ERURJ, 2023, 2, 1,121-160

(Review)

Pomegranates bioactive metabolites and biological activities: an updated review

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Received 24th January 2023, Revised 29th January 2023, Accepted 30th January 2023

DOI:10.21608/ERURJ.2023.283010

ABSTRACT

Pomegranate (*Punica granatum* L.) is a fruit that is widely consumed as fresh fruit and juice. The use of pomegranate fruit dates from ancient times and literature about its therapeutic qualities has echoed throughout the ages. Several *in vitro* and *in vivo* studies have shown how this fruit acts as antioxidant, antidiabetic, and hypolipidemic and shows antibacterial, antiparasitic antifungal, anti-inflammatory, antiviral, and anti-carcinogenic activities. The fruit also potentiates cardiovascular and oral health. These beneficial physiological effects may also have preventive applications in a variety of diseases such as (renal and hepatic toxicity). The health benefits of pomegranate have been attributed to its wide range of chemicals and extracts, which are predominantly polyphenols,

including primarily hydrolysable ellagitannins, anthocyanins, sugars, organic acids and other polyphenols. The aim of this review is to present an updated overview of the functional, medical, chemical and physiological properties of this fruit &/or its agri-food residues aiming to figure out the significance of pomegranates.

Keywords: Pomegranate; polyphenols; ellagitannin, anthocyanins, agri-food residue

Abbreviations

DAD	Diode Array Detector
DPPH	2,2-diphenyl-1-picrylhydrazyl
ESI	Electrospray Ionization
FRAB	Ferric reducing antioxidant power
GC	Gas Chromatography
HPLC	High-performance liquid chromatography
HR	High-resolution
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IR	Infrared spectroscopy
LC	Liquid chromatography
MS	Mass Spectrometry
TLC	Thin Layer Chromatography
UV-VIS	Ultraviolet-visible spectroscopy

1. Introduction

The pomegranate (*Punica granatum* L.) is an old fruit; it has been widely consumed in various cultures for thousands of years. The use of pomegranate fruit dates back to Biblical times and reports of its therapeutic qualities have echoed throughout the history of humanity. The Persians believed that the seeds conferred invincibility on the battlefields, while for the ancient Chinese the seeds symbolized longevity and immortality. The pomegranate belongs to the family Punicaceae. It is native from the area of Iran to the Himalayas in northern India and has been cultivated and naturalized over the entire Mediterranean region since ancient times. Actually,

pomegranate is widely cultivated throughout Iran, India, Mexico, Montenegro, Turkey (Antalya), Mediterranean countries especially Morocco, the drier parts of Southeast Asia as

Jordan, Malaysia, the East Indies, and tropical Africa and, to some extent, in the United States (drier parts of California and Arizona), China, Japan, and Russia. Extraction is performed mostly from parts which are higher in active constituents such as (peels, barks, seeds, flowers, and, anthers), also content and type of liquids and solvents used in extraction changes according to the extraction procedure, geographical origin and analysis method. Methods of analysis mainly include UV VIS (high-performance, ultra-performance liquid chromatography or gas chromatography (HPLC, UPLC, GC) followed by ultraviolet-visible spectroscopy and mass spectroscopy (1-5). The edible parts of pomegranate fruits are consumed fresh or used for the preparation of fresh juice, canned beverages, jelly, jam, and paste and also, for flavouring and colouring beverage products. In addition, it is widely used in therapeutic formulas, cosmetics, and food seasonings. Since ancient times, pomegranate has been regarded as a "Healing food" with numerous beneficial effects for several diseases. Indeed, the pomegranate was commonly used in folk medicine, for eliminating parasites, as an anthelmintic anti-giardiasis and vermifuge, and to treat and cure ulcers, diarrhoea, acidosis, dysentery, hepatic and renal toxicity, haemorrhage, and bronchial asthma. It was also used as an antipyretic (6-12).

Recent years have seen increased interest on the part of consumers, researchers, and natural products in how natural products can help maintain health; and the role that crude drugs play in the prevention and treatment of many illnesses has become widely accepted). Physiological benefits of pomegranate play an important role in disease prevention or slow the progress of chronic diseases (cancer (antioxidant effect on tumours and prevention of invasion), microbial, fungal and parasitic infections, diabetes Miletus (amylases inhibitors), anti-viral (its prophylactic effect against COVID 19) (7-9, 13-20).

The aim of this review is to present an updated overview of the functional, medical, and physiological properties of pomegranates.

2. Review Methodology

Relevant literature was conducted through the Reaxys database (https://www.reaxys.com/) with the keyword (*Punica granatum*) filtering the results to research articles published in 2022-2023.

2. Bioactive metabolite described in pomegranate

A wide array of metabolites was described in pomegranates. They were distributed in different pomegranates parts and analyzed via several techniques (Tables 1-6, Figure 1).

Chemical analysis of Anthocyanins

Anthocyanins occurred in peels and seeds. The extracts Fifty mg of the peel powder was dissolved in 1 mL of double distilled water (DDW) containing 0.1% HCl. The tubes were placed on a shaker rotating at 500 rpm at room temperature for 1 h. The extract was then centrifuged at 14,000 x g for 20 min and the supernatant was filtered through a 0.22-µm filter to a vial for metabolite analysis. Five µL of the extract was injected into an Ultimate 3000 ultra-performance liquid chromatography (UPLC) system (Thermo-Fisher Scientific, Waltham, MA, USA) with a photodiode array detector (DAD). (1)

One g of each comminuted sample was dissolved in 10 ml of 80% modifications, in an ultrasonic water bath at a constant temperature of 50 °C with a frequency of 50 KHz in a thirty-minute time interval. The extracts were filtered through Whatman filter paper and stored in sealed tubes in a refrigerator at 4° C until research. (2)

Chemical analysis of Vitamin C (Ascorbic acid)

About 2.5 g of individual juice samples (from rind and seeds) was dissolved in 10 ml of water (pH 2.5) and made up to 25 ml. The sample was filtered through 0.45µ syringe filters and 10 µl was injected (HPLC). (3)

Chemical analysis of essential elements

Analysis was performed by inductively coupled –plasma-optical emission spectrometry (720 ICP-OES). Samples (from rind and seeds) were digested with 5 ml of concentrated nitric acid (65%) in MARS Xpress microwave digester for 15 mins at 200 °C. Diwere t vessels allowed to cool down to 50 °C and then made up the volume to 25ml; 100 ppm of mixed standards stock was prepared, linear standards ranging from 0.1 to 5 ppm were aspirated in 720 the ICP-OES Agilent and response of detector was calibrated every time. (3)

Chemical analysis of Organic compounds

Pomegranates were stripped from the skin and the membranes and then granulated. The granules were pressed manually in a cheesecloth for juice extraction. The juices were then stored in at freezer (−20 ˚C) until analysis. In the study, contents of succinic acid, oxalic acid, citric acid, malic acid, fumaric acid, tartaric acid, acetic acid and lactic acid were determined in the fruit juices. The method of Bevilacqua and Califano (1989) was modified and used to extract organic acids. Mixtures containing 5 mL pomegranate juice and 20 mL 0.009 N H2SO4 homogenized (Heidolph Silent Crusher M, Germany). The mixtures were blended by a shaker (Heidolph Unimax 1010, Germany) for 1 h and then were centrifuged for 15 min at 15,000 rpm. The supernatants were filtered first through filter paper and then twice through a 0.45 _m membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) before being passed through a SEP-PAK C18 cartridge. An Aminex column (HPX-87 H, 300 mm×7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) was used in the HPLC system, and the instrument was controlled by a PC with Agilent software. The DAD detector in the system (Agilent, USA) was set at wavelengths of 214 and 280 nm. The mobile phase was 0.009 N H2SO4 that had been filtered through a 0.45 _m membrane filter. (4)

Chemical analysis of Hydroxy methyl furfural

Plant samples were shade dried and powdered. 10 g of prepared sample were dissolved in 100 ml solvent (methanol and ethyl acetate) and rotated in an incubator shaker for 48 hrs. It was then filtered, lyophilized, and stored for further use.

Gas Chromatography-Mass Spectrometry (GC-MS) with nonpolar column Rxi-5Sil MS (30m×0.25mm Id×0.25µm film thickness) was performed. (5)

Chemical analysis of Citric acid and malic acid

It was extracted from the peel by a mixture of ethanol and hydrochloric acid and distilled water with a ratio of 60:5:35 for 30 minutes and analyzed by thin-layer chromatography, high-pressure liquid chromatography, and UV-visible spectroscopy. (6)

Chemical analysis of Flavonoids

1. Twenty mg of the peel powder was placed in a 2-mL Eppendorf tube followed by the addition of 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl). The extraction was conducted in dark at room temperature with shaking at 500 rpm for 1 h. The extract was then centrifuged at 14,000 x g for 20 min, and the supernatant passed through a 0.22-μm filter and kept frozen until used. Five μL of the extract was injected into an UPLC connected to a DAD and an MS/MS detector. (1)

2. Ten grams of dried peels of *P. granatum* L. were crushed in the electric blender machine to make a fine powder. Methanolic extract of *P. granatum* L. peel was prepared through the Soxhlet apparatus method in 500ml of methanol solvent High Performance Liquid Chromatography (HPLC) was performed. (7)

Chemical analysis of Quercetin

One g of each comminuted sample was dissolved in 10ml of 80% modifications, in an ultrasonic water bath at a constant temperature of 50°C with a frequency of 50 KHz in a thirty-minute time interval. The extracts were filtered through Whatman filter paper and stored in sealed tubes in a refrigerator at 4° C until research. (2)

Chemical analysis of flavones (Tricetin and Tricetin 4'-O-glucoside)

The ground pomegranate floral tissues were freeze-dried, and 50 mg of the lyophilized tissue was extracted with 1 mL of 70% methanol under sonication. After centrifugation at 13,000 rpm for 10 min, the supernatant of the extract was passed through a syringe filter (Millipore Sigma, Burlington, MA, USA) and subjected to LC-HR-ESI-MS analysis on an (UPLC) (Waters, Milford, MA, USA) coupled to a Q Exactive mass spectrometer (Thermo Scientific, Waltham, MA, USA). (8)

Chemical analysis of hydrolysable tannins

Twenty mg of the peel powder was placed in a 2-mL Eppendorf tube followed by the addition of 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl). The extraction was conducted in dark at room temperature with shaking at 500 rpm for 1 h. The extract was then centrifuged at

14,000 x g for 20 min, and the supernatant passed through a 0.22-μm filter and kept frozen until used. Five μL of the extract was injected into an UPLC connected to a DAD and an MS/MS detector. (1)

Chemical analysis of phenolic compounds:

1. Phenolic compound contents in pomegranate juices were determined by HPLC. 13 phenolic compounds were examined in the pomegranate juice samples in the study. Among these, gallic acid contents ranged between 0.190 and 6.361 g L−1, catechin contents between 0.533 and 3.176 g L−1, chlorogenic acid contents between 0.0375 and 0.5473 g L−1, caffeic acid contents between 0.0162 and 0.0960 g L−1, syringic acid contents between 0.0214 and 0.0609 g L−1, p-coumaric acid contents between 0.0200 and 0.2456 g L−1, ferulic acid contents between 0.0446 and 0.2326 g L−1, o-coumaric acid contents between 0.0325 and 0.5514 g L−1, phloridzin contents between 0.0414 and 1.2155 g L−1, protocatechuic acid contents between 0.0169 and 0.4489 g L−1, vanillic acid contents between 0.0061 and 0.1708 g L−1. (4)

2. Ten grams of dried peels of *P. granatum* L. were crushed in the electric blender machine to make fine powder. Methanolic extract of *P. granatum* L. peel was prepared through the Soxhlet apparatus method in 500 ml of methanol solvent High Performance Liquid Chromatography (HPLC) was performed. (7)

Chemical analysis of Gallic acid:

1. One g of each comminuted sample was dissolved in 10ml of 80% methanol in a test tube. Extraction was performed with certain modifications, in an ultrasonic water bath at a constant temperature of 50°C with a frequency of 50 KHz in a thirty-minute time interval. The extracts were filtered through Whatman filter paper and stored in sealed tubes in a refrigerator at 4 °C until research. (2)

2. Fruits were manually peeled, and then rinsed with distilled water. Peel and seed were dried in an oven with air circulation at 40 °C, and they were finely ground in a laboratory grinder. The dried sample was then stored at 20 °C until further use. The peel and seed powder (10 g) were extracted with 60 mL of methanol by magnetic stirring at room temperature for 24 h. The extracts

were filtered through filter paper for the removal of particles. The residue was re-extracted with 50 mL of methanol and filtered. The pool of extracts was concentrated under a vacuum at 40 °C to obtain the final extracts. (9)

3. Ten grams of dried peels of *P. granatum* L. were crushed in the electric blender machine to make fine powder. Methanolic extract of *P. granatum* L. peel was prepared through Soxhlet apparatus method in 500mlof methanol solvent High Performance Liquid Chromatography (HPLC) was performed. (7)

4. Extracted from peel by a mixture of ethanol and hydrochloric acid and distilled water with a ratio of 60:5:35 for 30 minutes, and analyzed by thin layer chromatography, high pressure liquid chromatography, and UV-visible spectroscopy. (6)

Chemical analysis of Ellagic acid and Punicalagins (A, B):

1. The peels of the fruits were dried in the shade and then grinded. About 100 g of the grounded peels was extracted in 1000 mL ethanol by the Soxhlet extraction technique for 10 h. The extract was filtered, and the ethanol was completely evaporated at 40 ºC in a rotary evaporator. Then, the extract was prepared in different concentrations (100, 200,300, 400, 500, 600 μg/mL) with 96% ethanol. (10)

2. Extracted from peel by a mixture of ethanol and hydrochloric acid and distilled water with a ratio of 60:5:35 for 30 minutes, and analyzed by thin layer chromatography, high pressure liquid chromatography, and UV- visible spectroscopy. (6)

3. Phenolics (hydrolysable ellagitannins) punicalagins are extracted from the bark with a mixture of toluene and ethanol then analyzed by mass and ultraviolet spectroscopy. (11)

Chemical analysis of sugars (glucose, sucrose)

Sugars are extracted from peel by a mixture of ethanol and hydrochloric acid and distilled water with a ratio of 60:5:35 for 30 minutes, and analyzed by thin-layer chromatography, high-pressure liquid chromatography, and UV-visible spectroscopy. (6).

Table 1. Anthocyanins described in pomegranates

Compound	Molecular formula	Occurrence	Geographical origin	Extraction procedure	Method of analysis	Reference
Anthocyanin 1	$C_{78}H_{89}O_{44}^{+}$	fruit juice	Turkey	methanol–acetic acid–water (10:2:88)	HPLC	(4)
Cyanidin 3,5-diglucoside	$C_{27}H_{31}O_{16}$	outer peels	Palestine, Central Asia & California	50mg /1ml DDW(0.1%HCl)	UPLC-DAD-MS/MS	(1)
Cyanidin 3-glucoside	$C_{21}H_{21}O_{11}$	outer peels	Palestine, Central Asia & California	50mg /1ml DDW(0.1%HCl)	UPLC-DAD	(1)
		peels and seeds	Montenegro	80% methanol	FC-FRAP-DPPH	(2)
Delphinidin 3,5-diglucoside	$C_{27}H_{31}O_{17}$	outer peels	Palestine, Central Asia & California	50mg /1ml DDW(0.1%HCl)	UPLC-DAD	(1)
Delphinidin 3-glucoside	$C_{21}H_{21}O_{12}$	outer peels	Palestine, Central Asia & California	50mg /1ml DDW(0.1%HCl)	UPLC-DAD	(1)
Pelargonidin 3,5-diglucoside	$C_{27}H_{31}O_{15}$	outer peels	Palestine, Central Asia & California	50mg /1ml DDW(0.1%HCl)	UPLC-DAD	(1)
Pelargonidin 3-glucoside	$C_{21}H_{21}O_{10}$	outer peels	Palestine, Central Asia & California	50mg /1ml DDW(0.1%HCl)	UPLC-DAD	(1)

Table 2. Hydrolysable Tannins described in pomegranates

Compound	Molecular formula	Occurrence	Geographical origin	Extraction procedure	Method of analysis	Reference
Punicalagin-α	$C_{48}H_{28}O_{30}$	outer peels	Palestine, Central Asia & California	20 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Punicalagin-β	$C_{48}H_{28}O_{30}$	outer peels	Palestine, Central Asia & California	21 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Punicalin	$C_{34}H_{22}O_{22}$	outer peels	Palestine, Central Asia & California	22 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Pedunculagin	$C_{34}H_{24}O_{22}$	outer peels	Palestine, Central Asia & California	23 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
1,2,3,4,6-Pentagalloyl glucose (PGG)	$C_{41}H_{32}O_{26}$	outer peels	Palestine, Central Asia & California	26 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Brevifolin carboxylic acid	$C_{13}H_{8}O_{8}$	outer peels	Palestine, Central Asia & California	27 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Valoneic acid dilactone	$C_{21}H_{10}O_{13}$	outer peels	Palestine, Central Asia & California	28 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Castalagin	$C_{41}H_{26}O_{26}$	outer peels	Palestine, Central Asia & California	29 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Casuarinin	$C_{41}H_{28}O_{26}$	outer peels	Palestine, Central Asia & California	30 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)

β-Glucogallin	$C_{13}H_{16}O_{10}$	outer peels	Palestine, Central Asia & California	31 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Casuarictin	$C_{41}H_{28}O_{26}$	outer peels	Palestine, Central Asia & California	32 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Hippomanin A	$C_{27}H_{22}O_{18}$	outer peels	Palestine, Central Asia & California	33 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
3,4,3-Tri-O-methylellagic acid	$C_{17}H_{12}O_8$	outer peels	Palestine, Central Asia & California	34 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Eschweilenol C	$C_{20}H_{16}O_{12}$	outer peels	Palestine, Central Asia & California	35 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)

Table 3. Phenolic acid, Flavonoids, and other phenolic compounds described in pomegranates

Compound	Molecular formula	Occurrence	Geographical origin	Extraction procedure	Method of analysis	Reference
Vanillic acid	$C_8H_8O_4$	outer peels	Palestine, Central Asia & California	35 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Protocatechuic Acid	$C_7H_6O_4$	outer peels	Palestine, Central Asia & California	36 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
p-Coumaric acid glucoside	$C_{15}H_{18}O_8$	outer peels	Palestine, Central Asia & California	37 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Neochlorogenic Acid	$C_{16}H_{18}O_9$	outer peels	Palestine, Central Asia & California	38 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
(E)-Ferulic acid	$C_{10}H_{10}O_4$	outer peels	Palestine, Central Asia & California	39 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
4-Hydroxyphenylpyruvate	$C_9H_8O_4$	outer peels	Palestine, Central Asia & California	40 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
trans-Caffeic acid	$C_9H_8O_4$	outer peels	Palestine, Central Asia & California	41 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Dihydromyricetin	$C_{15}H_{12}O_8$	outer peels	Palestine, Central Asia & California	42 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Procyanidin B2	$C_{30}H_{26}O_{12}$	outer peels	Palestine, Central Asia & California	43 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)

Leucodelphinidin	$C_{15}H_{14}O_8$	outer peels	Palestine, Central Asia & California	44 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Leucocyanidin	$C_{15}H_{14}O_7$	outer peels	Palestine, Central Asia & California	45 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Cyanidin	$C_{15}H_{10}O_6$	outer peels	Palestine, Central Asia & California	46 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Taxifolin	$C_{15}H_{12}O_7$	outer peels	Palestine, Central Asia & California	47 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Naringenin	$C_{15}H_{12}O_5$	outer peels	Palestine, Central Asia & California	48 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Apiforol	$C_{15}H_{14}O_5$	outer peels	Palestine, Central Asia & California	49 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Luteoforol	$C_{15}H_{14}O_6$	outer peels	Palestine, Central Asia & California	50 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Eriodictyol	$C_{15}H_{12}O_6$	outer peels	Palestine, Central Asia & California	51 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Kaempferol	$C_{15}H_{10}O_6$	outer peels	Palestine, Central Asia & California	52 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Epigallocatechin	$C_{15}H_{14}O_7$	outer peels	Palestine, Central Asia & California	53 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Catechin	$C_{15}H_{14}O_6$	outer peels	Palestine, Central Asia & California	54 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)

Quercetin	$C_{15}H_{10}O_7$	peel	Pakistan	sample:methanol (10:500,w/v)	HPLC	(7)
		outer peels	Palestine, Central Asia & California	55 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
		peels and seeds	Montenegro	80% methanol	FC-FRAP-DPPH	(2)
Gallocatechin	$C_{15}H_{14}O_7$	outer peels	Palestine, Central Asia & California	56 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Astragalin	$C_{21}H_{20}O_{11}$	outer peels	Palestine, Central Asia & California	57 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	outer peels	Palestine, Central Asia & California	58 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Hirsutrin	$C_{22}H_{20}O_{12}$	outer peels	Palestine, Central Asia & California	59 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Gallic acid	$C_7H_6O_5$	peel	Pakistan	sample:methanol (10:500,w/v)	HPLC	(7)
		fruit juice	Turkey	methanol–acetic acid–water (10:2:88)	HPLC	(4)
		outer peels	Palestine, Central Asia & California	25 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
		Peel	Turkey	EtOH:HCl:Dw 60:5:35 for 30minutes	HPLC UV vis RF	(6)
		Bark	Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)
		Peel	Mexico san pedro	100ml Ethanol: water (7:3 v/v) then 10ml 4M NAOH then 4M HCL then freeze drying	UPLC-DAD	(13)

		peels and seeds	Morocco	methanol	FC-DPPH-ABTS-FRAP	(9)
		peels and seeds	Montenegro	80% methanol	FC-FRAP-DPPH	(2)
Ellagic acid	$C_{14}H_6O_8$	Peel	Turkey	EtOH:HCl:Dw 60:5:35 for 30minutes	TLC HPLC UV vis RF	(6)
		Bark	Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)
		peels	Anatlya	Ethanol	GC_MS	(10)
		outer peels	Palestine, Central Asia & California	24 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Punicalagins A ,B	$C_{48}H_{28}O_{30}$		Turkey	EtOH:HCl:Dw 60:5:35 for 30minutes	TLC HPLC UV vis RF	(6)
			Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)
			Tunisia	Extraction ethanol and toluene mixture	UV-Vis IR	(11)
			Anatlya	Ethanol	GC_MS	(10)
			Jordan	deionised water, 99% acetone(aq) and 75% ethanol	LC–MS, MS	(14)
chlorogenic acid	$C_{16}H_{18}O_9$	fruit juice	Turkey	methanol–acetic acid–water (10:2:88)	HPLC	(4)
Caffeic acid	$C_9H_8O_4$	fruit juice	Turkey	methanol–acetic acid–water (10:2:88)	HPLC	(4)
		peel	Pakistan	sample:methanol (10:500,w/v)	HPLC	(7)
		Bark	Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)
Benzoic acid	C7H6O2	peel	Pakistan	sample:methanol (10:500,w/v)	HPLC	(7)

P-coumaric acid	$C_9H_8O_3$	peel	Pakistan	sample:methanol (10:500,w/v)	HPLC	(7)
		Bark	Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)
M-coumaric acid	$C_9H_8O_3$	peel	Pakistan	sample:methanol (10:500,w/v)	HPLC	(7)
Ferulic acid	$C_{10}H_{10}O_4$	peel	Pakistan	sample:methanol (10:500,w/v)	HPLC	(7)
		peel	Morocco oudja	100ml Ethanol: water (7:3 v/v) then 10ml 4M NAOH then 4M HCL then freeze drying	UPLC-DAD	(13)
		Bark	Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)
		Peel	Mexico san pedro	100ml Ethanol: water (7:3 v/v) then 10ml 4M NAOH then 4M HCL then freeze drying	UPLC-DAD	(13)
Cinnamic acid	$C_9H_8O_2$	peel	Pakistan	sample:methanol (10:500,w/v)	HPLC	(7)
syringic acid	$C_9H_{10}O_5$	fruit juice	Turkey	methanol–acetic acid–water (10:2:88)	HPLC	(4)
catechin	$C_{15}H_{14}O_6$	Peel	Mexico san pedro	100ml Ethanol: water (7:3 v/v) then 10ml 4M NAOH then 4M HCL then freeze drying	UPLC-DAD	(13)
Chlorogenic acid	$C_{16}H_{18}O_9$	Peel	Mexico san pedro	100ml Ethanol: water (7:3 v/v) then 10ml 4M NAOH then 4M HCL then freeze drying	UPLC-DAD	(13)
Vanillin	$C_8H_8O_3$	Bark	Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)
Vanillic acid	$C_8H_8O_4$	Bark	Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)
phlorizin	$C_{21}H_{24}O_{10}$	Bark	Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)

Rutin	$C_{27}H_{30}O_{16}$	Peel	Mexico san pedro	100ml Ethanol: water (7:3 v/v) then 10ml 4M NAOH then 4M HCL then freeze drying	UPLC-DAD	(13)
		Bark	Morocco oudja	Hexane 2.22% , chloroform 2.59% , acetone 7.62% , menthol 5.13%, water 20.67%	HPLC-DAD and GC-MS	(12)
		outer peels	Palestine, Central Asia & California	60 mg pd/ 1 mL of extraction buffer (80% methanol in DDW, and 0.1% HCl)	LC-MS/MS	(1)
Tricetin	$C_{15}H_{10}O_7$	anthers in pomegranate Flower	Shanghai	liquid nitrogen , 70% methanol	HPLC,LC-HR-ESI-MS,MS,UPLC, NMR	(8)
Tricetin 4-O-glucoside	$C_{23}H_{24}O_{12}$	anthers in pomegranate Flower	Shanghai	liquid nitrogen , 70% methanol	HPLC,LC-HR-ESI-MS,MS,UPLC, NMR	(8)

Table 4. Organic compounds described in pomegranates

Compound	Molecular formula	Occurrence	Geographical origin	Extraction procedure	Method of analysis	Reference
Succinic acid	$C_4H_6O_4$	fruit juice	Turkey	5ml juice+20ml 0.009N H2SO4 homogenized	The method of Bevilacqua and Califano (1989)	(4)
Oxalic acid	$C_2H_2O_4$	fruit juice	Turkey	5ml juice+20ml 0.009N H2SO4 homogenized	The method of Bevilacqua and Califano (1989)	(4)
Citric acid	$C_6H_8O_7$	fruit juice	Turkey	5ml juice+20ml 0.009N H2SO4 homogenized	The method of Bevilacqua and Califano (1989)	(4)
		Peel	Turkey	EtOH:HCl:Dw 60:5:35 30	TLC HPLC UV vis RF	(4)
Malic acid	$C_4H_6O_5$	fruit juice	Turkey	5ml juice+20ml 0.009N H2SO4 homogenized	The method of Bevilacqua and Califano (1989)	(4)
		Peel	Turkey	MeOH:HCl:Dw 80:5:15 30	TLC HPLC UV vis RF	(6)
Fumaric acid	$C_4H_4O_4$	fruit juice	Turkey	5ml juice+20ml 0.009N H2SO4 homogenized	The method of Bevilacqua and Califano (1989)	(4)
Tartaric acid	$C_4H_6O_6$	fruit juice	Turkey	5ml juice+20ml 0.009N H2SO4 homogenized	The method of Bevilacqua and Califano (1989)	(4)
Lactic acid	$C_3H_6O_3$	fruit juice	Turkey	5ml juice+20ml 0.009N H2SO4 homogenized	The method of Bevilacqua and	(4)

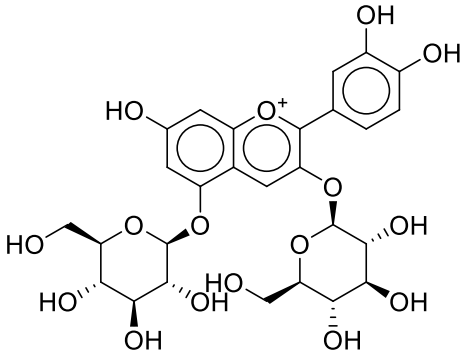
					Califano (1989)	
Acetic acid	CH_3COOH	fruit juice	Turkey	5ml juice+20ml 0.009N H2SO4 homogenized	The method of Bevilacqua and Califano (1989)	(4)
Hydroxymethyl furfural	$C_6H_6O_3$	peel	Rohtak city of Haryana, India	methanol and ethyl acetate	GC-MS analysis	(5)
Ascorbic acid	$C_6H_8O_6$	Rind-seed	India	sample:water (25:100,w/v)	HPLC Agilent-1200-UV- C18	(3)

Table 5. Essential Elements described in pomegranates

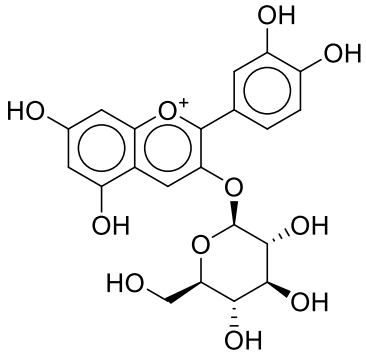
Compound	**Molecular formula**	**Occurrence**	**Geographical origin**	**Extraction procedure**	**Method of analysis**	**Reference**
Sodium	Na	Rind-seed	India	5 ml Suprapur nitric acid (65%)in MARS-Xpress micro wave digester	ICP-OES	(3)
Potassium	K	Rind-seed	India	6 ml Suprapur nitric acid (65%)in MARS-Xpress micro wave digester	ICP-OES	(3)
Iron	Fe	Rind-seed	India	7 ml Suprapur nitric acid (65%)in MARS-Xpress micro wave digester	ICP-OES	(3)
Calcium	Ca	Rind-seed	India	8 ml Suprapur nitric acid (65%)in MARS-Xpress micro wave digester	ICP-OES	(3)
Magnesium	Mg	Rind-seed	India	9 ml Suprapur nitric acid (65%)in MARS-Xpress micro wave digester	ICP-OES	(3)
Phosphorous	P	Rind-seed	India	10 ml Suprapur nitric acid (65%)in MARS-Xpress micro wave digester	ICP-OES	(3)

Table 6. Sugars described in pomegranates

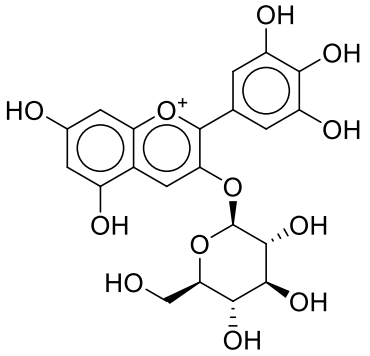
Compound	Molecular formula	Occurrence	Geographical origin	Extraction procedure	Method of analysis	Reference
Glucose	C6H12O6	Peel	Turkey	MeOH:HCl:Dw 80:5:15 30	TLC HPLC UV vis	(6)
Sucrose	C12H22O11	Peel	Turkey	EtOH:HCl:Dw 60:5:35 30	TLC HPLC UV vis	(6)



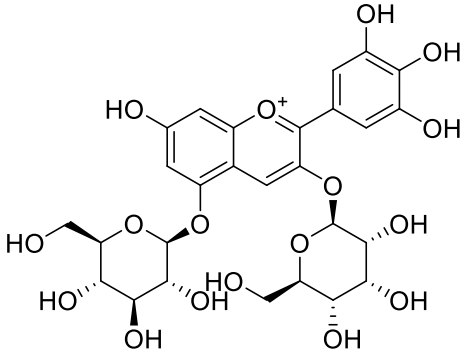
Cyanidin 3,5- diglucoside



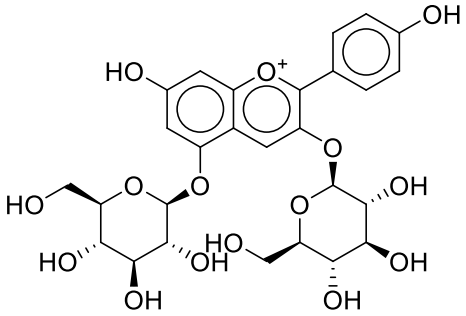
Cyanidin 3-glucoside



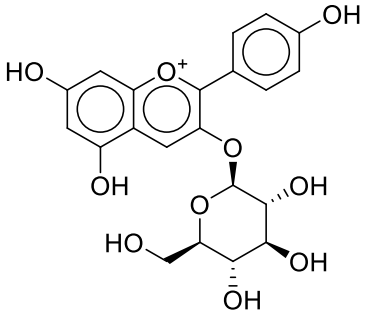
Delphinidin 3- glucoside



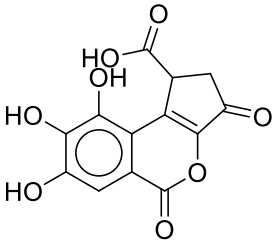
Delphinidin 3,5- diglucoside



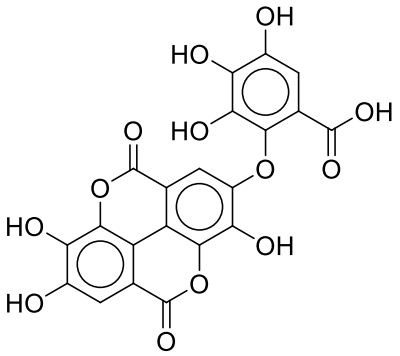
Pelargonidin 3,5- diglucoside



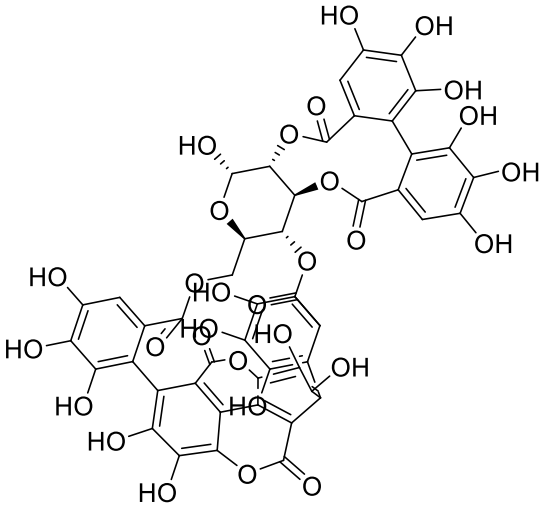
Pelargonidin 3- glucoside



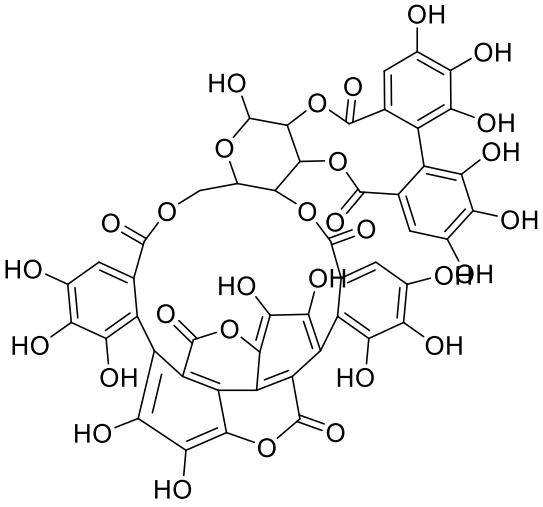
Brevifolin carboxylic acid



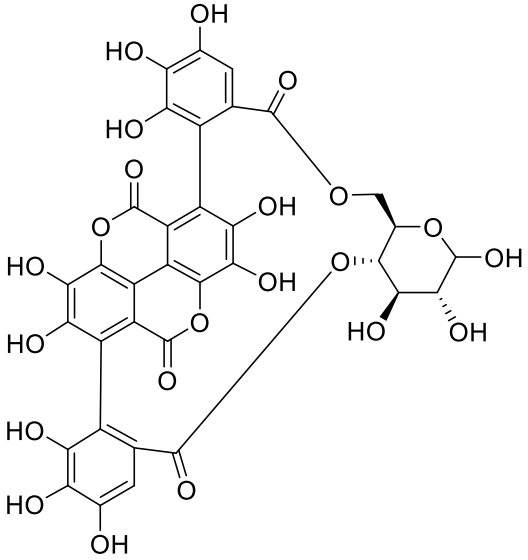
Valoneic acid dilactone



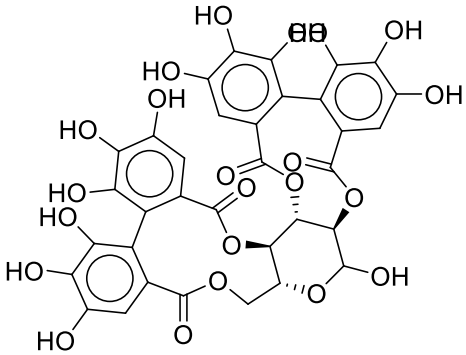
Punicalagin-α



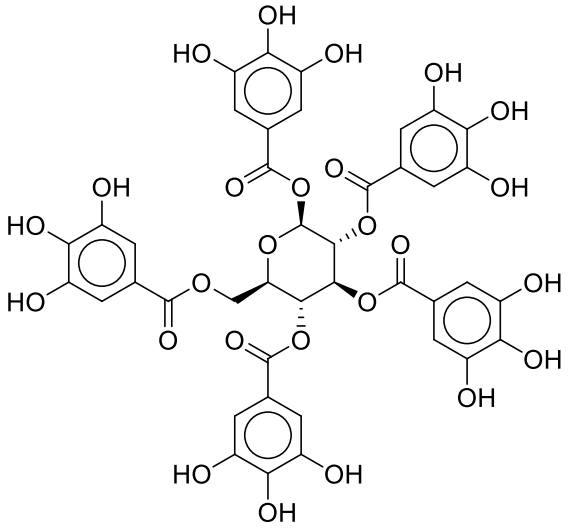
Punicalagin-β



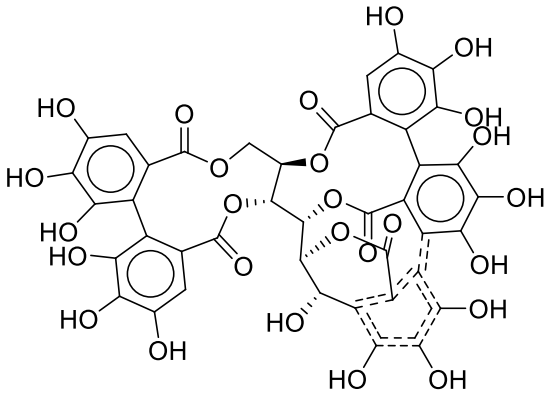
Punicalin



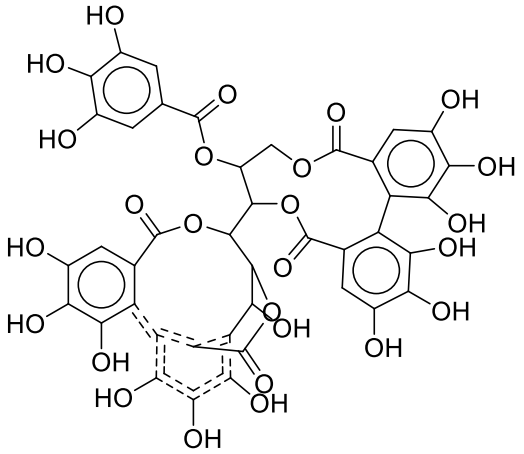
Pedunculagin



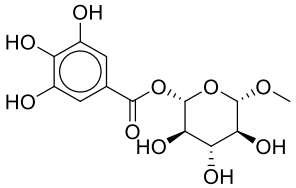
1,2,3,4,6-Pentagalloyl glucose



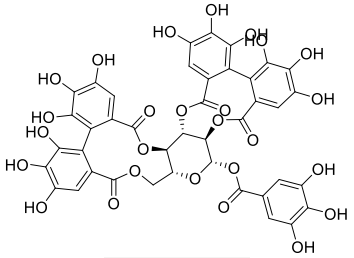
Castalagin



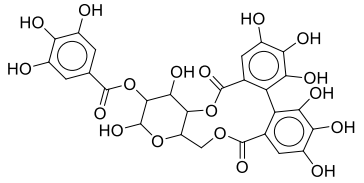
Casuarinin



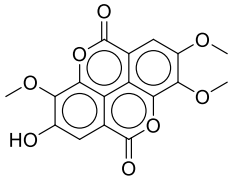
β-Glucogallin



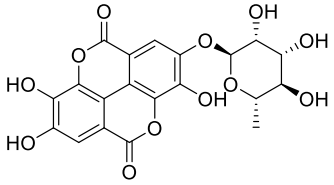
Casuarictin



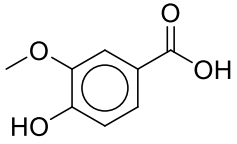
Hippomanin A



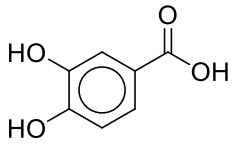
3,4,3-Tri-O-methylellagic acid



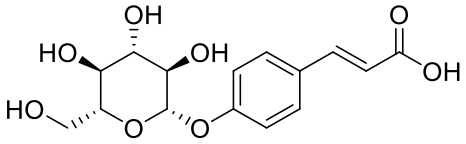
Eschweilenol C



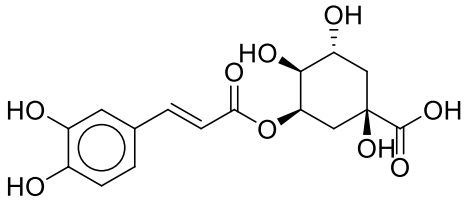
Vanillic acid



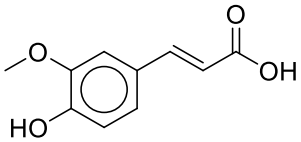
Protocatechuic Acid



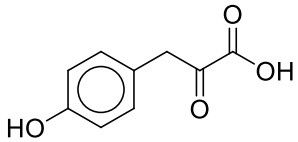
p-Coumaric acid glucoside



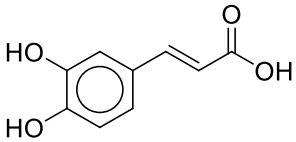
Neochlorogenic Acid



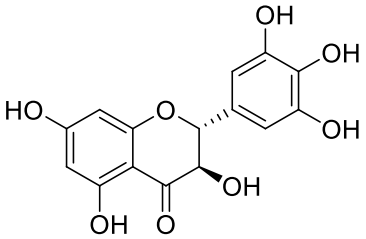
(E)-Ferulic acid



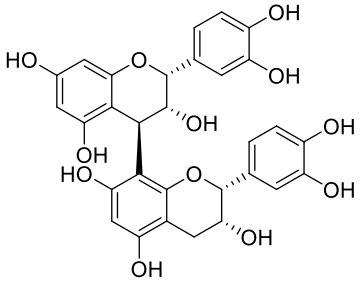
4-Hydroxyphenylpyruvate



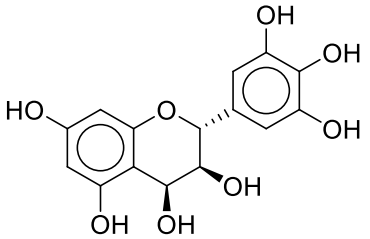
trans-Caffeic acid



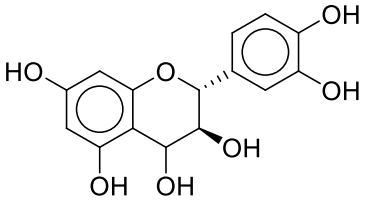
Dihydromyricetin



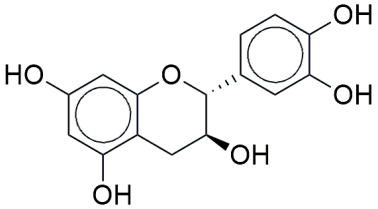
Procyanidin B2



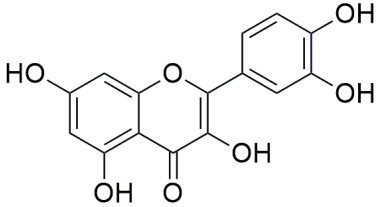
Leucodelphinidin



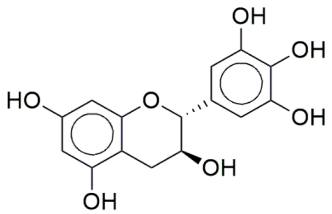
Leucocyanidin



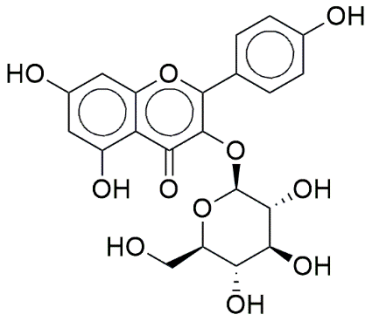
Catechin



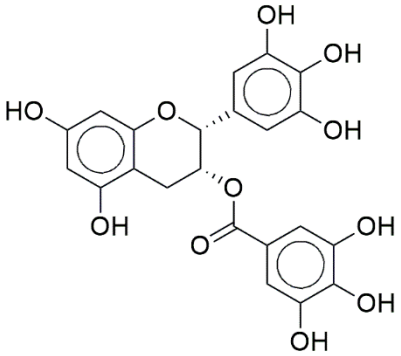
Quercetin



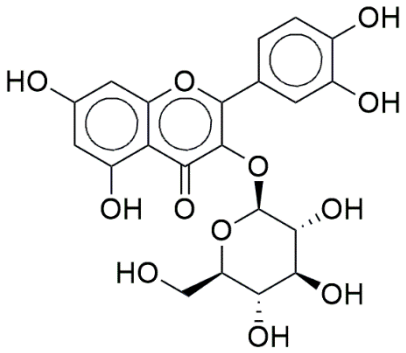
Gallocatechin



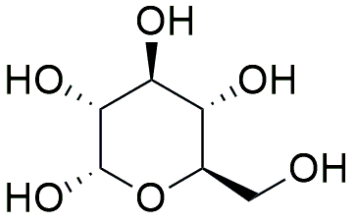
Astragalin



Epigallocatechin gallate



Hirsutrin



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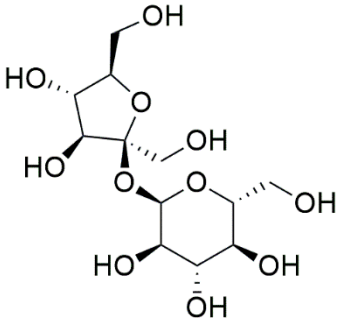
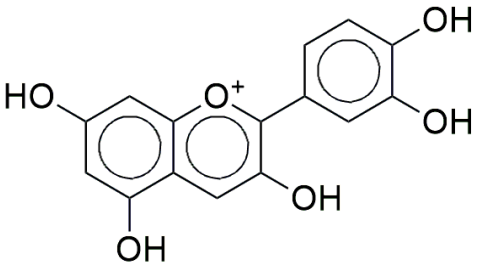
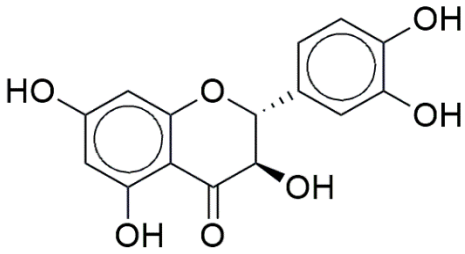


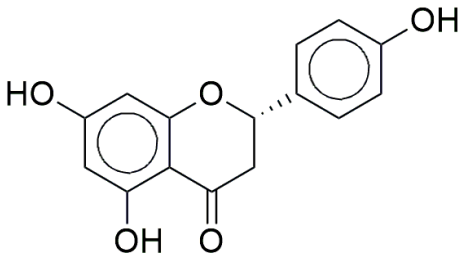
Sucrose



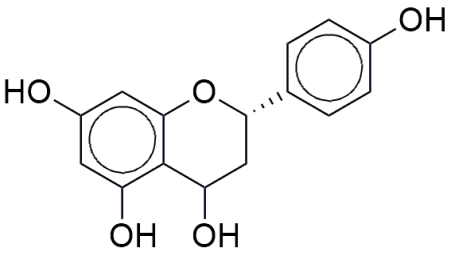
Cyanidin



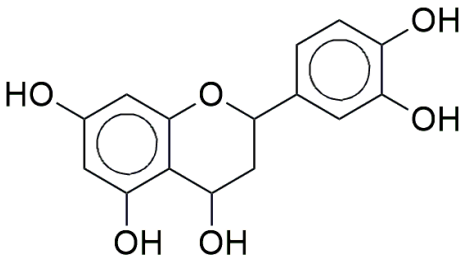
Taxifolin



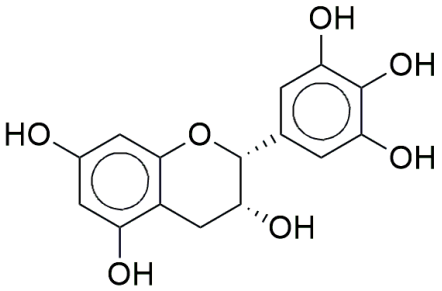
Naringenin



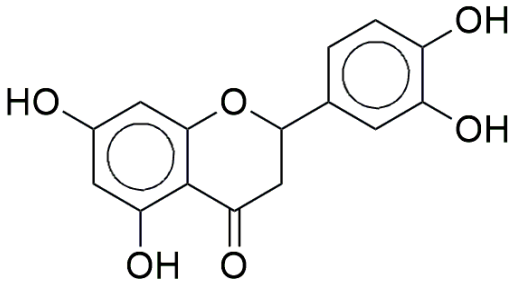
Apiforol



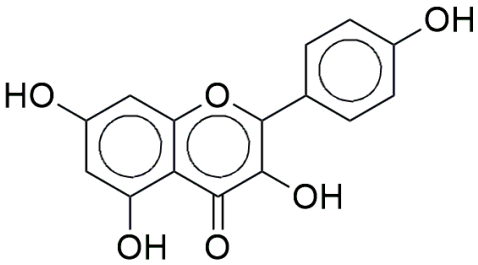
Luteoforol



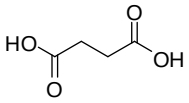
Eriodictyol



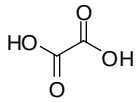
Kaempferol



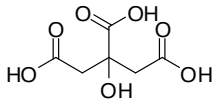
Epigallocatechin



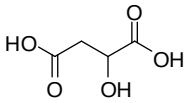
succinic acid



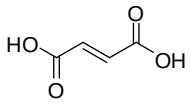
oxalic acid

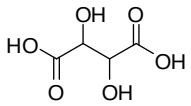


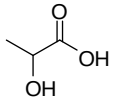
citric acid



malic acid



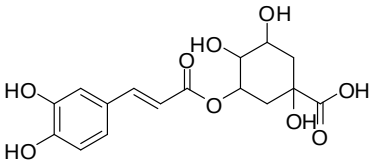




fumaric acid

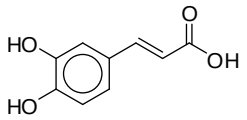
acetic acid

tartaric acid

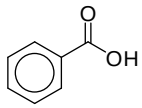


chlorogenic acid

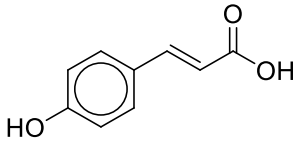
lactic acid



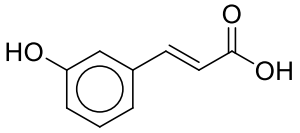
Caffeic acid



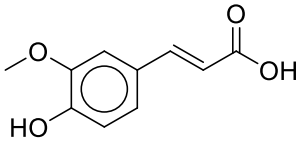
Benzoic acid



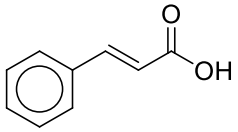
p-coumaric acid



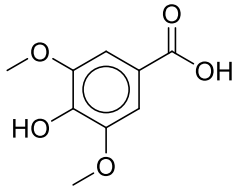
m-coumaric acid



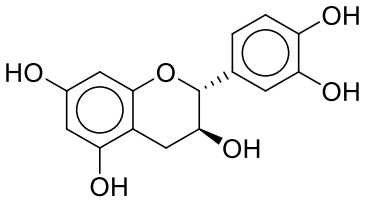
Ferulic acid



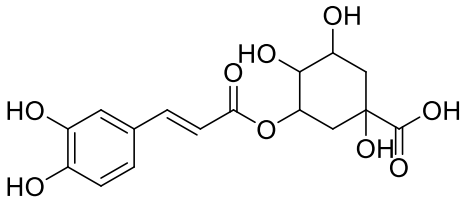
Cinnamic acid



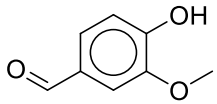
Syringic acid



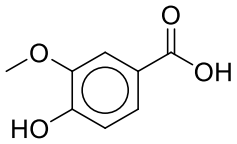
catechin



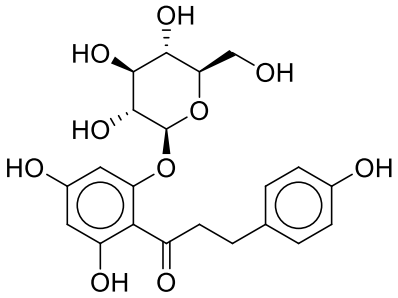
Chlorogenic acid



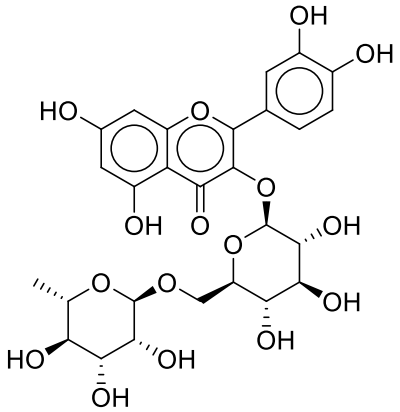
Vanillin



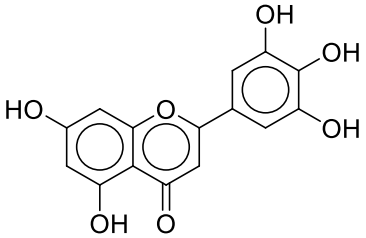
Vanillic acid



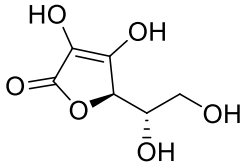
phloridzin



Rutin



tricetin



Ascorbic acid

Figure 1. Chemical structure of metabolites described in pomegranates.

3. Biological activities

Anticancer

Shells collected from *Punica granatum* were used to synthesize gold, they were characterized by UV-Vis, FT-IR, and TEM. Gold nanoparticles exhibited significant antioxidant and anti-human oral squamous cell carcinoma properties against the human oral squamous cell carcinoma cell line. These nanoparticles could likely be administered as chemotherapeutic agents. Nanoparticles as a convenient and safe material. (15)

In Acute myeloid leukemia cells: Pomegranate fruit extract using absolute ethanol would present new methods (multi target/ pathways) for treating AML patients, with less side-effects/ toxicity compared to chemotherapy, by impeding tumorigenesis, helping tumor suppression, affecting cancer cell replication, and altering gene regulation (upregulate p21 and p27 whereas downregulate cyclin-cdk network). Pomegranate extracts contains several valuable constituents including polyphenols, especially anthocyanins (Ats), and hydrolysable tannins (HTs), which have tumor inhibition activities. In vitro studies showed anti-proliferative and apoptosis inducing capacities (through the modulation of Bcl-2 proteins). These phytochemicals induce a complex set of activities that are involved in the signal transduction pathways pertaining to kinases, as MAPKs (mitogen-activated protein kinases), CDKs (cyclin dependent kinases), SphKs (sphingosine kinases, they also interact with several kinases, including CDKs, DNA PK, and Abl kinase, particularly strong binding with CDK5 and CDK8, as CDKs are one of the first kinases that contribute in AML. The extract inhibited the AML cell proliferations with GI50 values of 195.5 μg/ml, 289.1 μg/ml, and 353.5 μg/m in THP-1, THP-1, and HL-60 cells, respectively. (16)

The TGF-superfamily plays an important role in cell proliferation, which leads to tumor promoting activities at cancer. So inhibiting its pathway may help in cancer treatment. The active extract *Punica granatum* (leaves) 0.5mg/ml was chosen due to their high anti-proliferative activity against cancer cell lines (> 50%). The binding of TGF ligand to its receptor TGFR2 results in activation of TGFR1 triggered by TGFR2 and phosphorylation of TGFR1 while TGFR3 aids in ligand availability. Current research TGF receptors were studied for their relative expression in the HUH cell line using *Punica granatum* extracts. (17)

The anticancer activity of AgNPs and Ag/GO nanocomposites were tested against human breast cancer cell line (MCF-7) which has a dose dependent activity with half minimum inhibitory concentration of 100 and 150 μg/ml. (18)

Newly Synthesized Punicalin and Punicalagin Nano-Prototypes enhance the anti-cancer activity through induction of cytotoxicity in breast cancer Through ROS-Mediated apoptosis. (19)

Antiparasitic activity

Renata et al. investigated the anthelmintic activities of the saline extract of peels of fruits of *Punica granatum*, which come from the secondary metabolites found, mainly phenols, which can affect the decoupling of the oxidative phosphorylation responsible for ATP production, interfering with the energy production, which is fatal to this organism. Tannins are important too, synergistic interaction with enzymes, such as lipases, proteases, and aminopeptidases, which have vital role in the hatching and disintegration of eggshell. Tannins can also increase cell permeability, which favors their interaction with free proteins or cuticle glycoproteins of parasites, hindering nutrient absorption, mobility, and reproduction, consequently, causing death. Anthraquinones induces apoptosis, intercalation and binding with DNA, and inhibition of the enzyme topoisomerase. It also has antibacterial activities by inhibiting or interfering with redox processes inside the cell, this effect is not ruled out for parasites. The acute toxicity test showed the LC50 at the conc. of 6.19 mg mL-1. (20)

Punicalagin had better anticoccidial effects. The results of Elisa's studies on anticoccidial mechanisms, antioxidant studies, and pathological observations show that punicalagins alleviated appendicitis, improved immunoglobulin expression in cecal tissue, improved cecal integrity, and that her Indicates that the redox state has been restored. 16S rRNA sequence analysis also showed that punicalagins maintained fecal flora health during E. tenella infection by slightly increasing the proportion of Lactobacillus and Faecali bacterium. We selected her two monomers extracted from the pericarp of *Punica granatum*. Clinical results have shown that punicalagin has superior anticoccidial effects to ellagic acid. (21)

Studies show that pomegranate peel which is rich with phenolic compounds has antiparasitic activities used in treatment of *Giardia lamblia* is one of the most common flagellates in the intestinal tract. *Giardia lamblia* is one of the most common flagellates in the intestinal tract. In

vivo studies showed antigiardiasic potential by reducing cyst shedding and protecting intestinal cells however, they did not identify the compounds or elucidate any mechanism of action in the parasite. The in vitro antigiardial potential of polyphenolic extract was evaluated on *Giardia lamblia* trophozoites. The effect of polyphenolic extract on growth and adhesion capacity particularly on cells treated with 200 μg/mL, was determined by parasite kinetics; morphological damage was evaluated by SEM, alteration on a-tubulin expression and distribution were analyzed by western blot and immunofluorescence, respectively. (22)

Antimicrobial activity

Hydroalcoholic leaf extract of *Punica granatum* (HEPg) alone or in combination with calcium hydroxide ($Ca(OH)_2$)has antimicrobial activity against Enterococcus faecalis and Candida albicans in isolation and in mono- and polymicrobial biofilms. Microdilution tests in broth and assays for inhibition of biofilm formation were carried out to evaluate the antimicrobial properties of HEPg and HEPg + $Ca(OH)_2$ against Enterococcus faecalis and Candida albicans. The cytotoxicity of HEPg in HaCaT cells was evaluated by MTT assay. HEPg and HEPg + $Ca(OH)_2$ exerted significant antimicrobial activity against planktonic cells and mono- and polymicrobial biofilms. (23)

In the synthesis of Ag NPs, *Punica granatum* extract functions as a reducing and capping agent. Gram-positive and gram-negative bacteria were both significantly resistant to the antibacterial effects of Ag NPs/PG, although gram-negative bacteria were more so. (24)

Pomegranate peel extract (100 gm) was used as a reducing and stabilizing agent in the synthesis of ZnO-NPs. ZnO-NPs have both anti-microbial and antioxidant activity. The antimicrobial effect of biosynthesized ZnO-NPs can be interpreted in several ways, including (1) the generation of reactive oxygen species (ROS); (2) ZnO-NPs interacting with microbe cell walls, resulting in oxidative stress and cell death. ZnO-NPs inhibit Gram-negative bacteria and fungi more than Gram-positive bacteria and fungi. (25)

Anti-bacterial activity

Algerian pomegranate extracts have respectable antibacterial activities against both of environmental and clinical isolates of β_lactamase producing MRSA and ESBL-producing

Enterobacteriaceae. Methanolic and acetonic extracts of pomegranate are more effective against bacteria than the petroleum ether extracts. The presence of phytochemical compounds in such quantities in Algerian pomegranate extracts. Antibacterial traits of *Punica granatum* peel extracts-mediated Ag and Ag/GO nanocomposites. The green synthesized nanoparticles were found to have excellent antibacterial efficacy against both gram positive and gram-negative bacterial strains. The results showed that Ag/G nanocomposites showed better antibacterial activity than pure AgNPs with maximum inhibitory zone of 28 mm for Escherichia coli. (18)

NPP45 and NPP90 methanolic extracts of *Punica granatum* peel exhibited as strong antibacterial activity compared to CPP extracts. The most sensitive tested bacterial strains; *B. subtilis*, *St. aureus*, L. Monocytogenes and Spud. Aeruginosa with minimum inhibitory concentrations (MICs) values ringing (6-8 mg/ml) in the presence of both CPP and NPP45 methanolic extracts and with values of MICs (4 mg/mL) in the presence of the methanol extract NPP90. Finally, CPP, NPP45 and NPP90 represent unique natural materials rich in phenolics with strongly antibacterial efficiency (26)

This study was designed to determine the antibacterial activity of *Punica granatum* (*P. granatum* L.) (pomegranate) peel extract against Enterobacteriaceae [*Escherichia coli* (*E. coli*), *Salmonella Typhimurium* (*S. Typhimurium*) and *Shigella Dysenteriae* (*S. Dysenteriae*)] and gram-positive bacterium [*Staphylococcus aureus* (*Staph aureus*)]. (27)

Anti-oxidant and anti-inflammatory effect

Isolated several phenolic compounds through Methanol fraction from peel of pomegranate, which has the most increased total antioxidant activity. (150 mg/kg) daily for 3-4 weeks of extract administrated by rats, significantly rises the levels of SOD, GSH, and CAT, and decreased the levels of MDA in the blood. Also, it improves liver function; reduces inflammatory responses; and modulates of COX2, TGF-B1, and caspase-3, along with a significant upregulation of the expression of HO-1, Bcl2, and Nrf2 (which activates genes protecting against oxidative stress), and. It decreases IL-1β and elevates levels of IL-10 which reduces inflammation by inhibition of many inflammatory cytokines and chemokines, or by blocking the activities of TNF-α, with modulating inflammatory cell signaling. (28)

The results of this study showed that the hydroalcoholic extract of *P. granatum* fruit peel has a significant dose-dependent inhibitory activity against ulcerative colitis. The results obtained demonstrated the efficacy of *Punica granatum* fruit peel against inflammatory bowel disease, possibly due to its anti-inflammatory and antioxidant properties. The hydroalcoholic extract of *P. granatum* fruit peel (PGPE) was found to be safe at a dose of 2000 mg/kg body weight (29)

Anti-inflammatory effect

P. granatum has been widely used in treating inflammation-related diseases, such as cardiovascular diseases and cancer. Polyphenols were obtained from the peels their bioactivity of suppressing lipopolysaccharide stimulated inflammatory cytokines and Mediators in RAW 264.7 Cells via Activating p38 MAPK and NF-κB Signaling Pathways. (30)

It was reported that the Plant flower extracts were tested for their anti-inflammatory effects by determining the ability to inhibit the activity of lipoxygenase and proteinase. They are characterized by the ability to inhibit lipoxygenase at a level above 80% and proteinase at the level of about 55%. (31)

Anti-oxidant effect

It was studied that the Plant flower extracts of *Punica granatum* were tested for their antioxidant activity. Also, the extracts were tested for their cytotoxic effect on skin cells, using Alamar Blue and Neutral Red tests. The ability to inhibit the activity of enzymes responsible for the destruction of elastin and collagen was also studied. Research has shown that extracts have no toxic effect on skin cells, are a rich source of antioxidants and show the ability to inhibit the activity of elastase and collagenase enzymes. (31)

The present meta-analysis provides evidence that pomegranate can effectively improve some oxidative stress factors. Due to high content of polyphenols, flavonoids, and several other types of antioxidant compounds, Oxidative stress is related to many chronic diseases such as type 2 diabetes, cancers, hypertension, and heart diseases. (32)

Antioxidant activity of *Punica granatum* peel powder incorporated fat replaced low- calorie chhana podo. The DPPH radical scavenging activity (RSA) has been generally used to quantify the antioxidant activity of food products and the stable radical. Chhana podo with PPP has

enhanced potential to gain popularity with its reduced- calorie and fat content, improved antioxidant properties, and other nutritive benefits. (33)

Anti-diabetic activity

Pomegranate showed maximum amount of amylase inhibitory activity. The alpha amylase inhibitors interfere with enzymatic action which prevents the liberation of α-D glucose from oligosaccharides and disaccharides resulting in delayed glucose absorption and decrease in postprandial glucose levels. Amylase activity in pomegranate = 30 %. (34)

Punica granatum peel extract (100gm) was used in this study as a reducing agent for green synthesizing silver nanoparticles (AgNPs). In vitro AgNPs has the ability to inhibit carbohydrate digesting enzymes, so it has anti-diabetic activity. AgNPs can undergo against -amylase and -glucosidase, as well as antidiabetic efficacy, indicating that they are suitable for use in medical applications. (35)

Antifungal activity

Chemotherapeutic treatment of fungal fish diseases with chemicals and malachite green was proved to possess carcinogenic and teratogenic effects in fish and human health. Hence, the current study aimed to investigate antifungal activities of *Punica granatum* extracts against the causative agent of saprolegniasis, Saprolegnia parasitica, as an alternative fungicide.

Ethanolic extracts of *Punica granatum* showed a potential efficiency in preventing mycelial growth of *S. parasitica* at a concentration of 0.5 mg/ml recording inhibition zone diameters of 16.5 mm. Acute fish toxicity of the potential plant extracts revealed that P. granatum exhibited low toxicity to experimental fish. (36)

Pomegranate peels has activity against fungi without considerably affecting the tensile strength and elongation at break, by reducing the mechanical parameters. The films' mechanical strength and antifungal activity against *Candida albicans*, *C. glabrata*, *C. krusei*, and *C. tropicali* were sufficient. (37)

Bronchial asthma treatment

In the current study, priority was given to assess the anti-asthmatic effects of transdermal administration of freshly prepared Org (Org1) using the PSO-Sap fraction of the BW structure in

rats. Org1 showed potent protective and therapeutic effects in modulating OV-induced airway remodelling manifested by airway oxidative stress, inflammation, fibrosis and morphological changes in lung tissue. Suppression of oxidative stress in ovalbumin-sensitized rats by a novel organ gel of the saponified fraction of *Punica granatum* seed oil. Regulation of the NF-κB signalling pathway and IL-13 in asthmatic rats by aerosol inhalation of the combined active constituents of *Punica granatum* juice and peel. (38)

Healing colitis

Arpit et al. revealed that 50% ethanolic extracts of *Punica granatum* dried peel have significant healing Properties in acetic acid (AA) induced colitis observed by decrease rats as in colonic mucosal damage score, adhesions and weight (confirmed by macroscopic study of colon) and decrease in diarrhea, fecal output, presence of fecal mucous/blood and increase in body weight suggestive of beneficial effects of treatment. Further, PGE is safe, not produce any lethal effect, and with activity similar to sulfasalazine (SS). As acute toxicity study indicated no mortality or other adverse effects even with 1000 mg/kg dose (10 times of effective dose). (39)

Antiviral activity

The juice of pomegranate (*Punica granatum* L.) was tested against the respiratory virus's adenovirus type 5, influenza A virus, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reducing viral loads after infection or preventing viral influx could ease symptoms, stop spread to the lower airways, or stop transfer to the next person. (40)

Growth performance

Punica granatum (pomegranate; peel) was mixed with other herbal extracts (CPRE) to make effect on Ruminal fermentation, nutrient utilization, growth performance, nitrogen and phosphorus excretion, and methane emission. All were studied in growing buffaloes. To determine an optimal dose of CPRE for an in vivo study on buffaloes, in vitro tests for ruminal fermentation and feed degradability were performed using ruminal fluid as inocula and CPRE at 0 to 40 g/kg strate. (41)

Hepatic and renal toxicity

The administration of lead acetate caused hepatic and renal toxicity, as evidenced by changes in liver and kidney function markers, antioxidant status, and tissue integrity. Lead administration caused increases in serum and liver transaminases, increase in serum, increase in lipid peroxidation levels in both the liver and the kidney. and kidney function markers (p 0.05). There was also a decrease in average body weight, antioxidant markers in the liver and kidney. We can compact these toxins by soaking Pomegranate powder (500 g) in 1 Littre of ethanol for three days before being concentrated and freeze dried to produce *Punica granatum* L. ethanol extract (PLEE). PLEE administration at various doses significantly modulated these changes. (42)

Healing activity

Extraction and isolation of bioactive compound from the peel of *Punica granatum* Linn has wound healing activity. It was also expanded to assess wound healing activity in vitro using excision and incision models. In incision wound model, the highest wound healing strength was 201.83 ± 4.98 for isolated 10% w/w punicalagin ointment. In excision model the highest wound healing strength was 88 ± 0.78. Punicalagin and punicalin from *Punica granatum* peel can therefore be employed as powerful wound-healing agents. (43)

Anti-Giardiasis

We found that *Punica granatum* peel extract has effect against experimental giardiasis compared to metronidazole (MTZ) in Giardia intestinalis-infected mice.

Methanolic extracts extracted from the shell of *P. granatum* have been shown to be a promising candidate for the prevention and treatment of *G. lamblia* infection in laboratory mice and for giardiasis, where pomegranate is considered promising, due to its various functional properties. It may be used excellently in dietary supplements, for any of the alternative therapies. It has an anti-inflammatory effect and serves as the basis for further research into pomegranate's efficacy against other parasites. (44)

4. Conclusion

The use of pomegranate has been widely suggested due to its physiological health benefits.

Pomegranate and derivatives, such as juice, peel, and seeds, are rich sources of several high-value compounds with potential beneficial physiological activities. The rich bioactive profile of pomegranate makes it a highly nutritious and desirable fruit crop. Research offers evidences that routine supplementation with pomegranate juice or extract may protect against and even improve several diseases, including diabetes and cardiovascular disease; it may even help to prevent and arrest the development of certain cancers, in addition to protecting the health of the mouth and skin. Side effects are very rare, for this need we have to keep an eye on these crude drugs and natural products Using concentrated, low-cost pomegranate juice or standardized pomegranate extract capsules offers consumers a way of achieving the broad spectrum of health benefits of this fruit.

- **Conflict of Interest**

  The Authors declare no conflict of interest.

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