Antioxidant and Anti-diabetic Activities in Commercial and Homemade Pomegranate Molasses in Lebanon

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List of abbreviations

2,2’ –azino-bis(3-3thylbenzothiazoline-6-sulphonic acid ..... ABTS

Diphenyl picrylhydrazyl assay ..... DPPH assay

Ferric reducing antioxidant power assay ..... FRAP assay

Gas chromatography ..... GC

High pressure liquid chromatography ..... HPLC

Ethanol ..... EtOH

Pomegranate molasses ..... PM

mg Gallic Acid Equivalent/g pomegranate molasses ..... mg GAE/g PM

mg Quercetin Equivalent/g pomegranate molasses ..... mg QE/g PM

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Abstract

Pomegranate fruit and its derived products are rich sources of bioactive compounds that were shown to have many biological activities. Pomegranate molasses (PM) is a thick traditional Middle Eastern syrup used in many recipes for international cuisines from around the world, including the Lebanese cuisine. It is considered to be a highly nutritious product which makes it of great interest to researchers. The purpose of this study is to assess the phytochemical composition, antioxidant and anti-diabetic properties of homemade and commercial PM consumed in Lebanon using samples collected from households in rural areas (n=4) and the market (n=28), respectively. The objectives of this study are to determine their total phenolic content using the Follin-Ciocalteu method, their total flavonoid content using aluminum chloride method, their antioxidant activity using DPPH radical scavenging as well as ferrous ion chelating assays and their anti-diabetic activity using α-amylase and α-glucosidase inhibitory activities while using acarbose, a known anti-diabetic drug as the standard reference. Homemade PM samples exhibited a higher antioxidant activity than commercial samples, with the most active homemade PM having IC_{50} of DPPH: 0.09 mg/ml and % of chelation: 46.78. Also, homemade PM samples were found to have a higher anti-diabetic activity than commercial samples, with the most active homemade PM having IC_{50} against α-amylase: 0.63 mg/ml and IC_{50} against α-glucosidase: 0.405 mg/ml and being almost as active as acarbose with both enzymes (1.5 times). Significant inverse strong/moderate correlations were observed between total phenols content ($r = -0.542$)/total flavonoids content ($r = -0.483$) and IC_{50} of DPPH scavenging activity and significant negative moderate correlations were observed between total phenols/flavonoids contents and alpha amylase inhibitory activity (r: -0.436, p-value: 0.013 and r: -0.445, p-value: 0.011 respectively). Further investigation should be performed in order to identify and isolate the bioactive metabolites responsible for each activity.

Keywords

Pomegranate molasses • commercial • homemade • total phenolic content • flavonoid content • antioxidant activity • DPPH radical scavenging assay • ferrous ion chelating assay • anti-diabetic activity • α-amylase inhibitory assay • α-glucosidase inhibitory assay

Introduction

Fruits are rich sources of bioactive compounds with antioxidant potentials associated with diverse medicinal properties and health benefits (Li *et al.*, 2006). Recent studies have tackled those health benefits along with the composition and biological activities of fruits such as pomegranate and its derived products (Derakhshan *et al.*, 2018).

Phytochemicals and antioxidants abundantly present in fruits including flavonoids, tannins, and phenolic acids are known to help prevent several life-threatning diseases such as cardio and cerebro-vascular diseases and different types of cancers. (Li *et al.*, 2006; Derakhshan *et al.*, 2018). Bioactive compounds with antioxidant capacities dispel free radicals from cells to prevent lipid peroxidation reactions and any oxidative damage in order to preserve the cell structure and function and prevent food deterioration (Zou *et al.*, 2016).

Oxidative stress is the underling cause of many chronic diseases, in particular diabetes mellitus being associated with its onset and progression (Rains *et al.*, 2011). Diabetes mellitus (type 1 and 2) is a metabolic syndrome characterized mainly by hyperglycemia due to defects in the secretion and/or action of insulin with type 2 diabetes mellitus being the most common type spread worldwide. It is estimated that the number of type 2 diabetes mellitus patients in the world will increase from 415 million patients in 2015 to 642 million patients in 2040 (Khajebishak *et al.*, 2019). Since it is mainly linked to obesity and unhealthy eating habits, type 2 diabetes can be prevented by consuming more of natural bioactive compounds like the ones in fruits and vegetables due to their various properties such as antioxidant, anti-inflammatory anti-apoptotic and anti-diabetic effects (Rains *et al.*, 2011; Li *et al.*, 2019).

Pomegranate (*Punica granatum* L.) is an ancient fruit that belongs to the family Punicaceae and is widely grown in tropical and subtropical regions of the world. It is cultivated in Iran, India and Mediterranean countries such as Turkey, Tunisia, Egypt, Morocco and Lebanon, hence, it is commonly consumed in this region with its derived products (Erdrich *et al.*, 2015; Faour-Klingbeil & Todd, 2018). Pomegranate fruit is consumed fresh or as a juice or after processing into products such as pomegranate molasses that is added to salads and many dishes (Incedayi *et al.*, 2010).

Since ancient times, pomegranate has been broadly used in many cultures, being part of the folk medicine, as a "healing food" used to expel parasites and worm infections, to reduce fever and to treat ulcers, diarrhea, aphtae, acidosis, hemorrhage, dysentery, respiratory pathologies and microbial infections (Akpinar-Bayizit *et al.*, 2016). Also, it has been used as a traditional remedy for other illnesses like cancer, dental conditions, bacterial infections, antibiotic resistance, to repair skin damaged by ultraviolet radiations in addition to male infertility, Alzheimer's disease, arthritis, obesity and infant brain ischemia (Jurenka, 2008). In Lebanon, pomegranate extract was used in the treatment of diabetes mellitus (Raafat & Samy, 2014).

For many years, the pomegranate fruit has attracted the interest of researchers who aimed to identify the active secondary metabolites of its different parts due to its abundance and use in folk medicine. Based on the literature, the most common identified metabolites in the pomegranate fruit are ursolic acid and punicic acid in pomegranate seeds, hydroxycinnamic acids and ellagitannins (punicalin and punicalagin) in pomegranate juice and peels, apigenin in the fruit leaf and maslinic acid in its flower; in addition to other phenolic compounds, anthocyanins, hydrolysable tannins and flavonoids (Incedayi *et al.*, 2010; Lansky & Newman, 2007; Derakhshan *et al.*, 2018). Pomegranate molasses is considered to be a highly nutritious product since it is a concentration of pomegranate juice so it is assumed to have two to three folds higher mineral and antioxidant

content than fresh pomegranate juice which makes it of great interest to researchers to analyze and study in terms of secondary metabolites and biological activities (Yilmaz *et al.*, 2007). Several studies have been done on pomegranate fruit extract, juice, peel, seeds, flower, leaves and bark which confirmed their biological activities; pomegranate fruit and its parts were shown to have antioxidant activities, anti-diabetic, anti-inflammatory, anti-carcinogenic, anti-microbial, anti-diarrheal and neuro-protective properties (Elfalleh *et al.*, 2009; Hontecillas *et al.*, 2009; Sturgeon & Ronnenberg, 2010; Endo *et al.*, 2010; Zhao *et al.*, 2018; Bekir *et al.*, 2013). Fewer studies were conducted to determine the antioxidant activity of pomegranate molasses where this activity was associated to its high phenolic content and only one study was done to reveal its neuro-protective capacity (Derakhshan *et al.*, 2018, Hussein *et al.*, 2018).

Pomegranate molasses is a famous traditional condiment used in Lebanon with many commercial brands distributed in the market in addition to homemade pomegranate molasses made in rural areas. Only few commercial brands have been evaluated in terms of total phenolic content and antioxidant activity but to the best of our knowledge, pomegranate molasses hasn't been tested for its flavonoids content and anti-diabetic activity yet. Therefore, the purpose of this study is to assess the phytochemical composition, antioxidant and anti-diabetic capacities of homemade and commercial pomegranate molasses. The objectives of this study are to:

1) determine the total phenolic content of pomegranate molasses (commercial and homemade) samples using the Follin-Ciocalteu method and total flavonoids using aluminium chloride method

2) assess their antioxidant activity using DPPH radical scavenging as well as ferrous ion chelating assays and their anti-diabetic activity using α-Amylase and α-Glucosidase inhibitory activities

Chapter I Generalities

I.1. Introduction: botanical description and distribution of *Punica granatum* L.

Punicaceae is a plant family that comprises one genus named *Punica*. It was previously classified as a separate family but was recently placed as a subfamily under the family of Lythraceae by Angiosperm Phylogeny Group in 2009 (Allaby, 2012).

Several morphological features characterize the genus *Punica* L., primarily its fruit having a leathery pericarp and its pulpy seeds with edible sarcotesta (seed coat) (Narzary *et al.*, 2016). There are two species belonging to the genus *Punica* L., *Punica protopunica* Balf. fil. and *Punica granatum* Linn. (pomegranate). *Punica protopunica* Balf. f. is endemic in occurrence to Socotra Island, Yemen, whereas *Punica granatum* Linn. is more widespread; it originated from the Middle East, spread to Mediterranean countries, eastward to China, India, Japan and Russia and then to the American Southwest California and Mexico with over 1000 cultivars (Narzary *et al.*, 2016; Lansky & Newman, 2007; Levin, 1994). In addition, *Punica protopunica* Balf. f. differs by having pink flowers instead of the red flowers of *Punica granatum* L., and smaller, less sweet fruit (Levin, 1994). The global production of pomegranate was approximately 1,500,000 tons in 2009 and Iran is considered to be the primary origin of pomegranate contributing to around 47% to the total of world pomegranate production (FAO, 2012; Derakhshan *et al.*, 2018).

The taxonomic classification of Punica granatum is as follows (Haque *et al.*, 2015):

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Myrtales

Family: Punicaceae

Genus: *Punica*

Species: *granatum*

Synonyms: *Granatum punica* St Lag, *Punica florida* Salisb, *Punica multiflorida* Hort,exSiebold and Voss, *Punica nana* Linn, *Punica spinosa* Lam, and *Punica grandiflora* Hort.exSteud.

Vernacular name in Arabic: Rumman, رمّان

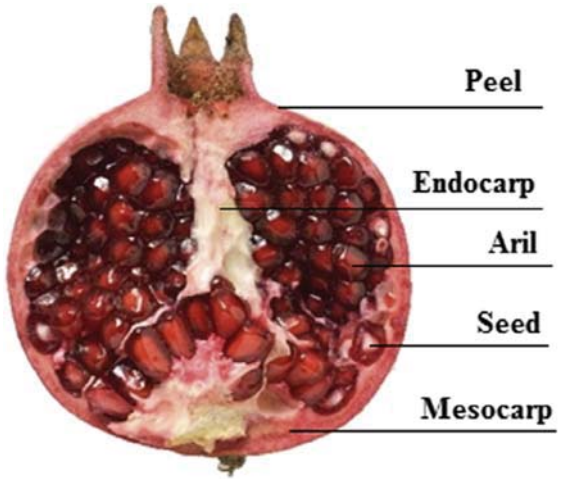

Fig. 1: Anatomical parts of pomegranate fruit

Pomegranate plant (*Punica granatum* L.) is considered a small tree or a large shrub with a stem that can reach five to ten meters high; it is very branchy and twisted with red-grey bark and thorny branches (Lansky & Newman, 2007; Russo *et al.*, 2012). The pomegranate rind is more or less arched, hard and brittle with a xanthous or reddish-brown color (Haque *et al.*, 2015). The leaves are deciduous, oblong, mostly opposite, stiff, shiny and 2.5-6.3cm long. The scarlet flowers are 3.8-5 cm long and they blossom at the ends of the branches from May to July (Lansky & Newman,

2007; Russo *et al.*, 2012, Haque *et al.*, 2015). Pomegranate fruit is a large round berry with a 3.8-7.5 cm diameter consisting of three parts: the seeds weighing 3% of the fruit with 20% oil, the juice weighing about 30% of the fruit and the peels weighing the rest including the interior network of membranes (Lansky & Newman, 2007). It is coated by a leathery pericarp and it is divided in 7-15 internal cavities where the seeds are surrounded by a sour and sweet, pink juicy pulp, forming the arils (Russo *et al.*, 2012). Thin membranes, with a pungent taste and yellow-chromatic color, spread into the interior of the fruit from the pericarp in order to suspend the arils (Lansky & Newman, 2007). Pomegranate fruit ripens in the fall and its cultivars vary in taste from sweet to sour depending on their organic acids and sugars content and nature (Russo *et al.*, 2012).

I.2. Pomegranate molasses description, production and use

Pomegranate molasses is a thick traditional Middle Eastern syrup, called “Dibs El Rumman” in Arabic, made from cooked down pomegranate juice; it is a sweet and sour condiment having a slight astringent taste and a deep dark ruby/red color (Yilmaz *et al.*, 2017; Yadav *et al.*, 2006). The level of its sweetness depends on the concentration of the fruit’s natural sugars (such as glucose, fructose, sucrose and maltose) and organic acids composition (such as citric and malic acids); the fruit sugar content is influenced by the variety of the cultivar, the climate and the degree of maturation of the fruit (Yilmaz *et al.*, 2017).

Fig. 2: Pomegranate molasses

The commercial production steps of pomegranate molasses include cleaning and crushing the pomegranate fruits, extraction, filtration, clarification and finally concentration of pomegranate juice by evaporation in an open container or under vacuum (Yilmaz *et al.*, 2017; Mahmoud *et al.*, 2015). The clarification step is commonly excluded since customers prefer the bitter and sour taste caused by phenolic substances and organic acids (Akpinar-Bayizit *et al.*, 2016). In rural areas, homemade pomegranate molasses is still produced using traditional methods without adding additives (including sugar) and with or without addition of citric acid (Mahmoud *et al.*, 2015). In general, about four cups of pomegranate juice are heated in a pan for 45 minutes in order to thicken without being overcooked in order to make half a cup of pomegranate molasses which is recommended to store is in air tight containers in the refrigerator (Dhumal *et al.*, 2014). The production process makes pomegranate molasses a highly nutritious product since it is then concentrated in minerals and antioxidants. The differences in the phenolic contents among different commercial brands or samples of homemade pomegranate molasses are due to those small variations in the production of pomegranate molasses (Mahmoud *et al.*, 2015; Dhumal *et al.*, 2014).

Pomegranate and its derivatives are abundantly cultivated, spread and added to many recipes in cuisines around the world. The pomegranate molasses is commonly used in cooking in countries such as Turkey, Iran as well as other Mediterranean countries like Lebanon and Syria. It is used as a flavoring agent like when used to flavor chutneys and curries, as a salad dressing, as a soft drink ingredient and to varnish or tenderize meat products due to its proteolytic enzymes (Yilmaz *et al.*, 2017; Dhumal *et al.*, 2014; Janbi & Al-Said, 2014). Thus, researchers have had a growing interest in screening and extracting their various secondary metabolites by running chemical analysis.

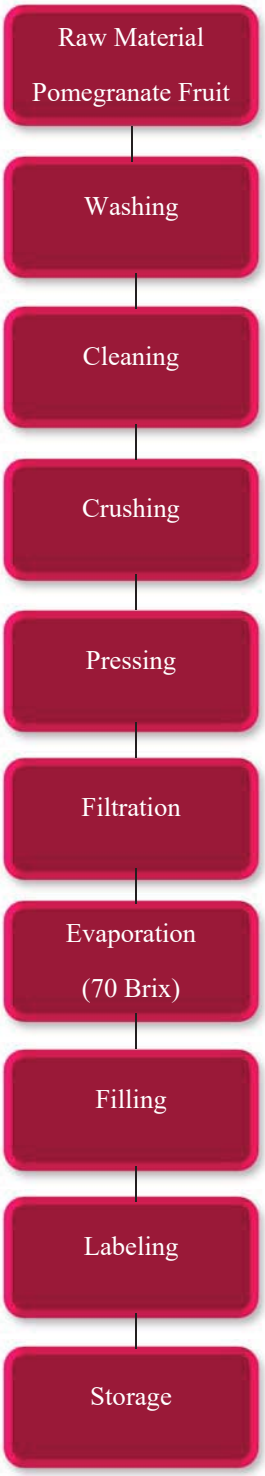

Fig. 3: The process of commercial pomegranate molasses production (Akpinar-Bayizit *et al.*, 2016)

I.3. Phytochemicals constituents of pomegranate fruit, juice and molasses

The phytochemical screening of pomegranate fruit (edible and non-edible parts), pomegranate juice, pomegranate molasses and others indicated the presence of various secondary metabolites such as phenolic acids, flavonoids, tannins, lignans, terpenoids and phytosterols, alkaloids, saponins, resins, fatty acids and lipids (Pande *et al.*, 2009; . Xie *et al.*, 2008; Fischer *et al.*, 2012; Ito *et al.*, 2012; Abd El Wahab *et al.*, 1998; Heftmann *et al.*, 1966; Neuhofer *et al.*, 1993; Fatope *et al.*, 2002; Yusuph *et al.*, 1997).

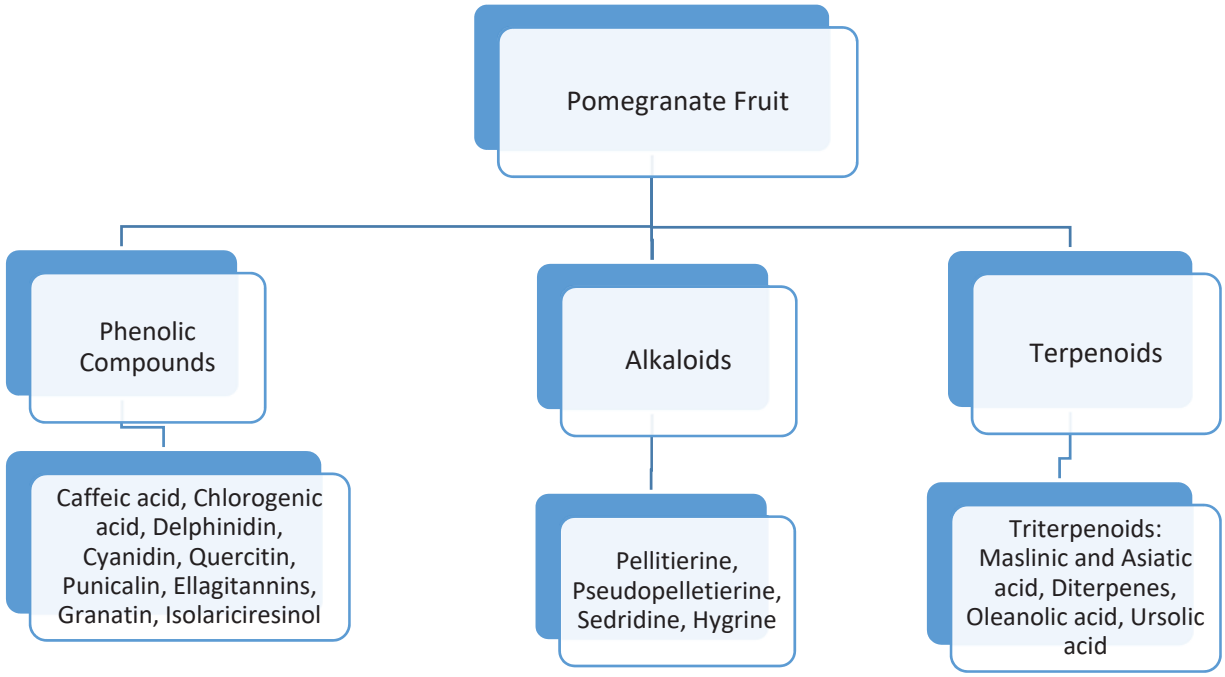

Fig. 4: Phytochemical composition of pomegranate fruit

I.3.1. Phenolic Compounds:

Having a wide range of structures and functions, phenolic compounds are secondary metabolites molecules widely found in fruits, synthesized in normal and tough circumstances such as UV

radiation and stress, respectively (Haminiuk *et al.*, 2012). They have an aromatic ring which holds one or more hydroxyl groups varying from simple phenols to highly polymerized compounds. They have antioxidant properties and are mostly represented by phenolic acids, flavonoids, tannins, lignans, saponins, resins (Haminiuk *et al.*, 2012).

I.3.1.1. Phenolic Acids/Organic Acids:

Phenolic acids are present in a bound form in fruits, comprise third of the dietary polyphenols and have one carboxylic group (-COOH) as their basic chemical structure responsible for their acidic character (Haminiuk *et al.*, 2012). They are divided into two subgroups: hydroxycinnamic and hydroxybenzoic acids. Being considered as derivatives, hydroxycinnamic acids have a C_6-C_3 skeleton such as caffeic acid, *p*-coumaric acid and ferulic acid. However, hydroxybenzoic acids have a C_6-C_1 skeleton and they are widely found in fruits as esters such as vanillic, ellagic, gallic and syringic acids (Haminiuk *et al.*, 2012); ellagic acid and gallic acid are two of the most extracted phenolic acids from pomegranate (*punica granatum* L. parts and extracts). Phenolic acids are shown to have antioxidant, anti-inflammatory, anti-diabetic, anti-carcinogenic and neuro-protective activities in several studies (Singh *et al.*, 2018; Lansky and Newman, 2007; Banihani *et al.*, 2013; Akpinar-Bayizit *et al.*, 2012; Hartman, *et al.*, 2006). Table 1 lists studies done on phenolic acids extracted from *Punica granatum* L. and its extracts/parts.

Table 1: Phenolic Acids and Other Phenolic Compounds Extracted from *Punica granatum* L.

Phytoconstituents	Part of Pomegranate	Country	References
Caffeic acid	Juice	India; Japan	Jurenka, 2008; Amakura *et al.*, 2000a
	Peel	Japan	Amakura *et al.*, 2000a
	Seed	USA	Pande *et al.*, 2009
	Leaf	Japan	Artik *et al.*, 1998
Chlorogenic acid	Juice, peel	Germany; Japan	Fischer *et al.*, 2011; Amakura *et al.*, 2000a

Hydroxycaffeic acid	Outer skin	USA	Ambigaipalan *et al.*, 2016
Caffeic acid hexoside	Outer skin and mesocarp	USA	Ambigaipalan et al., 2016
Protocatechuic acid	Outer skiN/Peel, Juice	Germany; USA	Fischer *et al.*, 2011; Ambigaipalan *et al.*, 2016
Quinic acid	Juice, peel	Japan	Amakura *et al.*, 2000a
Vanillic acid	Outer skin and mesocarp/peel, Juice	Italy; USA	Calani *et al.*, 2013; Ambigaipalan *et al.*, 2016
Ferulic acid	Juice, seed, peel, leaf	Italy; USA	Calani et al., 2013; Pande et al., 2009
Ferulic acid hexoside	Outer skin and mesocarp	USA	Ambigaipalan *et al.*, 2016
5-O-caffeoylquinic acid 353 6.2 111, 173, 191	Outer skin and mesocarp	USA	Ambigaipalan *et al.*, 2016
Citric acid	Outer skin and divider membrane	USA	Ambigaipalan *et al.*, 2016
p-hydroxybenzoic hexoside	Mesocarp	USA	Ambigaipalan *et al.*, 2016
Ascorbic acid	Leaf, seed, peel, juice	USA	Pande et al., 2009
Citric acid	Juice, leaf, peel, seed	Turkey; Italy; USA	Poyrazoğlu *et al.*, 2002; Mena *et al.*, 2012; Pande et al., 2009
Fumaric acid	Juice	Turkey; Spain	Poyrazoğlu *et al.*, 2002; Melgarejo *et al.*, 2000
L-malic acid	Juice, leaf, peel, seed	Turkey; Italy; USA	Poyrazoğlu *et al.*, 2002; Mena *et al.*, 2012; Pande et al., 2009
Oxalic acid	Juice, leaf, peel, seed	USA; Turkey	Pande et al., 2009; Poyrazoğlu *etal.*, 2002
Quinic acid	Pomegranate molasses: Darna, juice	Lebanon; Japan	Nasser *et al.*, 2017; Artik *et al.*, 1998
Succinic acid	Juice, leaf, peel, seed	Turkey; USA; Turkey	Poyrazoğlu *et al.*, 2002; Pande et al., 2009;
Tartaric acid	Juice	Turkey	Poyrazoglu *et al.* ,2002
Cinnamic acid	Juice	Japan	Artik *et al.*, 1998
O-Coumaric acid	Juice	Japan	Artik *et al.*, 1998
p-Coumaric acid	Peel, juice, seed, leaf	Germany; USA; USA	Fischer *et al.*, 2011; Ambigaipalan *et al.*, 2016; Pande *et al.*, 2009
Cis-p-Coumaric acid	Peel	USA	Ambigaipalan *et al.*, 2016

Coutaric acid	Peel	USA	Ambigaipalan *et al.*, 2016
7,8-Dihydroxy-3-carboxymethylcoumarin-5-carboxylic acid	Flower	China	Yuan *et al.*, 2013
Gallic acid	Peel, juice, flower	Italy; Germany; India	Calani *et al.*, 2013; Fischer *et al.*, 2011; Bagri *et al.*, 2010
Methyl gallate	Heartwood	Egypt	El Toumy *et al.*, 2001
Neochlorogenic acid (5-O-caffeoylquinic acid)	Peel, juice	Germany; USA	Fischer *et al.*, 2011; Ambigaipalan *et al.*, 2016
Coniferyl 9-O-[β-Dapiofuranosyl(1→6)]-O-β-Dglucopyranoside	Seed	China	Wang *et al.*, 2004
Sinapyl 9-O-[β-D-apiofuranosyl(1→6)]- O-β-D-glucopyranoside	Seed	China	Wang *et al.*, 2004

I.3.1.2. Flavonoids

Flavonoids are bioactive compounds with a C_6-C_3-C_6 carbon backbone, phenylbenzopyran structure and they are highly present in fruits as glycosides and sometimes as esters instead of free radicals (Haminiuk *et al.*, 2012).

They constitute almost two-third of the dietary phenolic compounds, having six subclasses: flavonols, flavan-3-ols, flavanones, isoflavones, flavones and anthocyanins and being responsible for most of the red, yellow and blue colors in fruits (Haminiuk *et al.*, 2012). Flavonoids are a natural potent source of antioxidants due to their free radical scavenging effect and metal chelating effect as shown in the *in vitro* tests (Singh *et al.*, 2018; Haminiuk *et al.*, 2012). Many studies reported other biological activities of flavonoids such as anti-diabetic, anti-proliferative, anti-inflammatory, anti-carcinogenic and neuro-protective effects (Zhou *et al.*, 2018; Seraam *et al.*, 2005; Akpinar-Bayizit, *et al.*, 2012; Hartman *et al.*, 2006). Table 2 lists studies done on flavonoids extracted from *Punica granatum* and its different parts/extracts.

Table 2: Flavonoids Extracted from *Punica granatum* L.

Phytonutrient	Part of Pomegranate	Country	References
Anthocyanins	Pomegranate fruit	Spain, Turkey	Perez-Vincente *et al.*, 2004; Incedayi *et al.*, 2010
	Juice	India, Italy, Palestine	Jurenka, 2008; Rinaldi *et al.*, 2010; Saad, 2015
	Pericarp	India, Lebanon	Jurenka, 2008; Chalfoun-Mounayar *et al.*, 2012
Delphinidin	Peel, juice	USA, India	Noda *et al.*, 2002; Chauhan *et al.*, 2001
Myrtillin (Delphinidin-3-O-glucoside) Delphinidin-3,5-di-O-glucoside	Pomegranate Fruit Extract (PFE)	USA	Afaq *et al.*, 2004
	Juice	Spain; Italy	Hernandez *et al.*, 1999; Gómez *et al.*, 2013
	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
Cyanidin	Peel, juce	USA	Noda *et al.*, 2002;
Chrysanthemin (Cyanidin-3-O-glucoside) Cyanin (Cyanidin-3,5-di-O-glucoside)	Pomegranate Fruit Extract (PFE)	USA	Afaq *et al.*, 2004
	Juice	Spain; Italy	Hernandez *et al.*, 1999; Gómez *et al.*, 2013
	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
Cyanidin pentoside derivatives	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
Antirrhinin (Cyanidin-3-O-rutinoside)	Outer skin and divider membrane; juice	USA; Italy	Ambigaipalan *et al.*, 2016; Gómez *et al.*, 2013
Catechin-cyanidin-3-hexoside	Juice	Itally	Gómez *et al.*, 2013
Pelargonidin	Peel, Juice	USA; Italy	Noda *et al.*, 2002; Gómez *et al.*, 2013
Callistephin (Pelargonidin-3-O-glucoside) Pelargonin (Pelargonidin-3,5-di-O-glucoside)	Pomegranate Fruit Extract (PFE)	USA	Afaq *et al.*, 2004
	Juice	Spain; Italy	Hernandez *et al.*, 1999; Gómez *et al.*, 2013
	Outer skin and divider membrane	USA	Ambigaipalan *et al.*, 2016
Catechins	Pomegranate fruit	Saudi Arabia	Al Hazzani *et al.*, 2013
	Juice	India; Palestine; Spain; USA	Jurenka, 2008; Saad, 2015; de Pascual-Teresa *et al.*, 2000; Ambigaipalan *et al.*, 2016

	Pericarp	India, Lebanon	Jurenka, 2008, Chalfoun-Mounayar *et al.*, 2012
	Outer skin/peel	USA; Spain; Italy	Ambigaipalan *et al.*, 2016; de Pascual-Teresa *et al.*, 2000; Mena *et al.*, 2012
	Leaf	USA	Pande *et al.*, 2009
Epicatechins	Juice, peel, leaf, seed	Spain; Italy; USA; USA	de Pascual-Teresa *et al.*, 2000; Mena *et al.*, 2012; Ambigaipalan *et al.*, 2016; Pande *et al.*, 2009
Gallocatechin-(4→8)-catechin Gallocatechin-(4→8)-gallocatechin Catechin-(4→8)-gallocatechin	Peel	UK	Plumb *et al.*, 2002
Gallocatechin	Juice	Palestine	Saad, 2015
	Outer skin	USA	Ambigaipalan *et al.*, 2016
Dihydroxygallocatechin	Outer skin	USA	Ambigaipalan *et al.*, 2016
Epicatechin gallate	Outer skin/Peel	USA	Ambigaipalan *et al.*, 2016
Epigallocatechin-3-O-gallate	Fruit	India	Chauhan *et al.*, 2001
Epigallocatechin galate (EGCG)	Juice	India; Spain	Jurenka, 2008; de Pascual-Teresa *et al.*, 2000
	Juice	Spain	de Pascual-Teresa *et al.*, 2000
Gallocatechin hexoside	Outer skin	USA	Ambigaipalan *et al.*, 2016
Quercetin	Juice; Pericarp (peel, rind), leaf, seed	India; Netherlands; USA	Jurenka, 2008; van Elswijk *et al.*, 2004; Pande *et al.*, 2009
Quercetin 3-O-rhamnoside	Outer skin, mesocarp and divider membrane/peel	USA	Ambigaipalan *et al.*, 2016
Quercetin hexoside	Outer skin and divider membrane	USA	Ambigaipalan *et al.*, 2016
Quercetin-3,4'-dimethyl ether 7-O-α-	Stem bark	Spain	de Pascual-Teresa *et al.*, 2000

Larabinofuranosyl(1-6)-β-Dglucoside			
Hirsutrin (Quercetin-3-O-glucoside)	Peel	USA	Ambigaipalan *et al.*, 2016
Quercimeritrin (Quercetin-7-O-glucoside)	Peel	USA	Ambigaipalan *et al.*, 2016
Rutin (Quercetin-3-O-rutinoside)	Juice; Pericarp (peel, rind)	India	Jurenka, 2008
	Pomegranate molasses	Saudi Arabia	Kamal *et al.*, 2018
	Juice	Japan	Artik *et al.*, 1998
Flavones	Pericarp (peel, rind)	India	Jurenka, 2008
Flavone glucosides: Luteolin and Apigenin	Leaves	India	Jurenka, 2008
Luteolin	Peel, flower	Netherlands; Japan; India	van Elswijk *et al.*, 2004; Xie *et al.*, 2008; Lal *et al.*, 2011
Luteolin 7-O-glycoside	Peel	Netherands	van Elswijk *et al.*, 2004
Luteolin 3'-O-β-glucopyranoside Luteolin 4'-O-β-glucopyranoside	Peel, leaf	Netherands; Egypt	van Elswijk *et al.*, 2004; Nawwar *et al.*, 1994a
Cynaroside (Luteolin 7-O-glycoside)	Peel	Netherlands	van Elswijk *et al.*, 2004
Luteolin 3'-O-β-xylopyranoside	Peel, leaf	Netherands; Egypt	van Elswijk *et al.*, 2004; Nawwar *et al.*, 1994a
Apigenin	Leaf, peel	Egypt; China	Nawwar *et al.*, 1994a; Zhao *et al.*, 2014
Apigenin 4'-O-β-glucopyranoside	Leaf	Egypt	Nawwar *et al.*, 1994a
Flavonones	Pericarp (peel, rind)	India	Jurenka, 2008
Naringin (Naringenin-7-O-rhamnoglucoside)	Peel	Korea	Kim *et al.*, 2002
Flavonols	Pericarp (peel, rind)	India	Jurenka, 2008
Flavan-3-ol	Peel and juice	Spain	de Pascual-Teresa *et al.*, 2000
Phlorizin	Outer skin, flower	USA; China	Ambigaipalan *et al.*, 2016; Yuan *et al.*, 2013
Kaempferol	Peel	Netherlands	van Elswijk *et al.*, 2004
Astragalin (Kaempferol 3-O-glucoside)	Outer skin, mesocarp and divider membrane/Peel	USA; Netherlands	Ambigaipalan *et al.*, 2016; van Elswijk *et al.*, 2004

Kaempferol 3-O-rhamnoglycoside	Peel, juice	Netherlands; Italy	van Elswijk *et al.*, 2004; Mena *et al.*, 2012
cis-dihydrokaempferol hexoside	Outer skin and mesocarp	USA	Ambigaipalan *et al.*, 2016
Syringetin hexoside	Outer skin and divider membrane	USA	Ambigaipalan *et al.*, 2016
Pelargonidin-3-O-glucoside	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
Hovetrichoside C	Flower	China	Yuan *et al.*, 2013
Phloretin	Juice	Italy	Mena *et al.*, 2012
Eriodictyol-7-O-α-L-arabinofuranosyl (1-6)-β-D-glucoside	Stem bark	India	Srivastava *et al.*, 2001
Granatumflavanyl xyloside	Flower	India	Bagri *et al.*, 2010
Naringenin-4'methyl ether 7-O-α-Larabinofuranosyl(1-6)-β-D-glucoside	Stem bark	India	Srivastava *et al.*, 2001
Pinocembrin	Juice	Italy	Mena *et al.*, 2001
Punicaflavanol	Flower	India	Bagri *et al.*, 2010
Tricetin	Flower, peel	Japan; India	Xie *et al.*, 2008; Lal *et al.*, 2001
Daidzein	Seed	Egypt	Moneam *et al.*, 1988
Genistein	Seed	Egypt	Moneam *et al.*, 1988
Amurensin (Noricaritin 7-β-D-glucopyranoside)	Juice	Italy	Gómez *et al.*, 2013
Myricetin	Peel	China	Zhao *et al.*, 2014
Phellatin	Juice	Italy	Gómez *et al.*, 2013
Procyanidin A2	Peel	USA	Ambigaipalan *et al.*, 2016
Procyanidin B1	Peel	USA	Ambigaipalan *et al.*, 2016
Procyanidin B2	Peel	USA	Ambigaipalan *et al.*, 2016
Procyanidin B2	Peel	USA	Ambigaipalan *et al.*, 2016

I.3.1.3. Tannins:

Tannins are polyhydroxy-flavan-3-ol oligomers and polymers of phenolic compounds, C-C bonds between favanol subclasses, commonly found in fruits giving them an astringent and bitter taste. Tannins are of different molecular weights and they are divided into two main types: condensed tannins such as proanthocyanidins present in grapes (astringent) and hydrolysable tannins such as tannic acid found in tea (soluble in water) and both types were found in the pomegranate fruit (Haminiuk *et al.*, 2012). Studies have shown that tannins exert different biological activities including antioxidant, anti-inflammatory, anti-carcinogenic neuro-protective and anti-diabetic activities: punicalagin (Seraam *et al.*, 2004; Husain *et al.*, 2019; Koren-Gluzer *et al.*, 2011). Some tannins found in pomegranate parts and extracts are listed in table 3.

Table 3: Tannins Extracted from *Punica granatum* L.

Phytoconstituents	**Part of Pomegranate**	**Country**	**References**
Ellagitannins	Pomegranate fruit	Spain; Turkey	Perez-Vincente *et al.*, 2004; Incedayi *et al.*, 2010
	Pomegranate Fruit Extract	USA	Afaq *et al.*, 2004
Ellagic acid	Peel	Egypt	Nawwar *et al.*, 1994b
	Leaf	China	Wang *et al.*, 2006
	Pomegranate fruit	Saudi Arabia	Al Hazzani *et al.*, 2013
	Juice	India; Italy; Japan; China	Jurenka, 2008; Rinaldi. *et al.*, 2010; Amakura *et al.*, 2000b; Wang *et al.*, 2004
	Pomegranate seed oil	India; Japan; China	Jurenka, 2008; Amakura *et al.*, 2000b; Wang *et al.*, 2004
	Pomegranate peel extract	Lebanon; Japan; China	Chalfoun-Mounayar *et al.*, 2012; Amakura *et al.*, 2000b; Wang *et al.*, 2004

	Pomegranate molasses	Saudi Arabia	Kamal *et al.*, 2018
3,3′-Di-O-methylellagic acid	Seeds	China	Wang *et al.*, 2004
3,3′,4′-Tri-O-methylellagic acid	Seeds	China	Wang *et al.*, 2004
3-O-methylellagic acid	Heartwood	Egypt	El Toumy et al., 2003
4,4′-Di-O-methylellagic acid	Heartwood	Egypt	El Toumy et al., 2003
3′-O-methyl-3,4-methylenedioxy-ellagic acid	Heartwood	Egypt	El Toumy *et al.*, 2001
Eschweilenol C (Ellagic acid 4-O-α-L-rhamnopyranoside)	Heartwood	Egypt	El Toumy *et al.*, 2001
Ellagic acid pentoside (include in derivatives with ones below?)	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
Ellagic acid deoxyhexoside	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
Ellagic acid hexoside	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
Ellagic acid derivatives	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
	Seed	China	Wang *et al.*, 2004
Corilagin	Peel and leaf	Japan; Egypt; Japan	Satomi *et al.*, 1993; Nawwar *et al.*, 1994b; Tanaka *et al.*, 1985
Isocorilagin	Flower	China	Yuan *et al.*, 2012
Hippomanin A	Flower	China	Yuan *et al.*, 2013
Gemin D	Flower	China	Yuan *et al.*, 2013
Gallagyldilacton	Peel	Japan	Satomi *et al.*, 1993
Pedunculagin	Peel	Japan	Satomi *et al.*, 1993
Tellimagrandin I	Peel	Japan	Satomi *et al.*, 1993
1,2,3-Tri-*O*-galloyl-β-^{4}C1-glucose	Leaf	Egypt	Nawwar *et al.*, 1994b
Punigluconin 2,3-di-O-galloyl-4,6-	Bark and roots	Japan	Tanaka *et al.*, 1986b

(S)hexahydroxydiphenoylglu conic acid			
Punicalin	Pomegranate Fruit Extract	USA	Afaq *et al.*, 2004
	Peel	Egypt	El Toumy *et al.*, 2002
	Aril	Japan	Ito *et al.*, 2014
	Juice	Japan	Tanaka *et al.*, 1986a;
	Heartwood	Germany	Fischer *et al.*, 2011
	Leaf	Japan; USA	Tanaka *et al.*, 1986a; Gil *et al.*, 2000
	Roots, stem bark and leaves	India; Japan; USA	Jurenka, 2008; Tanaka *et al.*, 1986a; Gil *et al.*, 2000
Punicalagin A Punicalagin B	Peel, stem bark, aril, juice, roots, leaf	Japan; Japan; Japan; Peru; USA; USA	Xie *et al.*, 2008; Ito *et al.*, 2014; Tanaka *et al.*, 1986a; Fischer *et al.*, 2011; Ono *et al.*, 2012; Gil *et al.*, 2000
Punicalagin derivatives	Pomegranate Fruit Extract	USA; Japan; USA	Afaq *et al.*, 2004; Tanaka *et al.*, 1986a; Gil *et al.*, 2000
	Pericarp, roots, bark	India	Jurenka, 2008
	Pomegranate fruit	Turkey	Incedayi *et al.*, 2010
	Juice	Italy, Palestine	Rinaldi. *et al.*, 2010; Saad, 2015
	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
Granatin A	Peel	Japan	Tanaka *et al.*, 1990
	Pomegranate fruit	Japan	Tanaka *et al.*, 1985
	Leaf	USA	Steinmetz *et al.*, 2010
Granatin B	Peel	Japan	Tanaka *et al.*, 1990
	Pomegranate fruit	Japan	Tanaka *et al.*, 1985
	Leaf	USA	Steinmetz *et al.*, 2010
Punicafolin	Leaf	India; Egypt	Jurenka, 2008; Nawwar *et al.*, 1994b; Tanaka *et al.*, 1985
Gallotannins	Pomegranate fruit	Saudi Arabia; Turkey	Al Hazzani *et al.*, 2013; Incedayi *et al.*, 2010
Hydrolysable tannins	Pomegranate fruit extract	Saudi Arabia	Al Hazzani *et al.*, 2013
Pedunculagin	Pomegranate fruit extract	USA	Afaq *et al.*, 2004
	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016

Galloyl hexoside derivatives	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
Valoneic acid bilactone	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
	Juice, Peel	Germany	Fischer et al., 2011
HHDP hexoside	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan et al., 2016
Monogalloyl-diglucose	Outer skin and divider membrane	USA	Ambigaipalan et al., 2016
HHDP-diglucoside (esterified)	Outer skin	USA	Ambigaipalan et al., 2016
bis-HHDP-hexoside	Divider membrane	USA	Ambigaipalan et al., 2016
Galloylglucopyranose (tri, tetra, penta)	Outer skin (tri and tetra only) and divider membrane	USA	Ambigaipalan et al., 2016
Galloyl-HHDP-glucoside derivatives	Mesocarp	USA	Ambigaipalan et al., 2016
Brevifolin	Leaf	Egypt	Nawwar *et al.*, 1994b
Brevifolin carboxylic acid	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
	Leaf	Egypt	Hussein *et al.*, 1997; Nawwar *et al.*, 1994b
	Flower	China	Yuan *et al.*, 2013
	Heartwood	Egypt	El Toumy et al., 2003
Brevifolin carboxylic acid 10-monopotassium sulphate	Leaf	Egypt	Hussein *et al.*, 1997
Ethyl brevifolincarboxylate	Flower	China	Wang *et al.*, 2006
Castalagin	Stem bark	Japan	Tanaka *et al.*, 1986b
Casuariin	Stem bark	Japan	Tanaka *et al.*, 1986b
Casuarinin	Stem bark and peel	Japan; Japan	Tanaka *et al.*, 1986b; Satomi *et al.*, 1993
Diellagic acid rhamnosyl(1→4) glucopyranosid	Heartwood	Egypt	El Toumy *et al.*, 2002
1,2-Di-O-galloyl-4,6-O-(S)-hexahydroxydiphenoyl β-Dglucopyranoside	Flower	Japan	Xie *et al.*, 2008
Eucalbanin B	Aril	Japan	Ito *et al.*, 2014
Eucarpanin T1	Aril	Japan	Ito *et al.*, 2014
Pomegraniin A	Aril	Japan	Ito *et al.*, 2014
Pomegraniin B	Aril	Japan	Ito *et al.*, 2014
Gallic acid 3-O-β-D-(6′-O-galloyl)- glucopyranoside	Flower	China	Yuan *et al.*, 2013

Gallic acid	Pomegranate fruit and extract	Saudi Arabia	Al Hazzani *et al.*, 2013
	Juice, pericarp (peel, rind) and flower	India; Japan; Australia and Japan	Jurenka, 2008; Amakura *et al.*, 2000b; Huang *et al.*, 2005b
	Pomegranate peel extract	Lebanon	Chalfoun-Mounayar *et al.*, 2012
	Pomegranate molasses	Saudi Arabia	Kamal *et al.*, 2018
	Outer skin, mesocarp and divider membrane	USA	Ambigaipalan *et al.*, 2016
6-O-galloyl-2,3-(S)-hexahydroxydiphenoyl-D-glucose	Stem bark	Japan	Tanaka *et al.*, 1986a
	Juice	Italy	Calani *et al.*, 2013
5-Galloylpunicacortein D	Heartwood	Egypt	El Toumy *et al.*, 2002
2-O-galloylpunicalin (2-O-galloyl-4,6-(S,S)-gallagyl-Dglucose)	Heartwood	Egypt	El Toumy *et al.*, 2002
	Stem bark	Japan	Tanaka *et al.*, 1986a
2,3-(S)-hexahydroxydiphenoyl-D-glucose	Stem bark	Japan	Tanaka *et al.*, 1986a
	Juice	Italy	Calani *et al.*, 2013
Lagerstannin B	Peel	Germany	Fischer *et al.*, 2011
Lagerstannin C	Juice	Japan	Calani *et al.*, 2013
3-O-methylellagic acid 4-O-α-Lrhamnopyranoside	Heartwood	Egypt	El Toumy *et al.*, 2002
3,4'-O-dimethylellagic acid 4-O-α-Lrhamnopyranoside	Heartwood	Egypt	El Toumy *et al.*, 2002
Oenothein B	Aril	Japan	Ito *et al.*, 2014
Pedunculagin I	Peel	Japan	Tanaka *et al.*, 1986a
	Stem bark	Italy	Calani *et al.*, 2013
Pedunculagin II	Juice	Italy	Calani *et al.*, 2013
1,2,3,4,6-Penta-O-galloyl-β-D-glucose	Leaf	Japan	Tanaka *et al.*, 1985
3,4,8,9,10-Pentahydroxydibenzo[b,d] pyran-6-one (Urolithin M-5)	Leaf	Egypt	Nawwar *et al.*, 1994b
Phyllanthusiin E	Flower	China	Wang *et al.*, 2006
Pomegranatate	Flower	China	Wang *et al.*, 2006
Punicacortein A	Stem bark and roots	Japan	Tanaka *et al.*, 1986b
Punicacortein B	Stem bark and roots	Japan	Tanaka *et al.*, 1986b
Punicacortein C	Stem bark and roots	Japan	Tanaka *et al.*, 1986b
	Peel	Japan	Ito *et al.*, 2014
Punicacortein D	Stem bark and roots	Japan	Tanaka *et al.*, 1986b
Punicatannin A Punicatannin B	Flower	China	Yuan *et al.*, 2012
Punigluconin	Stem bark	Japan	Tanaka et al., 1986b

Strictinin [1-O-galloyl-4,6-(S) -hexahydroxydiphenoyl-D-glucose]	Leaf	Japan	Tanaka *et al.*, 1985
Tercatain [1,4-Di-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-β-glucopyranose]	Leaf	Egypt	Hussein *et al.*, 1997
Terminalin (Gallagyl dilactone)	Stem bark	Japan	Tanaka *et al.*, 1986a
1,2,4,6-Tetra-O-galloyl-β-D-glucose	Leaf	Japan	Tanaka *et al.*, 1985
1,2,3-Tri-O-galloyl-β-glucopyranose	Leaf	Egypt	Nawwar *et al.*, 1994b
1,2,4-Tri-O-galloyl-β-glucopyranose	Leaf	Egypt	Hussein *et al.*, 1994
1,2,6-Tri-O-galloyl-β-glucopyranose	Leaf, Flower	Egypt; Japan	Nawwar *et al.*, 1994b; Xie *et al.*, 2008
1,3,4-Tri-O-galloyl-β-glucopyranose	Leaf	Egypt	Hussein *et al.*, 1994
1,4,6-Tri-O-galloyl-β-glucopyranose	Leaf	Egypt	Nawwar *et al.*, 1994b
3,4,6-Tri-O-galloyl-β-glucopyranose	Flower	China	Yuan *et al.*, 2013
Valoneic acid dilactone	Juice, peel	Germany	Fischer *et al.*, 2011

I.3.1.4. Lignans:

Lignans are glycosides that have two phenypropanoid dimers linked through their side chain C_8 carbons. They are one of the main phytoestrogens, found in fruits but not in very high amounts since fruits are not their main dietary source (Haminiuk *et al.*, 2012). Table 4 summarizes some lignans derivatives found in pomegranate fruit parts and its extracts.

Table 4: Lignans Derivatives Extracted from *Punica granatum* L.

Phytoconstituents	**Part of Pomegranate**	**Country**	**References**
-	Pomegranate juice	Italy	Rinaldi. *et al.*, 2010
Coniferyl 9-O-[β-D-dapiofuranosyl(1→6)]-*O*-β-D-glucopyranoside	Seed	China	Wang *et al.*, 2004
Sinapyl 9-O-[β-D-dapiofuranosyl(1→6)]-O-β-D-glucopyranoside	Seed	China	Wang *et al.*, 2004
Conidendrin	Juice	Germany	Fischer *et al.*, 2012

Isohydroxymatairesinol	Peel	Germany	Fischer *et al.*, 2012
Isolariciresinol	Juice, peel	Germany	Fischer *et al.*, 2012
Matairesinol	Wood knot	Italy	Bonzani *et al.*, 2009
Medioresinol	Juice, wood knot, seed	Italy	Bonzani *et al.*, 2009
Phylligenin	Peel	Germany	Fischer *et al.*, 2012
Pinoresinol	Peel	Italy	Mena *et al.*, 2012
Secoisolariciresinol	Peel, juice	Germany	Fischer *et al.*, 2012
Syringaresinol	Juice, wood knot, peel, seed	Italy	Mena *et al.*, 2012
Pomegralignan	Aril, peel	Japan	Ito *et al.*, 2014
Punicatannin C	Flower	China	Yuan *et al.*, 2013

I.3.1.5. Other Compounds:

Table 5: Other Compounds Extracted from *Punica granatum* L.

Phytoconstituents	**Part of Pomegranate**	**Country**	**References**
Coumestrol	Juice	Japan	Artik *et al.*, 1998
Coumestrol	Seed	Egypt; USA	Moneam *et al.*, 1988; Micheli *et al.*, 1962
Icariside D1	Seed	China	Wang *et al.*, 2004
Phenethyl rutinoside	Seed	China	Wang *et al.*, 2004
Syringaldehyde	Juice	Italy	Mena *et al.*, 2012

I.3.2. Terpenes

Terpenoids are derived from isoprenoid compounds formed through the concentration of C5 isoprene by catalyzing different terpene synthases as substrates and leading to the formation of hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20) and others (Rodríguez *et al.*, 2014).

Terpenoids volatiles are low molecular terpenes responsible for the floral scents of fruits, vegetables and essential oils. They are produced by plants in green tissues to repel herbivores and attract carnivores and by flowers/mature fruits to attract pollinators (Rodríguez *et al.*, 2014). Table 5 represents a summary of some terpenoid derivatives extracted from pomegranate fruit (parts and extracts).

Table 6: Terpenoid Derivatives Extracted from *Punica granatum* L.

Phytoconstituents	Part of Pomegranate	Country	References
Diterpenes	Pomegranate molasses: Darna, Dates	Lebanon	Nasser *et al.*, 2017
Ursolic acid	Flower	India; Taiwan; Japan	Jurenka, 2008; Huang *et al.*, 2005c; Xie *et al.*, 2008
	Seed		Ahmed *et al.*, 1995
Beta-carotene	Juice	Palestine	Saad, 2015
Oleanolic acid	Flower	Australia; Japan	Huang *et al.*, 2005a; Xie *et al.*, 2008
Maslinic acid	Flower	Iran; Japan; India	Batta and Rangaswami (1973); Xie *et al.*, 2008; Jurenka, 2008
Asiatic acid	Flower	Iran; Japan; India	Batta and Rangaswami (1973); Xie *et al.*, 2008; Jurenka, 2008
Daucosterol	Seed, flower	China	Wang *et al.*, 2004; Wang *et al.*, 2006
Campesterol	Seed	Egypt	Abd El Wahab *et al.*, 1998
Stigmasterol	Seed	Egypt	Abd El Wahab *et al.*, 1998
β-Sitosterol	Seed, flower	Egypt; Japan	Abd El Wahab *et al.*, 1998; Xie *et al.*, 2008
β-Sitosterol laurate	Peel	India	Lal *et al.*, 2011
β-Sitosterol myristate	Peel	India	Lal *et al.*, 2011
Cholesterol	Seed	Egypt	Abd El Wahab *et al.*, 1998
17-α-Estradiol	Seed	Korea; Netherlands	Kim *et al.*, 2002; Lansky *et al.*, 2005a
Estrone	Seed	USA; UK; Egypt	Heftmann *et al.*, 1966; Dean *et al.*, 1971; Abd El Wahab *et al.*, 1998
Testosterone	Seed	Egypt	Abd El Wahab *et al.*, 1998
Estriol	Seed	Egypt	Abd El Wahab *et al.*, 1998
Punicanolic acid	Flower, peel	Japan, India	Xie *et al.*, 2008; Lal *et al.*, 2011

I.3.3. Alkaloids

Alkaloids are natural compounds characterized by a ring structure and a nitrogen atom where the nitrogen is located in most cases inside the ring. Alkaloid-containing plants were known to be used

mainly as poisons, insecticides, medicines and stimulants since former ages and are still incorporated in our regular diet especially in the Western diet like in vegetables and tea (Koleva *et al.*, 2012). Table 6 summarizes some alkaloid derivatives present in pomegranate fruit (parts and extracts).

Table 7: Alkaloid Derivatives Extracted from *Punica granatum* L.

Phytoconstituents	**Part of Pomegranate**	**Country**	**References**
Pelletierine	Pomegranate peel, stem, root bark	Yogosavia (now Croats, Slovenes); Cuba	Neuhofer *et al.*, 1993, Vidal *et al.*, 2003
N-methylpelletierine	Stem, root bark	Yogosavia (now Croats, Slovenes)	Neuhofer *et al.*, 1993
Pseudopelletierine	Stem, root bark	Yogosavia (now Croats, Slovenes)	Neuhofer *et al.*, 1993
Norpseudopelletierine	Stem, root bark	Yogosavia (now Croats, Slovenes)	Neuhofer *et al.*, 1993
Sedridine	Root bark	Yogosavia (now Croats, Slovenes)	Neuhofer *et al.*, 1993
2-(2'-Hydroxypropyl)Δ^{1}-piperideine	Root bark	Yogosavia (now Croats, Slovenes)	Neuhofer *et al.*, 1993
2-(2'-Propenyll)Δ^{1}-piperideine	Root bark	Yogosavia (now Croats, Slovenes)	Neuhofer *et al.*, 1993
N-(2',5'Dihydroxyphenyl) pyridium chloride	Leaf	Egypt	Nawwar *et al.*, 1994a
Hygrine	Root bark	Yogosavia (now Croats, Slovenes)	Neuhofer *et al.*, 1993
Norhygrine	Root bark	Yogosavia (now Croats, Slovenes)	Neuhofer *et al.*, 1993
N-(2',5'-dihydroxyphenyl)pyridinium chloride	Leaf	Egypt	Nawwar *et al.*, 1994a
Punigratane (2,5-Diheptyl-N-methylpyrrolidine)	Peel	India	Rafiq *et al.*, 2016
Melatonin	Fruit extract	Egypt	Badria, 2004
Serotonin	Fruit extract	Egypt	Badria, 2004
Tryptamine	Fruit extract	Egypt	Badria, 2004

I.3.4. Fatty acids and lipids:

Table 8: Fatty acids and lipids Extracted from *Punica granatum* L.

Phytoconstituents	**Part of Pomegranate**	**Country**	**References**

Cerebroside	Pomegranate seed	Japan	Tsuyuki *et al.*, 1981
Caprylic acid (Octanoic acid)	Juice	Spain	Andreu-Sevilla *et al.*, 2013
Capric acid (Decanoic acid)	Juice	Spain	Andreu-Sevilla *et al.*, 2013
Lauric acid (Dodecanoic acid)	Seed	Iran	Akbari *et al.*, 2015
Myristic acid (Tetradecanoic acid)	Fruit, Seed	USA	Pande *et al.*, 2009
Myristoleic acid (9-cis-Tetradecanoic acid)	Seed	Iran	Akbari *et al.*, 2015
Palmitic acid (Hexadecanoic acid)	Fruit, seed	USA; Spain	Pande *et al.*, 2009; Fernandes *et al.*, 2015
Palmitoleic acid (Hexadec-9-enoic acid)	Fruit, seed	USA	Pande *et al.*, 2009
Punicic acid (9Z, 11E, 13Z-octadecatrienoic acid)	Seed	Spain	Fernandes *et al.*, 2015
Linoleic acid (cis, cis-9,12-Octadecadienoic acid)	Fruit, seed	USA; Spain	Pande *et al.*, 2009; Fernandes *et al.*, 2015
α-Linolenic acid (All-cis-9,12,15-octadecatrienoic acid)	Fruit, seed	USA	Pande *et al.*, 2009
γ-Linolenic acid (All-cis-6,9,12-octadecatrienoic acid)	Fruit, seed	USA	Pande *et al.*, 2009
Oleic acid (9Z-octadecenoic acid)	Fruit, seed	USA; Sultanat Oman	Pande *et al.*, 2009; Fatope *et al.*, 2002
Stearic acid (Octadecanoic acid)	Fruit, seed	USA; Sultanat Oman	Pande *et al.*, 2009; Fatope *et al.*, 2002
α-Eleostearic acid (9Z, 11E, 13E-octadecatrienoic acid)	Seed	Turkey	Topkafa *et al.*, 2015
β-Eleostearic acid (9E, 11E, 13E-octadecatrienoic acid)	Seed	Turkey	Topkafa *et al.*, 2015
Catalpic acid (9E, 11E, 13Z-octadecatrienoic acid)	Seed	Turkey	Topkafa *et al.*, 2015
Arachidic acid (Eicosanoic acid)	Fruit, seed	USA; Sultanat Oman	Pande *et al.*, 2009; Fatope *et al.*, 2002
Gadoleic acid (9Z-icosenoic acid)	Seed	Turkey	Topkafa *et al.*, 2015
Behenic acid (Docosanoic acid)	Seed	Turkey	Topkafa *et al.*, 2015
Nervonic acid (cis-15-Tetracosenoic acid)	Seed, fruit	USA	Pande *et al.*, 2009

1-O-9E,11Z,13E-octadecatrienoyl glycerol	Seed, peel	India; Sultanate Oman	Lal *el al.*, 2011; Fatope *et al.*, 2002
1-O-isopentyl-3-O-octadec-2-enoyl glycerol	Seed, peel	India; Sultanate Oman	Lal *el al.*, 2011; Fatope *et al.*, 2002
Tri-O-punicylglycerol	Seed	UK	Yusuph *et al.*, 1997
Di-O-punicyl-O-octadeca-8Z, 11Z, 13Etrienylglycerol	Seed	UK	Yusuph *et al.*, 1997
N-palmitoyl cerebroside	Seed	Japan	Tsuyuki *et al.*, Japan

I. 4. Traditional uses of pomegranate fruit parts and pomegranate molasses in folk medicine

Since ancient times, pomegranate has been broadly used in many cultures, being part of the folk medicine, as a "healing food" used to expel parasites and worm infections, to reduce fever and to treat ulcers, diarrhea, aphtae, acidosis, hemorrhage, dysentery, respiratory pathologies and microbial infections (Akpinar-Bayizit *et al.*, 2016). In the traditional Arab medicine and for a long time, pomegranate has been used in treating many illnesses such as sore throat, inflammation and rheumatism and these uses are also common in the Mediterranean region, Iran and India where the fruit is widespread (Saad, 2015). It was also known to resolve diarrhea and colic, remove worms in children, treat bladder disturbances, smooth mouth ulcers and strengthen gums, specifically the pomegranate seeds and peels (Saad, 2015). Additionally, mixing pomegranate seed, juice and peel have been known to prevent abortion and conception (Lansky & Newman, 2007). Also, the Ayurvedic medicine used the bark and roots of pomegranate due to their anthelmintic and vermifuge benefits, the pomegranate peels due to their ability to heal oral aphtae, diarrhea and ulcers, as well as the pomegranate juice as a "blood tonic" (Jurenka, 2008). In Egypt, pomegranate fruit has been used in treating different infections (Howel & D'Souza, 2013). In addition, the Jordan folk medicine used the bark and the rind of the pomegranate fruit to cure dysentery,

diarrhea, and bronchitis, piles, as an anthelmintic and to reduce cardiovascular diseases (Qnais *et al.*, 2007). Also, pomegranate molasses has been used as a traditional remedy for other illnesses like cancer, dental conditions, bacterial infections, antibiotic resistance, Alzheimer's disease, arthritis, obesity, male infertility, infant brain ischemia as well as to repair skin damaged by ultraviolet radiations (Jurenka, 2008). In the Unani and Ayurvedic systems of medicine, also practiced in the Middle East and India, pomegranate flowers has been served as a remedy for diabetes (Jurenka, 2008; Akpinar-Bayizit *et al.*, 2016). Similarly, in Lebanon, pomegranate extract was used in the treatment of diabetes mellitus (Raafat & Samy, 2014).

I.5. Pharmacological studies on pomegranate fruit parts and pomegranate molasses

Fruits such as pomegranate and its derived materials are increasingly consumed due to their micronutrients, phytochemicals and medicinal compounds contents (Mouly *et al.*, 2017). This has led to widespread investigation about their pharmacological effects. Several studies have been conducted on pomegranate fruit extract, juice, peel, seeds, flower, leaves and bark and confirmed their biological activities. Pomegranate fruit and its derived materials were shown to have antioxidant activities, anti-diabetic, anti-inflammatory, anti-carcinogenic, anti-microbial, anti-diarrheal and neuro-protective properties (Elfalleh *et al.*, 2009; Hontecillas *et al.*, 2009; Sturgeon & Ronnenberg, 2010; Endo *et al.*, 2010; Zhao *et al.*, 2018; Bekir *et al.*, 2013).

For instance, pomegranate flower has been found to have potent antioxidant and anti-diabetic effects as shown by their high free radical scavenging activity; that is due to the synergistic effect of different polyphenols present in the flower such as gallic acid and anthocyanins: pelargonidin 3,5-diglucoside, pelargonidin 3-glucoside, cyanidin (Huang *et al.*, 2005; Zhang *et al.*, 2011).

Moreover, pomegranate seeds have been shown to have anti-carcinogenic and neuro-protective properties because they have high levels of punicic acid (Grossmann *et al.*, 2010; Mizrahi *et al.*, 2014). Few studies were conducted to determine the antioxidant activity of pomegranate molasses and only one was done to reveal its neuro-protective capacity (Derakhshan *et al.*, 2018, Hussein *et al.*, 2018).

Table 9 summarizes findings of pharmacological studies done on different parts of pomegranate fruit and pomegranate molasses.

Table 9: Pharmacological Uses of Phytochemicals Present in Different Parts or Derived Materials of Pomegranate Fruit

Pomegranate Component	**Identified Compound**	**Pharmacological Use**	**Reference**
Pomegranate fruit/fruit extract	Total phenolics	Antioxidant activity, anti-carcinogenic against skin cancer	Ozgen *et al.*, 2008; Pacheco-Palencia *et al.*, 2008
	Total anthocyanins	Antioxidant activity, anti-tumor against skin cancer, anti-inflammatory effect	Ozgen *et al.*, 2008; Afaq *et al.*, 2005; Sturgeon & Ronnenberg, 2010
	Tannins: punicalagins and ellagic acid	Antioxidant activity, anti-tumor against skin cancer	Bialonska *et al.*, 2009; Afaq *et al.*, 2005
	Ellagitannins: ellagic acid and gallagic acid	Anticancer, anti-atherosclerotic properties, anti-angiogenesis, anti-estrogenic activity, anticarcinogenic effect against colon cancer	Adams *et al.*, 2010, Sturgeon & Ronnenberg, 2010; Larossa *et al.*, 2006
	Ellagic acid, caffeic acid, luteolin, punicic acid	Antitumor effect against prostate cancer, anticancer against colon cancer	Lansky *et al.*, 2005a; Sharma *et al.*, 2010
	NA	Chemopreventive/therapeutic potential against lung cancer	Khan *et al.*, 2007
	Punicalagin	Antiviral properties	Haidari *et al.*, 2009
Juice	Phenolic acids: gallic acid, caffeic acid, chlorogenic acid, ferulic acid, and coumaric acid	Antioxidant ativity (total phenols)	Krueger, 2012; Elfalleh *et al.*, 2009
	Tannins: ellagitannins (punicalagins and granatins) and gallotannins	Anti-atherogenic activity (punicalagin), anti-cancer against colon cancer, anti-inflammatory, antioxidant activities, chemo-preventive effect against colon cancer (ellagitannins and urolithins)	Fischer *et al.*, 2011; Seeram *et al.*, 2008, Fuhrman *et al.*, 2010; Adams *et al.*, 2006; Kasimsetty *et al.*, 2010

	Total pomegranate tannins	Anti-proliferative, apoptotic, antioxidant activities (anti-inflammatory)	Seeram *et al.*, 2005
	Ascorbic acid	Antioxidant activity	Medjakovic & Jungbauer, 2013
	Flavonoids: catechin, quercetin, phloridzin, flavan-3-ols or flavanols (catechin, epicatechin, and epigallocatechin)	Antioxidant activity (total flavonoids), anti-inflammatory, cardioprotective activities	de Pascual-Teresa *et al.*, 2000; Elfalleh *et al.*, 2009, Schubert *et al.*, 1999
	NA	Anti-atherogenic activity, reduce total cholesterol and LDL, anti-lipid peroxidation effects	Rozenberg *et al.*, 2006; Esmailzadeh *et al.*, 2004 ; Basu & Penugonda, 2009
	Ellagic acid	Body fat reduction, Anti-atherogenic activity, Anti-proliferative, apoptotic, antioxidant activities (anti-inflammatory), anti-angiogenesis	Makino-Wakagi, *et al.*, 2012; Fuhrman *et al.*, 2010, Seeram *et al.*, 2005; Sturgeon & Ronnenberg, 2010
	NA	Cancer chemo-preventive, cancer chemotherapeutic activities against prostate cancer in humans	Malik *et al.*, 2005; Albrecht *et al.*, 2004
	NA	Anti-atherogenic, reverse progression of CHD, cardio-protective effect	Sumner *et al.*, 2005; Razani *et al.*, 2017
	NA	Anti-cholinesterase activity	Hartman *et al.*, 2006
	NA	Anti-carcer effect against skin cancer	Afaq *et al.*, 2009
	NA	Anti-angiogenic potential, chemopreventive effect against breast cancer, anti-carcenogenic effect against colon cancer, anti-carcinogenic effect against leukemia	Toi *et al.*, 2003; Kim *et al.*, 2002; Adams *et al.*, 2006; Kawaii & Lansky, 2004
Pomegranate seed (seed oil)	NA	Anti-diabetic activity (methanolic seed extract)	Das et al., 2001
	Punicic acid (seed oil)	Anti-diabetic activity, anti-inflammatory effect, chemo-preventive agent against skin cancer, antiproliferative effect on human breast cancer, lipid peroxidation, neuro-protective effect	Hontecillas *et al.*, 2009; Hora *et al.*, 2003; Grossmann *et al.*, 2010; Mizrahi *et al.*, 2014
	Total flavonoids	Antioxidant cardioprotective, anti-inflammatory activities	Schubert *et al.*, 1999
	NA	Antitumor activity against prostate cancer, anticancer effect against leukemia	Albrecht *et al.*, 2004; Suziki *et al.*, 2001
	NA	Anti-angiogenic potential, chemo-preventive/anti-proliferative effect against human breast cancer	Toi *et al.*, 2003; Kim *et al.*, 2002; Tran *et al.*, 2010

	α-eleostearic acid	Anti-proliferative effect against human breast cancer	Tran *et al.*, 2010
	Linolenic acid	Antitumor activity against colon cancer	Kohno *et al.*, 2004a,b
Pomegranate pericarp (peel and rind)	NA	Anti-diabetic effect, anti-lipid peroxidation effects, antitumor against prostate cancer, anti-carcinogenic effect against leukemia	Šavikin et al., 2018; Parmar & Kar, 2008, 2007; Albrecht *et al.*, 2004; Kawaii & Lansky, 2004
	Total phenols	Antioxidant activity, wound healing activity	Elfalleh *et al.*, 2009; Murthy *et al.*, 2004;
	Total flavonoids	Antioxidant activity	Elfalleh *et al.*, 2009
	Punicalagin	Antimicrobial activity (such as against Staphyloccocus aureus and Pseudomonas aeruginosa)	Opara *et al.*, 2009; Endo *et al.*, 2010; Santos *et al.*, 2009
	Punicalagin, corilagin, and ellagic acid	Antidiarrheal effect	Zhao *et al.*, 2018
	NA	Antioxidant, anti-coccidial properties, anthelmintic activity	Dkhil, 2013
	NA	Neuro-protective effect	Morzelle *et al.*, 2016
Pomegranate leaves	Total phenols, total flavonoids, total anthocyanins, total tannins	Antioxidant, anti-inflammatory, anti-cholinesterase activities	Bekir *et al.*, 2013
Pomegranate flower (extract)	NA	Hypoglycemic activity, decrease cardiac fatty acid uptake and oxidation, antioxidant activity, wound healing activity, improve learning and memory performances	Jafri *et al.*, 2000; Huang *et al.*, 2005a; Bagri et al., 2009; Pirbalouti *et al.*, 2010; Cambay *et al.*, 2011
	Gallic acid	Anti-diabetic activity, antihyperlipidemic properties	Huang *et al.*, 2005b; Li *et al.*, 2008
	Anthocyanins: pelargonidin 3,5-diglucoside, pelargonidin 3-glucoside, cyanidin	Antioxidant, antitumoral activities	Zhang *et al.*, 2011; Chaturvedula *et al.*, 2011
	Oleanolic acid	Anti-hyperlipidemic properties	Li *et al.*, 2008
	Ursolic acid	Ant-hyperlipidemic properties	Li *et al.*, 2008
	NA	Anti-cholinesterase, anti-hyperglycemic activities	Bekir *et al.*, 2016
Pomegranate bark	Hexane extract of stem	Antifungal activity	Johann *et al.*, 2010
Pomegranate molasses	Total phenols	Antioxidant activity	Kamal *et al.*, 2018
	NA	Neuro-protective effect	Hussein *et al.*, 2018

I.6. Antioxidant studies done on (Punica granatum L.) pomegranate fruit, juice and pomegranate molasses

Antioxidants are natural or synthetic chemicals found in food, they dispel free radicals from cells to prevent lipid peroxidation reactions and any oxidative damage in order to preserve the cell structure and function and prevent food deterioration and they can lead to other biological functions such as anti-inflammation and anti-cancer (Zou *et al.*, 2016). Natural antioxidants like the ones found in fruits and vegetables can prevent chronic diseases such as diabetes, cardiovascular diseases and cancer (Zou *et al.*, 2016). The main natural bioactive compounds responsible for this free radical scavenging activity and therefore providing the health benefits mentioned are phenolic compounds and flavonoids (Gülçin, 2012). Several studies were done on the antioxidant activity of different parts of pomegranate fruit and its derived materials such as pomegranate juice and molasses.

Derakhshan *et al.* conducted a study on pomegranate peels, juice and seeds collected from three regions of Natanz, Shahreza, and Doorak of Iran to determine their antioxidant activity, total phenolic, flavonoids, and flavonols contents. The pomegranate fruit seeds, peels and juices were separated, dried in the incubator then powdered by grinding and extracted with 1000 ml ethanol (80%) then filtered and dried in the oven. The antioxidant activity was tested using the β-carotene bleaching test; the pomegranate peel showed higher activity level than the seeds and juices and peels from Doorak region had a significantly higher antioxidant activity by 58% compared the two other peels. Total phenolics were determined using Folin–Ciocalteu method, total flavonoids and flavonols were estimated and determined by aluminum chloride colorimetric technique. All three phenolics were reported much higher in peels than in seeds and juice and a significant positive correlation was shown between antioxidant activity and total phenolics (Derakhshan *et al.*, 2018).

Similar results were shown by Elfalleh *et al.*, when they conducted a study on six pomegranate ecotypes collected from Tunisia to determine the phenolic, tocopherol contents and antioxidant capacities of their fruits juices, peels, seed oils and pulps after undergoing methanolic extraction. Four phenolic compounds: two hydrobenzoic acids (gallic and ellagic acids) and two hydrocinnamic acids (caffeic and *p*-coumaric acids) were identified and quantified in the peels and pulps using the high-performance liquid chromatography/ultraviolet method and the tocopherol contents were found in the dry seeds of pomegranate using HPLC with fluorescence detection. Antioxidants of the juices, peels and seed oils were evaluated by ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) methods and both methods recorded the highest values in peels. High correlation was found between the antioxidant capacities of pomegranate extracts especially their peel hydrobenzoic acids and their phenolic contents, also identified tocopherols seem to contribute to the antioxidant activity in seed oil (Elfalleh *et al.*, 2011)

In another study done in Italy, five genotypes of pomegranate juices were collected ('Mollar', 'Kingdom', 'Dente di Cavallo', and two old populations 'Francofonte' and 'Santa Tecla') where the intact arils of the fruits of each genotype were removed manually, squeezed mechanically then centrifuged and filtered to get their juices. The aim of this study was to assess their anthocyanin and non-anthocyanin phenolic compound contents using the ultrahigh performance liquid chromatography (UHPLC)–Orbitrap-mass spectrometry (MS) and to evaluate their total antioxidant activity using a 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay. Among the twenty-three phenolic compounds identified, including tannins, flavonoids and phenolic acids (non-anthocyanin phenolic compounds) cyanidin-3,5-O-diglucoside and pelargonidin-3,5-O-diglucoside were mostly present as anthocyanins in all genotypes where

the Santa Tecla population had the highest content of these anthocyanins and the Francofonte population had the highest levels of ferulic acid hexocide compound (non-anthocyanin phenolic compound). Also, the Santa Tecla pomegranate population (genotype) indicated a high antioxidant activity which confirms the positive correlation between the phenolic content in pomegranate juice and antioxidant activity (Di Stefano *et al.*, 2018).

Also, Li *et al.* conducted a study on pomegranate peel and pulp extracts of ripened pomegranates obtained from Lintong, Shanxi Province of China. The pomegranate peel and pulp were separated manually and each was extracted using ethanol, methanol and acetone and powdered to determine their total phenolic content using the Folin-Ciocalteu method and their flavonoids, proanthocyanidins and ascorbic acid contents using aluminum chloride technique, vanillin-hydrochloric acid method and 2,4-dinitrophenylhydrazine spectrophotometric method respectively. Moreover, four procedures were done to determine the antioxidant properties of the peel and pulp extracts: the Ferric reducing antioxidant power assay (FRAP), the superoxide radical (O_2^-)-scavenging activity, the hydroxyl radical (OH) prevention activity the peroxyl radical (ROO^-)-scavenging activity and the inhibition of low density lipoprotein (LDL) oxidation assay. The study reported a higher antioxidant capacity in the pomegranate peel extract than the pulp extract in the four procedures performed (scavenging capacity against superoxide anion, hydroxyl and peroxyl radicals as well as in preventing LDL oxidation). Similarly, higher contents of total phenolic, flavonoids and proanthocyanidins was shown in the peel extract than the pulp extract which may be due to its potent antioxidant effect. In addition, both peel and pulp extracts contained a small amount of ascorbic acid so it could not be an important antioxidant, either in the peel or pulp extract obtained. Further studies were suggested to support using pomegranate peel extract, which is rich in antioxidants, as a potential natural health supplement (Li *et al.*, 2006).

Another study was done by Akhavan *et al.* in 2015 on ten juices from both arils and whole pomegranate cultivars grown in Iran; pomegranate juices were prepared by being pressed using a self-made press then filtered and analyzed. The aim of this study was to determine (1) the anthocyanins compounds present in each juice by separating them using an Acquity HSS-T3 RP18 column, (2) the punicalagin and ellagic acid compounds present in each juice by separating them using an Acquity BEH Phenyl column, (3) their total phenolic content using Folin-Ciocalteu method with gallic acid as a reference and (4) their antioxidant capacity using the free radical scavenging capacity by DPPH radical, ABTS radical and the ion-reducing capacity/FRAP method. The main phenolic compounds that were found in the juices were punicalagin A, B and ellagic acid (2). Also, the anthocyanins reported were cyanidin 3- glucoside, pelargonidin 3,5-diglucoside + delphinidin 3-glucoside, delphinidin 3,5- diglucoside, pelargonidin 3-glucoside, and cyanidin-pentoside. Cyanidin-pentoside-hexoside, delphinidin-pentoside and cyanidin-pentoside were detected for the first time in Iranian pomegranates. Phenolic contents and antioxidant activities of each of the ten juices from whole fruit were significantly higher than the juices from the pomegranate arils due to the added phenolic compounds from the rind parts of pomegranate to the juices. Moreover, a positive correlation was observed between total phenolic content and antioxidant activity (ABTS and FRAP methods) in all pomegranate juices and the phenolic content of the juices were more influenced by the cultivar than the extraction method (Akhavan *et al.*, 2015).

In 2017, Nasser et al., conducted a study on six samples of pomegranate molasses (5 commercial samples and 1 homemade/artisanal sample) obtained from different sources in the Lebanese market to identify their total phenolic content using the Folin-Ciocalteu method, their flavonoids content using the aluminum chloride method and their antioxidant (free radical scavenging)

activity using DPPH assay. The study reported the presence of many secondary metabolites like phenols, flavonoids, saponins and resins in all samples tested besides having potent antioxidant capacity especially the homemade sample which can be associated to the presence of phenolic compounds. Thus, pomegranate molasses can be used in the medical field such as in the food, cosmetic and pharmaceutical industry (Nasser *et al.*, 2017).

In another study done by Chalfoun-Mounayar *et al.* on fresh pomegranate juice and manually produced (homemade) pomegranate molasses made from bitter pomegranate fruits cultivated in Lebanon, their total phenolic content was measured using the Folin-Ciocalteu reagent with gallic acid as standard and their antioxidant effect was measured *in vitro* using electrolysis as a free radical generating system. Compared to fresh pomegranate juice and at a very low concentration (100 to 600 µl), pomegranate molasses had the strongest antioxidant properties *in vitro*, also the total phenolic content in pomegranate molasses was three times higher than the juice (252.28 and 79.49 mg of Gallic Acid equivalent/L respectively). Another part of the study was adding pomegranate juice or molasses to the drinking water of mice for 11 weeks; their weights were observed and biochemical assays were done. Pomegranate juice and molasses led to a significant decrease in the weights of the mice in the active intervention group compared to the mice in the control group, and a significant decrease of their triglycerides and lipid peroxidase levels in the heart, liver and lungs whereas the superoxide dismutase, which is a cellular detoxification system from superoxide radicals, increased. This study confirms that pomegranate molasses has more potent antioxidant properties and weight loss effect in mice than the juice and it also indicates that high temperature does not alter the antioxidant activity of pomegranate molasses against reactive oxygen species; instead it helps in the release of polyphenols from the fruit cells (Chalfoun-Mounayar *et al.*, 2012).

A study was done on pomegranate molasses samples purchased from a local markets in Bursa, Turkey to determine their total phenolic content using the Folin–Ciocalteu colorimetric method and their antioxidant activity using the DPPH assay. The water soluble dry matter content (brix) of the samples was expressed as g $100g^{-1}$ by using an Abbe refractometer. Antioxidant activity was determined as the percentage of DPPH decrease and the inhibition (%) was calculated according to trolox calibration curve as "µmol trolox equivalent per gram of sample". The study showed that the total phenolic content of all samples ranged between 118.28 to 828.15 mg of gallic acid equivalent per gram of pomegranate molasses sample, and their antioxidant activity was found to be between 560.23 to 1885.23 µmol trolox equivalent per gram of sample. The difference in antioxidant activity level of pomegranate molasses depends on several factors, such as cultivar and climatic conditions during fruit maturation (Akpinar-Bayizit *et al.*, 2016).

Kamal *et al.*, conducted a study on three different commercially available pomegranate molasses samples obtained from supermarkets in Riyadh, Saudi Arabia to investigate their content of four major antioxidant markers (vitamin C, gallic acid, ellagic acid and rutin) using a matrix solid phase dispersion method (MSPD) and HPLC quantification (C_{18} column was used for the separation). These methods along with the validation parameters studied such as linearity, accuracy, robustness (found acceptable as per ICH guidelines) were successfully employed for the quantification of the antioxidant markers. The peaks in the sample chromatograms were identified by comparing the retention time of each marker and its quantity in each sample was calculated from the calibration curve. Sample 1 was the highest in rutin (mean content (% w/w) ± SD (n = 3) of 0.054 ± 0.003), sample 2 was the highest in ascorbic acid and ellagic acid (mean content of 0.250 ± 0.002 and 0.139 ± 0.0033 respectively) and sample 3 was the highest in gallic acid (mean content of 0.611 ± 0.014). By quantifying these four antioxidant makers, these results confirmed the direct

association between the phenolic compounds in pomegranate molasses and its strong antioxidant properties (Kamal *et al*., 2018).

In 2010, Incedayi *et al*. led a study on seven different pomegranate molasses brands collected from the local markets of Bursa, Turkey to analyze their total polyphenol content using the Folin-ciocalteu reagent and the gallic acid standard and their antioxidant activity using the DPPH radical scavenging assay. Moreover, other tests were performed to analyze their viscosity using a rotary viscosimeter, their total acidity using titration, pH using a Nel-pH 890 model pH meter, the hydroxymethyl furfural (HMF) using the color variation technique, protein by burning and distilling fresh samples, invert and total sugars using the Luff-School method and minerals using the wet ashing method. The results showed that the total polyphenol content ranged between 551.61 and 9695.17 mg/kg depending on the pomegranate cultivar and the production technique used to make pomegranate molasses. Their antioxidant activity ranged between 0 and 46.31% which confirms the effect of long thermal processing of the samples on lower antioxidant activity and the effect of the production technique used to make pomegranate molasses on the phenolic concentration and therefore the high antioxidant activity. In addition, the study showed that some pomegranate molasses samples were very high in HMF, pH and the total acidity and all samples were found to to be rich in minerals such as potassium, magnesium and calcium (compared to the pomegranate molasses standard published by the Turkish Standardization Institute in 2001 and USDA National Nutrient Database for Standard Reference, released in 2002). Based on these results, authors suggested to improve and optimize the production techniques of pomegranate molasses and test for adulteration in order to preserve its nutritional and health values and to limit HMF levels specifically due to its adverse health effects resulting from long thermal processing (Incedayi *et al.*, 2010).

I.7. Anti-diabetic studies done on (Punica granatum L.) pomegranate fruit, juice and pomegranate molasses

Diabetes mellitus (type 1 and 2) is a metabolic syndrome characterized mainly by hyperglycemia due to defects in the secretion and/or action of insulin with type 2 diabetes mellitus being the most common type spread worldwide. It is estimated that the number of type 2 diabetes mellitus patients in the world will increase from 415 million patients in 2015 to 642 million patients in 2040 (Khajebishak *et al.*, 2019). Alpha amylase is an essential amylolytic enzyme contributing to the breakdown of dietary starch, being the most common carbohydrate in nature (Zhang *et al.*, 2017); also, alpha glucosidase is responsible for the final step in the breakdown of the dietary starch and glycogen (Liu *et al.*, 2016). Therefore, inhibiting both enzymes can potentially delay postprandial glucose absorption and lower the level of blood glucose (Liu *et al.*, 2016). Pomegranate has been used in folk medicine to treat diabetes but to our knowledge, no studies have been conducted on the anti-diabetic activity of pomegranate molasses.

A study was done on pomegranate peel powder, where pomegranate fruits were collected from three different villages in Serbia and the peels were manually removed from the seeds, cut small, air-dried and then grounded to pomegranate peel powder to undergo extraction. The aim of this study was to determine the phenolic composition and biological activities of the pomegranate peel extracted with 70% ethanol solution (named pomegranate peel 70% ethanol extract) and its fractions (petroleum ether, ethyl acetate, butanol and water) using the liquid/liquid extraction. Using the Folin-Ciocalteu method followed by HPLC analysis, the highest levels of phenolic content (gallic acid and ellagic acid) were present in the ethyl acetate fraction. Antioxidant activity was tested using four different methods showing that butanol and ethyl acetate fractions showed more activity using the DPPH and ABTS assays, while the water fraction showed more activity

using the FRAP and β-carotene methods. Anti-diabetic activity was evaluated using the α-amylase and α-glucosidase inhibition assays, and all fractions revealed better inhibition activity (most active) on α-glucosidase than α-amylase with ethyl acetate fraction as the most active. Also, acetylcholinesterase (AChE) and tyrosinase (TYR) inhibitory activity assays were used to test their anti-neurodegenerative activity and 70% ethanol extract presented the most effective inhibitory effect on both enzymes. According to previous studies, these activities can be attributed to high levels of gallic and ellagic acids (Šavikin *et al.*, 2018).

In another study done in Australia, pomegranate flowers were collected from Maharashtra, India and pomegranate fruits were collected from Victoria, Australia. Different parts of pomegranate (juice, peels, seeds and flowers) were extracted using different methods like freeze drying for the pomegranate juice, peel (methanolic peel extract) and seeds (ethyl acetate and methanolic seed extracts), air-drying for the pomegranate flower and methanolic extraction (ethyl acetate soluble fraction and water soluble fraction) also for the pomegranate flower. The aim of the study was to evaluate and compare their anti-diabetic activity using the α-amylase and α-glucosidase inhibition assays. The study showed that the flower extract with the strongest activity was the one undergoing water-ethyl acetate partition and the ethyl acetate fraction was more active than the water fraction in inhibiting both enzymes. The presence of gallic acid and ellagic acid in the ethyl acetate fraction was confirmed using the HPLC-DAD and HPLC-HESI-MS and they revealed an inhibition against the α-glucosidase alone. The methanolic flower extract inhibited both enzymes (α-amylase and α-glucosidase) but the methanolic peel extract inhibited α-glucosidase alone. Thus, pomegranate flowers and peels have anti-hyperglycemic (anti-diabetic) properties (Kam *et al.*, 2013).

In 2014, Banihani *et al.* conducted a study on the effect of pomegranate juice on fasting serum glucose and insulin levels in type 2 diabetes patients between 37 and 60 years of age. Blood

samples were collected from 85 participants with type 2 diabetes after fasting for 12 hours, then after 1 and 3 hours of ingesting 1.5 ml/kg body weight of pomegranate juice, serum glucose was identified using the BS-200 Chemistry Analyzer and human insulin was measured using commercial immunoassay kits. After 3 hours of pomegranate juice ingestion, results showed a decrease in fasting blood glucose, an increase in β-cell function and a decrease in insulin resistance among the type 2 diabetes participants. However, participants with a lower initial fasting blood glucose levels presented better hypoglycemic response compared to those who had higher fasting blood glucose levels, also the effect of pomegranate juice was not affected by the gender of the participants and was less effective with the increase in their age. Therefore, pomegranate juice can influence and control glucose levels in type 2 diabetes patients (Banihani *et al.*, 2014).

I.8. Food recipes for pomegranate molasses

Pomegranate is an ancient fruit cultivated in Iran, India and Mediterranean countries such as Turkey, Tunisia, Egypt, Morocco and Lebanon, thus it is commonly consumed in this region with its derived products (Erdrich *et al.*, 2015; Faour-Klingbeil & Todd, 2018). Nowadays, pomegranate molasses is broadly present in the international gastronomic markets originated from the Middle Eastern supermarkets and cooking, following people's taste in food and the nutritional values and biological activities it holds (Faour-Klingbeil & Todd, 2018).

Pomegranate Barbecue Sauce Recipe: *Use like any Barbecue sauce* (Ashton *et al.*, 2006).

Ingredients: - 1 cup thick ketchup

- 1/3 cup pomegranate molasses

-1 teaspoon of cumin

- 2 teaspoons of powdered garlic or minced garlic

-1 teaspoon of Louisiana hot sauce

In saucepan combine all ingredients and simmer 5 minutes on low heat. You want just enough heating to bring out the flavors. Stir to prevent sticking. It can be used immediately. You can adjust these ingredients to taste (Ashton *et al.*, 2006).

Fig. 5: Pomegranate barbecue sauce

Many villages in Lebanon preserved the tradition of making and consuming homemade pomegranate molasses while others buy commercial ones from the markets. Pomegranate is cultivated and the arils are separated and juiced, then concentrated by boiling to obtain the pomegranate molasses. It is suggested that pomegranate and its derived materials such as pomegranate molasses can contribute to the advantages linked to the diets in the Mediterranean region (Erdrich *et al.*, 2015).

In Lebanon, pomegranate molasses is traditionally used by chefs in restaurants and in households as a condiment and it is mainly added as a dressing to salads such as Fattoush and Tabbouli or as a sauce to sausages for an original sweet and sour taste. It can also be added to appetizers such as Mtabbal.

“Fattoush” is one of the most famous Lebanese salads and besides its variety in vegetables, pomegranate molasses is responsible of its special taste being the main ingredient of its dressing.

Ingredients (serves 6 people): - 1 chopped small lettuce

- 2 cups of cutted cabbage (thin chop)

- 1 bundle of chopped parsley (rough chop)

- 1 bundle of chopped fresh mint (rough chop)

- 4 medium tomatoes cut into small cubes

- 5 cucumbers cut into round pieces

- 8 radishes cut into cubes

- 1 cup of green bell pepper cut into small cubes

- 1 bundle of purslane

- ½ cup of chopped red onion

- ½ cup of chopped green onion

- 2 loafs of fried white Arabic bread cut into cubes or small rolls

Dressing Ingredients: - ¼ cup of pomegranate molasses

- ¼ cup of lemon juice

- ½ cup of olive oil

- 1 tablespoon of sumac

- Salt to taste

- ½ teaspoon of black pepper

- 2 crushed garlic cloves (optional)

In a big bowl, combine the main ingredients; lettuce, cabbage, parsley, fresh mint, tomatoes, cucumbers, radishes, green bell pepper, purlsane, red onion, green onion. Mix the ingredients of the dressing, add them to the main ingredients and blend them together. Add the fried Arabic bread on top and serve.

Fig. 6: A bowl of traditional Lebanese fattoush salad

“Makanek” are small Lebanese sausages made with ground lamb meat, beef meat, or a combination of both. They are seasoned with cumin, cinnamon, nutmeg, pine nuts and vinegar. The seasoned ground meat is stuffed into sheep casings. The sausages can be either fried with vegetable oil or grilled and pomegranate molasses is traditionally added before serving.

Fig. 7: Traditional Lebanese “Makanek” sausages

Chapter II Materials and Methods

II.1. Introduction

After presenting this literature review, we can conclude that pomegranate molasses has high levels of phytochemicals with antioxidant and neuro-protective capacities. In Lebanon, only few brands of pomegranate molasses have been evaluated in terms of total phenolic content and antioxidant activity but to the best of our knowledge, pomegranate molasses hasn't been tested for its flavonoids content and anti-diabetic activity yet; therefore, the objective of our study is to determine the total phenols and flavonoids content, antioxidant and anti-diabetic activities of different homemade samples and samples of different commercial pomegranate molasses brands.

To evaluate the antioxidant activity of pomegranate molasses, the DPPH radical scavenging assay and the ferrous ion chelating assay will be used and both tests are based on single electron transfer reactions. Compared to other tests, DPPH is a rapid, simple, reproducible and convenient test model and it is commonly used to determine the antioxidant activity of phenolic compounds extracted from plants, fruits and vegetables (Russo *et al.*, 2015). The DPPH test is based on the reduction of the methanol DPPH solution in the presence of an antioxidant donating a hydrogen. The reaction leads to a decrease in the absorbance of free radical species detected through the change of color from purple to yellow, color of diphenylpicrylhydrazine (Russo *et al.*, 2015; Kosakowska *et al.*, 2018). However, using the ferrous ion chelating assay and in the presence of chelating agents in the sample tested, a disruption in the formation of complexes between ferrozine and ferrous ion (a metal-induced free radical) will occur which leads to a decrease in the red color that is usually formed by the complex and the measurement of the color reduction gives an estimation of the binding ability of the coexisting chelating agent (Yusof *et al.*, 2013; Adjimani *et al.*, 2015).

To determine the anti-diabetic activity of pomegranate molasses, α-amylase inhibition and α-glucosidase inhibition assays will be used. Treating diabetes is achieved by decreasing post-prandial hyperglycemia and this is done by delaying the absorption of glucose by inhibiting the role of both enzymes in breaking down starch into glucose during digestion (Liu *et al.*, 2016). So both methods aim to test if a pomegranate molasses brand has this inhibitory effect and therefore an anti-diabetic capacity.

II.2. Materials and Methods

Raw material - Sampling

Pomegranate molasses samples were produced in the fall of 2018 and collected in the spring of 2019; the commercial ones (28 samples: Al Barakah, Al Rabih, Annabil, Al Wadi Al Akhdar, Aoun, Ashka, Baydar, Boulos, Chtoura Gardens, Chtoura Fields, Cortas, Gardenia, Houkoul Khadraa, Jana, Jeita, Kortbawi, Lumiere, Maxim's, Maymouna, Mechaalani, Mrouj Chtoura, Monzer Basha, Salloum, Spinneys, Taj, Tiba, Terrois, Yamama) from main supermarkets in Mount Lebanon and homemade ones (4 samples) from few random households in Mount Lebanon. The homemade samples were traditionally and manually produced by cleaning the pomegranate fruits, crushing it to extract the pomegranate juice, filtrating and then concentrating the juice by boiling and evapotration in open containers without adding further sugar, additives or citric acid. Commercial and homemade samples were stored in the refrigerator of the Chemistry Laboratory of Notre Dame University – Louize, Lebanon between 0-4ºC until getting tested from freshly opened air tight containers (bottles) to ensure precision which started after one month of storage.

Total Phenol Content for pomegranate molasses

Total phenols in the extracts will be assessed by a modified Folin-Ciocalteu method (Koivikko et al., 2005). Briefly, 0.5 mL of the diluted sample will be mixed with 0.5 mL of 1N Folin-Ciocalteu reagent. The mixture will stand for 3 min, after which 1 mL of 20% sodium carbonate (Na_2CO_3) was added. Samples will be incubated in the dark at room temperature for 45 min then centrifuged (5 min at 2400g). Absorbance of the supernatants will be measured at 730 nm using a Jenway 6405 UV/Vis spectrophotometer. Total phenol content will be expressed as mg of gallic acid equivalents (GAE) per 100 mg of sample. All measurements will be performed in duplicate.

Total Flavonoid Content for pomegranate molasses

Total flavonoids in the extract will be estimated using the modified aluminum chloride method (Shams Ardekani, *et al.*, 2011) where quercetin will be used as a standard and the flavonoid content will be measured as quercetin equivilant so the quercetin calibration curve will be needed. An aliquot (1 mL) of sample or standard solution of quercetin (20, 40, 60, 80 and 100 mg/L) will be added to 10 mL volumetric flask containing with 4 mL of double distilled water. Then 0.3 ml 5% $NaNO_2$ will be added to the flask and after 5 min, 0.3 mL $AlCl_3$ (10%) will also be added. At the 6th min, 2 mL NaOH (1 M) will added and the total volume will be made up to 10 mL with double distilled water. The solution will be mixed completely and the absorbance level will be measured versus prepared reagent blank at 510 nm. Total flavonoid content is expressed as mg of quercetin equivalents (QE) per 100 mg of sample. All measurements were performed in duplicates.

Biological activities

DPPH radical scavenging assay

The scavenging effects of the extracts for DPPH radical will be determined by the method of Yan and Chen (1995) with slight modifications. Serial dilutions of the extracts will be prepared in EtOH. The basic procedure was to add an aliquot (1 mL) of test sample to 1 mL of DPPH 0.15 mM EtOH solution. The mixture will be vortexed for 1 min and then left to stand at room temperature for 30 min in the dark. The absorbance will be read at 517 nm using a UV/Vis spectrophotometer, and the calculations of the scavenging activity (%) (SA) is as follows: SA (%): [1- (Asample - Asample blank) /Acontrol] x 100. Sample solution (1 mL) plus EtOH (1 mL) is used as a sample blank and DPPH solution (1 mL) plus EtOH (1 mL) is used as a negative control. Ascorbic acid is used as the positive control. Stock solutions of ascorbic acid (0.8 mg/mL) and ascorbic acid (0.8 mg/mL) will be diluted with EtOH to give concentrations ranging from 1.5 to 20 μg/mL. All measurements will be performed in duplicate or triplicate.

Ferrous ion chelating assay

The ferrous ion chelating activity was determined according to Lim et al (2007). Equal volumes of 0.12 mM $FeSO_4$, test sample (at different concentrations), and 0.6 mM ferrozine will be mixed. The solutions were allowed to stand for 10 min at room temperature, and the absorbance of Fe^{2+}-ferrozine complex was measured at 562 nm using UV/Vis spectrophotometer. Ultra-pure water instead of sample solution was used as a negative control. Ultra-pure water instead of ferrozine solution is used as a blank, which is used for error correction because of unequal color of the sample solutions. EDTA-Na_2 is used as the positive control. The ability of the sample to chelate ferrous ions is calculated by using the following formula: $A_0 - (A_{s\ tested} - A_{s\ alone} / A_0) \times 100$ where

A_0 is the absorbance of the negative control, $A_{s\ tested}$ is the absorbance of the sample tested and A_{alone} is the absorbance of the sample alone. All measurements were performed in duplicate.

α-Amylase Inhibitory Activity

α-Amylase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification (Ademiluyi,& Oboh, 2013). In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl α–amylase (2 U/ml), and 20 µl of varying concentrations of extract and fractions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 1 mg/ml) was pre-incubated at 37°C for 20 min. Then, the 20 µl of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37°C for 30 min; 100 µl of the DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate ELISA Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was used as a standard. Without test (extract and fractions) substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula, Inhibitory activity (%) = $(1 - A_s / A_c) \times 100$; where, A_s is the absorbance in the presence of test substance and A_c is the absorbance of control.

α-Glucosidase Inhibitory Activity

α-glucosidase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification (Shai et al., 2011) In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl alpha-glucosidase (1 U/ml), and 20 µl of varying concentrations of extract and fractions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 1 mg/ml) was pre-incubated at 37°C for 15 min. Then, 20 µl P-NPG (5 mM) was added as a substrate and incubated

further at 37°C for 20 min. The reaction was stopped by adding 50 µl Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate ELISA Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. Without test substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula, Inhibitory activity (%) = $(1 - A_s / A_c) \times 100$; where, A_s is the absorbance in the presence of test substance and A_c is the absorbance of control.

Statistical analysis

All the statistical analysis was carried out using the statistical software SPSS version 23. The statistical analysis performed in the present study included a general descriptive exploration of data. First, the mean values of each method used were calculated for the samples taken from commercial pomegranate molasses (28) and samples taken from homemade pomegranate molasses (4). So, we had a total of six mean values for samples taken from commercial pomegranate molasses and six mean values for samples taken from homemade pomegranate molasses (two mean values for the total phenol content, two mean values for the total flavonoid content, four mean values for the antioxidant activity and four mean values for the anti-diabetic activity). Then, Mann-Whitney U test was carried out to compare the mean values between commercial and homemade samples of pomegranate molasses per method used. Finally, the determination of Spearman's correlation analyses was performed to examine the correlation between total phenol/total flavonoid contents and anti-diabetic/antioxidant activities per method used.

II.3. Results and Discussion

Total phenols and flavonoids content in pomegranate molasses

Fruits are recognized by their high levels of nutrients, vitamins, minerals and bioactive compounds associated with their health benefits that were recently studied along with their composition and biological activities (Li *et al.*, 2006; Derakhshan *et al.*, 2018). Pomegranate is a fruit that is widely cultivated in the Mediterranean region where it is consumed with its derived products (Faour-Klingbeil & Todd, 2018). Its abundance and use in folk medicine has attracted the interest of researchers to identify the active secondary metabolites present in its different parts. They were found to have many biological activities including antioxidant activities, anti-diabetic, anti-inflammatory, anti-carcinogenic, anti-microbial, anti-diarrheal and neuro-protective properties (Elfalleh *et al.*, 2009; Hontecillas *et al.*, 2009; Sturgeon & Ronnenberg, 2010; Endo *et al.*, 2010; Zhao *et al.*, 2018; Bekir *et al.*, 2013). Pomegranate molasses, the subject of our study, is a famous traditional condiment produced and consumed in Mediterranean countries including Lebanon; it is present in the market as commercial brands and homemade ones produced in rural areas to be added to salads and many other dishes (Incedayi *et al.*, 2010). Since limited studies are conducted on pomegranate fruit parts, derived products and pomegranate molasses in Lebanon, studies done in other countries were stated so we should take into account factors such as differences in cultivars, soil and climate.

The total phenols (TPC) and total flavonoids (TFC) content of pomegranate molasses were determined by Folin-ciocalteu method and aluminum chloride test for 32 commercial and homemade samples and results were expressed in mg GAE/g of PM and mg QE/g of PM respectively.

Phenolic compounds are widely found in plants and food, including fruits, ranging from simple to highly polymerized compounds with different structures and functions which affect their identification and extraction (Haminiuk *et al.*, 2012). They are recognized by having at least one aromatic ring with one or more hydroxyl groups attached and they are found as complexes with sugars, acids or alkyl groups (Cartea *et al.*, 2010). They are responsible for the color, astringent and bitter taste of pomegranate (Akpinar-Bayizit *et al.*, 2016).

Our results showed that total phenols content (table 9) in commercial pomegranate molasses samples ranged between 9.86 and 78.28 mg GAE/g PM and that of homemade samples ranged between 21.19 and 44.32 mg GAE/g PM. Table 10 displayed that the mean value of total phenols content in homemade pomegranate molasses samples (37.27 ± 10.79 mg GAE/g PM) is higher than that of commercial samples (26.13 ± 14.32 mg GAE/g PM); however, this difference is not statistically significant (*p*-value: 0.087). This finding might indicate that the phenolic content in homemade pomegranate molasses samples may be more effectively preserved which is assumed to be due to the production techniques used; by not adding any preservatives and crushing/squeezing the whole fruit or we might have a fraud in some of the commercial brands by having other than pomegranate as a main ingredient. In a study conducted by Incedayi *et al.* in Turkey, results reported that total phenolic content in commercial pomegranate molasses samples, tested using Folin-Ciocalteu method and a spectrophotometer measuring at a 765nm, ranged between 551.61 and 9695.17 mg GAE/kg (0.55161 – 9.69517 mg GAE/g PM) which are lower levels than those found in the commercial samples in this study (Incedayi *et al.* in 2010). In another study conducted in Lebanon on fresh pomegranate juice and molasses from six different bitter pomegranate varieties, the mean level of total polyphenols in molasses (252.28 ± 33.67 mg GAE/L) was found to be three times higher than that of pomegranate juice (79.49 ± 25.25 mg

GAE/L) since pomegranate molasses is a concentrate of pomegranate juice; due to the evaporation of water during processing (Chalfoun-Mounayar *et al.*, 2012). Also, Yilmaz *et al.* reported that total phenols content of commercial pomegranate molasses, determined according to the Folin-Ciocalteu method, was five to ten times higher than fresh pomegranate arils (52.6 mg GAE/g) which is higher than that of commercial pomegranate molasses in our samples too (Yilmaz *et al.* 2007). In a study conducted by Akpinar-Bayizit *et al.*, total polyphenols content of commercial pomegranate molasses was determined spectrophotometrically at 725 nm according to modified Folin–Ciocalteu method and it varied between 118.28 to 828.15 mg GAE/g of PM which is much higher than the amounts found in our study; this might be due to having fraud in some of our commercial samples (Akpinar-Bayizit *et al.*, 2016). However, Ergin findings did not support the findings of our study; since the average phenolic content of commercial pomegranate molasses samples tested also using Folin-Ciocalteu method was found to be higher (490.22 ±278 mg GAE/L) than the traditional pomegranate molasses samples (24.11 ±4.06 mg GAE/L); which was attributed to lack of support and awareness about the hygienic conditions during production stages for the traditional pomegranate molasses (Ergin, 2020). The differences in total phenols content of pomegranate molasses among these studies are expected to be due to factors such as cultivars, climate, soil and production techniques.

Table 9 displayed the total flavonoids content of all samples; for commercial pomegranate molasses samples it ranged between 12.15 and 90.92 mg QE/g PM and for homemade samples it ranged between 28.73 and 48.28 mg QE/g PM. The mean value of total flavonoids content (Table 10) in homemade pomegranate molasses samples (40.09 ± 8.41 mg QE/g PM) was found to be significantly higher (*p*-value: 0.035) than that of commercial ones (29.47 ± 17.61 mg QE/g PM). These findings were supported by the findings of a study conducted by Nasser *et al.*, in Lebanon

where the values of total flavonoids content of commercial pomegranate molasses samples, determined using aluminum chloride method, ranged between 54.34 and 74 mg Rutin Equivalent/g of PM and the homemade/artisanal pomegranate molasses sample tested had the highest value of 137.74 mg Rutin Equivalent/g of PM (Nasser *et al.*, 2017). Compared to pomegranate juice (8.54–23.99 mg QE/100g) tested by Kaur *et al.*, total flavonoids content of pomegranate molasses in our study is much higher since it is a concentrate of the juice (Kaur *et al.*, 2017). Another study done on pomegranate juice samples of twelve varieties and five clones in Italy showed that they contain a high amount of flavonoids such as pelargonin and catechin-cyanidin-3-hexoside despite the fact that flavonoids content differ between varieties and cultivars (Gómez *et al.*, 2013).

Flavonoids' free radical scavenging effect and metal chelating effect indicate their antioxidant effect besides other biological activities including anti-diabetic, anti-proliferative, anti-inflammatory, anti-carcinogenic and neuro-protective effects (Singh *et al.*, 2018; Haminiuk *et al.*, 2012; Zhou *et al.*, 2018; Seraam *et al.*, 2005; Akpinar-Bayizit, *et al.*, 2012; Hartman *et al.*, 2006).

Pomegranate Brand Name	Total Phenols Content (mg GAE/g PM)	Total Flavonoids Content (mg QE/g PM)
Al Barakah	18.48	28.38
Al Rabih	35.32	26.06
Annabil	34.45	42.54
Alwadi	22.61	13.78
Aoun	22.57	29.82
Ashkar	28.24	21.37
Baydar	22.79	22.09
Boulos	10.68	13.31
Chtoura Gardens	21.98	36.73
Chtoura Fields	14.81	15.45
Cortas	21.85	26.81
Gardenia	10.08	37.38
HM Dima 1	44.32	48.28
HM Dima 2	41.60	39.32
HM Mira	41.97	44.03
HM Salwa	21.19	28.73
Houkoul Khadraa	15.26	26.17
Jana	27.42	24.92

Jeita	13.47	12.92
Kortbawi	78.28	90.92
Lumiere	22.06	21.07
Maxim's	34.86	24.28
Maymouna	24.30	23.72
Meshaalani	21.64	19.36
Monzer Basha	36.80	35.27
Mrouj Chtoura	22.74	27.27
Salloum	37.02	30.02
Spinneys	9.86	12.15
Taj	11.88	12.37
Tiba	23.84	25.75
Terrois	52.46	69.07
Yamama	36.02	56.26

Table 9: Total phenols and total flavonoids contents of our commercial and homemade pomegranate molasses samples

	Type of pomegranate molasses	Mean	Standard Deviation	*p*-value
Total phenols content (mg GAE/g PM)	Commercial PM	26.13	14.32	0.087
	Homemade PM	37.27	10.79	
Total flavonoids content (mg QE/g PM)	Commercial PM	29.47	17.61	0.035
	Homemade PM	40.09	8.41	

Table 10: Means and *p*-values of total phenols and total flavonoids contents of our commercial and homemade pomegranate molasses samples

Antioxidant activities of pomegranate molasses using DPPH assay and Fe^{2+} chelating assays

DPPH assay is an easy and highly reproducible test that has a widespread use in the free radical scavenging assessment (Akpinar-Bayizit *et al.*, 2016). DPPH is a stable free radical characterized by a deep violet color, it is dissolved in ethanol to form a DDPH solution with a spectrophotometric absorption at about 520nm. It is based on the reduction of methanol DPPH solution in the presence of a hydrogen donating antioxidant. A change in the color of the solution from purple to yellow detected indicates a decrease in the absorbance of free radical species (Russo *et al.*, 2015).

The DPPH scavenging activity exhibited by commercial and homemade pomegranate molasses samples tested was expressed by IC_{50} (Table 12) which is defined as the concentration of substrate that causes 50% loss of the DPPH activity. The values shown in Table 12 were determined using the regression equations obtained from concentration-activity curves.

Our results showed that IC_{50} of DPPH scavenging activity (Table11) in commercial pomegranate molasses samples ranged between 0.09 and 0.76 mg/ml and that of homemade samples ranged between 0.09 and 0.26 mg/ml. After comparing the mean values of IC_{50} of commercial and homemade PM samples we can notice that homemade pomegranate molasses samples have higher DPPH scavenging activity (IC_{50} = 0.15 ± 0.07 mg/ml) than commercial samples (IC_{50} = 0.23 ± 0.15 mg/ml); since a lower IC_{50} means that a lower concentration of PM was needed to achieve 50% of the enzyme inhibition and therefore indicating a stronger antioxidant activity. However, this difference was not found to be statistically significant (*p*-value: 0.231). Thus, homemade pomegranate molasses samples were shown to be the most potent towards DPPH free radical. Comparing our results with ascorbic acid, the commonly used reference compound that is known to have a radical scavenging activity at IC_{50} of 0.03 mg/ml, we noticed that both commercial and homemade pomegranate molasses samples exhibited an activity close to the reference group that is a very potent antioxidant agent which is logical especially that pomegranate molasses contains sugars. These findings are supported by the findings of the study conducted by Nasser *et al.* where antioxidant capacity was stronger in homemade/artisanal than in commercial pomegranate molasses (Nasser *et al.*, 2017). However, a study done by Ergin showed that in average commercial pomegranate molasses samples tested had higher antioxidant content than homemade pomegranate molasses samples which he attributed to the lack of support and knowledge in the homemade PM production and in terms of commercial samples' production, pomegranate molasses standards

should be applied in details to prevent adulterations such as colorants, thickener and antioxidant additions that may have changed the results (Ergin, 2020). A study done by kamal et al. confirmed the presence of strong antioxidant markers in pomegranate molasses which was associated with the presence of vitamin C, gallic acid, rutin and ellagic acid (Kamal *et al.*, 2018). In a previous study conducted by Chalfoun-Mounayar *et al.*, pomegranate molasses has shown the strongest antioxidant properties *in vitro* compared to pomegranate juice which indicated that high temperature does not affect the antioxidant activity of pomegranate molasses against ROS; instead it is assumed that it helps the cells of pomegranate fruit to release polyphenols especially that there is no extraction with a solvent during the preparation of pomegranate molasses (Chalfoun-Mounayar *et al.*, 2012). In another study done by Incedayi *et al.*, results showed that pomegranate molasses have good antioxidant activity which is an important aspect for human health but opposing to the previous study, long term thermal processing of pomegranate molasses samples is assumed to affect their antioxidant activity (Incedayi *et al.*, 2010). In addition, the study conducted by Akpinar-Bayizit *et al.* showed that pomegranate molasses has potent antioxidant activity (560.23-1885.23 μmol trolox equivalent per gram of sample) and the difference in its antioxidant activity level between samples is affected by several factors, such as cultivar and climatic conditions during fruit maturation (Akpinar-Bayizit *et al.*, 2016).

Ferrous ion chelating assay is the ability of antioxidants in food, in this case pomegranate molasses, to chelate transition metal ions (Fe^{2+}) which leads to a decrease in Fe^{2+} concentration and provide protection against oxidative damage by catalyzing the Fenton-type reactions in a biological system, resulting in the generation of hydroxyl radicals (OH•). Ferrozine can quantitatively form complexes with ferrous ion (a metal-induced free radical) yielding a red color (Yusof *et al.*, 2013). However, in the presence of chelating agents, there is disruption of the formation of the complex

which leads to a decrease in the red color and the measurement of color reduction gives an estimation of the binding ability of the coexisting chelating agent. The ferrous ion was monitored by measuring the formation of a red ferrous ion-ferrozine complex at 562 nm; the higher the absorbance, the weaker the ferrous iron binding strength of the chelating agent (Adjimani *et al.*, 2015). The percentage inhibition of Fe^{2+} - ferrozine complexess formation was calculated.

In our study, pomegranate molasses samples exhibited Fe^{2+} chelating activity in a concentration dependent manner. As shown in table 11 and using the highest concentration for all samples (1 mg/ml), the percentage of inhibition of ferrous ion chelation of commercial pomegranate molasses samples ranged between -40.52% and 41.93% and that of homemade samples ranged between 28.47% and 46.78%. The mean percentages of inhibition of ferrous ion chelation (table 12) of homemade and commercial pomegranate molasses samples were 34.87% ± 8.24 and -2.06% ± 17.81 (*p*-value: 0.03), respectively, indicating that commercial pomegranate molasses show significantly lower chelating activity compared to homemade ones and that commercial pomegranate molasses didn't exhibit an activity which might be due to the presence of iron in some of the commercial samples initially, forming complexes with the antioxidants in PM; thus they don't exhibit a chelation power. In a study conducted by Viuda-Martos *et al.* in Spain ferrous ion chelating assay was performed on pomegranate arils and peel combined (WFB) and arils alone (AB) with different concentrations (10 g/L – 100 g/L); AB samples showed chelating activities ranging between 0 and 35.59% ± 0.35 and WFB samples showed a chelating effect ranging between 23.71% ± 0.35 and 42.87% ± 0.36. These results showed that pomegranate arils and peels have a lower chelating activity than that of homemade pomegranate molasses samples in our study; 34.87% ± 8.24 at a lower concentration of 1 mg/ml (Viuda-Martos *et al.*, 2011). This can be attributed to the concentration of phenolic compounds in pomegranate molasses produced

traditionally. Another study done by Fawole *et al.* testing the chelating ability of methanolic extracts of pomegranate peel on ferrous ion where, on average, low ferrous ion chelating activity was exhibited at the lowest concentration (10 µg/ml) of methanolic extracts of pomegranate peel but the activity of almost all extracts increased above 50% at a concentration of at 100 µg/ml which indicated that the pomegranate fruit peel contains constituents that inhibit oxidation through a mechanism other than radical scavenging activity, DPPH (Fawole *et al.*, 2012). Compared to our results, pomegranate fruit peel show a stronger chelating activity on ferrous ion than pomegranate molasses.

Pomegranate Brand Name	IC_{50} DPPH	% of inhibition of ferrous ion chelation
Al Barakah	0.19	9.88
Al Rabih	0.21	-9.36
Annabil	0.09	-26.49
Alwadi	0.23	-5.93
Aoun	0.3	-2.78
Ashkar	0.09	9.79
Baydar	0.26	-18.92
Boulos	0.3	13.08
Chtoura Gardens	0.1	-26.54
Chtoura Fields	0.43	4.19
Cortas	0.11	-.28
Gardenia	0.15	7.86
HM Dima 1	0.09	33.84
HM Dima 2	0.13	46.78
HM Mira	0.12	30.40
HM Salwa	0.26	28.47
Houkoul Khadraa	0.46	-40.52
Jana	0.15	.05
Jeita	0.16	13.13
Kortbawi	0.13	26.92
Lumiere	0.11	-16.94
Maxim's	0.2	-15.95
Maymouna	0.1	13.13
Meshaalani	0.29	-26.12
Monzer Basha	0.34	-10.49
Mrouj Chtoura	0.24	.85
Salloum	0.1	2.16
Spinneys	0.76	9.74
Taj	0.45	11.48

Tiba	0.22	-4.42
Terrois	0.14	41.93
Yamama	0.12	-17.18

Table 11: IC_{50} of DPPH and % of inhibition of ferrous ion chelation of our commercial and homemade pomegranate molasses samples

	Type of pomegranate molasses	Mean	Standard Deviation	*p*-value
IC_{50} DPPH	Commercial PM	0.23	0.15	0.231
	Homemade PM	0.15	0.07	
% of inhibition of ferrous ion chelation	Commercial PM	-2.06	17.81	0.003
	Homemade PM	34.87	8.24	

Table 12: Means and *p*-values of IC_{50} of DPPH and % of inhibition of ferrous ion chelation our commercial and homemade pomegranate molasses samples

Correlation between phytochemical constituents and antioxidant activity

Correlation coefficients between the assessed phytochemical constituents (phenols and flavonoids) and both DPPH radical scavenging activity and Fe^{2+} metal chelating activity are reported in Table 13. Total phenols content (r = -0.542) / total flavonoids content (r = -0.483) and IC_{50} of DPPH scavenging activity were found to have an inverse (negative) strong/moderate statistically significant correlation (*p*-value: 0.001; *p*-value: 0.005, respectively). These inverse correlations indicate that whenever the total phenols or flavonoids contents increase, IC_{50} of DPPH decreases; a decrease in IC_{50} means that a lower concentration of PM is needed to achieve 50% of the enzyme inhibition indicating a higher antioxidant activity. Therefore, they are involved in the DPPH scavenging activity and they have strong anti-radical scavenging activity and capacity to reduce oxidative stress.

On the other hand, a weak positive correlation was observed between total phenols content (r = 0.152) / total flavonoids content (r = 0.146) and Fe^{2+} chelating activity, expressed in percentage of inhibition of ferrous ion chelation (*p*-value: 0.407 and *p*-value: 0.424 respectively) but these correlations were found to be not statistically significant which could be explained by having a small sample size of homemade pomegranate molasses samples.

A study done by Kaur *et al.* on pomegranate varieties in India showed a significant strong correlation between total phenols content (DPPH $R^2 = 0.9$), total flavonoids content (DPPH $R^2 = 0.85$) and antioxidant activity as assessed by three *in vitro* assays including the DPPH radical scavenging assay; these findings indicate that both total phenols and total flavonoids contents are strong determinants of the powerful antioxidant activity attributed to pomegranate (Kaur *et al.*, 2014). In contrast, Ergin conducted a study on total phenols content and antioxidant activity of pomegranate molasses in Turkey using the DPPH scavenging activity assay and found no significant correlation between phenolic content and antioxidant activity which he attributed to difference in cultivars/species of pomegranate in addition to various additives used in pomegranate molasses production especially commercial ones that may have affected the results (Ergin, 2020). The Ambigaipalan *et al.* study revealed that pomegranate peel contained 79 phenolic compounds, including 16 phenolic acids and 12 flavonoids. Phenolic acids were the major phenolic compounds in pomegranate peel followed by flavonoids, hydrolysable tannins, proanthocyanidins (Ambigaipalan *et al.*, 2016). This study can prove how the phenolic content in pomegranate molasses is increased and its health benefits improved whenever the whole pomegranate fruit is used during pomegranate molasses production, including the peel and not only the juice part. In another study conducted by Derakhshan *et al.* on pomegranate peel, juice and seeds extracts, a positive significant correlation was reported between antioxidant activity tested using the β-

carotene bleaching test and phenolic (r = 0.78), flavonoids (r = 0.95), and flavonol (r = 0.89) contents in all samples, using Folin-Ciocalteu and aluminum chloride calorimetric methods, respectively (Derakhshan *et al.*, 2018).

		IC_{50} DPPH	% of inhibition of ferrous ion chelation
Total phenols content	Correlation coefficient	-0.542	0.152
	p-value	0.001	0.407
	N	32	32
Total flavonoids content	Correlation coefficient	-0.483	0.146
	p-value	0.005	0.424
	N	32	32

Correlation is significant at the 0.05 level (2-tailed).

Table 13: Correlation between total phenols and total flavonoids contents and antioxidant activity assays

Anti-Diabetic activities of pomegranate molasses using alpha amylase and alpha glucosidase inhibitory assays

The anti-diabetic activity of commercial and homemade pomegranate molasses samples was determined using the alpha-amylase and alpha-glucosidase inhibitory assays. The results were expressed as IC_{50} (Table 15) and acarbose, a known anti-diabetic drug, similar to Glucobay and Precose, was used as the reference standard for both assays each with IC_{50} values of 0.42 mg/ml for alpha-amylase assay and 0.28 mg/ml alpha-glucosidase inhibitory assay. We should note that a lower IC_{50} means that a lower concentration of PM was needed to achieve 50% of the enzyme inhibition and therefore indicating a higher anti-diabetic activity.

Table 14 displayed the IC_{50} of alpha amylase of commercial pomegranate molasses samples ranging between 0.68 and 28.24 mg/ml and of homemade samples ranging between 0.63 and 2.06 mg/ml. Data obtained (Table 15) showed that the homemade pomegranate molasses samples (IC_{50}

= 1.19 ± 0.61 mg/ml) were more active against alpha amylase than commercial samples (IC_{50} = 3.98 ± 5.14 mg/ml) meaning that it is more effective against diabetes and this difference was found to be statistically significant (*p*-value: 0.014). Moreover, homemade pomegranate molasses is very close in activity to the reference standard acarbose; only 1.5 to 5 times (on average ~2.8 times) less active than acarbose (IC_{50} = 0.42 mg/ml) in spite the fact that pomegranate molasses contains natural sugars that can interfere with this assay and activity against alpha amylase. Also, table 14 displayed the IC_{50} of alpha glucosidase of commercial pomegranate molasses samples ranging between 0.32 and 4.05 mg/ml and of homemade samples ranging between 0.4 and 1.83 mg/ml. Results showed that the homemade pomegranate molasses samples (IC_{50} = 0.78 ± 0.7 mg/ml) were more active against alpha glucosidase compared to commercial samples (IC_{50} = 1.47 ± 0.77 mg/ml); meaning a higher effectiveness against diabetes for homemade samples, but this difference was not found to be statistically significant (*p*-value: 0.077). Similarly, homemade pomegranate molasses were found to be very close in activity to the reference standard acarbose; only 1.4 to 6.5 times (on average ~2.8 times) less potent than acarbose (IC_{50} = 0.28 mg/ml) despite the presence and concentration of natural sugars in pomegranate molasses affecting the results.

A previous study done by Kam *et al.* on seven extracts from different parts of pomegranate (juice, flower, peel and seeds) to determine their inhibitory activities against alpha amylase and alpha glucosidase enzymes showed that the strongest inhibitory effect of the flower extracts on both enzymes followed by the peel extract on alpha glucosidase alone. These results were associated with the presence of gallic acid and ellagic acid and led to the conclusion that pomegranate flowers and peels have an anti-diabetic activity (Kam *et al.*, 2013). In addition, Šavikin *et al.* showed that all pomegranate peel extracts/fractions revealed a better inhibition activity against alpha glucosidase enzyme than alpha amylase and according to previous studies this anti-diabetic

activity was also attributed to high levels of gallic and ellagic acids (Šavikin *et al.*, 2018). Another study conducted by Les, *et al*. showed that pomegranate juice exhibited an inhibitory effect against alpha glucosidase enzyme with a similar profile to acarbose, which is a reference inhibitor of this enzyme, with polyphenols such as punicalagin, urolithin-A, ellagic acid being responsible for the inhibition of the enzyme and therefore the presence of an anti-diabetic activity (Les *et al.*, 2018).

Pomegranate Brand Name	IC_{50} alpha amylase inhibitory assay	IC_{50} alpha glucosidase inhibitory assay
Al Barakah	1.38	2.45
Al Rabih	2.34	1.44
Annabil	4.74	1.86
Alwadi	4.11	4.05
Aoun	1.8	2.41
Ashkar	1.25	0.55
Baydar	2.84	0.89
Boulos	5.01	2.01
Chtoura Gardens	1.36	0.82
Chtoura Fields	2.26	1.12
Cortas	1.69	1
Gardenia	8.67	0.94
HM Dima 1	0.94	0.405
HM Dima 2	2.06	0.41
HM Mira	0.63	0.49
HM Salwa	1.14	1.83
Houkoul Khadraa	7.96	1.45
Jana	5.32	1.49
Jeita	4.09	2.02
Kortbawi	1.13	1.8
Lumiere	3.24	0.94
Maxim's	28.24	1.54
Maymouna	2.23	0.32
Meshaalani	4.34	1.67
Monzer Basha	1.41	1.21
Mrouj Chtoura	3.56	1.4
Salloum	1.35	2.61
Spinneys	2.52	1.1
Taj	1.71	1.4
Tiba	3.21	1.25
Terrois	0.68	0.45
Yamama	3.04	1

Table 14: IC_{50} of α-amylase inhibitory assay and IC_{50} of α-glucosidase inhibitory assay of our commercial and homemade PM samples

	Type of Pomegranate Molasses	Mean	Standard Deviation	*p*-value
IC_{50} alpha amylase inhibitory assay (mg/ml)	Commercial PM	3.98	5.14	0.014
	Homemade PM	1.19	0.61	
IC_{50} alpha glucosidase inhibitory assay (mg/ml)	Commercial PM	1.47	0.77	0.077
	Homemade PM	0.78	0.7	

Table 15: Means and *p*-values of the IC_{50} of α-amylase inhibitory assay and IC_{50} of α-glucosidase inhibitory assay of our commercial and homemade pomegranate molasses samples

Correlation between phytochemical constituents and anti-diabetic activity

Spearmen correlation coefficients between total phenols, total flavonoids contents and alpha amylase inhibitory activity and alpha glucosidase inhibitory activity are shown in table 16. Significant moderate negative correlations were observed between total phenols/flavonoids contents and alpha amylase inhibitory activity (r: -0.436, *p*-value: 0.013 and r: -0.445, *p*-value: 0.011 respectively). In addition, weak negative correlations were found between total phenols/flavonoids contents and alpha glucosidase inhibitory activity; however, these correlations were not found to be statistically significant (r: -0.284, *p*-value: 0.116 and r: -0.247, *p*-value: 0.173, respectively). This lack of statistical significance could be attributed to the small sample size of homemade samples. Thus, these results reveal a potential anti-diabetic activity of phenolic compounds found in the pomegranate molasses.

		IC_{50} alpha amylase inhibitory assay	IC_{50} alpha glucosidase inhibitory assay
Total phenols content	Correlation coefficient	-0.436	-0.284
	p-value	0.013	0.116
	N	32	32
	Correlation coefficient	-0.445	-0.247

Total flavonoids content	*p*-value	0.011	0.173
	N	32	32

Correlation is significant at the 0.05 level (2-tailed).

Table 16: Correlation between total phenols and total flavonoids content and anti-diabetic activity assays

Conclusion

The screening of commercial and homemade pomegranate molasses samples for total phenols, flavonoids, and antioxidant and anti-diabetic activities was performed. Homemade pomegranate molasses samples exhibited higher antioxidant activity than commercial samples against DPPH radical scavenging activity and Fe^{2+} chelating assay. Similarly, homemade pomegranate molasses samples exhibited higher anti-diabetic activity using alpha amylase and alpha glucosidase inhibitory assays, noting that homemade samples were very close in activity to the reference standard acarbose; only 1.5 to 5 times (~2.8 times) less potent than acarbose in activity against alpha amylase enzyme and 1.44 to 6.5 times (~2.8 times) less potent than acarbose in inhibiting alpha-glucosidase, despite the presence and concentration of natural sugars in pomegranate molasses affecting the results. Moreover, total phenols and flavonoids contents were found to be correlated with antioxidant and anti-diabetic activities.

The existing findings can serve as a building block for further research studies to validate these activities using other methods or have a bigger sample size of homemade pomegranate molasses samples, to quantify more classes of phytochemicals, and to perform a bio-guided fractionation then isolate and identify the bioactive molecules that are responsible for each biological activity.

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