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A Chemical Study By Using GC-Mass Spectrometry Of The Peel And Seeds Of Punica Granatum L. Plant

[1]* Arkan Ali Al-Tai [2] * Taiba Falih Al-Mayyahi

1,2...Al-Qadisiyah Education Directorate
Corresponding author: Arkan Ali Al-Tai E-mail: arkanarkan8585@gmail.com

ABSTRACT

This study was conducted in gas Chromatography-Mass spectrometers (GC-MS) / Department of Environment and Water / Ministry of Science and Technology during 2019. To determine the concentration and percentage of active chemical compounds in the ethanol extract of pomegranate seeds and seeds (Punic granatum L.) Salami variety In Iraq using GC-MS gas chromatography. The study showed that the ethanol content of pomegranate shells contained 28 chemical compounds, As recorded 2H-Pyran-2-one, Hexadecanoic acid, ethyl ester showed the highest ratios, respectively, As for The compound 1,12-Bis (2-nítrophenoxy) dodecane was the lowest ratio of 0.02%,The results showed that the ethanol content of pomegranate seeds contained 33 chemical compounds, 5-hydroxymethylfurfural, furfural, 4-fluorobenzyl alcohol, and the highest concentrations were respectively, and record the compound 1-ethyl-2-hydroxy methyl imidazole had the lowest ratio of 0.08. Proved GC-MS analysis of pomegranate peel and seeds demonstrated the presence of many compounds including phenol such as 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-, 4-Mercaptophenol, and Terpenes such as Octalcane, Nonacosane and Carboxylic acids such as Acetic acid and fatty acids such as Oleic Acid, Linoleic Acid Ethyl ester and esters such as Cyclopropanecarboxylic acid and other compounds. This means that the Salami variety pomegranate cultivated in Iraq is effective against many diseases and can be used for medical purposes.

Keywords: Punica granatum L .Salami variety. GC-MS. Phytochemicals. Phenols

Correspondence:
Arkan Ali Al-Tai
Al-Qadisiyah Education Directorate

*Corresponding author: Arkan Ali Al-Tai email-address:
arkanarkan8585@gmail.com

INTRODUCTION

Punica granatum L. This name means the red fruit with many seeds belonging to the Punicaceae family, which consists of one genra, Punica, which contains several species of pomegranate [1].Pomegranate is one of the oldest fruits to be eaten, which is mentioned in the holy books of the Jews, Christians and the Holy Quran, and known the trees and fruits of pomegranates in ancient Egyptians and found painted on the walls of their graves, and planted trees in the Hanging Gardens in Babylon[2] The pomegranate is a monoculture, carrying two types of flowers. The first is small, bell-shaped, sterile, short-pitted and ovarian flowers with few eggs. The second type is a large, The vase-shaped vase, with a sophisticated and long-lasting ovary, develops after fertilization and fertilization into fruits The ratio between these two species varies according to the different categories[3,4].The cultivars currently present in Iraq show a clear variation in the shape, colour, taste, colour of seeds and crust thickness. Salimi is the most prevalent species in the central and southern regions of Iraq[2] (Mars, 2000). The sources did not indicate any difference in the Salimi tree of the specifications of pomegranate trees, but the distinction is based on the characteristics of fruit at maturity and described by the sources as a large fruit, The color of the green colour is red and the dark red colour includes all the fruit at the end of the season and the red berries are a lot of juice and tasty. As the fruits mature, the sweetness increases and the acidity decreases[5]. The importance of pomegranate cultivation comes from the beginning of its early fruits in the third year of agriculture, its high productivity and its ease of reproduction. As well as the high nutritional value of its fruits, and the use of pomegranate fruits in several areas, such as fresh consumption and the preparation of some foods and in the manufacture (molasses pomegranate) The peel of fruits and their residues are used as pigments in the manufacture and tanning of leather. Pomegranate fruits are of economic importance due to the long shelf life of the pomegranate and its ability to store in many ways and for relatively long periods and the possibility of transporting them over long distances. Its nutritional importance comes as a result of its high levels of vitamins, mineral salts, pigments and fats. Carbohydrates, sugars, acids, fibre, proteins and other nutrients, which vary in proportion to different species[6,7,8]. Generally, every 100 grams of pomegranate contains 81.3% water, 0.8% g protein, 0.7% fat, 0.5% ash, 2% fiber, 8.2 - 19.7% sugar, 10 mg calcium, 24 mg phosphorus, 0.6 mg iron and 0.07 mg Thiamine, 0.02 mg riboflavin, 0.9 mg niacin, 8 mg vitamin C and 0.46 - 3.6% citric acid [9]. Although Iraq is one of the major pomegranates of the Middle East [10] the available information on the production and production of pomegranates in Iraq indicates that its numbers are low and its productivity is fruit [11].According to information obtained from the Central Bureau of Statistics in Iraq, pomegranate production in Iraq reached 98683 tons in 2015 and production increased rapidly from year to year[10]

Description of *Punica granatum* L. plant

The Punicaceae family consists of one species, Punica, which has several species. first one: *Granatum* includes species that are cultivated globally, and second species: *P. protopunica* Balf is particularly cultivated in Yemen, especially the island of Storra The Third species: *P. Granatum Florepleno* This species is cultivated for decoration to the beauty of its bright flowers resembling red carnations[12,13] Pomegranate is a shrub up to 6 meters high with branches hanging, with spines on its edges, branches and leaves tend to red. Its bright red flowers are beautiful scenery. The spherical fruit bears a crown, while the fruit crust. Leather Textures. The fruit contains many red seeds or tends to whiteness sometimes, but often a reddish colour, The leaves fall in autumn so the pomegranate tree is not evergreen. The

pomegranate flowers are called gulnar, a word for the Persian word Planar, meaning pomegranate. Pomegranate is known in the Pharaonic language as "Raman" and in Coptic "Erman"[2,6].The area extending from Iraq and Iran to the Himalayas in northern India is the original home of pomegranates, and from there moved to the Arabian Peninsula. There are also wild and agricultural flowers in the Mediterranean region since ancient times. Pomegranate is a tropical and sub-tropical plant that adapts significantly to the conditions of the Mediterranean basin because of its drought resistance [14].The number of pomegranate varieties in the main regions of the world is more than 130 varieties, including Salimi, which is one of the most important species and its spread in the central and southern regions of Iraq because of environmental conditions in these regions, Gabsi and Zehri in Tunisia, Beynar and Hicazna in Turkey, Robab and Schahva in Iran, Moller and Tendral in Spain, Fleshman and Wonderfull in the state of California and Occupied Palestine [15]

The medical importance of *Punica granatum* L. plant

The care of medicinal and aromatic plants is due to the fact that it is the first major source of access to medicines since the beginning of creation. Human beings use plants in their diet, relieve their pain and treat their various diseases through the use of one or all parts of the plant after it is soaked, boiled,) Without ascertaining it and searching for the substance containing it, which causes the therapeutic act or the part of the plant of therapeutic interest, but only on the basis of experience, and the progress of science and technology has become able scientists and researchers to separate the active substances from the plants that contain and eliminate Here a way that fits their use and pathological condition used for which[16,17,18].The pomegranate fruit is widely used in Indian folk medicine and ancient Greek medicine. Pomegranate husks, seeds and lipids are used as antioxidants and gastrointestinal ulcers because they contain a high percentage of phenols, tannins, which alter the nature and kill germs of bacteria. Where epithelial layer proteins are deposited to act as a protective layer over damaged tissue[19,20]

Pomegranate syrup and peeling are used in the treatment of diarrhoea and dysentery because it changes the nature of proteins and reduces the leakage of fluids as well as it kills germs and stings toxins. Pomegranate juice is used to treat gastric pain, intestinal and colon infections, and pomegranate juice is used to treat vaginal secretions due to its deadly bacteria and fungi [21.Pomegranate extracts are used to expel parasites and kill microorganisms. This deadly activity is due to alkaloids and tannins. Pomegranate is also used as a diuretic, tonic, and cooling agent for the body's heat[22,23].

previous studies

Various studies have shown that some pomegranate species contain various chemical compounds such as phenols, turbines, flavonoids, alkaloids, acids, sugars and other chemicals in different parts of the plant, such as [24]. On Pomegranate juice by GC-MS analysis showed that the juice contained 40 chemical compounds, including Gallic acid, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6methyl and Furfural, and 43.25, 4.65 respectively, and most of the compounds were phenolic, which means that pomegranate has a large activity as an antioxidant. The study of [25] on two species of *P.granatum* and *P.protopunica* by GC-MS analysis showed that the containment of the seeds of the first type of 21 chemical compounds, notably Benznepropanoic acid and benzenedicarboxylic acid-1,2 and propanol-2 (propylthio) (CAS) -1 and 17.04, 11.32, and 6.49 respectively, while the seeds of the second species contained 14 chemical compounds, most notably Di-2 (ethylhexyl) phthalate (27.71) and Benzoic acid (24.05) and Dimethlamine (6.98). The study by [26] showed GC-MS analysis on *P. granatum* shells containing 20 chemical compounds, most notably Pyrogallol (41.88), Hydroxymethylfurfural-5 (14.10) and D-Allose (9.17). The analysis of GC-MS by[27] showed that the ethylene extract of pomegranate peel contains 26 chemical compounds, including Glycerin, Guanosine, Pyrogallol, and 27.03, 13.34 and 6.45 respectively. And other studies that proved that pomegranate plant rich in chemicals and effectiveness, which have great nutritional and therapeutic importance.

Materials and methods of work

Collect plant samples

The fruit of the pomegranate plant was collected from one of the orchards of the Sunni area of Qadisiyah city, The seeds were then isolated from the crust and placed in the shade to dry, first grinded the ceramic mortar and then used a small electric mill to get a fine powder. Place the powder in clean, dry plastic bags, marked with the name of parts of the plant used for study and date of collection, and then kept in a dark and dry place at room temperature until use.

Preparation of alcohol extract for pomegranate peel and seeds

The botanical extract of plant samples (peel and pomegranate seeds) was prepared according to the[28] method, By taking 50 g of dry powder and then extracting the substance from the Saxholate extract using 500 ml of ethyl alcohol at a concentration of 99% for 24 hours, after which the alcohol extract was placed in sealed, sealed bottles in the refrigerator until use.

Sample analysis in GC-MS device

The active compounds were diagnosed and evaluated by (Model Agilent 5977A gas chromatograph mass spectrometry)system manufactured by the GC Clarus 500 Perkin Elmer system, which includes the AutoSampler [AOC-20i + s] for compounds and the bound gas chromatography By mass spectrometry.chromatograms supplied with the device, all this information is automatically programmed on the machine.

Determination of raw chemical compounds

Active compounds were determined based on GC-MS mass spectrometry, and after obtaining the mass spectrometry of each compound, the results were processed by GCMS solutions and Peaks curves separated by The National Institute of Standardization and Technology (NIST) database, which contains more than 62,000 known patterns, The resulting spectrum of the unknown component was compared with a range of known components stored in the National Library to confirm the name, molecular weight, and structure of the components of the test materials. This was done in GC-MS / Environment and Water Service / Ministry of Science and Technology.

RESULTS AND DISCUSSION

The analysis of GC-MS was observed by GC-MS and the mass spectrometry of the chemical content of the plant parts under study. Sixty-one compounds were recorded, varying between phenols, terpenes, alkanes, alkaloids, esters, steroids and acids. The ethanolic extract of pomegranate extracts contained 28 chemical compounds Figur(1) Table(1), and the ethanolic extract of seeds contained 33 chemical compounds Fagur(2) Table(2). In addition, the recurrence of some chemical compounds was recorded at different times and for the same plant part. The compounds that were repeated in the peel extract were Furfural, 2H (1H) -Pyridinone, 4H-Pyran-4-one, 2,3-dihydro-3,5 -Di hydroxy-6-methyl- and 5-Hydroxymethylfurfural, while Furfural and 5-Hydroxymethylfurfural were repeated in the seed extract. The study also showed that 14 chemical compounds were found among the studied plant parts: Furfural, 2H-Pyran-2-one, Furan, Methyl 2-furoate, 2H (1H) -Pyridinone, Furancarboxylic acid, methyl ester-3, 5-Hydroxymethylfurfural, 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-Benzenemethanol, 3-fluoro-4-Mercaptophenol, 4-Fluorobenzyl alcohol and 9-Octadecenoic acid (Z) -, methyl ester, Hexadecanoic acid, ethyl ester and Octadecanoic acid, ethyl ester. It was also observed that some compounds appeared in high proportions such as compound 2H-Pyran-2-one at 25.27 in shell extract and 5-hydroxymethylfurfural compound (37.33) in seed extract. While some of the other compounds appeared at very low rates such as the compound 1,12-Bis (2-nítrophenoxy) dodecane at the concentration (0.02) and the compound 1-ethyl-2-hydroxy methyl imidazole at the concentration (0.08). The current study shows that phenolic compounds are the most prominent in the analysis of GC-MS, the results of the study stated that the plant extracts are rich in beneficial chemicals, including flavonoids, which recent studies have proven, including[29]study, that it has anti-cancer, anti-inflammatory and anti-oxidant efficacy. it found biological functions for some of the major compounds in GC-MS analysis study,

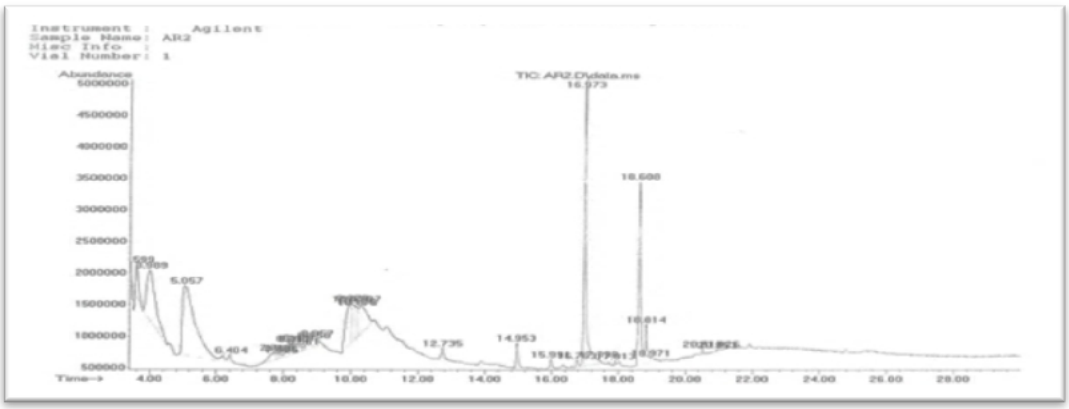


Figure (1) GC-MS chromatogram of an extract of peel of *Punica granatum*. L

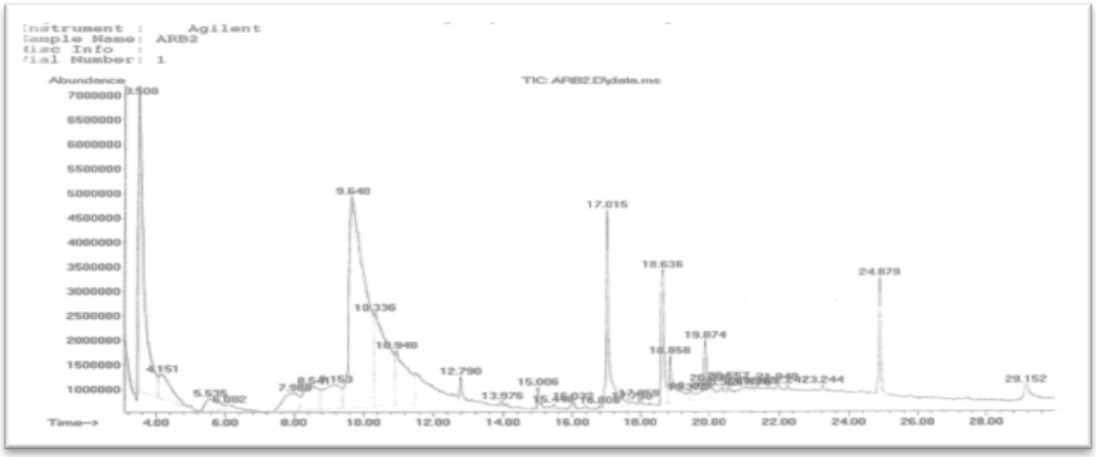
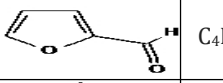
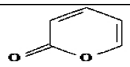
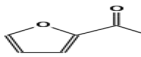
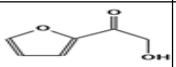
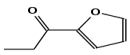
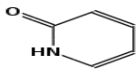
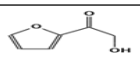
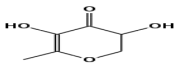
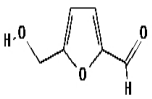


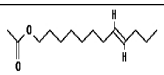
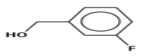
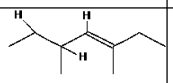
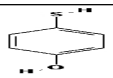
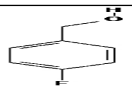
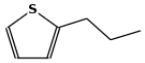
Figure (2) GC-MS chromatogram of an extract of seeds of *Punica granatum* L

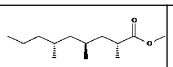
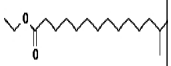
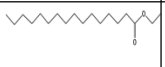
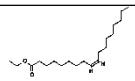
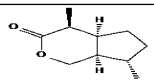
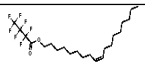
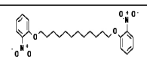
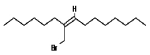
Then resulting give its importance medical for *Punica granatum.* The chemical compounds obtained are of medical importance and have many medical uses, including 4H-Pyran-4—one, 2,3—dihydro—3,5—di hydroxy—6—methyl—that has efficacy Anti-tussive, antibacterialactivities and antioxidant[30] anti-inflammatory and antiasthma activites [31] and Furan as; anti-inflammatory, antifungle, antiviral, and antibacterial.[32] and Oleic acid as: Antimicrobial activity[33], and 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) as: Reductase inhibitor, , Antiarthritic Hepatoprotective, Insectifuge-Anti-inflammatory, Cancer preventive, Anticoronary, Nematicide Antiandrogenic , Insectifuge, and Hypocholesterolemic.[34]. which means that pomegranate has great medical importance against many diseases as an antioxidant. Through the analysis of GC-MS, many biologically active chemical compounds have been successfully detected in the ethanolic extract of pomegranate husks and pomegranate seeds and this is of great importance in the use of pomegranate extracts in subsequent microbial studies.

Table(1) Analysis GC-MS of an extract of the peel of *Punica granatum* L.

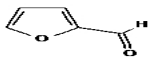
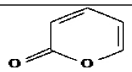
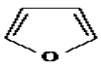
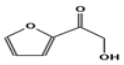
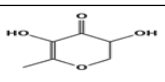
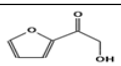
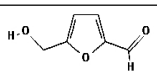
P. N	Compound Name	Structure	Molecular Formula	Peak Area %	Retenton timie (min)	CAS	Qual
1	Furfural		C_4H_3OCHO	1.81	3.599	000098—01—1	86
2				12.12	3.986		94
3	2H-Pyran—2—one		C5H4O2	25.27	5.056	000504—31—4	90
4	Furan		C4H4O	0.71	6.407	000110—00—9	72
5	Furyl hydroxymethyl ketone		C6H6O3	1.70	7.712	017678-19—2	46
6	Methyl 2-furoate		C6H6O3	0.40	7.826	000611—13—2	52
8	l—Propanone, 1-(2-furanyl)—		C7H8O2	0.19	7.925	003194—15—8	47
7	2(1H)—Pyridinone		C5H5NO	0.12	7.887	000142—08—5	55
10				0.47	8.304		42
9	3-Furancarboxylic acid, methyl ester		C6H6O3	1.60	8.236	013129-23-2	35
11	4H-Pyran-4—one, 2,3—dihydro—3,5—di hydroxy—6—methyl—		C6H8O4	1.00	8. 418	028564—83—2	41
12				0.23	8.562		30
13	5—Hydroxymethylfurfural		C6H6O3	1.25	8.858	000067—47—0	22
18				1.08	10.179		89

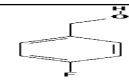
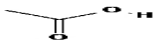
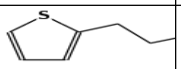
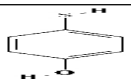
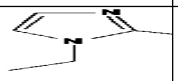
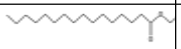
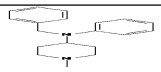
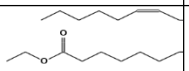
supplement Table(1) Analysis GC-MS of an extract of peel of *Punica granatum* L.

P.N	Compound Name	Structure	Molecular Formula	Peak Area %	Retenton timie (min)	CAS	Qual
14	E-8-Methyl-7-dodecen-1—ol acetate		C14H26O2	1.09	8.957	1000131-32—2	27
15	Benzenemethanol, 3—fluoro—		C7H7FO	6.58	9.981	000456-47—3	53
16	3,5-Dimethyl-3-heptene		C9H18	3.31	3.31	059643—68—4	64
17	4-Mercaptophenol		C6H6OS	1.15	10.125	000637—89-8	50
19	4-Fluorobenzyl alcohol		C7H7FO	5.71	10.285	000459-56—3	49
20	Thiophene, 2—propyl-		C7H10S	0.77	12.736	001551-27—5	45

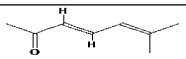
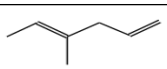
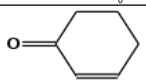
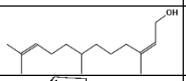
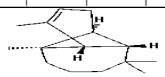
21	Nonanoic acid, 2,4,6-trimethyl-, methyl ester, (2R,4S,6R)-(-)—		C13H26O2	1.46	14.952	018450-82—3	46
22	Ethyl 13—methyl-tetradecanoate		C17H34O2	0.63	15.992	1000336-61—5	83
23	9-Octadecenoic acid (Z)-, methyl ester		C19H36O2	0.48	16.766	000112-62—9	42
24	Hexadecanoic acid, ethyl ester		C18H36O2	16.32	16 970	000628—97—7	99
25	l—Docosanethiol		C22H46S	0.33	17.395	007773—83—3	38
26	Heptadecanoic acid, ethyl ester		C19H38O2	0.09	17.911	014010-23—2	80
27	Ethyl oleate		C20H38O2	10.89	18.610	000111—62-6	98
28	Octadecanoic acid, ethyl ester		C20H40O2	2.42	18.814	000111—61—5	99
29	Iridomyrmecin		C10H16O2	0.52	18.974	000485-43—8	90
30	Oleyl alcohol, heptafluorobutyrate		C22H35F7O2	0.21	20.514	1000352—68—1	68
31	1,12—Bis(2—nítrophenoxy)dodecane		C24H32N2O6	0.02	20.954	155056—69—2	78
32	Pentadec-7-ene, 7-bromomethyl—		C16H31Br	0.05	21.023	1000259—58-5	95

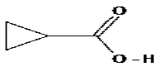
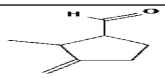
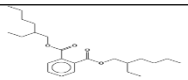
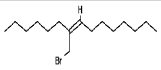
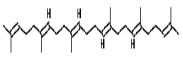
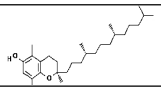
Table(2) Analysis GC-MS of an extract of seeds of *Punica granatum* L.

P.N	Compound Name	Structure	Molecular Formula	Peak Area %	Retento n timie (min)	CAS	Qual
1	Furfural		C_4H_3OCHO	15.91	3.504	000098—01—1	95
2				2.89	4.149		90
3	2H-Pyran—2—one		C5H4O2	1.45	5.538	000304 — 31— 4	76
4	Furan		C4H4O	0.26	6.084	000110—00—9	62
5	3-Furancarboxylic acid, methyl ester		C6H6O3	2.74	7.989	013129—23—1	46
6	4H-Pyran-4—one, 2,3—dihydro—3,5—di hydroxy—6—methyl—		C6H8O4	3.32	8.543	028564—83—2	38
7	Methyl 2-furoate		C6H6O3	3.67	9.150	000611—13—2	30
8	5—Hydroxymethylfurfur		C6H6O3	31.42	9.651	000067—	94
10				5.91	10.949		96

	al					47—0	
9	4-Fluorobenzyl alcohol		C7H7FO	11.42	10. 334	000459-56—3	94
11	Acetic acid		CH3COOH	0.59	12.793	1000186 - 7 5 - 5	46
12	Thiophene, 2— propyl-		C7H10S	0.12	13.976	001551 — 27 — 5	53
13	Octadecane		C18H38	0.45	15.008	000593 — 45 — 3	35
14	Benzenemethanol, 3-fluoro-		C7H7FO	0.12	15.449	000546 — 47 — 3	52
15	4-mercaptophenol		C7H7FO	0.22	16.033	000637 — 89 — 8	38
16	1-ethyl-2-hydroxy methyl imidazole		C6H10N2	0.08	16.807	063634 — 44 — 6	42
17	Hexadecanoic acid, ethyl ester		C18H36O2	5.85	17.012	000628— 97—7	98
18	Bamipine		C19H24N2	0014	17.748	004945 — 47 — 5	56
19	Oleic Acid		C18H34O2	0.09	17.960	000112 — 80 — 1	45
20	Linoleic acid ethyl ester		C20H36O2	3.65	18.636	000544 — 35 — 4	95
21	Octadecanoic acid, ethyl ester		C20H40O2	2.26	18.856	000111— 61—5	98

supplement Table(2) Analysis GC-MS of an extract of seeds of *Punica granatum* L.

P.N	Compound Name	Structure	Molecular Formula	Peak Area %	Retento n timie (min)	CAS	Qua l
22	2-Hexyne		C6H10	0.37	21.057	000764—35— 2	56
23	6-Methyl-3,5-Heptadien-2-One		C8H12O	0.15	19.372	001604—28 —0	52
24	1,4-Hexadiene, 4-methyl-		C7H12	0.41	19.455	001116—90— 1	46
25	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-		C20H34O2	1.67	19.873	001191—41— 9	93
26	2-Cyclohexen-1-one		C6H8O	0.49	20.032	056051—94— 6	58
27	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (Z)-		C15H28O	0.37	20.385	20576—57—2	81
28	Longipinane		C15H24	0.27	20.557	1000156— 14—5	55

29	Cyclopropanecarboxylic acid		C4H6O2	0.13	21.178	0080360—57—2	80
30	Cyclopentanecarboxaldehyde, 2-methyl-3-methylene-		C8H12O	0.09	21.368	1000154—24—0	76
311	Bis(2-ethylhexyl) phthalate		C24H38O4	0.19	21.952	000117—81—7	46
32	Pentadec-7-ene, 7-bromomethyl-		C16H31Br	0.09	22.248	1000259—58—5	90
33	Nonacosane		C29H60	0.14	23.242	000630—03—5	90
34	Squalene		C30H50	2.41	24.881	000111—02—4	99
35	beta-Tocopherol		C28H48O2	0.92	29.154	000111—02—4	93

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