IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 10, Issue 10 Ver. III (October 2017), PP 48-53
www.iosrjournals.org

GC-MS Analysis ofPhytochemical Constituents in Ethanoic Extract of Pomegranate (Punica granatum L.) "Salami variety") grown in Iraq

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ABSTRACT: *This study has been performed in the biotechnology laboratory of Agriculture faculty- Basrah University during 2016. The purposeof it is to determine the concentration and ratio of the Phytochemical Constituents inEthanoic Extract of Pomegranate (Punica granatum L.)in Iraq using GC MS. Salami variety was chosen as a sample of the grown plant. There are forty phytochemical constituents were obtained from juice by analysis using GC MS. The main compounds classified as phenolic compounds. Which means that the pomegranate grown in Iraq has high activity as an antioxidant and could use for medical purposes.*

Keywords:*Pomegranate, Salami variety, Phytochemical Constituents, antioxidant.*

Date of Submission: 13-10-2017 Date of acceptance: 31-10-2017

I. Introduction

The Pomegranate (*Punica granatum* L.) is one of the oldestrecognized edible fruits [1] belongs to the Punicaceae family. It is widely grown in many tropical and subtropical countries [2].

The first record of pomegranate cultivation in about 2200 B.C. comes to us from the ancient civilization of Sumer, located in the lower Tigris and Euphrates valley, approximately the area of present day Basra, Iraq. It is believed that the pomegranate wasbrought into the area by people who were migrating from the Zagros Mountains on the present day Iran and Iraq border. From there, the pomegranate spread east to Asia Minorin about 1300 B.C. when the Phoenicians started to cultivate it[3]. The Phoenicians were great traders who sent many ships to ports in the Mediterranean Basin. By 1000 B.C., pomegranates were growing in Carthage and Egypt[4]. Today there are more than 1000 cultivars of Punica granatum exist, originating from the Middle East, extending throughout the Mediterranean, eastward to China and India, and onto the American Southwest, California and Mexico in the New World [5].

Because of the high levels of phenolic compounds, flavonoids, anthocyanins, tannins, ascorbic acids and gallic acid present in pomegranates which means it has a great antioxidant potential[6]. The inherent properties of these compounds act in the prevention of various diseases [7, 8]. Pomegranate is also a rich source of natural compounds, such as polyphenols, mostly punicalagin and ellagic acid [9]. These components have been isolated mainly from the pomegranate rind, and their biological activity has been studied, especially its antioxidant capacity [6, 10],and can reduce the risk of major chronic diseases[11].

The pomegranate has garnered increasing interest over the world due to its high nutritional value [12], and the phytochemical and medicinal properties of its juice [13-15].

The prevouse Studies have shown that juice from pomegranate (PJ) has a high benefit to human health. The juice hashigh polyphenol and total phenols content [16], these components play role as scavengers of free radicals and reactive oxygen species [17]. Pomegranate juice (PJ) has been shown to exert significant anticancer, anti-inflammatory, antidiabetic, antimicrobial, antioxidant, antiatherosclerotic, and antihypertensive effects[18]. Some studies have indicated that the significant antidiabetic effect associated with pomegranate may be attributable to the presence of oleanolic, ursolic, and gallic acids as chief chemical constituents [19].

Although Iraq is one of the origin and the main pomegranate producers among the middle east countries[20], but the available information on pomegranates from Iraq is very littles [1]. According to the information obtained from the Central Organization of Statistics in Iraq, The pomegranate production in Iraq was about 98,683 tons in 2015and production has rapidly increased from year to year[20].

A number of local types of pomegranate which are seedling progenies are cultivated in different growing regions of Iraq. Most of them are known by the names of places where they are popular. The varieties presently grown in Iraq show distinct variation in fruit shape, colour, taste, colour of aril, rind thickness, etc.

There are more than 23 cultivars of pomegranate have been grown in different places of Iraq. Salami is one of the most common varieties cultivated especially in the center and south region of Iarq because of the suitable environmental conditions in these regions.

There is a limit information is available related to the medical benefit of this cultivar and the ratio of the antioxidant compounds present in the peel and juice of the pomegranate. Due to the presence of these compounds in the fruit and their importance in maintaining a healthy body, this study aims to identify the phytochemical constituents of ethanolic extract of Punica granatum vs Salami using of GCMS technique to evaluate to enrich commercial juices with dried extract obtained from pomegranate peels.

II. Experimental work and material

2.1 Plant and juice preparation

This study was conducted in the Biotechnology Laboratories of the Faculty of Agriculture, University of Basrah during 2016. Samples of pomegranate (Salami variety)were collected from the field located in the Alnoumanya districtin Wassit province near the Tigris river as shown in Fig. 1. After soil analysis a suitable quantity from a mature fruit were harvested from pomegranate trees eight years old and transported to the laboratory by special cans. Then the samples were washed in cold tap water and left to dry. After that they were cut open by a hand and remove the outer leathery skin. The arils were separated from the peel and mixed by a mixer, the mixture was filtered to remove coarse and suspended mattes. 100 mL from the mixture was obtained and centrifuged at 10000 rpm for 2 min. Finally the juice filled by a seal dark glass vial and and kept frozen at18 °C upon analysis[21].

2.1 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The component identification of methanolic extract of pomegranate juice (Salami variety) was done using Shimadzu GC-2010 gas chromatography coupled with QP2010 mass spectrometer. The sample was injected into the GC-MS on a 30 m glass capillary column with a film thickness of 0.25 μm (30 m × 0.25 μm).Helium as carrier gas was used at 1 ml/min constant flow mode. Injector and detector temperatures were kept at 250 °C. The GC temperature programme was 60°C - 280°C at 15°C/min. Split ratio 1:30. The total GC running time is at 35 min. The MS was taken at 70 eV. The MS scan parameters included a mass range of m/z 40-1000, a scan interval of 0.5 s, a scan speed of 1000 amu s^{-1}, and a detector voltage of 1.0 kV. Identification of compounds was conducted using the database of NIST08, WILEY8 and FAME Libraries. The mass spectrum of the individual unknown compound was compared with the known compounds stored in the software database Libraries. The name, molecular weight and structure of the components of the test materials were ascertained.

2.2 Statistical analysis

One-way analysis of variance was used to analyze the data using Anova: Single actor through Microsoft Office Excel 2010, the determining the value of L.S.D for significant value 5%.

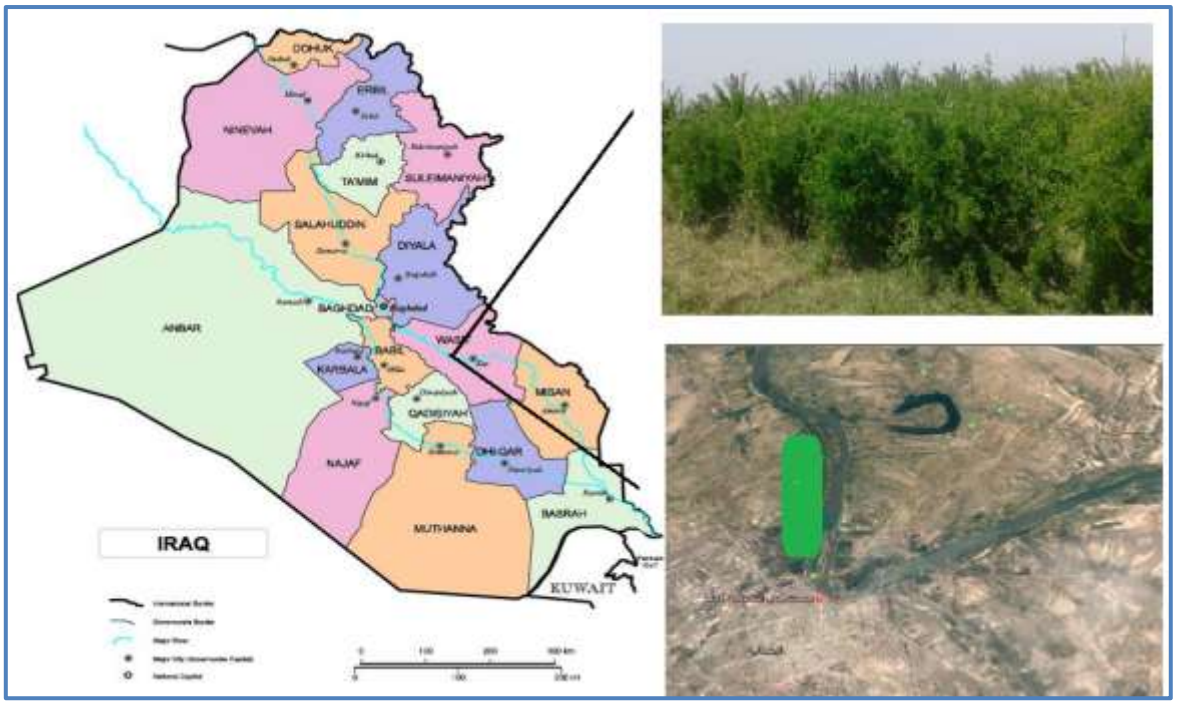


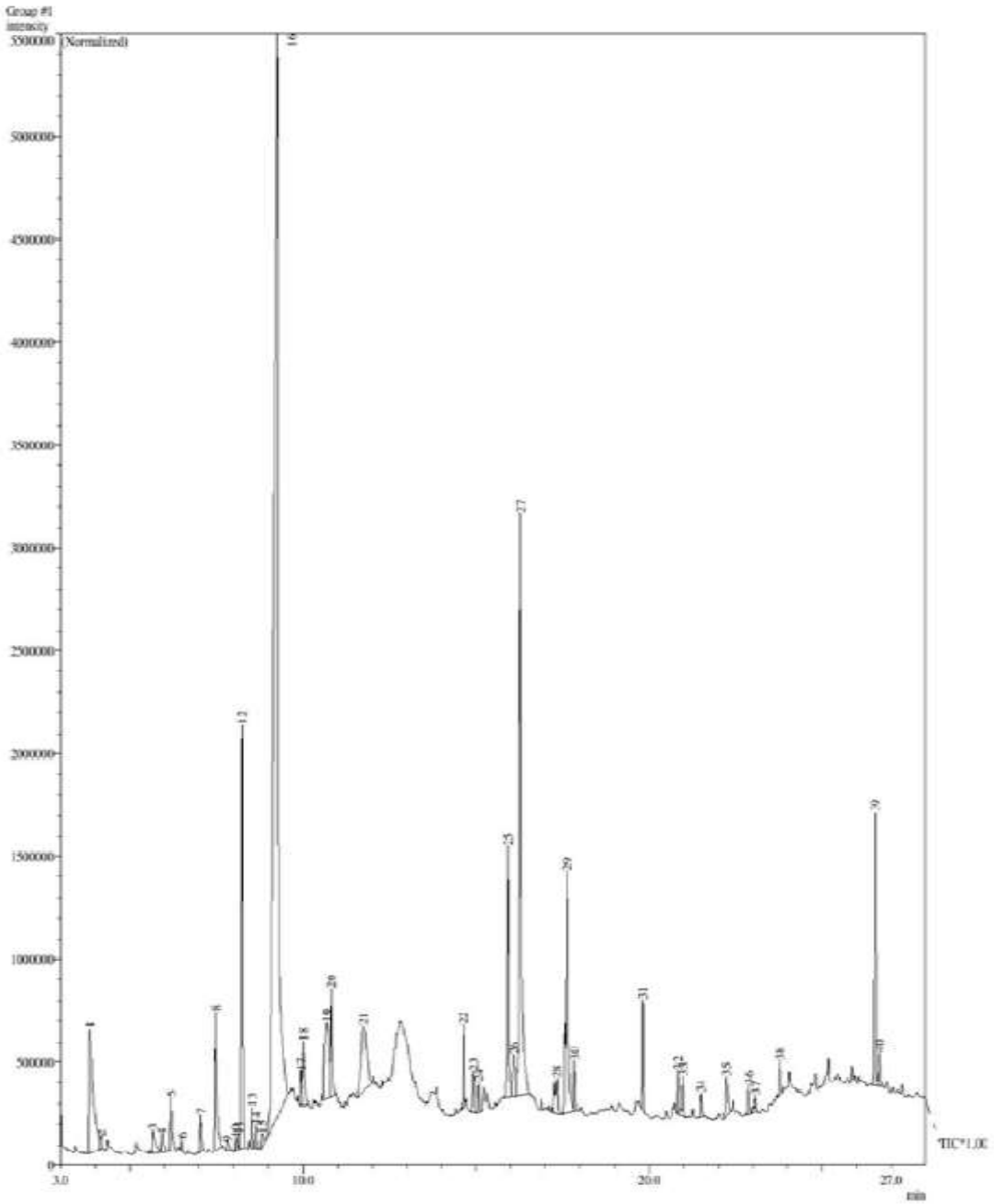
Fig. 1 The location of the pomegranate sample used in the study

III. RESULTS AND DISCUSSION

3.1 Identification of the components by GC-MS

The components identified in GC-MS analysis of the ethanolic extract from pomegranate juice as shown in Fig.2 there are 40 peaks indicating the presence of forty phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST08, WILEY8 and FAME libraries the forty phytochemical constituents were characterized and identified (Table 1).

The major phytochemical constituent's mass spectra are Gallic acid with ratio of 43.25 followed by Quercetin (10%), third consitituent is 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(6.07) and Furfural (4.6%), followed by the Oleic Acid with ratio (4.45%), the other major phytochemical constituent are D-Allose (3.56%), gamma.-Sitosterol (3.52%),Thiamine (3.17%), Ascorbic acid (3.16%), Pyrogallol (2.25%), the ratio of other components are small compared with the major phytochemical constituent, and as shown in table 1.



chromatogram of GC- MS

Figure 1 ***GC-MS Chromatogram of ethanolic extract of the Punica granatum peel***

Table 1 ***Phytochemicals identified in the ethanolic extracts of the Punica granatum Juice by GC-MS***

Peak#	R.Time	Component Name	Area%	Molecular Formula	Molecular weight
1	3.849	Furfural	4.65	$C_5H_4O_2$	96
2	4.192	2-Furfurylthiol	0.22	C_6H_9Cl	116
3	5.667	2,5-Furandione, 3-methyl- (2-Furoic acid)	0.7	$C_5H_4O_3$	112
4	5.925	2-Furancarboxaldehyde, 5-methyl-	0.41	$C_5H_6No_2$	110

5	6.193	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.73	$C_5H_6O_5$	146
6	6.508	1,3,2-Oxazaborolane-4-carboxylic acid, 2-butyl-, methyl ester, L-	0.09	$C_8H_{16}BNo_3$	185
7	7.043	2-Thiazolamine, 4,5-dihydro-	0.44	$C_6H_{12}O_3$	132
8	7.48	Pyrogallol	2.25	$C_6H_6O_3$	126
9	7.817	4-Pyrimidinol, 5-methoxy-	0.16	C5H6N2O2	126
10	8.058	4,4-Dimethyl octane	0.17	$C_{10}H_{22}C$	142
11	8.15	Ethanamine, N-ethyl-N-nitroso-	0.18	$C_4H_{10}N_2O$	102
12	8.249	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.07	C6H8O4	144
13	8.525	Pyrimidine, 4-chloro-5-ethoxy-2-methyl-	0.44	$C_7H_9ClN_2O$	172
14	8.653	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	0.32	$C_6H_6O_4$	142
15	8.825	2,3-Dihydrooxazole, 2-t-butyl-3-pivaloyl-	0.25	$C_{12}H_{21}NO_2$	211
16	9.266	Gallic acid	43.25	$C_6H_6O_3$	126
17	9.942	Cyclohexane, 1,4-diethoxy-, trans-	0.49	$C_{10}H_{20}O_2$	172
18	10.01	Punicalin -	1.05	$C_{16}H_{34}B_2O_8$	376
19	10.692	Thiamine	3.17	$C_5H_6NOO_2$	126
20	10.833	Hexadecaneperoxoic acid, 1,1-dimethyl-3-[(1-oxohexadecyl)oxy]propyl ester	1.24	$C_{37}H_{12}O_5$	596
21	11.742	D-Allose	3.56	$C_6H_{12}O_6$	180
22	14.651	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.69	$C_{20}H_{40}O$	296
23	14.926	Hexanedioic acid, dioctyl ester	0.63	$C_{22}H_{42}O_4$	370
24	15.09	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.31	$C_{20}H_4OO$	296
25	15.928	Ascorbic acid	3.16	$C_{38}H_6O_8$	652
26	16.098	3-Pentanamine, N,N'-1,2-ethanediylidenebis[2,4-dimethyl-	1.28	$C_{16}H_{32}N_2$	252
27	16.285	Quercetin	10	$C_6H_8O_3$	128
28	17.35	1-Tetradecanol, 14-chloro-	0.9	$C_{14}H_{29}ClO$	48
29	17.632	Oleic Acid	4.45	$C_{18}H_{34}O$	66
30	17.849	Octadecanoic acid	0.5	$C_{18}H_{36}O_2$	84
31	19.827	Hexanedioic acid	0.98	$C_{22}H_{42}O_4$	370
32	20.842	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.48	$C1_9H_{38}O_4$	330
33	20.983	1,2-Benzenedicarboxylic acid, diisooctyl ester	0.49	$C_{24}H_{38}O_4$	390
34	21.512	2-Furaldehyde azine	0.31	$C_{10}H_8N_2O_2$	188
35	22.242	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	0.89	$C_{21}H_{40}O_4$	356
36	22.881	3-(2-Fluoro-benzylsulfanyl)-1H-[1,2,4]triazole	0.4	$C_9H_{28}O_2$	52
37	23.075	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	0.25	$C_{30}H_{50}$	410
38	23.781	Cyclopropylpyrrol4-[3-(1H-imidazol-4-yl)propoxy] phenylmorphomethanone oxime	0.43	$C_{16}H_{19}N_3O_2$	177
39	26.545	.gamma.-Sitosterol	3.52	$C_{29}H_{50}O$	414
40	26.671	Fucosterol	0.48	$C_{29}H_{48}O$	412
			100		

3.2 Quantitation of Phenolic Compounds in theJuices and Antioxidant Activity

The main phenolic compounds present inpomegranate juice can be arranged into four groups. Afirst group includes the anthocyanin pigments, whichare easily quantified by GC MS. The second group includes thehydrolyzable tannins of the gallagyl type. This group includes thepunicalagin isomers, punicalin (gallagylglucose),and other related compounds, and they werequantified as punicalagin by GC MS. The third group ofpomegranate juice phenolics includes ellagic acid and its glycosides. The fourth group of pomegranate phenolics includes avery wide group of hydrolyzable tannins. Thesecompounds are different combinations of glucose, gallicacid, hexahydroxydiphenic acid (which gives rise toellagic acid after hydrolysis), and tertgallic acid. These results showed that the pomegranates grown in Iraq have the same antioxidant potential to other varieties are grown in different region over the world.

IV. Conclusion

In the present study related with pomegranate in Iraq Salami variety there are forty constituents have been identified from the ethanolic extract of the Punica granatum juice by GC-MS analysis. The presence of

various bioactive compounds, especially phenolic compounds reverses the antioxidant capacity of this plant. Some compounds were present in high amount such as Gallic acid, while others appear in low ratio. However, further studies are undertaken to animal model to evaluate their bioactivity.

Acknowledgments

This research was self-supported by the author. The authors thank the Department of Horticulture, College of Agriculture, Baghdad University, and the Faculty of Agriculture, University of Basrahfor using of facilities in the Biotechnology Laboratories.

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Falah Hassan Almiahy GC-MS Analysis ofPhytochemical Constituents in Ethanoic Extract of Pomegranate (Punica granatum L.) "Salami variety") grown in Iraq." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), vol. 10, no. 10, 2017, pp. 48-53.