic, citric, fumaric, and malic acids, all of which are simple organic acids. Additionally, it contains proline, methionine, and valine in small quantities of all amino acids. (Aida Zarfeshany, et al,.2014)

The chemical structure of the seeds is about 18% of dried and cleaned white seeds are oil. The oil is substantial in punicic acid (65%), which is a triple-conjugated 18-carbon fatty acid as shown in figure1.

The chemical structure of Pomegranate juice is a pleasant source of fructose, sucrose, and glucose. In addition, it has some common organic acids including fumaric acid, citric acid, ascorbic acid, and malic acid. Besides, it includes small quantities of all amino acids, especially proline, valine, and methionine.

Polyphenols are abundant in both the juice and the peel. The main groups are tannins and flavonoids (Figure 2), whose peculiar antioxidant and preservation properties suggest the pomegranate's medicinal potential. A particular kind of tannin called ellagitannin may be converted into hydroxybenzoic acids like ellagic acid. Due to its antioxidant properties, it is frequently utilized in plastic operations to prevent the death of skin flaps. Punicalagin and penicillin are two additional ellagitannins that are present in pomegranate juice and peel. Anthocyanins, flavan 3-ols, and flavonols are a few of the classes of pomegranate flavonoids. Both the juice and peel of the pomegranate have significant antioxidant activity catechins. They are essential compounds of anthocyanin's production with antioxidant and inflammatory roles. Anthocyanins cause the red color of the juice, which is not found in the peel. All pomegranate flavonoids show antioxidant activity with indirect inhibition of inflammatory markers such as tumor necrosis factor-alpha (TNF-α)

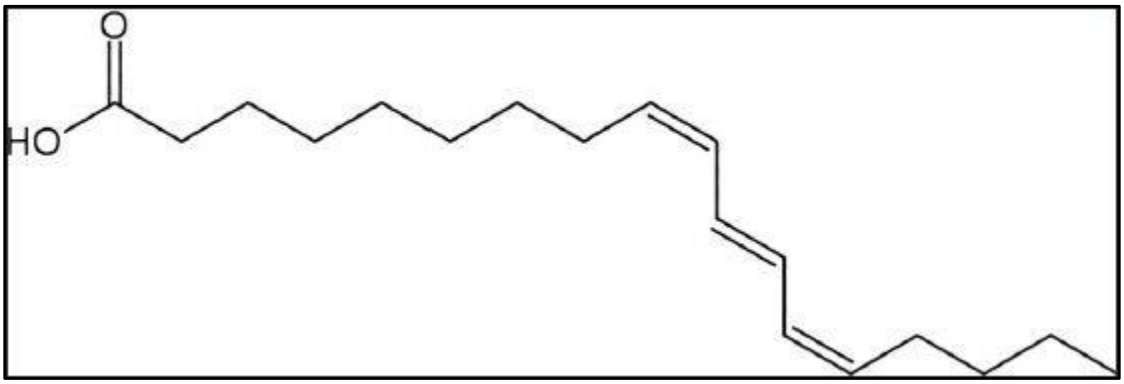



**Figure 1** Chemical structure of punicic acid (9Z,11E,13Z-octadeca- 9,11,13-trienoic acid)( (Aida Zarfeshany,et al,.2014)

Finally, these studies focused primarily on the use of the pomegranate peel and juice extract in an effort to discover a natural remedy for all health issues, particularly hypercholesterolemia and cardiovascular disease.

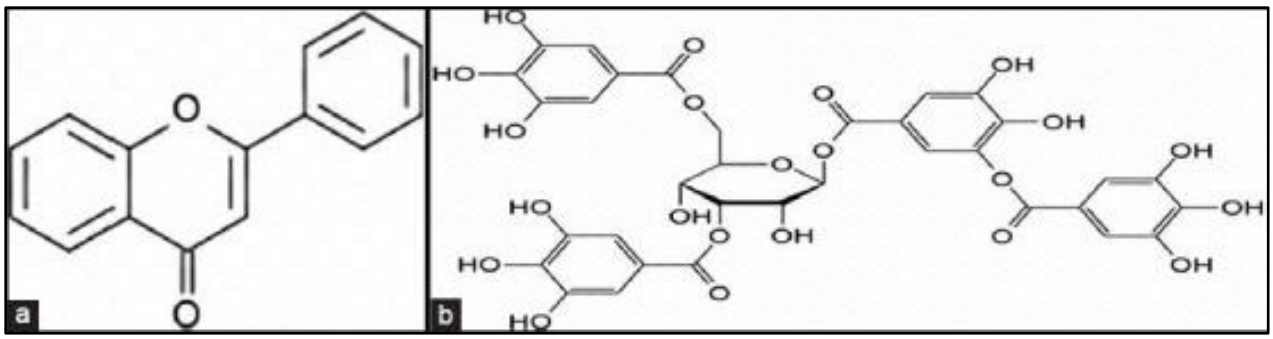



**Figure 2** (a) Flavone backbone (2-phenyl-1, 4-benzopyrone). (b) Tannic acid((Aida Zarfeshany,et al,.2014)

## 1.1.1 Phytochemical constituents of pomegranate:

Numerous cultures around the world have included pomegranate, which has always been valued highly for its health-promoting properties. Bioactive compounds like phenolics, tannins, anthocyanins, flavonoids, and organic acids and terpenoids are abundant in pomegranates. (Anna Montefusco, et al ,.2021;Nhlanhla Maphetu, et al ,.2022)

Pomegranate peel has a greater level of antioxidant activity than the eaten portion and is a significant source of phenolics, flavonoids, ellagitannins, hydroxybenzoic acids, and many other bioactive compounds. Additionally, kernels contain a wealth of nutrients, including vitamins, minerals, proteins, fibers, and, most importantly, fatty acids that are good for your health. Due to the presence of non-steroidal phytoestrogens, pomegranate peel and kernel extracts also exhibited estrogenic action. (Anna Montefusco, et al ,.2021)

One of the most highly recommended drinks is pomegranate juice, which is made from the fruit's sweet red arils, cores, and peels and contains vitamin C. The phenolic content of pomegranate juice is thought to come from it. Pomegranate juice extracts had polyphenolic compounds like punicalagin and punicalin removed from them. Additionally, the juice has been identified as a source of copper, manganese, zinc, potassium, and phosphorus. (Nhlanhla Maphetum, et al,.2022)

## 1.1.2 Extract Techniques of pomegranate peel:

The Soxhlet technique is considered one of the oldest techniques in extraction methods for the solid parts of wild plants. In this step, Soxhlet was the filtration technique generally used in antiquity. It is currently the primary standard by which other filtration methods are evaluated. This method generally depends on the solvent, temperature, and extraction time effect on the template. The increase in the extraction temperature supports the mass transfer and spread of the compounds present in the template to the solvent and enhances the solubility of the extracted compounds. (Lara Campos, et al,.20222, MD Luc de Castro et al. 1998)

In a conventional Soxhlet, as shown in figure1.1.2, the sample (pomegranate peel) is placed in a thimble holder, and during the process, it is gradually filled with fresh, condensed solvent from the distillation beaker. When the liquid extends the excess level, a solute thimble carrier solute is sucked up and emptied appear into the distillation flask, transporting the extracted analyte towards the bulk liquid. This procedure is repeated until all of the material has been extracted. This achievement makes Soxhlet a continuous or intermittent hybrid technology. (MD Luc de Castro et al. 1998; M.A.López-Bascón, et al,.2020)

**Figure 1.1.2** Soxhlet extraction apparatus

## 1.2 Bioactivity effects of pomegranate peel and juice:

Pomegranate (P. granatum) has a long history of which can be used and has been used for medicinal use and nutritional value (as they contain bioactive phytochemicals, which have health benefits like reducing inflammation and oxidative stress. (Gundogdu and Yilmaz, 2012; Teixeira da Silva et al., 2013; ShemaDidi et al., 2012). Numerous bioactive compounds are present in the juice and plant of the pomegranate, which has therapeutic, nutritional, and health benefits. The plant can be utilized for its peels, seeds, seed oils, roots, trunk (barks), wood spout, leaves, flowers, and fruit rinds given the certification range. The pomegranate is considered drought-tolerant because of its adaptability to arid and semi-arid regions worldwide. (Nhlanhla Maphetu, et al ,.2022;Galindo et a., 2017; Rodríguez et al., 2012)

Presently, pomegranate fruit contains numerous bioactive components that, in fact, function as antioxidant and anti-inflammatory molecules. They are an excellent tool for preventing cancer, heart disease, Alzheimer's disease, diabetes, arthritis, obesity, male infertility, bacterial infections, and radiation-induced tissue damage. (Miriana Durante, et al,.2021)

### 1.2.1 Antioxidant effect:

Today, the majority of antioxidants are used to slow the rate at which food fats oxidize. Primary protection against such oxidative declination is provided by antioxidants. As a result, strong antioxidants with less toxicity and more activity are required. Due to their safety in food formulation, natural antioxidants have attracted the attention of food researchers at recent conventions. These are plant extracts and essential oils that positively influence nutrient oxidation reactions. (Ahmad Pedram Nia, et al,.2020)

The antioxidant properties of the pomegranate peels against breast cancer were investigated. Pomegranate peels also contain over 48 chemical compounds, including alkaloids, anthocyanins, anthocyanidins, tannins, flavonoids, phenolics, proanthocyanidins, sterols, terpenes, and xanthonoids. Furthermore, finding pomegranate strip concentrate can improve the cell reinforcement properties of films and coatings because of its high phenolic content (PC). More specifically, punicalagin, the pomegranate's predominant ellagitannin, improved antioxidant activity during 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging tests to 59.74 percent at 1% w/w pomegranate peel and 71.82 percent at 5% w/w pomegranate peel due to the high PC of the rind. (Valeria Sorrenti, et al,.2019; Katharine Ko,et al,.2021 Nhlanhla Maphetu, et al,.2022)

Different groups of polyphenols, including ellagic acid, gallotannins, ellagitannins, and flavonoids like anthocyanins, were found to be associated with pomegranate juices *in vitro* antioxidant activity. It was determined that pomegranate juice had an antioxidant activity *in vitro* that was three times higher than that of red wines and green teas and two to six times stronger than that of other natural beverages. (Lilach Shema-Didi, et al,.2012)

### 1.2.2 Antibacterial effect:

Pomegranates are an antibacterial because they contain flavonoids and tannins, which can combat a variety of microorganisms. The fruit of pomegranate is frequently utilized in traditional medicine. The pathogenic bacteria *Streptococcus sanguis (S. sanguis), Escherichia coli (E. coli), Pseudomonas aeruginosa (Pa), Clostridia,* and *Staphylococcus aureus (S. aureus)* may all grow substantially slower than helpful bacteria like *Lactobacillus spp*. and *Bifidobacterium spp*. (Valeria Sorrenti, et al., 2019; S. Bialonska et al., 2009)

Due to its antibacterial properties, pomegranate peel extracts are useful against bacteria that are resistant to medication. However, there is a link between the antioxidant and antimutagenic activities of total phenolics found in medicinal plants. Given the range of chemically and physiologically active substances found in pomegranates. In order to assess the antioxidant activity of successively extracted P. granatum peel extracts, a number of in vitro experiments were employed. (Maryam Zahin, et al,.2010)

So, a variety of bioactive substances, including epicatechin, catechin, ellagitannins, and others, may be found in pomegranate peel. These bioactive chemicals have a wide range of biological effects, including scavenging reactive oxygen species (ROS), preventing oxidation and microbial development, and reducing the risk of chronic illnesses including cancer and cardiovascular ailments.(Lilach Shema-Didi, et al,.2012)

### 1.2.3 Glycation of Hemoglobin in blood and type 2 diabetes

In diabetes, hyperglycemia contributes to complications and damages tissue through a variety of mechanisms. As a result, the primary cause of diabetes complications is oxidative stress. One mechanism that contributes to tissue damage caused by hyperglycemia is the increased production of glycated end products (AGEs). Non-enzymatic reduction of polysaccharides reacts with the free amino groups of the protein in diabetes to form AGEs, which are different groups of protein-binding proteins. AGE signaling modifies plasma proteins when it reaches AGE sensors on cells, such as vascular endothelial, vascular smooth muscle, and macrophage cells. ROS are produced as a result of this binding, which causes numerous pathological changes in gene expression. In type 2 diabetes (T2D), peripheral artery disease is a macrovascular complication of diabetes, an indicator of reactive oxygen species (ROS), and associated with AGEs. Planning to reduce the risk of pathological complications may be successful with antioxidant phytochemical dietary supplements. (Golbon Sohrab,et al,.2015)

Pomegranate products also contribute to the inhibition of carbohydrate digestive enzymes, and pomegranate peel extract (PPE), in particular peel extract, may have a positive effect on insulin sensitivity and glucose tolerance. Due to the phenolic content of Punica granatum (PG) peel, it may also have an antihyperglycemic effect. The methanolic extract of PP has the ability to inhibit oxidative stress and histopathological changes in the liver and kidneys, which is related to its antiapoptotic and antioxidant properties. When compared to commonly consumed polyphenol-rich beverages, pomegranate juice (PJ) has the highest antioxidant capacity. A few clinical studies have shown that taking PJ supplements can lower risk factors for atherosclerosis and improve hypertension. (Zahra Amri, et al, 2020; Khadija A., et al,.2019; Golbon Sohrab, et al,.2015)

### 1.2.4 Skin sensitivity

Atopic dermatitis, eczema, and psoriasis, as well as chronic lesions like diabetic foot and bed sores, are examples of skin conditions that are becoming more common throughout the world and have an estimated 1-year prevalence of up to 20% in children and 2-10% in adults. Beyond a genetic predisposition, several variables, including food, infections, inflammatory processes, alcoholism, smoking, ionizing and solar radiation (UVA and UVB rays), air pollution, medicines, and generally hazardous chemicals, subscribe to the development of dermatitis and chronic wounds. These stressors trigger oxidative stress, or an excessive formation of reactive oxygen species (ROS), which sets off pathogenic processes that change the physiological characteristics of cells as they normally would. During metabolic activities and under normal settings, the body's cells create ROS. (Marianna Barbalinardo, et al,.2022)

Pomegranate belongs to the Punica L. genus, Punicaceae family. Products for skin health can contain pomegranate peel. ellagic acid and punicalagin, two bioactive components found in pomegranate peel, both improve skin health by preventing tyrosinase from doing its job and starting the anti-inflammatory and anti-fungal benefits it causes. Pomegranate seed oil (PSO), which is punicic acid-rich, has anti-inflammatory and protective properties that make it effective against UV-induced radiation. PSO can also serve as a glycation inhibitor, preventing aging-induced glycation and harming skin elasticity. The following lists some of the pomegranate extract's prospective medical and beauty uses, including its ability to cure UV-induced hyperpigmentation, weakened skin elasticity, and skin wrinkling. (Katharine Ko, et al,.2021)

### 1.2.5 HMG-CoA reductase inhibitory activity of pomegranate peel and juice

One of the main factors causing cardiac conditions like myocardial infarction is atherosclerosis, which is brought on by hypercholesterolemia. The major cause of hypercholesterolemia, which can also result in other disorders including obesity, diabetes, and cancer, is elevated plasma cholesterol, especially low-density lipoprotein (LDL) and triglyceride levels. 1,2 The rate-ending enzyme in cholesterol production is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which catalyzes the transformation of HMG-CoA to mevalonate. The inhibition of HMG-CoA reductase successfully reduces cholesterol levels in humans and most other animals by activating sterol regulatory element-binding protein-2, which in turn upregulates the LDL receptor and HMG-CoA reductase. 3 Notwithstanding being notable HMG-CoA reductase inhibitors, statins have serious negative aftereffects that incorporate. (Gunasekaran Baskaran, et al,.2015)

One of the most commonly used fruits for their therapeutic properties is the pomegranate (Punica granatum L). The pomegranate fruit has been shown to have positive effects on human health. According to human studies, pomegranate juice lowers serum levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). Pomegranate peel (PP) and pomegranate peel extract (PPE) have been shown to have high antioxidant and hydroxyl radical scavenging activity in vitro. Superoxide anion scavenging ability is also enhanced by PPE's antioxidant activity. (Mahdiyeh KhademHaghighian,et al,.2020)

## 1.3 Objective of this study

This study's primary objective is to investigate the impact of pomegranate peel and juice on HMO-CoA reductase inhibitory activity by examining the qualities and advantages of pomegranate peel and juice, which are described as follows:

1. To test the antibacterial effectiveness against four clinical infections.
2. antioxidant activity (AA)
3. Phenolic content all-in-one (TPC).
4. Pomegranate peel at wave length 280 nm HPLC chromatography.
5. The amount of pomegranate's total flavonoids (TFC).
6. Type 2 diabetes and blood glycation of hemoglobin

# Chapter Two:
# Literature review

## 2.1 The composition of pomegranate peel and juice

**Deeba N. Syed, et al, (2013)**Numerous researchers have been interested in the pomegranate fruit's distinctive chemical makeup due to its abundance of antioxidant tannins and flavonoids (figure 2.1.1). The potential chemopreventive and/or cancer therapeutic effects of polyphenol-rich fractions produced from pomegranate fruit have been investigated in a number of animal models. To find out whether pomegranate can join our arsenal in fighting against cancer, well-designed clinical trials in humans are required to conduct ***in vitro*** and ***in vivo*** research.

**The same finding by Sheng Wu, et al, (2017).** Found in pomegranate (Punica granatum) juice and fruit have polyphenols, particularly anthocyanins ***(ATs)*** and hydrolyzable tannins ***(HTs)***, to the health-promoting activities of pomegranate juice and fruit extracts by examining the composition and analytical information of the diverse phytochemical (figure 2.1.2) that have been identified in different pomegranate tissues and findings on the function and molecular mechanism of ATs as well as urolithins, the intestinal microbial derivatives of pomegranate HTs, on human nutrition and health. Studying and understanding the structural diversity of pomegranate and phytochemicals accelerate their applications in dietary-based cancer chemoprevention and treatment in the future.

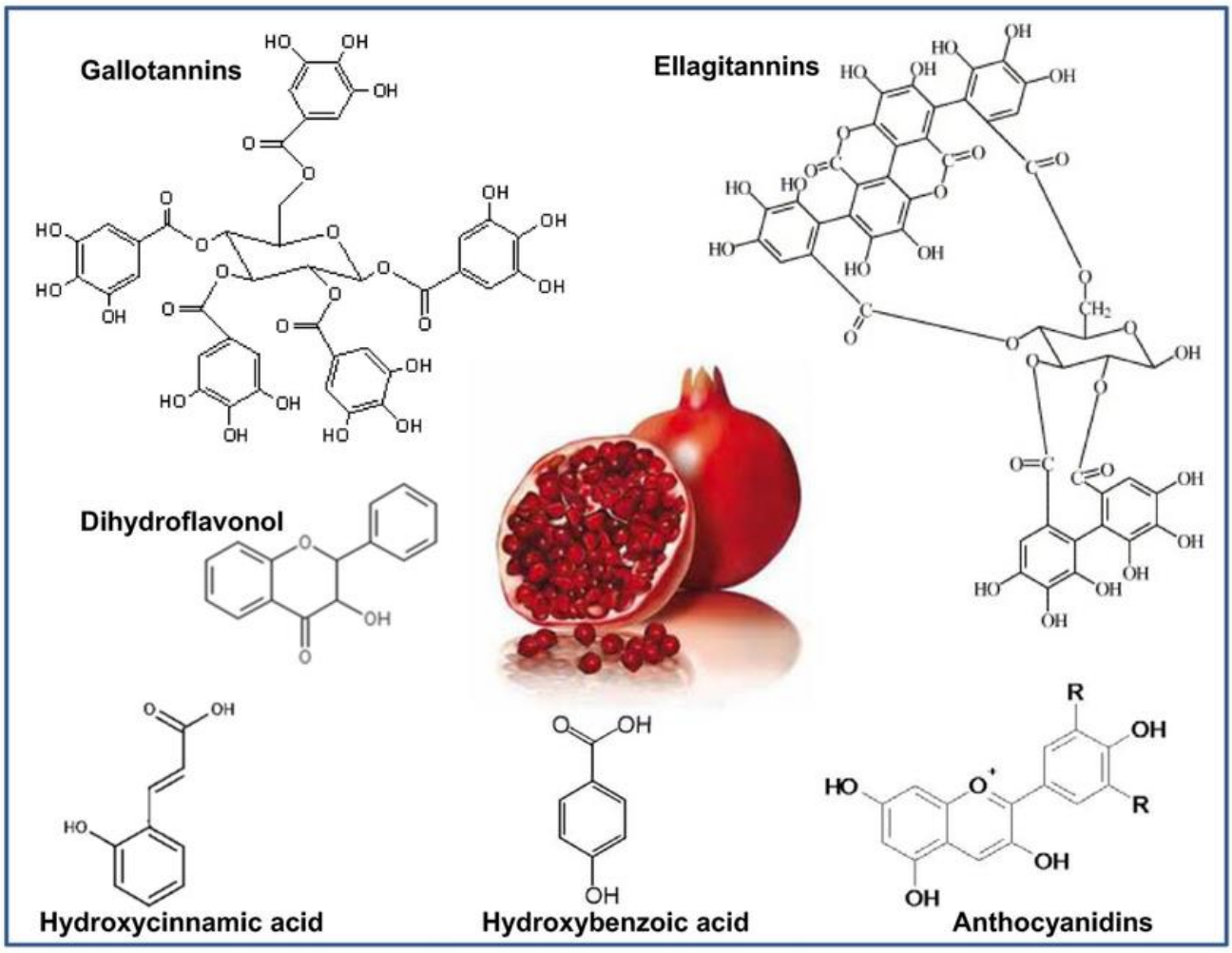



**Figure 2.1.1** Chemical constituents of pomegranate (Deeba N. Syed, et al,.2013)

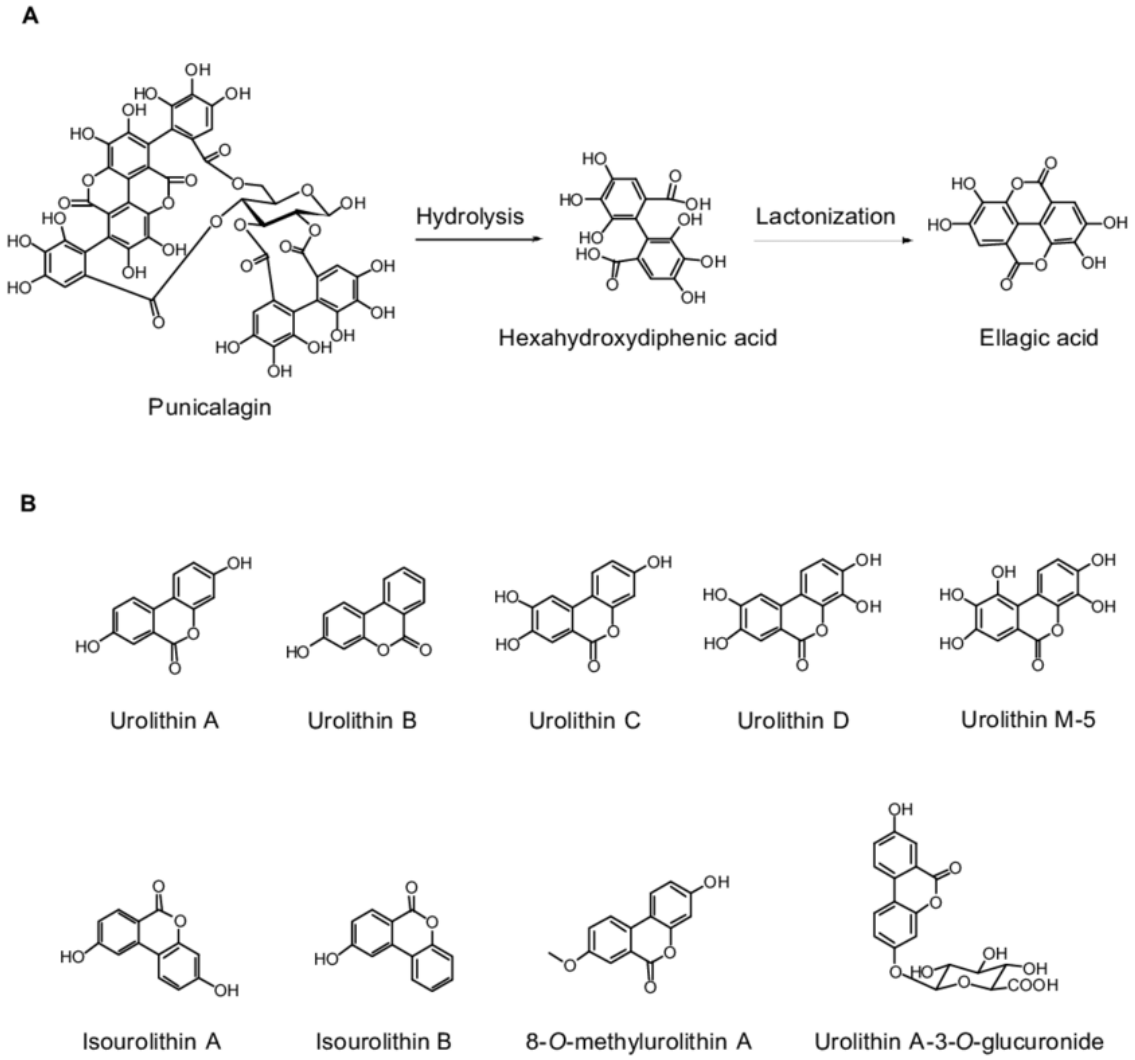



**Figure 2.1.2** Pomegranate ellagitannin-derived metabolites. (A) Punicalagin (a pomegranate ellagitannin) is hydrolyzed to hexahydroxydiphenic acid, which is lactonized to get ellagic acid; (B) Chemical structures of representative urolithins, the microfloral transformation products of ellagic acid. ( Sheng Wu,et al,.2017)

## 2. 2 Extraction Methods

**JAMES E. SIMON, et al, (2003).** The antioxidant components from the aqueous methanol extractions of artichoke heads and leaves were purified using pomegranate peel extract as an antioxidant activity-directed fractionation technique. Using NMR and MS, seven active polyphenolic compounds were isolated from pomegranate. Following the investigation, it was discovered that two of these substances, apigenin-7-rutinoside, and narirutin, are specific to artichoke heads. Following that, colorimetric and validated HPLC were used to test and compare the levels of these antioxidants and total phenols in dried pomegranate samples from the peel, Green Globe, and Violet.

In similar **the Samia Inayatullah, et al, (2011**). Aantioxidants from natural sources and used as medicine for many diseases by methanol extracts peels, after extraction had the highest total phenols content.

**By Zofia Niziol Lukaszewska, et al, (2018).** Plant extracts that are high in antioxidants can be used as active ingredients in a variety of products. The ultrasound-assisted extraction method (UAE) was used to make the plant extracts. Using the Folin-Ciocalteu method, the total phenolic and flavonoid compounds in the extracts were measured spectrophotometrically. The DPPH free radical scavenging assay was used to measure the extract's antioxidant activity, and the extracts' effects on the proliferation of fibroblasts (BJ) and keratinocytes (HaCaT) were also measured. Consequently, it was demonstrated that leaf extract contains more phenols and flavonoids than tuber extract.

**However, Grac¸a Miguel, et al, (2004**). The impact of the two extraction methods was evaluated on the stability and one of the method was used Soxhlet method as shown in (figure 2.2) and fineness of pomegranate juice. After isolating the seeds from the fruits, the first method used centrifuging. The second method involves using a fruit equity electric lemon squeezer. After being cooled for 72 hours at 4°C, the juices underwent tests to determine whether they contained any sugars, organic acids, or anthocyanins. It was discovered that delphinidin 3-glucoside was the main anthocyanin at 45–69 mg/L. Oxalic and tartaric acids regulate organic acids. Glucose and sucrose were the two major sugars discovered in pomegranate juice. The juices produced utilizing the two various extraction methods had identical concentrations of organic acids, sugars, and anthocyanins.

**Figure 2.2** Example for method extracting the pomegranate peels by ethanol

## 2.3 Bioactivity effects of pomegranate peel and juice:

### 2.3.1 Antioxidant:

**Marı´a I. Gil, et al, (2000).** Pomegranate juice's antioxidant activity was assessed using four distinct techniques (ABTS, DPPH, DMPD, and FRAP) and contrasted with that of red wine and a green tea infusion. Three times more antioxidant activity was found in trade pomegranate juices (18–20 TEAC) than in red wine and green tea (6-8 TEAC). The juices included the pomegranate tannin punicalagin (1500–1900 mg/L) according to HPLC-DAD and HPLC-MS studies of the juices. This demonstrates that some hydrolyzable tannins in the pomegranate rind are extracted during industrial processing. This might explain why trade juices have more antioxidant activity than experimental juices. inside pomegranate juices, anthocyanins, derivatives of ellagic acid, and hydrolyzable tannins were also found and measured.

**As the same as in Monica Viladomiu, et al, (2013**). Pomegranate fruit has powerful anti-inflammatory, antioxidant, anti-obesity, and anticancer effects. Its three components seeds, peel, and juice, all have medical uses. The presence of ellagic corrosive, ellagitannins (counting punicalagin), punicic corrosive, and other

unsaturated fats, as well as flavonoids, anthocyanidins, anthocyanins, estrogenic flavanols, and flavones, which are remedially invaluable parts, is associated with a great impact. Intestinal inflammation, obesity, insulin resistance, and cancer are just a few of the illnesses that have shown promising outcomes in therapy.

**Similar to Susanne M. Henning, et al, (2014).** The antioxidant activity (AA) of three to five DS each from pomegranate, milk thistle, green tea, grapes, goji, and acai using four widely used standard methods. The secondary objective was to determine the effects of in vitro digestion on their AA. The AA of the DS before digestion ranked as follows: pomegranate > resveratrol > green tea > grape seed > milk thistle and very low in goji and acai with significant group variability in AA.

## 2.3.2 Antibacterial:

**Sweetie R. Kanatt. et al,(2009).** Pomegranate seed extract and peel's antioxidant and antimicrobial properties were examined. With a minimum inhibitory concentration of 0.01%, pomegranate peel extract (PE) demonstrated excellent antioxidant and antimicrobial activity against *Staphylococcus aureus* and *Bacillus cereus*. While it was ineffective against *Escherichia coli* and *S. Typhimurium* at a higher concentration of 0.1%, ***Pseudomonas*** could be inhibited.

**At the same time Amy B, et al,(2013)**. Pomegranates have a number of health benefits, including having antimicrobial qualities as shown in (figure 2.3.2). Research indicates that pomegranates and extracts may function as natural substitutes due to their efficacy against a number of bacterial and viral infections. Studies have been done on the pomegranate plant's antimicrobial properties, juice, and peel. There are a variety of phytochemicals in pomegranates that have been found to have antimicrobial activity, but most of our studies found that ellagic acid and larger hydrolyzable tannins, such as punicalagin, had the greatest activities. The majority of the information about the antibacterial and antiviral activity of pomegranates against viruses that cause foodborne illness and other targeted disease species comes from ***in vitro***, cell-based testing. Given the positive clinical results for pomegranate and the decrease in oral microbes.

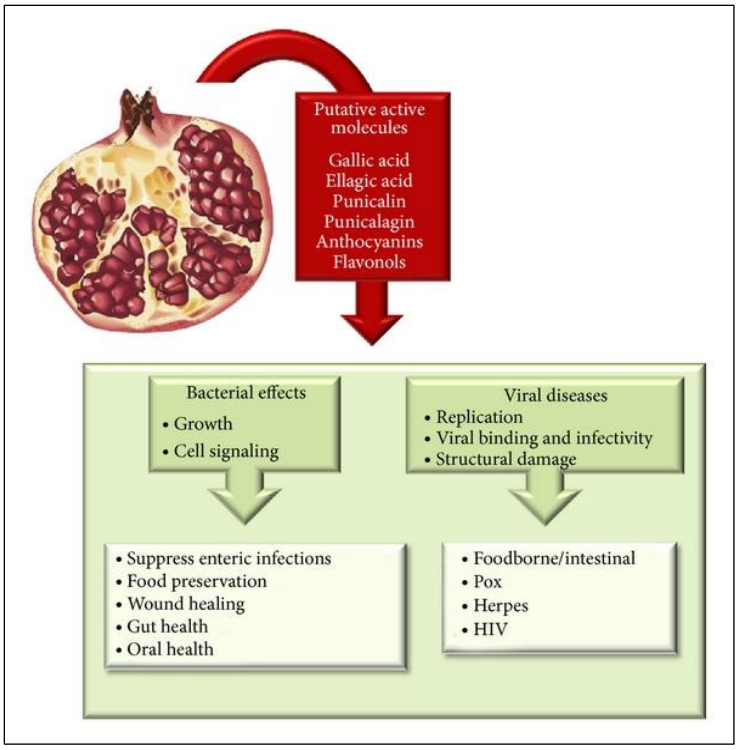



**Figure 2.3.2** Methanol extracts of pomegranate are rich in hydrolyzable tannins (punicalins and punicalagins), ellagic acid, a component of ellagitannins, and gallic acid, a component of gallotannins)( Amy B, et al, (2013)).

**A similar finding by Jing Chen, et al, (2020).** Pomegranate (Punica granatum L.) Bioactive compounds, phenolic acids, flavonoids, and hydrolyzable tannins are found in greater quantities in pomegranate fruit juice, seeds, and peels. The primary byproducts of pomegranate food processing are its peels, which are rich in antioxidants and broad-spectrum antimicrobial agents and can even prevent food from deteriorating. In order to provide a comprehensive guide for farmers, the food processing and storage industries, as well as academia, this study discusses the biochemical composition of the pomegranate peel extract (PPE), as well as its effectiveness in food preservation and antimicrobial activities.

## 2.3.3 Glycation of Hemoglobin in blood and type 2 diabetes

**Golbon Sohrab, et al, (2015).** This study's goal was to determine how pomegranate juice (PJ), which contains a natural antioxidant, affected individuals with type 2 diabetes(T2D) levels of plasma AGEs and lipid peroxidation. Split into two groups in a randomized, double-blind, placebo-controlled experiment. According to the study, PJ reduces lipid peroxidation. Consuming PJ may therefore postpone the start of T2D problems brought on by oxidative damage.

**In a similar context, Zahra Amr, et al, (2020)**. T**t**he current study's objective was to examine the inhibitory properties of pomegranate juice, peel, and leaf extracts on oxidative stress, insulin resistance, and obesity-inducing high-fat and high-fructose diet-induced obese rats. Rats were given a diet high in saturated fat and fructose (HFD) for a lengthy period (12 weeks), which caused them to gain weight. When compared to the control group (CG), which received chow die, the HFD group showed a significant rise in the fasted plasma levels of glucose (29.8%), insulin (45%), amylase (70%), and lipase (54%). High saturated fat and fructose (HFD) eating has also led to higher levels of protein carbonylation, lipid peroxidation, and antioxidant enzymes. According to these findings, consuming pomegranate extracts for an extended period of time could be an alternative strategy for preventing insulin resistance and oxidative stress caused by HFD. However, treatment with PL, PJ, and PP significantly reduces amylase and lipase levels and prevents glucose intolerance, insulin resistance, and oxidative stress.

### 2.3.4 Skin sensitivity

**Marianna Barbalinardo,et al,(2022)**.In this research, we mixed an organic protein called fibroin with antioxidants that were water-extracted from pomegranate waste. We present the efficient and simple production of bioactive and environmentally friendly films that may be useful for skin restoration. They are also flexible, and stable under physiological settings and in the presence of trypsin for 12 days. Furthermore, Tests for the production of reactive oxygen species (ROS) revealed that our films have a strong capacity to lessen the oxidative stress in cells that causes a variety of skin diseases.

**Also, Katharine Ko, et al,(2021).** Researchers are now incorporating pomegranate peel and seed into bioplastics and edible coatings for food packaging since new studies have shown their important antioxidant and antibacterial properties when exploited as natural food additives. , in addition to using active packaging, these ingredients have safely increased the plasticizing effects on packing materials and the shelf life of food. Pomegranate seed oil and its bioactive ingredients have shown to be particularly efficient in reducing UV-induced stressors on animal skin, where cells and microbes are removed from the entire organism, even in skin health applications. They have also explained important anti-inflammatory, analgesic, and antibacterial activities and helped heal the injury. In the amount collected transformation of pomegranate biowaste, there are several current and pertinent uses for food and skin health that are highlighted in this research.

## 2.3.5 HMO-CoA reductase inhibitory activity of pomegranate peel and juice

**Gunasekaran Baskaran, et al, (2015).** In this work, the anti-HMG-CoA reductase activity of 25 methanol extracts of medicinal plants was examined. Gas chromatography with tandem mass spectrometry and reversed-phase high-performance liquid chromatography was used to detect the presence of phenol 2,6-bis(1,1-dimethyl ethyl). To confirm its potential as an alternative treatment for hypercholesterolemia and related cardiovascular diseases, additional research using in vivo models is required.

**In addition Mahdiyeh KhademHaghighian, et al, (2021).** Pomegranate peel extract (PPE) supplementation's effects on serum lipid profile and antioxidant status were the focus of this randomized, double-blind, placebo-controlled clinical trial on 66 overweight women with knee osteoarthritis (OA). Two groups were formed from the participants. In overweight women with knee OA, the results showed that short-term PPE supplementation had useful effects on blood scales of total cholesterol and triglyceride as well as antioxidant status.

**Additionally, Michael Aviram, et al,(2022)**. the Consumption of pomegranate juice affects atherosclerosis, macrophage atherogenicity, platelet aggregation, and the oxidation, aggregation, and retention of lipoproteins. Pomegranate juice's potent antioxidative effects on lipid peroxidation in whole plasma and separated lipoproteins (HDL and LDL) were later evaluated in humans and mice. Following pomegranate juice treatment, mouse peritoneal macrophage absorption of natural and oxidized LDL was 20% lower. Last but not least, compared to control mice fed water, the mice supplemented with pomegranate juice had a 44% decrease in both the size of their atherosclerotic lesions and the number of foam cells. Due to its antioxidative properties, pomegranate juice may have potent antiatherogenic effects on both healthy humans and animals with atherosclerosis.

# Chapter Three:
# Materials & Methods

## 3.1 Chemical Materials:

Materials were bought from Sigma Aldrich: Absolute Ethanol (EtOH)( 99.9%), Ethanol (35%), TCA (trichloro acetic acid), PBS (phosphate buffer saline), Ethyl acetate, $ALCl_3$, $[K_3Fe(CN)_6]$ Potassium ferricyanide, $NaNO_2$, $FeSo_4.6H2O$(ferrous iron standard), Flavonoid standards (quercetin) (HPLC), reference antibiotic disc, Gallic acid.

## 3.2 Equipment and Apparatus:

The following equipment and apparatus were used in chemistry, pharmacy, biology, and environmental sciences laboratory at Al-Quds university: analytical balance from sartorius CP, mechanical grinder, refrigerator, Soxhlet apparatus, rotary evaporator from IKA WEREK RV06-ML, oven, electronic balance, spectrophotometer (UV2550, Shimadzu, Kyoto, Japan), RP-HPLC, hot plate, wire brush, graduated cylinder, evaporating dish, plastic test tubes, test tubes, and test tubes rack, micro pipets, spatula, thermometer, funnel, beaker.

## 3.3 Methods:

### 3.3.1 The identification of plant and juice:

The plant of pomegranate take from the vegetables and fruits local market in the summer of 2021 in Al- Bireh, Palestine, and the juice get from the local market name Al-Silwadi in Ramallah, Palestine, the identification of the plant *Punica granatum L* as shown in ( figure 3.1)

**Figure 3.1** *Punica granatum L, juice*

## 3.3.2 Plant sample:

Five pomegranate pills were chosen, the peels were separated from the inner grains of the pomegranate, the peels were dried with tissues, the peels were cut into small pieces to dry more quickly, put in a tray, and placed in an oven at a temperature of 35-45 Celsius, stirring pomegranate peel while drying after six hours removed from the oven and cool it for six hours then grinding and preserving in the refrigerator.

## 3.3.3 Pomegranate peels extraction:

### 3.3.3.1 Preparation of pomegranate peels ethanolic extract:

By employing a Soxhlet apparatus (type FA-46) ( figure 3.2) extraction method using 15 gm of dried powder (obtained after grinding pomegranate peels) in various solvents with (ethanol 99.9%, ethanol 35%, ethyl acetate), it was possible to detect the chemical component in pomegranate peels. The plant's powder-to-solvent ratio was 1:9 (wt/vol) throughout the extraction procedure utilizing the Soxhlet equipment in (ethanol 99.9%, ethanol 35%, and ethyl acetate). The extract was filtered with MN 615, 110 mm filter paper after extraction. Then evaporate solvent by rotary evaporator.

**Figure 3.2** (Soxhlet apparatus)

Each sample of the different extracts (ethanol 99.9%, ethanol 35%, and ethyl acetate) is filtered before being processed using a rotary evaporator, which lowers the boiling point of liquids as pressure is reduced. Select a vacuum pump for rotavapor, as indicated in (Figure 3.3), to ensure increased evaporation efficiency. In comparison to boiling in a normal environment, this enables the solvent to evaporate at lower temperatures.


**Figure 3.3** Rotary evaporator

On a dry weight basis, the dried powder yielded was determined using the following equation:

$$\text{Percentage yield} = \frac{\text{Wt. of dried extracts}}{\text{Wt. of powder taken}} \times 100$$

After that put them in a plastic test tube and stored them in the refrigerator, to use them in future tests, and after squeezing juice stored them in the refrigerator to be used in different tests in the future.

### 3.3.4 Total phenolic content (Folin–Ciocalteu assay)

The Folin-Ciocalteu test was used to determine the total phenolic content. Folin-Cioacalteu regent reduces polyphone-containing samples, producing a colorful complex as a consequence. Pomegranate juice was dissolved in distilled water (20 ml), pomegranate peel extract (0.2 g) from ethanol 99.5%, 35%, and ethyl acetate, and 1 ml of the sample's and gallic acid (1000 ppm) was combined with 5 mL of the Folin-Ciocalteu reagent (diluted 10-fold) and 5 mL (7.5 g/L) sodium carbonate. The quantitative phenolic determination was carried out at 765 nm by a UV spectrophotometer (EMC-61PC-UV Spectrophotometer) following incubation at 25°C for 30 min. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve of 20 µg/ ml, 30µg/ml, 40 µg/ml, 50µg/ml, 60µg /ml, 70 µg/ml, and 80µg/ml. µg/mL ethanoic Gallic acid by putting the value of absorbance vs. concentration. And determine the TPC by the following equation:

TPC = C*V/M

Where C: is the concentration of the sample, V is: the volume of the solution, and M: is the mass of the sample

### 3.3.5 Total Flavonoid Content (TFC):

A percentage of 5% of quercetin was used to make the standard calibration curve. The standard solutions of quercetin were prepared by serial dilutions using distilled water ( 25 - 50- 100-200-300-400-500 µg / ml ).1 ml of diluted standard quercetin solutions and extracts (ethanol 99.9%, 35% and pomegranate juice PJ) was separately mixed with 3 ml of methanol, 200µl of 2% aluminum chloride solution, 200µl of 1M sodium acetate solution, and 5.6 ml of distilled water. The solution was incubated for 30 min at room temperature, The absorbance of the reaction mixtures was measured against blank at 420 nm wavelength with (EMC-61PC-UV Spectrophotometer). The following equation is used to determine the TFC:

TFC=C*V/M

Where C is the concentration of the solution, V is the volume of the solution, M is the mass of the sample

### 3.3.6 RP-HPLC analysis of flavonoids

The high-performance liquid chromatography (HPLC) technique is frequently used to assess the flavonoid concentration in natural extracts, for both separation, quantification, and identification of these chemicals. The crude raw extracts of the pomegranate peel were identified using HPLC, which is an effective instrument.

By dissolving 10 mg of extract in 10 ml of corresponding solvent 99.9 % ethanol, samples of extracts of pomegranate peel were generated at a concentration of 1 mg/ml. HPLC with UV detector method was used for quercetin analysis using a C18 column (15 cm with 4-micrometer particle size) at 280 nm wavelength and a mobile phase of water: methanol (50:50 v/v) at a flow rate of 1.0 ml per minute. standard of 0.01 g per 10ml was used.

### 3.3.7 Antioxidant assay

The antioxidant activity of the extract (PP) with ethanol 99.9%, ethanol 35%, and ethyl acetate and (PJ)was measured by determining the 1.1-diphenyl-2-picrylhdrazyl (DPPH) free radical scavenging capacity according to (Nishant Kumar, et al,.2021) 3 ml of 4% DPPH methanolic solution added to 1 ml of sample and 6ml methanol. the solution was incubated for 30 min at room temperature. Using an EMC-61PC-UV Spectrophotometer, the absorbance of the reaction mixtures was measured against a blank at 517 nm. The following equation was used to figure out the percentage of the DPPH scavenging effect. DPPH scavenging effect (%)

DPPH scavenging effect (%) = [(A0 control – A1 sample)/A0 control] × 100

Where A0 = absorbance of control. A1 = absorbance of standard.

### 3.3.8 Antibacterial assay

Agar disc diffusion was used to conduct the antibacterial test (Murray et al., 1999). Negative controls were made with identical solvents (99.9% ethanol) that were used to dissolve the samples. Positive controls for the examined bacteria included standard antibiotics Gentamicin (10 micrograms/disc) and Penicillin (10 units). The diameter of the zones of inhibition surrounding the disc against the tested microorganisms was used to assess antibacterial activity.

**3.3.8.1 Microorganism:** Clinical isolates of *Escherichia coli*, *Staphylococcus aureus, Methicillin-resistant staphylococcus aureus (*MRSA*), and Pseudomonas aeruginosa* were provided by Palestinian Medical Complex (PMC) laboratory medical serveries.

**3.3.8.2 Preparation of medium:** Mueller – Hinton Agar was supplied by the Department of Microbiology, Faculty of sciences and technology, Al Quds University.

Mueller Hinton Agar (MHA) was prepared and sterilized by autoclaving for about 30 minutes after the bacterial inoculum was prepared in broth. The media was then put into sterile Petri dishes for the next stage. They were left to harden for 10-15 minutes. Plates were stored in a plastic bag and turned upside down to prevent moisture from collecting on the surface of the medium.

**3.3.8.3 Culture of bacteria:** The bacteria were inoculated into the nutritional broth and incubated at 37°C for 24 hours, after which the culture was diluted to a concentration comparable to that of MacFarland nephelometer tube no. 108 CFU/ml) using a spectrophotometer set to 625 nm (optical density 0.08 to 0.1).

The inoculum was evenly distributed on the surface of MHA after it had solidified using a cotton swab. Carpet culture is another term for it.

### 3.3.8.4 Antibacterial:

The antibacterial activity of four different bacteria strains was examined using the disc diffusion method. The disc (5mm diameter) was autoclaved for around 30 minutes to disinfect it.

The produced bacterial species were spread into Muller Hinton agar plates. 28 As a positive control, a reference antibiotic disc was placed on the surface of MHA. After that, sterile discs were impregnated with (50 microliters) of plant extracts (at various concentrations) and negative controls (solvent) with 50 l in each disc. The plates were then incubated for 24 hours at 37°C.

## 3.3.9 Fluorescence-based assay of the inhibition of AGE formation

The approach was accomplished as formerly reported (Wang et al., 2009), with the following amendment:

## a. Preparation of Incubation media

1- A sodium phosphate monobasic monohydrate buffer (pH 7.4) (100mM) was generated.

2- In (100mM) sodium phosphate monobasic monohydrate buffer, a stock solution of (1 mg/ml) (BSA) resulted in (pH 7.4).

3- In (100mM) sodium phosphate monobasic monohydrate buffer, a stock solution of (100mM glucose/100mM fructose) combination was produced (pH 7.4).

### b. Preparation of extract samples

Five various concentrations of peel pomegranate and juice pomegranate were prepared using ethanol 99% and distilled water, and these concentrations began from 2.5 mg/ml to 50 mg/ml for each extract to determine concentration-dependent responses.

### c. Test Samples

The extract and control test samples were prepared in a test tube with a screw cap and a sample volume of (1000 mL), and each sample was replicated twice.

**Extract samples**

100 µl of BSA, 100 µl of sugar solution, 300 µl of phosphate buffer (pH 7.4), and 500 µl of each of five concentrations of peels pomegranate and juice pomegranate, were prepared in a Test tube with a screw cap and incubated in incubator shaker (Environmental Sciences Laboratory lab) at 37°C for 7 days.

**Positive control**

In test tubes, 100 µl of BSA, 100 µl of sugar solution, 300 µl of phosphate buffer (pH 7.4), and 500 µl of Quercetin standard (Q4951) were made with five various doses ranging from 2.5 mg/ml to 12.5 mg/ml, and incubated in an incubator shaker at 37°C for 7 days.

**Negative control**

In a test tube with a screw cover, 100 µl of BSA, 100 µl of sugar solution, 300 µl of phosphate buffer (pH 7.4), and 500 µl of Ethanol 99 percent were made and incubated at 37°C for 7 days in an incubator shaker.

**d. Fluorescence-based assay of the inhibition of AGE formation:**

Evolving of fluorescent antiglycation End-products (AGEs) in each sample was measured using a fluorometer (Albaraj lab, Al-Quds University) at excitation and emission wavelengths of 455 nm and 375 nm, respectively, after 7 days of incubation. In order to reduce baseline fluorescence, the experimental treatment (including BSA, sugar, and either extract or pure standard) and the negative control had their fluorescence values blanked against BSA, phosphate buffer, and the relevant extract blanks. The corrected fluorescence readings (F) for the negative control (F negative control) and experimental treatments (F experimental corrected) were used to calculate the percentage of inhibition of AGE production.

$$\% \text{ Inhibition} = \frac{(\text{F negative control} - \text{F experimentalcorrected})}{\text{F negative control}} * 100\ \%$$

### 3.3.10 Preparation of cream

The hand cream was produced according to the following researcher-developed formula:

**Phase A**: selected materials and determined their weights, and these materials are listed as follows: Cetylstearyl alcohol, Isopropyl Myristate, Glyceryl Monostearate, peels pomegranate.

**Phase B**: selected materials and determined their weights, and these materials are listed as follows: Glycerin, triethanolamine, sorbitol, and water.

**Additive C**: selected a proper perfume, methylparaben.

**Procedure**: materials mentioned in phase A and phase B were heated to 75 C° and then materials of phase B were added to materials of phase A, then the selected perfume was added to the mixture at the temperature of 40C°.

### 3.3.11 Enzyme assay

The enzyme and the substrate are less stable when added to the assay buffer. Hence, it is very important to add the different ingredients according to the order mentioned in this procedure to get the best results.

The HMG-CoA reductase stock solution had a concentration of 0.5–0.75 mg/mL. Each crude extract (50 µ g) was combined with a reaction mixture containing nicotinamide adenine dinucleotide phosphate (400µ M), HMG-CoA substrate (400µ M), and potassium phosphate buffer (100 mM, pH 7.4) consisting of potassium chloride (120 mM), ethylene diamine tetra acetic acid (1 mM), and dithiothreitol (5 mM), as well as HMG-CoA (Baskaran, et al., 2015).

Firstly, set the spectrophotometer at 37 °C and 340 nm, with a kinetic program:

• 1 ml sample: read every 20 seconds for up to

5 minutes.

The HMG-Co A reductase inhibition (%) was evaluated depending on the following formula:

$$\text{Inhibition } \% = \left(\frac{\Delta\text{Absormance control} - \Delta\text{ Absorbance test}}{\Delta\text{ Absorbance control}}\right) * 100$$

# Chapter Four:
# Result and Discussion

## 4.1 The pomegranate peels extract result:

In this study, the various extracts, the activity of pomegranate peels in various extracts (ethanol 99.9 percent, ethanol 35 percent, ethyl acetate, and juice), their relevance for the body, as well as the amounts of active components present in pomegranate peels and juice, are demonstrated as shown in (Figure 4.1).

Pomegranate dried peels weighing 25g were dissolved in 225 ml of ethanol 99,9%, 20g were dissolved in 180 ml of ethanol 35%, and 20g were dissolved in 180 ml of ethyl acetate. Soxhlet equipment was used to extract this whole solution (type FA-46) The solution was concentrated using an IKA WERKE RV06-ML rotary evaporator. The following table 4.1 displays the final weights:

Table 4.1: show the different

| No. | Type of extract (pomegranate peels) | Weight after evaporated | % active ingredient |
|---|---|---|---|
| 1 | ethanol 99.9% | 4.735 g | 26.3% |
| 2 | ethanol 35 % | 2.133 g | 14.2% |
| 3 | ethyl acetate | 1.638 g | 0.091% |
| pomegranate juice | juice taken from The pomegranate seeds was squeezed and produced 0.5 liters of them | | |

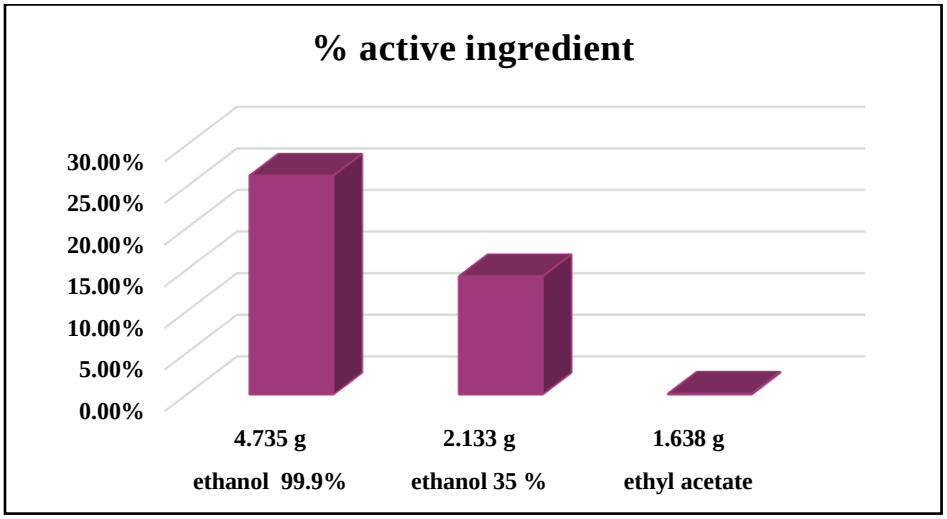



**Figure 4.1** show the percentage of active ingredient

## 4.2 The Total Phenols in pomegranate peels and juice:

Table 4.2 shows the TPC between different extract solutions using ethanol 99.9%, ethanol 35%, and ethyl acetate for peels pomegranate and juice pomegranate. And figure 4.2 show the absorbance of the standard of gallic acid and their concentration. The TPC found that pomegranate peels extracted with ethanol 99.9% (78.81 mg/g) are higher than those extracted with ethanol 35% (74.94 mg/g), and ethyl acetate (56.61 mg/g). The effect of pomegranate juice according to TPC analysis is less than that of the pomegranate peels, and this is due to the presence of a higher amount of polyphenols in the peels than in the juice. As several researchers found that ethanolic extract has higher TPC than another extract like ethyl acetate

**Table 4.2**: shows the result of total phenols for pomegranate peel extract with (ethanol 99%, ethanol 35%, ethyl acetate) and pomegranate juice

| Name of solvent | Sample solution volume (ml) | Weight of dry extract (g) | Conc. of solution (mg/ml) | Absorbance | TPC (mg/g) |
|---|---|---|---|---|---|
| Ethanol 99.9% | 20 ml | 0.2 g | 10 | 0.7824 ± 0.068 | 78.81 |
| Ethanol 35% | 40 ml | 0.219 g | 5.475 | 1.1493 ± 0.03 | 74.94 |
| Ethyl acetate | 20 ml | 0.194 g | 9.7 | 0.9684 ± 0.02 | 56.61 |
| Juice of pomegranate | 30 ml | 1.1675 g | 3.8 | 1.0345 ± 0.03 | 23.88 |

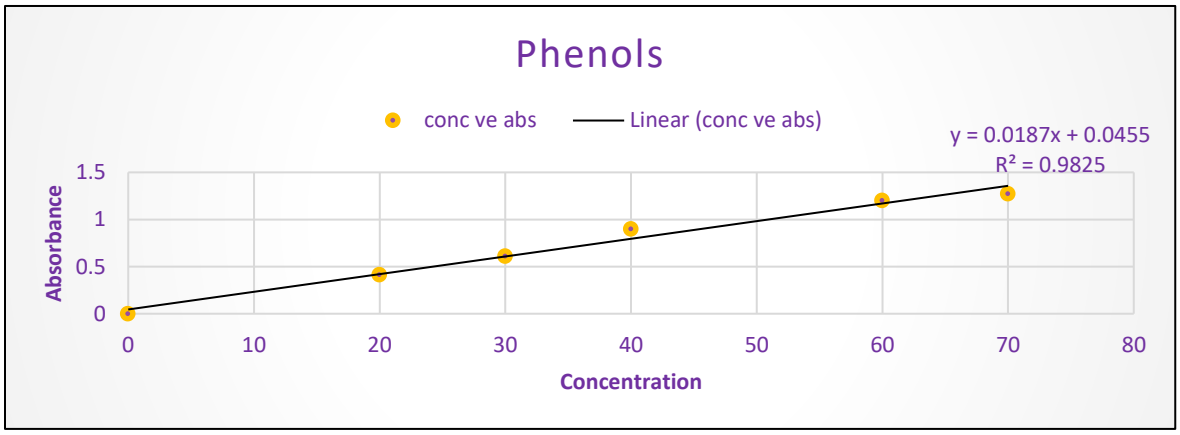



**Figure 4.2** show the absorbance of the standard of gallic acid and their concentration

## 4.3 The Total Flavonoids assay:

**Table 4.3:** at the bottom shows the result of total Flavonoids for peels pomegranate extract with ethanol 99.9%, ethanol 35%, and pomegranate juice

| Name of solvent | Sample solution volume (ml) | Weight of dry extract (g) | Conc. of solution (mg/ml) | Absorbance | TFC (mg/g) |
|---|---|---|---|---|---|
| Ethanol 99.9% | 50 ml | 0.2085 g | 4.17 | 0.2354 ± 0.005 | 7.95 |
| Juice of pomegranate | 10 ml | 1.000 g | 100 | 0.1495 ± 0.003 | 0.0547 |
| Ethanol 35% | 50 ml | 0.1 g | 2 | 0.2801 ± 0.004 | 18.09 |

The pomegranate peels were extracted with ethanol 99.9%, ethanol 35%, and pomegranate juice, according to the Aluminum chloride for test determined (TFC) results, which were shown in table 4.3 and the concentration of quercetin and their absorbance were shown in figure 4.3. (This study shows that the total phenol content has a higher value compering with their corresponding total flavonoid content due to the type of extract).

Some researchers found that the (TFC) in peeled pomegranate and juice is higher due to different extracts used in methanolic solvent to extract peeled pomegranate and different ways for the temperature of drying that they used.

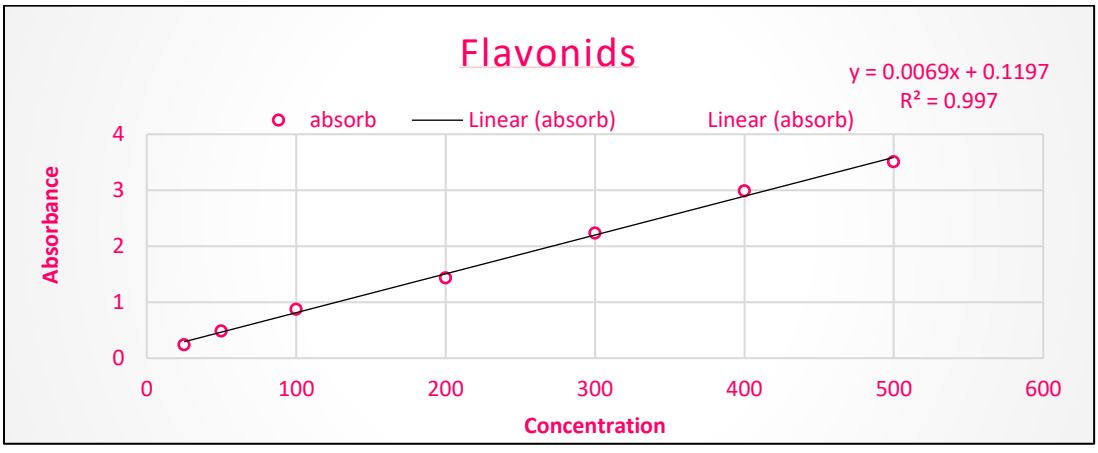
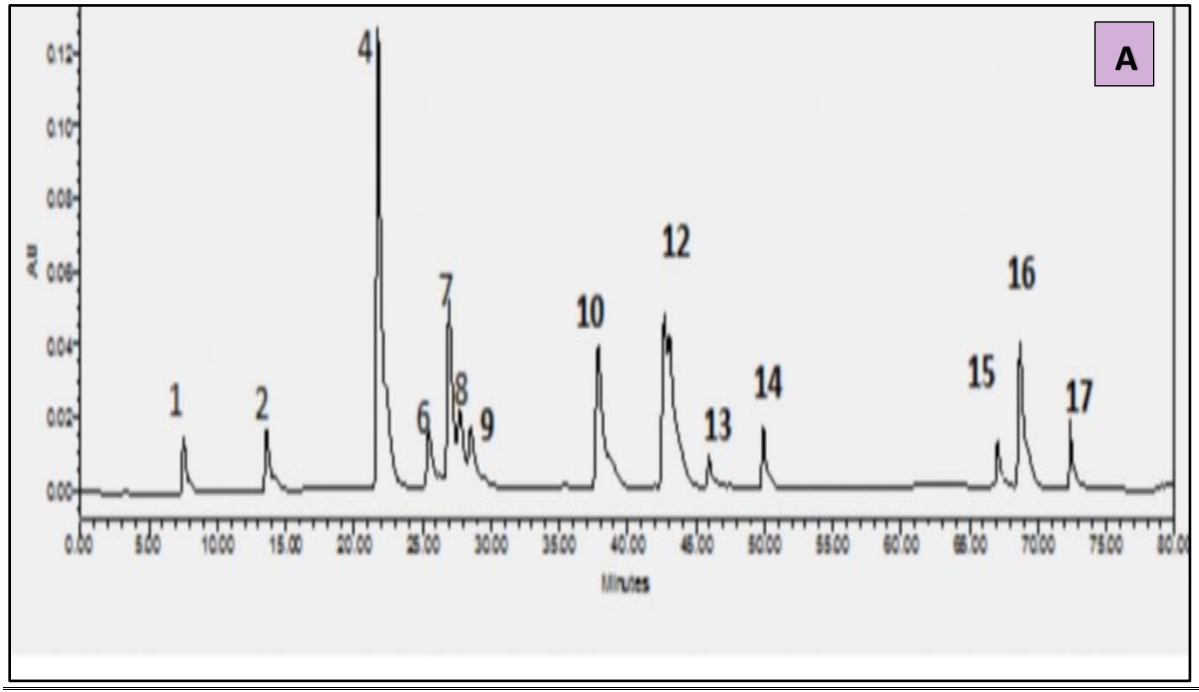
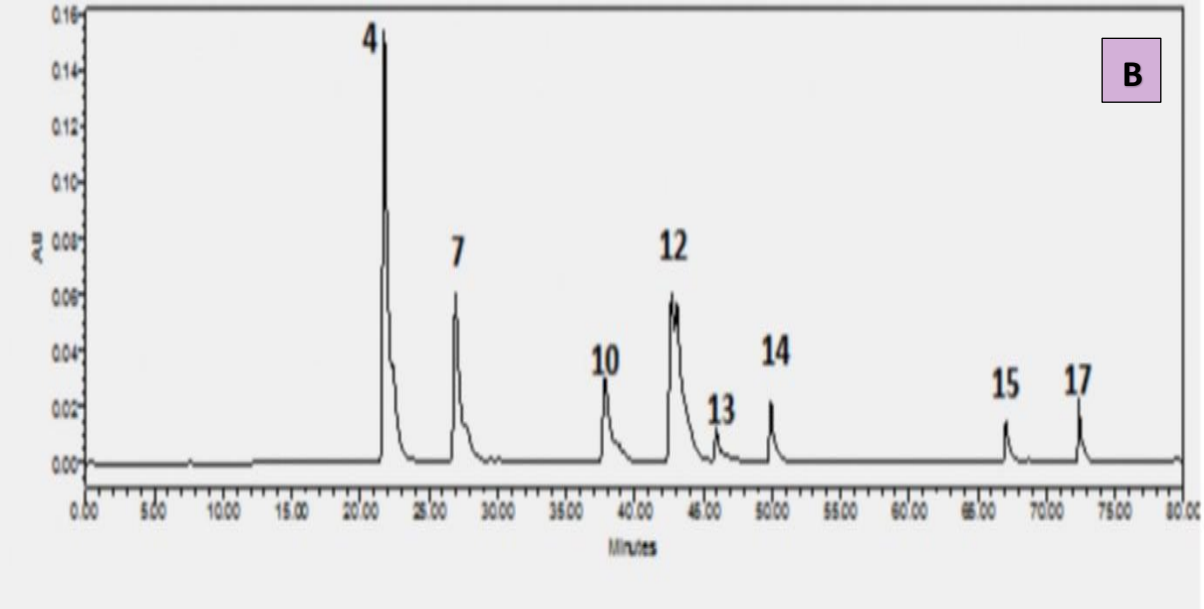



**Figure 4.3** The concentration of quercetin and its absorbance

## 4.4 HPLC analysis of the standards of polyphenolic compounds and flavonoids

The combination of 17 standards was infused (20 µL) into the HPLC chromatograph and examined using the RP-stage strategy depicted previously. The photodiode array detector was used at various wavelengths because each compound has its wavelength of maximum absorption (Table 4.4). The standard mixture's chromatograms at various wavelengths (300 nm (A), 323 nm (B), 280 nm (C), and 290 nm (D)) are illustrated in Figure 4.4.1. When different wavelengths were used, the 17 compounds were separated, as shown in Figure 4.4.1 (A-D). The maximum absorption wavelength for each standard is listed in Table 4.4, which provides a summary of the standards' retention times.

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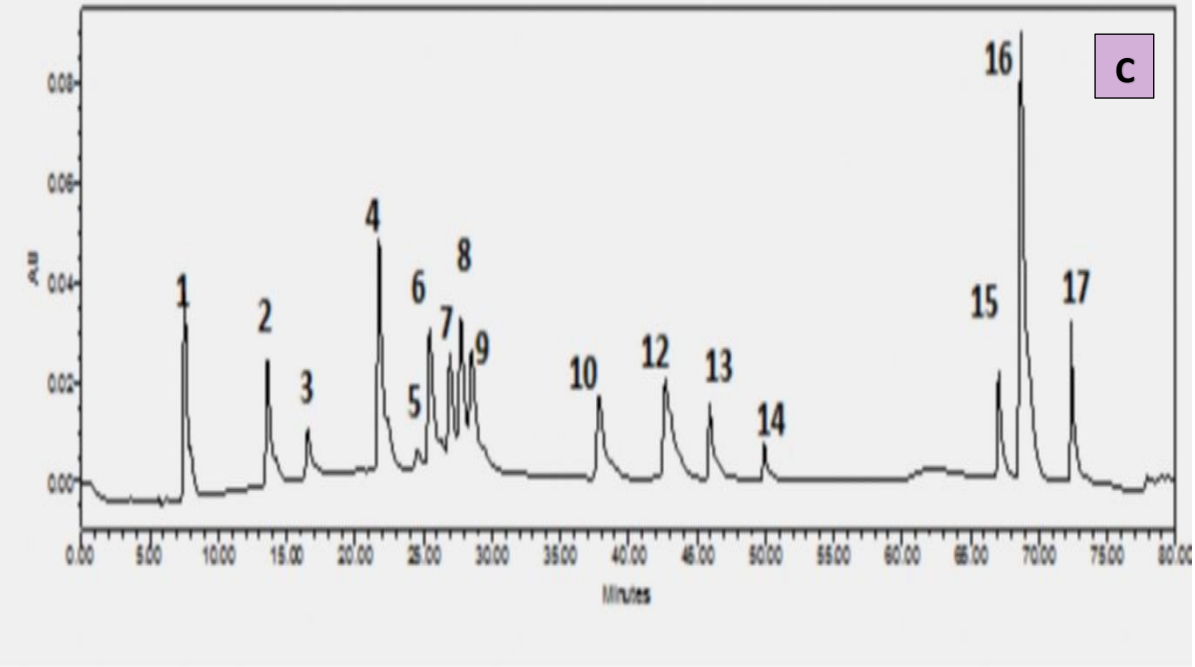
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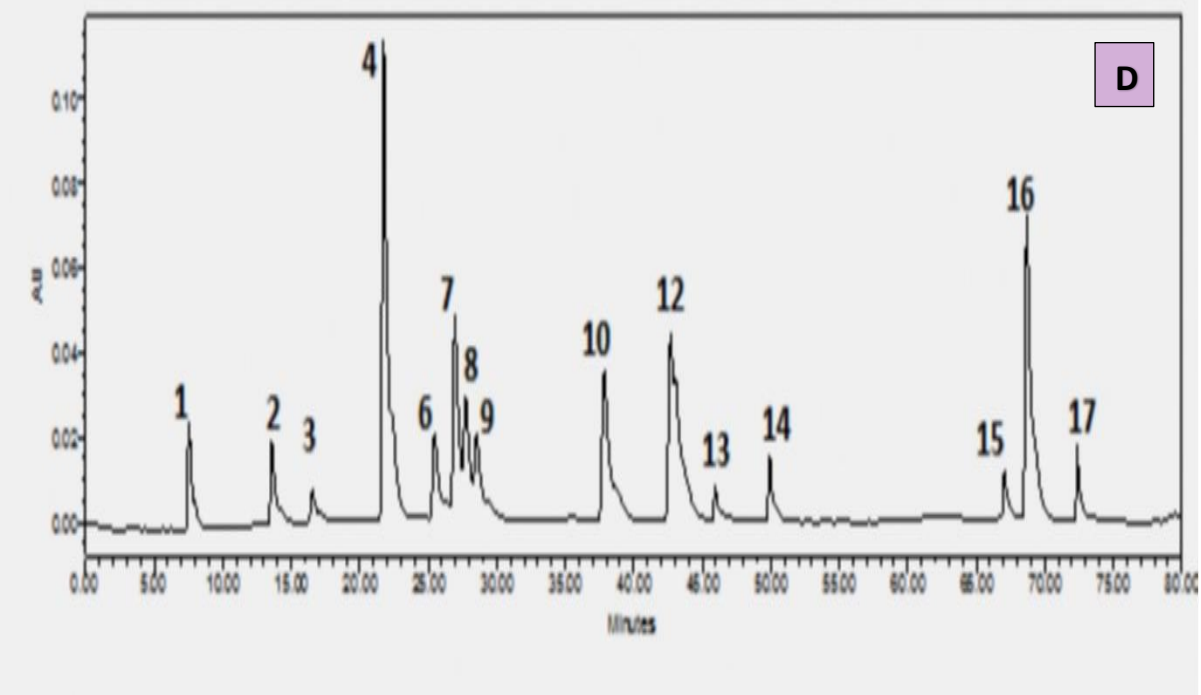



**Figure 4.4.1** HPLC chromatogram of polyphenolic and flavonoid standards examined applying the RP-HPLC method at 300 nm (A), 323 nm(B), 270 nm (C), and 290 nm (D).

**Table 4.4:** Standard compounds list analyzed under the RP-HPLC method with their retention times and maximum wavelength of absorption.

| Standard # | Standard name | Retention time (min) | Wavelength (nm) |
|---|---|---|---|
| 1 | Gallic acid | 8.26 | 271 |
| 2 | 3,4-Dihydroxybenzoic acid | 13.87 | 259 |
| 3 | 3,4-Dihydroxyphenylacetic acid | 16.57 | 280 |
| 4 | Chlorogenic acid | 21.64 | 323 |
| 5 | 4-hydroxyphenyl acetic acid | 24.55 | 274 |
| 6 | Vanallic acid | 25.42 | 260 |
| 7 | Caffeic acid | 26.92 | 322 |
| 8 | Syringic acid | 27.73 | 274 |
| 9 | Isovanallic acid | 28.55 | 259 |
| 10 | p-Coumaric acid | 37.82 | 309 |
| 11 | Ferrulic acid | 42.68 | 322 |
| 12 | Sinapic acid | 43.1 | 323 |
| 13 | Rutin | 45.99 | 255 |
| 14 | Verbascoside | 49.98 | 329 |
| 15 | Quercetin | 67.04 | 364 |
| 16 | Trans-cinnamic acid | 68.69 | 275 |
| 17 | Kaempferol` | 72.36 | 265 |

### 4.4.1 HPLC analysis of plant extracts

The plant extracts were analyzed using the method developed particularly for the standards. (Figure 4.4.2 shows the chromatogram extract at 280 nm). Polyphenolic compounds listed below were detected and identified by comparing the retention times of the peaks in the sample chromatogram of the extract with that of the standard:

1. Gallic acid

2. Rutin

As same as some researchers that found in (Ilham Hamid, et al,.2016)

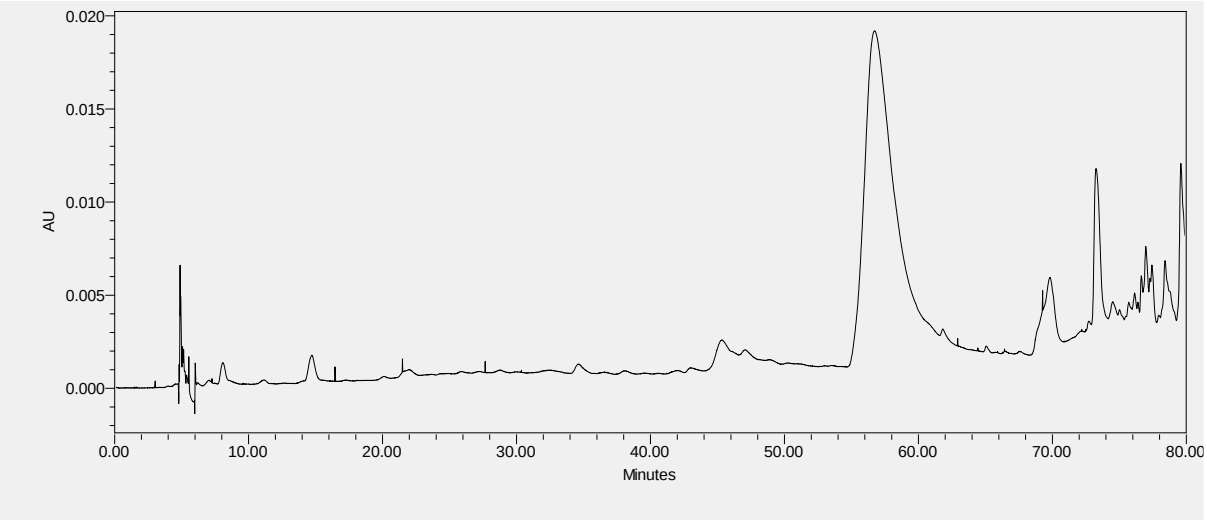



Figure 4.4.2 shows the chromatogram extract at 280 nm

## 4.5 Antioxidant assay:

**Table 4.5:** at the bottom shows the result of Scavenging activity for pomegranate peels extract with ethanol 99.9%, ethanol 35%, Ethyl Acetate, and pomegranate juice:

| Name of solvent | Weight of extract (g) | AS (Absorbance standard) | AC (Control) | AE (Absorbance Sample) | % Scavenging activity |
|---|---|---|---|---|---|
| Ethanol 99.9% | 0.534 | 0.4717 | 1.2912 | 0.2944± 0.026743 | 51.6% |
| Ethanol 35% | 0.507 | 0.4717 | 1.2912 | 0.5490± 0.044273 | 9.86% |
| Ethyl Acetate | 0.505 | 0.4717 | 1.2912 | 0.1412 ±0.026875 | 76.8% |
| Juice of pomegranate | 0.515 | 0.4717 | 1.2912 | 0.5068± 0.014534 | 16.79 % |

***Abbreviation: AS: absorbance standard; AC: Absorbance control; AE: absorbance sample**

Table 4.5 shows this research's antioxidant activity for peel pomegranate extract by 99.9 %, ethanol 35%, ethyl acetate, and pomegranate juice. Figure 4.5 shows the absorbance and concentration of ascorbic acid as a control. The data clearly show that the extraction with ethanol 99.9 % contains (51.6 mg/g). where comparative to the antioxidant activity of peel pomegranate extracted using ethanol 35%, ethyl acetate( 9.86 mg/g, 76.8 mg/g ), and pomegranate juice (16.79 mg/g ) the extract with ethyl acetate in this research, had higher antioxidant activity than that extracted using ethanol 99.9% and ethanol 35 %.

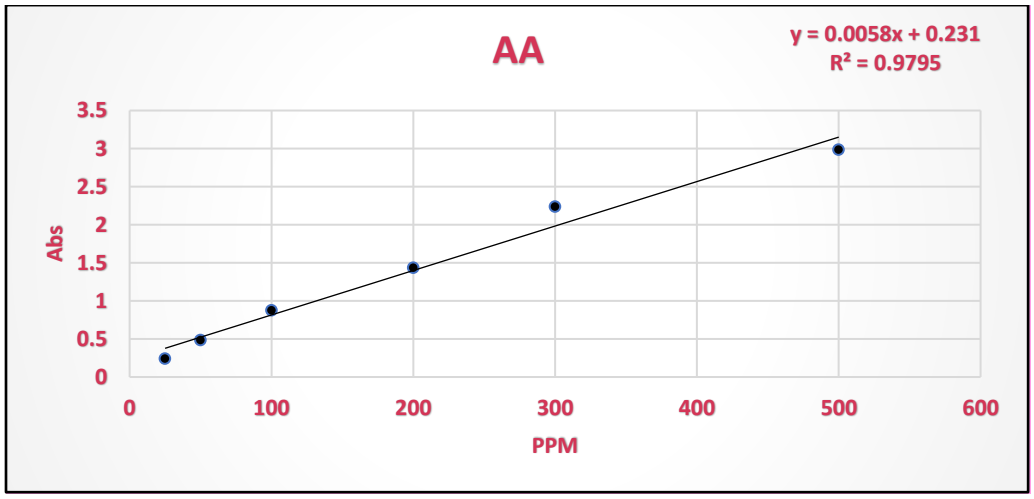
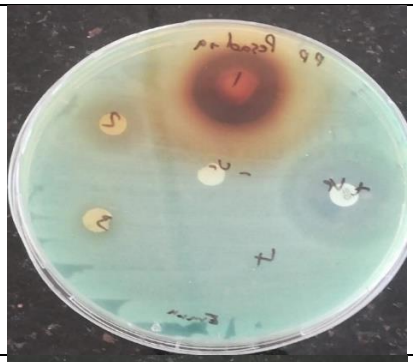



**Figure 4.5** shows the absorbance and concentration of ascorbic acid as a control

## 4.6 Antibacterial assay:

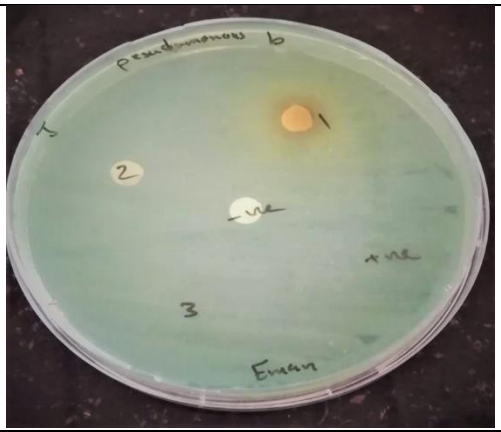
The extracts from peel pomegranate (extracted using ethanol 99.9%) demonstrated antibacterial effects against *Pseudomonas aeruginosa, Escherichia coli, Methicillin-resistant Staphylococcus aureus* **(**MRSA**),** *Staphylococcus aureus*. The existence of positive control (Gentamicin (10 micrograms/disc)) for *Methicillin-resistant Staphylococcus aureus* (MRSA), *Staphylococcus aureus*. and Penicillin (10 units) for *Pseudomonas aeruginosa, and Escherichia coli* by using the disc diffusion method. The zones of inhibition (Figure 4.6.a) were calculated and the average results of the zones of inhibition were presented in Table 4.6.

| Type bacteria | Result |
|---|---|
| *(Punica granatum L)* peels extract antimicrobial activity against ***Pseudomonas aeruginosa*** |  |
| *(Punica granatum L)* peels extract antimicrobial activity against ***Escherichia coli*** |  |

| | |
|---|---|
| *(Punica granatum L)* peels extract antimicrobial activity against ***Staphylococcus aureus*** |  |
| *(Punica granatum L)* peels extract antimicrobial activity against ***Methicillin-resistant Staphylococcus aureus (MRSA)*** |  |

**Figure 4.6 a**. Zone inhibition of pomegranate peels (*Punica granatum L*.) extract

and the result from pomegranate juice showed low antibacterial activity against ***Staphylococcus aureus and E.Coli*** only, but the juice does not have a direct effect on the bacteria, as the bacteria appeared diluted. The inhibition zones in (Figure 4.6.b) were calculated and the average results of the zones of inhibition were presented in Table 4.6.

| Type bacteria | Result |
|---|---|
| *(Punica granatum L)* juice antimicrobial activity against ***Pseudomonas aeruginosa*** |  |

| | |
|---|---|
| *(Punica granatum L)* juice antimicrobial activity against ***Escherichia coli*** |  |
| *Punica granatum L)* juice antimicrobial activity against ***Staphylococcus aureus*** |  |
| *Punica granatum L)* juice antimicrobial activity against ***Methicillin-resistant Staphylococcus aureus (MRSA)*** |  |

**Figure 4.6 b.** Zone inhibition of pomegranate juice (*Punica granatum L*)

**Table 4.6** the inhibition zone (mm) of the extract is described below:

| | | | | |
|---|---|---|---|---|
| Peel pomegranate extract with EOTH 99.9% | Bacteria type | Average zone concentration 1 of inhibition (mm) | Average zone concentration 2 of inhibition (mm) | Positive control |
| | *P.aeruginosa* | 18 | 15 | 17 mm |
| | *E-Coli* | 26 | 12 | 14 mm |
| | *S.aureus* | 36 | 15 | 15 mm |
| | *MRSA* | 26 | 13 | 13 mm |
| Pomegranate juice | Bacteria type | Average zone concentration 1 of inhibition (mm) | Average zone concentration 2 of inhibition (mm) | |
| | *P. aeruginosa* | 11 | ND | 17 mm |
| | *E-Coli* | 30 | ND | 14 mm |
| | *S.aureus* | 10 | ND | 15 mm |
| | *MRSA* | 9 | ND | 13 mm |
| | * ND: Not detected. | | | |

Researchers such as (Zeljko et al,.2020) confirmed that pomegranate peels can be antibacterial because they contain a high percentage of phenol, especially affecting Gram-positives such as *S.aureus.*

## 4.7 Antiglycation End Products (AGEs) Assay:

Take different concentrations (2.5mg/ml, 5mg/ml, 7.5mg/ml 10mg/ml, 12.5mg/ml) from pomegranate peels (PP) and take this concentration (7.5mg/ml, 10mg/ml, 12.5mg/ml) from pomegranate juice (PJ) the result shows in table 4.7

| Concentration (mg/ml) | Fluorescence Response | % Inhibition |
|---|---|---|
| For peel pomegranate (measurement count :1 Ex:455 Em:375 scaling Factor:1/1) | | |
| 2.5 | 2.607 | 51.7 |
| 5 | 2.591 | 52.01 |
| 7.5 | 2.500 | 53.7 |
| 10 | 2.345 | 56.5 |
| 12.5 | 2.317 | 57.09 |
| For pomegranate juice( measurement count :1 Ex:455 Em:375 scaling Factor:1/1) | | |
| 7.5 | 5.460 | N |
| 10 | 3.836 | 28.9 |
| 12.5 | 3.287 | 39.1 |

***N: negative result**

Advanced Glycation End-products (AGEs) play a key part in pathology in human diseases such as diabetes type 2, this study shows that peel pomegranate ethanolic extract was effective as an inhibitor for (AGEs) in different concentrations from the sample, the table shows that the pomegranate peel extract has high effects on (AGEs) due to containing high polyphenols like gallic acid. At the same time, samples of pomegranate juice with different concentrations were taken to measure their effectiveness as inhibitors, and results show that pomegranate juice is lower effective than peel pomegranate alcoholic extract. As shown in (Figure 4.7.a) the result of inhibitor for (AGEs) in different concentrations from the pomegranate peel extract and (Figure 4.7.b) the result of inhibitor for (AGEs) in different concentrations from the pomegranate juice

Also, after reviewing previous research concerning Anti-glycation activity, the results were better and more promising due to using clinical experiments (Yuya Kumagai. et al,.2015).

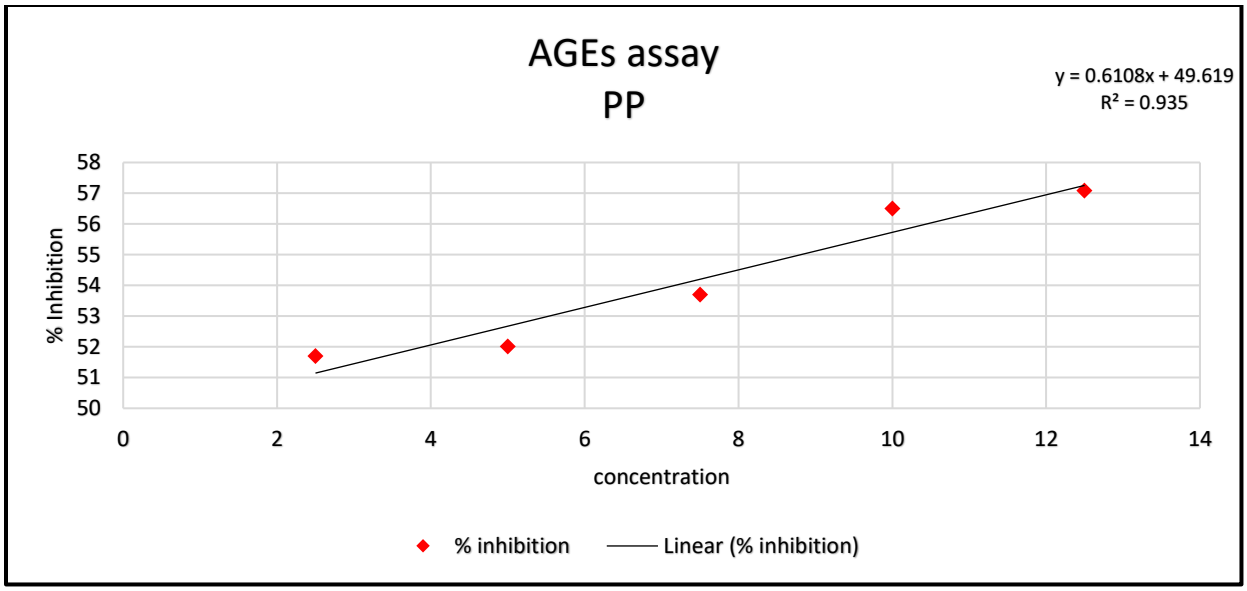



**Figure 4.7.a** the result of inhibitor for (AGEs) in different concentrations from the pomegranate peel extract

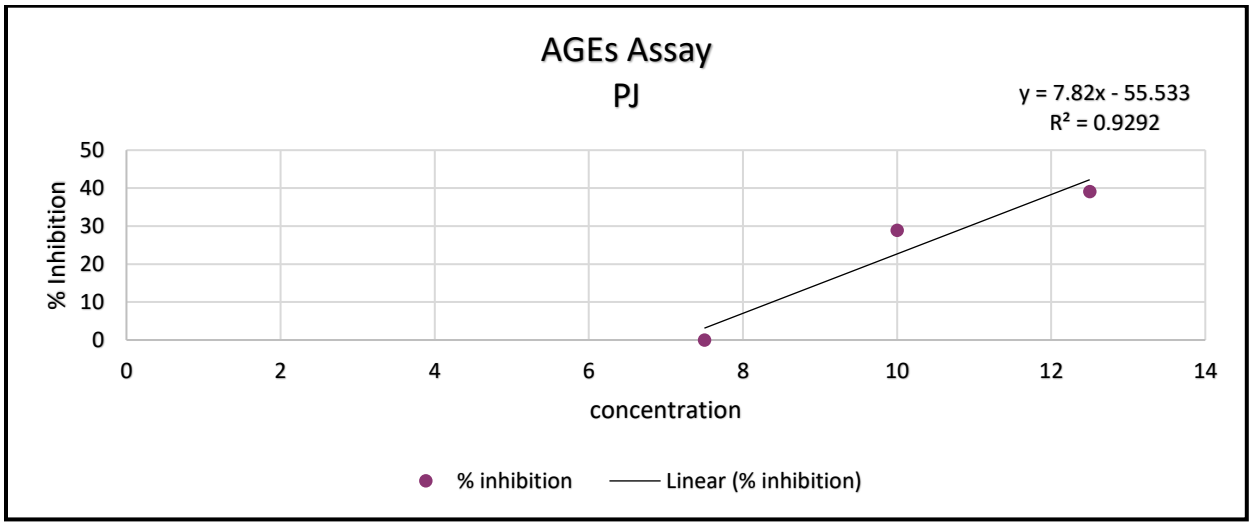



**Figure 4.7.b** the result of inhibitor for (AGEs) in different concentrations from the pomegranate juice

## 4.8 Preparation of cream:

When the cream is applied topically, the formula is moisturizing, smoothing, pleasant-smelling, rapidly absorbed (without leaving a film or any greasy feeling), non-irritating the skin with (ph= 5.5-6), has excellent color, and easy to spread on the skin.


**Figure 4.8.1** cream of peel pomegranate extract by EOTH 99.9%

The samples stored at 8°C and 25°C showed no signs of liquefaction. The sample remained stable in the evaporimeter at 40°C for seventeen days. Also, the formulation remained stable at different temperatures.

Measurement: The percentage of peel pomegranate extract in cream = 3.03/99.9*100% = 3.03 %

**Figure 4.8.2** Evaporimeter (left) creams at 40°C (middle)

## 4.9 Enzyme assay:

The HMG-CoA reductase inhibitory effect of the pomegranate peel and juice was examined based on Spectrophotometric measurements.

HMG-CoA reductase catalyzes the rate-limiting step in the synthesis of cholesterol. This study concluded that inhibition of the enzyme may reflect the potential of pomegranate peel and juice in cholesterol reduction.

**Table 4.8 Anti -HMG-CoA reductase activity of pomegranate peel extracted with ethanol 99% and juice**

| Scientific name | Family name | Inhibition % |
|---|---|---|
| Punica granatum L. | Punicaceae | 45% |
| (Punicagranatum) juice | Punicaceae | 74% |

***Abbreviation:HMG-CoA,hydroxy-3-methyl -glutaryl-coenzyme A**

Table 4.8 details the inhibition of pomegranate juice and peel (which was extracted using 99.9% ethanol), (Figure 4.9.a) the details of the absorbance of pomegranate peel were taken every 20 seconds even 5 minutes, and (Figure 4.9.b) the details of the absorbance of pomegranate juice were taken every 20 seconds even 5 minutes Simvastatin was used as the positive control in this study, and other investigations showed that pomegranate juice and peel had an impact on lowering cholesterol in mice and humans. (Mahdiyeh KhademHaghighian, et al,.2021; Michael Aviram, et al,.2022).

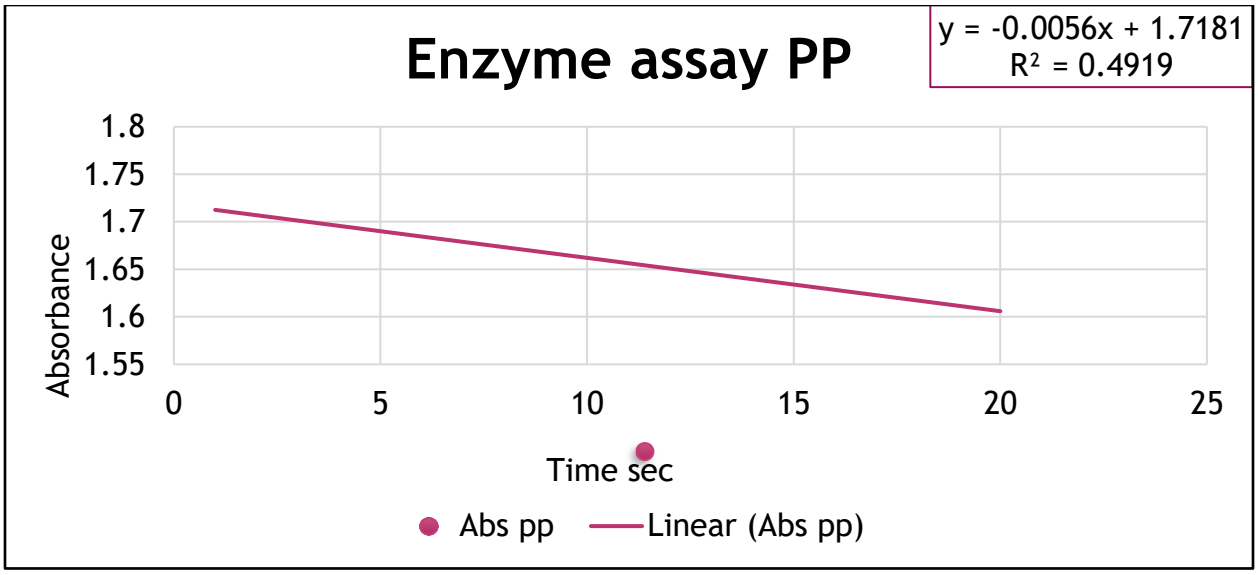



**Figure 4.9.a** the details of the absorbance of pomegranate peel was taken every 20 seconds even 5 minutes.

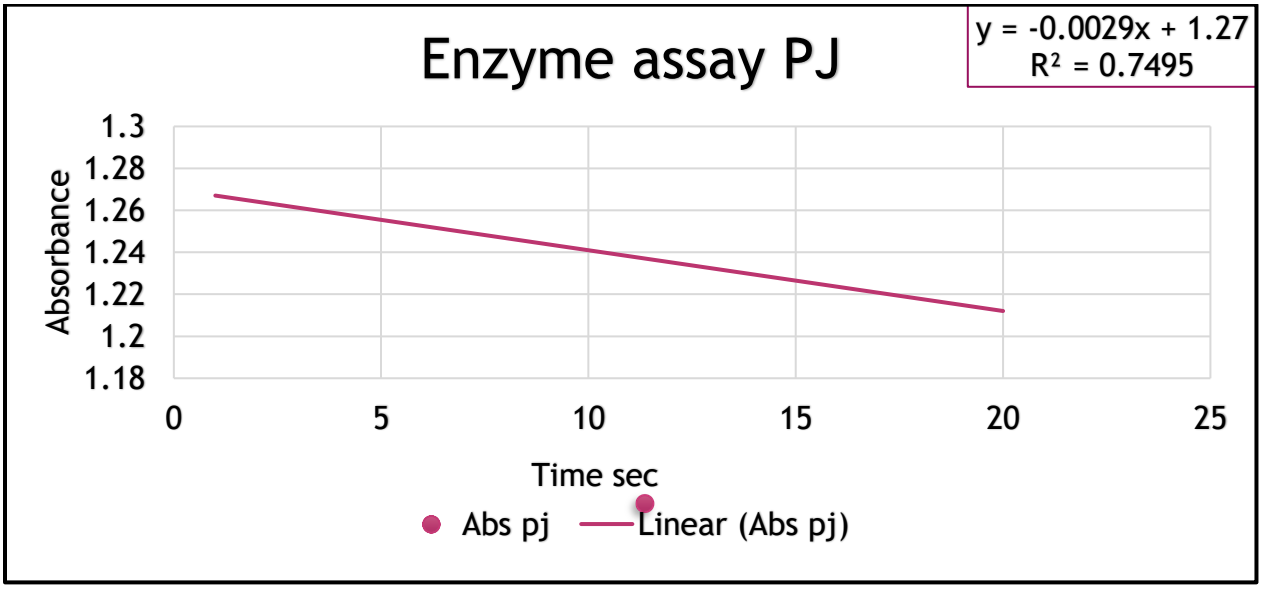



**Figure 4.9.b** the details of the absorbance of pomegranate juice were taken every 20 seconds even 5 minutes

# 5. Conclusion and Future work:

## 5.1 Conclusion:

Pomegranate juice and peel extract were studied for their chemical composition. This plant was harvested from Palestinian trees and processed in a dedicated natural juice store.

The antioxidant content of plant extracts has aroused interest since they may be used as natural additives in place of synthetic ones. The antioxidant efficiency of pomegranate peel extract was primarily associated with flavonoids, then phenolic acids. Pomegranate juice was shown to have a lower quantity of phenolic and flavonoid compounds than pomegranate peel extract.

Pomegranate peels exhibited a greater antibacterial impact than pomegranate juice when tested against various microorganisms.

Pomegranate peels were also analyzed using HPLC to figure out the presence of flavonoids in the extract.

Finally, pomegranate fruit has been shown to have positive effects on human health. In human research, it has been shown that pomegranate juice lowers blood levels of both LDL-C and total cholesterol (TC). Pomegranate peel (PP) and pomegranate peel extract (PPE) and juice (PJ) show excellent antioxidant and hydroxyl radical scavenging activities, according to in vitro research.

## 5.2 Future work

Discovering additional applications for pomegranate peels and juice and conducting further research on these items to better understand how they affect an enzyme that lowers blood pressure in vitro and Vivo and to eventually produce and use therapeutic pharmaceutical supplements for the treatment of people and improve the quality of their lives.

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# قشر الرمان مقابل العصير: الخصائص والفوائد

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**المشرف الرئيسي: الدكتور ابراهيم الكيالي**
**المشرف الثاني: الدكتور جواد شقير**

## الملخص

**يحتوي قشر وعصير الرمان على مجموعة متنوعة من المركبات الفعالة ولها فوائد طبية، بما في ذلك مضادات الأكسدة والبولي فينول. ويحتوي العصير على المعادن والفيتامينات تم استخدام العديد من المستخلصات منها مستخلص الكحولي بنسبة 99.9% و35% ومستخلص الأثيل اسيتات، وفي هذا البحث من الممكن فهم خصائص ومزايا الرمان حيث تساعد في علاج مجموعة من الأمراض و يساعد في تحضير ادوية لأمراض مختلفة.**

**في هذه الدراسة ، تم استخراج قشر الرمان باستخدام جهاز استخلاص Soxhlet ، والذي كان قادرًا بشكل أساسي على إزالة جميع المركبات الفعالة من هذا النبات**

**تم استخدام اختبار Folin-Ciocalteu لقياس المحتوى الفينولي الكلي (TPC) لمستخلصات قشر الرمان وعصيرها. تم إجراء جميع الاختبارات باستخدام مقياس الطيف الضوئي المرئي للأشعة فوق البنفسجية (spectrophotometer). وتبين أن عصير الرمان وقشره يحتويان على مضادات للأكسدة ومحتوى الفينول بنسبة عالية. بينما نتج المحتوى الفينولي الكلي (TPC) من قشر الرمان المستخلص من الإيثانول 99.9٪ نتيجة (78.81 ملجم / جم) (أعلى من محتوى قشر الرمان المستخلص مع الإيثانول 35٪ وهو (74.94 ملجم / جم) والإيثيل اسيتات (56.61 مجم / جم) وعصير (23.88 مجم / جم) ، حيث ان محتوى الفلافونويد الكلي (TFC) من مستخلص الرمان مع الإيثانول بنسبة 35٪ (18.09 مجم / جم) أعلى من المستخلص بالإيثانول 99.9٪ ( 7.95 مجم / جم) وعصير رمان (0.0547 مجم / جم).**

**تم الكشف عن تحليل HPLC لمعايير مركبات البولي فينول والفلافونويد في عينة كروماتوجرام لقشر الرمان (المستخلص باستخدام الإيثانول) . بعد إجراء تحليلHPLC أظهرت النتائج وجود حمض روتين وجاليك**

**تم التعرف على النشاط المضاد للأكسدة (AA) لمستخلصات قشر الرمان باستخدام طريقة DPPH تم استخلاص قشر الرمان باستخدام الإيثانول 99.9٪ والإيثانول 35٪ كمذيب ، مما أسفر عن نتائج (51.9 مجم / جم) ، (9.86 مجم / جم) ، وعصير (16.79 مجم / جم) ، بالمقابل (76.8 مجم / جم). ) عند استخدام أسيتات الإيثيل كمذيب.**

**تم تحديد النشاط المضاد للبكتيريا من مستخلصات قشر الرمان (المستخلص باستخدام الإيثانول بنسبة 99.9٪) في المختبر باستخدام طريقة (agar disc diffusion). أظهرت النتائج أن النشاط المضاد للبكتيريا ضد Pseudomonas aeruginosa و Escherichia coli و Staphylococcus aureus والعصير ليس له تأثير مباشر على البكتيريا حيث ظهرت البكتيريا مخففة.**

**تم تقييم التكوين المضاد السكري للمنتج النهائي باستخدام اختبار الألبومين (BSA)في المختبر. أظهرت نتائج الدراسة أن مستخلص قشر الرمان مع الإيثانول له تأثير مضاد للسكري، بينما عصير الرمان له تأثير قليل على تقليل نسبة السكر**

**تم صُنع الكريم باستخدام قشر الرمان المستخلص بالإيثانول 99.9% وله العديد من الميزات، بما في ذلك من ترطيب و تنعيم البشرة ، وله رائحة لطيفة ، و يمتصه الجلد بسرعة (دون ترك غشاء) ، ولا يسبب تهيجًا للبشرة (درجة الحموضة = 5.5-6) ، لون ممتاز ، يتوزع بسهولة على البشرة**

**إن إنزيم HMG-CoA (3-هيدروكسي-3-ميثيل غلوتاريل-أنزيم أ) ينتج الكوليسترول. و ينتج الكبد نسبة أقل من الكوليسترول عند وجود اختزال HMG-CoA. تم استخدام (spectrophotometer) لقياس فعالية قشر الرمان (المستخلص بنسبة 99.9٪ من الإيثانول) و عصير الرمان على تقليل نسبة الكوليسترول ووجد ان قشر الرمان له تأثير مثبط بنسبة 45٪ ، وعصير الرمان له تأثير مثبط بنسبة 74٪.**