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QUALITATIVE PHYTOCHEMICAL SCREENING OF *PUNICA GRANATUM* LEAF EXTRACTS

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The qualitative analysis of chemical compounds for the ethanol extracts of pomegranate leaves have been analyzed in this study. The aim of present study is to analyze bioactivity constituents of ethanol leaf extract from *Punica granatum* L. ("Goy nar" variety) by Gas chromatography–Mass spectroscopy (GC-MS). The characterization of the chemical composition was done using Agilent 6890N Network GC System. In the GC-MS analysis, totally 79 phytochemical compounds were identified in the leaf ethanol extract of "Goy nar" variety from which 47 compounds were identified, including phenolic compound such as Phenol, 2-[(1-methylpropyl)thio]-, pyrones – 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-, carboxylic acids such as Acetic acid, 2-propyltetrahydropyran-3-yl ester, Carbonic acid, allyl nonyl ester and fatty acids such as 2,5-Octadecadiynoic acid, methyl ester, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) and other compounds. The results of current study indicate the possibility of using pomegranate leaves of Goy nar variety cultivated in Azerbaijan as a good source of natural compounds intended for applications in pharmaceutical and food industry.

Keywords: "Goy nar" variety, leaves, qualitative composition, phytochemicals, ethanol extract, chromatography.

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Introduction

The pomegranate (*Punica granatum* L.) is a shrub that belongs to the *Lythraceae* family. It is between 5 and 10 m tall and is characterized by deciduous fruiting leaves. The genus name, *Punica*, was the Roman name for Carthage, where the best pomegranates were known to grow. Pomegranate is known by the French as grenade, the Spanish as granada, and literally translates to seeded ("granatus") apple ("pomum") [1, 2]. *Punica granatum* L. is usually recognized as pomegranate and is native to the Mediterranean area [3, 4]. Azerbaijan is one of the richest floristic regions and a major focus in the South-West Asian and it is the origin center of many cultivated plants including pomegranate [5].

The pomegranate culture began in prehistoric times. The introduction of pomegranate began around the Neolithic [6]. According to D. Zohari and P. Spiegel-Roy, pomegranate domestication began in the Transcaucasian-Caspian region and in northern Turkey [7]. Evidence of pomegranate use in the Middle East dates back over 5000 years. Pomegranate artifacts and relics dating back to 3000 BC have been found in Egypt, Israel and Mesopotamia [8]. Fossil remains of the plant were also found in Azerbaijan in the deposits of the Akchagyl stage of the Pliocene time [9]. According to Kh. Ragimov the pomegranate culture in Azerbaijan since ancient times is evidenced by the data of paleontological studies, archaeological excavations, according to which the remains of the pomegranate were found in a jug burial of the 4th-1st centuries BC [10].

Pomegranate (*Punica granatum* L.) has been used in the folk medicine of many cultures especially in the Middle East. In Azerbaijani folk medicine, the juice of sweet pomegranate fruits is used for pain in the kidneys and stomach, and the juice of sour pomegranate fruits is used for diabetes. For this, natural pomegranate juice is prepared

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under the local name "narrub" or condensed pomegranate juice of wild pomegranate fruits with sugar "narsharab", which is also used as a seasoning to improve appetite, with scurvy and other diseases [11].

Currently, interest in this plant is increasing as researchers increasingly begin to realize its benefits and potential in the treatment of various diseases.

Some clinical studies show that pomegranate juice may be useful for heart disease, Alzheimer's disease and cancer [12]. In the studies of T.M. Chaouche et al. reported that pomegranate juice is effective in preventing atherosclerosis, low-density lipoprotein oxidation, prostate cancer, platelet aggregation, and various cardiovascular diseases [13].

The results of studies in recent decades indicate that *P. granatum* and its preparations can be used in treatment of type 2 diabetes [14]. The antidiabetic effect is primarily due to the ability of its components to reduce the severity of oxidative stress in the body and influence the main digestive enzymes [15].

All the listed useful properties of pomegranate are due to the presence of bioactive compounds in its composition. Pomegranate is rich in antioxidants of polyphenolic class which includes tannins and anthocyanins and flavonoids [16, 17]. It is an ancient plant that is well known in folk medicine and is becoming increasingly popular as a functional food and nutraceutical source due to its high polyphenol content, not only in the edible part, but also in other parts of the fruit and plant, including the peel, bark, leaves, and flowers [18]. All parts of the pomegranate including fruits, bark, roots, and leaves reported to have antioxidant and therapeutic properties [19–21].

Pomegranate (*Punica granatum*) has been acclaimed for its health benefits, this fruit has long been cultivated and consumed as a fresh fruit or in beverage form especially in the Mediterranean region. Pomegranate fruit, juice and peel possess a marked antioxidant capacity [22] with a high content in polyphenols, in particular, ellagitannins, condensed tannins and anthocyanins [23]. The demand for pomegranate juices is increasing worldwide due to its documented health promoting effects which likely derive from phenolic compounds [24]. Pomegranate leaf extracts contain high total phenols, tannins, and triterpenoids [25]. The leaf extract of *Punica granatum* with its active components (flavonoids) could improve cellular membrane and organ functioning more profoundly and bring NQO1 gene expression levels towards the control values [26]. The methanolic extract of pomegranate leaves displayed high antioxidant, anti-inflammatory, anti-cholinesterase, and antiproliferative activities [27].

Currently, more than 500 varieties of pomegranate are known. Of these, about 60 varieties are most widely used in production in various countries of the world [28]. In Azerbaijan, such varieties as Goy nar, Shah nar, Guleisha pink, Guleisha red, Veles red, Bala Mursal, etc., are widely cultivated in home gardens, and in industrial plantations Vesna, Arash, Meyhosh, Respublika, etc. [28, 29].

The present study was aimed at performing GC-MS analysis of an ethanol extract from the leaves of pomegranate "Goy nar" variety in order to identify bioactive components that can be further used in the production of food supplements and medicine.

Materials and Methods

Preparation of Punica granatum leaf ethanolic extract. Plant samples (leaves of "Goy nar" varieties) were collected from the crop area of Institute of Genetic Resources of Azerbaijan National Academy of Sciences. The leaves dried in shade and powdered using an electric blender. Then the plant material was submitted to ethanolic extraction (95% ethanol) at room temperature by maceration for 24 hours [30]. The percentage of yield of ethanol extract were 14.5% w/w. The extracts were filtered, the solvent eliminated under vacuum and stored in air tight containers under refrigeration. All chemicals and solvents used were of analytical grade.

Gas Chromatography and Mass Spectrometry. The characterization of the chemical composition was done Agilent 6890N Network GC System, 5975 inert Series Mass Selective Detector (MSD) with a mass spectrometer as a detector Split / Splitless, Injection – Split, Inlet pressure 60.608 kpa, Split – 100 Low Mass – 40, High Mass – 400, Treshold 150. A 30-meter capillary quartz column "HP-5MS 5% Methyl Siloxane" with an internal diameter of 0.25 mm and a film thickness of the stationary phase of 0.25 μ was used. The analyses were carried out in the temperature programming mode from 50 to 280 °C at a speed of 15 °C/min.

The column temperature was set as follows:

- initial temperature 50 °C – 2 min stable;
- rise in temperature 15 °C/min up to 200 °C – 6 mins stable;
- rise in temperature 15 °C/min up to 280 °C – 10 mins stable;
- vacuum – HiVac – 3.38e-005.

Diluted with solvent methanol : chloroform (1 : 2).

Carrier gas (He) flow rate was 1 ml/min. Sample injection with current division (1 : 50). The duration of the analysis was 33 minutes. The identification of compounds was accomplished by: comparison of their retention time, peak area percentage and mass spectra fragmentation pattern (NIST) and also with published literature.

Results and Discussion

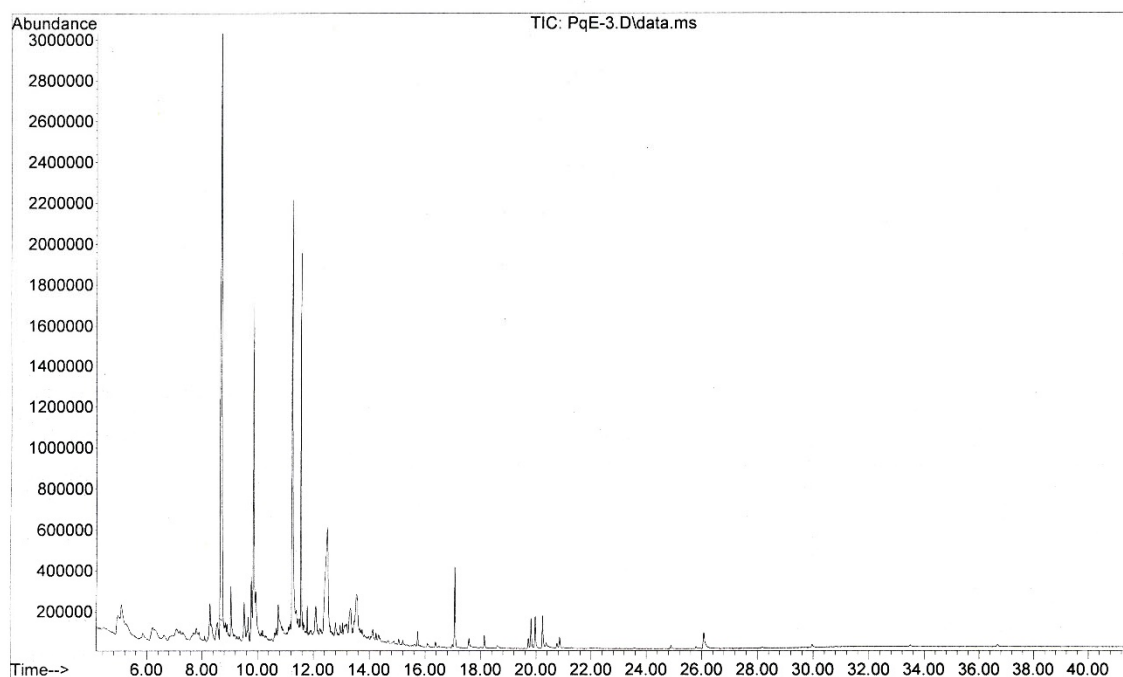
The results of determining the chemical composition of the leaves are presented in the table. The formula, structure, name, retention time, and molecular weight of the compounds were ascertained. As follows from the obtained chromatogram data the composition of the ethanol extract of Goy nar variety leaves revealed 79 peaks (Fig.).

The phytochemicals identified in the leaf extract of "Goy nar" variety

Molecular formula	Identification (IUPAC name)	CAS	RT (min)	Mol. weight
1	2	3	4	5
C ₅ H ₄ O ₂	Furfural	98-01-1	4.963 5.014	96.02
C ₆ H ₁₂ O ₂	2-Pentanone, 4-hydroxy-4-methyl	123-42-2	5.072	116.08
C ₂ H ₂ Cl ₄	Ethane, 1,1,2,2-tetrachloro	79-34-5	6.221 6.291	165.9
C ₁₀ H ₂₂ O ₃ Si	Methyl 2-[(triethylsilyl)oxy]propanoate		7.067	218.1
C ₉ H ₁₁ N ₅ O ₅	2'-azido-2'-deoxyuridine		7.793	269.07
C ₁₈ H ₃₃ ClN ₂ O ₅ S	Clindamycin	18323-44-9	8.287	424.2
C ₇ H ₈ O ₂	1-Propanone, 1-(2-furanyl)-	3194-15-8	8.324	124.05
C ₅ H ₆ N ₂ O ₂	4,6-Dihydroxy-5-methylpyrimidine	18337-63-8	8.555	126.04
C ₆ H ₆ O ₃	Maltol	118-71-8	8.702	126.03
C ₅ H ₆ N ₂ O ₂	5-Methylpyrimidine-4,6-diol	18337-63-8	8.815	126.04
C ₁₀ H ₁₈ O ₃	Acetic acid, 2-propyltetrahydropyran-3-yl ester		8.853	186.13
C ₁₃ H ₂₄ O ₃	Carbonic acid, allyl nonyl ester		8.901	228.17
C ₆ H ₈ O ₄	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	28564-83-2	9.040	144.04
C ₁₂ H ₁₄ N ₄ O ₄	2-Vinyl-9-[3-deoxy-β-d-ribofuranosyl]hypoxanthine	132121-65-4	9.517	278.1
C ₁₄ H ₂₂ O ₈	1,4-Diacetyl-3-acetoxymethyl-2,5-methylene-l-ramnitol		9.620	318.13
C ₈ H ₁₀ Cl ₂	2,3-Dichloro-7-methylenebicyclo[2.2.1]heptane		9.663	176.01
C ₈ H ₈ O	Benzofuran, 2,3-dihydro-	496-16-2	9.775	120.06
C ₆ H ₆ O ₃	5-Hydroxymethylfurfural	67-47-0	9.877 9.938	126.03
C ₈ H ₁₄ O ₇	6-Acetyl-β-d-mannose		10.616	222.07
C ₁₈ H ₃₂ O ₁₆	α-D-Glucopyranoside	597-12-6	10.678	504.17
C ₁₁ H ₁₈ O ₂	Methyl 2,6,6-trimethyl-1-cyclohexene-1-carboxylate	49815-58-9	10.747	182.13
C ₉ H ₁₄ O ₂ Si	Dimethoxymethylphenylsilane	17882-01-8	10.872	182.08
C ₁₁ H ₁₈ O ₂	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-	16778-27-1	11.169	182.13
C ₆ H ₆ O ₃	1,2,3-Benzenetriol	87-66-1	11.252	126.03
C ₁₀ H ₁₄ OS	Phenol, 2-[(1-methylpropyl)thio]-	29549-64-2	11.390	182.08
C ₁₀ H ₁₀ Cl ₂	Benzene, (2,2-dichloro-1-methylcyclopropyl)	3591-42-2	11.515	200.02
C ₁₅ H ₂₀ Si ₂	1,1,3,3-Tetramethyl-1,3-disilaphenalanane	32538-51-5	11.561	256.11
C ₁₀ H ₁₇ NO	2-Methyl-4,5-tetramethylene-5-ethyl-2-oxazoline	24337-58-4	11.652	167.13
C ₁₅ H ₂₈ O ₇	Methyl 4-O-acetyl-2,3,6-tri-O-ethyl-α-d-galactopyranoside		11.784	320.18
C ₁₂ H ₂₂ O ₁₁	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl	5965-66-2	12.089	342.12
C ₆ H ₁₂ O ₆	β-D-Allose	2595-97-3	12.500	180.06
C ₁₇ H ₃₂ O ₂	7-Methyl-Z-tetradecen-1-ol acetate		12.794	268.24
C ₈ H ₁₁ FN ₂ O ₃	ethyl 2-fluoro-1-(methoxymethyl)imidazole-4-carboxylate		12.958	202.07
C ₁₉ H ₃₀ O ₂	2,5-Octadecadiynoic acid, methyl ester	57156-91-9	13.044	290.22
C ₁₀ H ₁₇ NO ₆ S	Desulphosinigrin	5115-81-1	13.123 13.188	279.08
C ₁₂ H ₂₂ O ₁₁	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl	5965-66-2	13.337 13.452 13.551	342.12
C ₉ H ₁₆ O ₆	3,4-O-Isopropylidene-d-galactose		14.124	220.09
C ₂₀ H ₄₀ O	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	102608-53-7	15.733	296.31
C ₁₇ H ₃₄ O ₂	Hexadecanoic acid, methyl ester	112-39-0	17.068	270.26

End of table

1	2	3	4	5
C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid	57-10-3	17.577	256.24
C ₁₈ H ₃₆ O ₂	Hexadecanoic acid, ethyl ester	628-97-7	18.134	284.27
C ₁₉ H ₃₄ O ₂	Methyl 9-cis,11-trans-octadecadienoate		19.725	294.256
C ₁₉ H ₃₂ O ₂	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	301-00-8	19.817	292.24
C ₂₀ H ₄₀ O	Phytol	150-86-7	19.971	296.31
C ₁₉ H ₃₈ O ₂	Methyl stearate	112-61-8	20.240	298.28
C ₂₀ H ₃₄ O ₂	Ethyl 9,12,15-octadecatrienoate		20.859	306.25
C ₂₄ H ₃₈ O ₄	Bis(2-ethylhexyl) phthalate	117-81-7	26.075	390.27



GC-MS Chromatogram of ethanol extract of "Goy nar" variety leaf

The data for the leaves extract using GC/MS revealed 79 chemical compounds from which were identified 47 compounds, including: furfural (MW: 96.02), Desulphosinigrin (MW: 279.08), Acetic acid, 2-propyltetrahydropyran-3-yl ester (MW: 186.13), n-Hexadecanoic acid (MW: 256.24), phytol (MW: 296.31), Methyl stearate (MW: 298.28) etc. GC-MS analysis of the ethanolic leaf extract of *Punica granatum* showed the presence of various phytochemicals including phenolic compound such as Phenol, 2-[(1-methylpropyl)thio]-, pyrones – 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, carboxylic acids such as Acetic acid, 2-propyltetrahydropyran-3-yl ester, Carbonic acid, allyl nonyl ester and fatty acids such as 2,5-Octadecadienoic acid, methyl ester, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z). Analyzing the data obtained by us and comparing them with the literature data, it can be noted that Furfural, 5-Hydroxymethylfurfural and Hexadecanoic acid ethyl ester are characteristic components of the ethanol extract of *Punica granatum* leaf, seeds and peel, and Octadecanoic acid (16.89%), Furfural (14.62%), γ -Sitosterol (9.23%), Glycerin (7.74%), Heptasiloxane, hexadecamethyl- (3.14%), Pyrazole [4,5-b]imidazole, 1-formyl-3-ethyl-6- β -d-ribofuranosyl (2.39%), Lanosterol (1.82%), Cycloartenol acetate (1.64%), Cyclobutylamine (1.58%), Palmitic acid (1.18%), 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl (1.14%) and L-Glucose (1.07%) – methanolic extract of pomegranate peel [31, 32].

A previous study reported that Furfural, 5-hydroxymethylfurfural, 2-Furancarboxaldehyde, 5-methyl, 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl, γ -Sitosterol, Hexadecanoic acid, methyl ester and Octadecanoic acid have potential antioxidant, antimicrobial and antiproliferative activities [33]. Various studies have shown that 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl have efficient anti-tussive, antibacterial, antioxidant, anti-inflammatory and anti-asthma activities, furan has anti-inflammatory, antifungal, antiviral, and antibacterial activities, oleic acid has antimicrobial activity [34], 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) has reductase inhibitor,

antiarthritic, hepatoprotective, insectifuge-anti-inflammatory, cancer preventive, anticoronary, nematocide anti-androgenic, insectifuge, hypocholesterolemic [35] which means that pomegranate has great medical importance against many diseases as an antioxidant [31].

Conclusion

In conclusion, totally 79 phytochemical compounds were identified in the leaf ethanol extract of “Goy nar” variety from which 47 compounds were identified, including phenolic compound, pyrones, carboxylic acids, carbonic acid, allyl nonyl ester and fatty acids and other compounds were evaluated. The results of current study indicate the possibility of using pomegranate leaves of Goy nar variety cultivated in Azerbaijan as a good source of natural compounds intended for applications in pharmaceutical and food industry.

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Conflict of Interest

The authors of this work declare that they have no conflicts of interest.

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