توزع البوليفينولات الكلية في عصير الرمان وقشوره وبذوره من مناطق مختلفة في سورية

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ملخص

تم دراسة المحتوى الكلي للفينولات في مستخلصات عصير وقشور وبذور الرمان السوري (Punica granatum L.) من أربع مناطق سورية مختلفة (حلب وحماة والرقة ودرعا) .وتم تحديد المركبات الفينولية الكلية بطريقة فولين-سيوكالتيو اللونية . وأظهرت النتائج أن المحتوى الكلي للبوليفينولات يتغير تبعاً للمناطق وأجزاء الفاكهة .تم الحصول على أعلى محتوى كلي للبوليفينولات من مستخلصات القشور (٥٩٧٤,٠٥ إلى ٧٩٥٨,٤ ملغم/لتر) . و تراوح المحتوى الكلي للبوليفينولات في عصير الرمان ومستخلص البذور من ٩٩٩,٣ إلى ١٨٦٦ ملغم/لتر و١٢٦,٧ إلى ١٩٤,٨١ ملغم/لتر على التوالي

احتوى "رمان حلب" على أعلى نسبة من البوليفينول الكلي من بين المناطق الأربع التي تم اختبارها . وبالمقارنة مع الدراسات السابقة، وجد أن نسبة البوليفينول الكلي الموجودة في الرمان السوري أعلى من تلك الموجودة في الرمان التركي

الكلمات المفتاحية:

الرمان، البوليفينول الكلي، المناطق السورية، مستخلصات العصير والقشور والبذور.

Distribution of Total Polyphenols in Pomegranate Juice, Peels, and Seeds from different Syrian regions.

Abstract

The total phenolic content in juice, peel, and seed extracts of Syrian pomegranate *(Punica granatum L. Regions)* from four different Syrian regions (Aleppo, Hama, Raqqa and Daraa) was investigated. Total phenolic compounds were determined with Folin–Ciocalteu colorimetric method. The results showed that the total polyphenols content changes depending on regions and fruit parts. In all regions, the highest levels of total polyphenols content were obtained from the peel extracts, (5974.05 to 7958.4 mg/L). However, the total polyphenols content of pomegranate juice and seed extract ranged from 999.3 to 1866 mg/L and 126.7 to 194.81 mg/L, respectively. "Pomegranate of Aleppo" showed the highest amount of the total polyphenols content among the four regions tested.

In comparison with the Turkish studies, it was found that the percentage of Total Polyphenols found in the Syrian pomegranate is more than that found in the Turkish pomegranate.

Key words: Pomegranate, Total Polyphenols, Syrian regions, juice, peels and seeds extracts.

Introduction

Pomegranate (*Punica granatum L., Lythraceae*) is a tree native to the Middle East, now cultivated around the world, especially in Mediterranean countries, China, Southeast Asia, and other dry areas [1] , and it is one of the most beneficial fruits for human health, because it contains relative high concentration of nutritional compounds such as vitamins, minerals, and antioxidants [2]. Pomegranate has been widely consumed and used as therapeutic agents since ancient times [3]. It was also mentioned in ancient Indian medicine that pomegranate is a heart tonic, and blood purifier [4] , and it was considered an effective medicine for gastritis and pain. Also in Arab medicine, Pomegranate peel was considered an astringent to stop Diarrhea and bleeding [5].

This historical health importance of the pomegranate fruit is now supported by a lot of modern medical research, which showed that the different parts of the pomegranate fruit have different biological activities of great importance, as recent research has proven anti-carcinogenic effects [6-7], anti-bacterial [8] and anti-inflammatory effects [9], and researches have also shown that the pomegranate fruit contains estrogenic compounds that help treat cancers related to hormones such as breast cancer [10], also the role of pomegranate in improving bone density in menopausal syndrome [11]. In addition, researches have shown that extracts of pomegranate peels and seeds stimulated precollege synthesis in human skin cells [12].

Pomegranate is a rich source of a variety of phytochemical like (Phenols, Flavonoids alkaloids, Ellagic acid, Punicalagin, Anthocyanins, and Tannins), and Vitamin C and E, which are responsible for its strong anti-oxidative and anti-inflammatory activities [3]. A review of the previous literatures confirms that extracts obtained from different parts of this plant (fruit, peel, seeds, and leaves) have great health benefits in both vitro and vivo studies [3]. The anti-effects of diabetic, hypertensive, microbial and tumour, which belongs to pomegranate fruit, are of particular scientific and clinical interests [3].

Historical, Pomegranate plant was used not only for its nutritional benefits, but also it was used in the industrial, food fields (to enhance the nutritional value, taste, texture, flavour, and shelf life of foods), in leather tanning, ink fixation, and dye fixation [13].

In Syria, A pomegranate juice can be boiled to prepare a pomegranate molasses, and preserved to acidify some foods. Also, pomegranate seeds are used after drying and grinding to make Syrian zaatar. Thus, the reuse of pomegranate derivatives can improve the health benefits, the

quality of food products, and reduce environmental impact and costs associated with their disposal [13].

Polyphenols are secondary metabolites that naturally found in plants and food, such as vegetables, herbs, spices, tea, dark chocolate, wine and fruits [14]. Pomegranates contain three times more antioxidants and polyphenols than green tea or red wine [1]. Polyphenols may be involved in the bitterness, astringency, colour, flavour, odour and oxidative stability of food [15]. They composed of aromatic rings with hydroxyl groups (See figure 1), which make them relatively hydrophilic and extractable using water or polar organic solvents as methanol, ethanol and acetone [16]. Polyphenols have been classified according to their chemical structure into four main groups: Phenolic acids, Flavonoids, Stilbenes, and Lignans [14]. They can act as antioxidants, by suppressing the generation of free radicals that always present in our bodies. On the other hand, having an excessive amount of them can be harmful and contribute to a number of chronic diseases [14]. A high percentage of Total Polyphenols (TP) was found in fruits such as (pomegranate, berries, cherries, strawberries), vegetables such as (carrots, red and yellow onions, potatoes), spices such as (cinnamon, cloves), nuts, and grains [17].

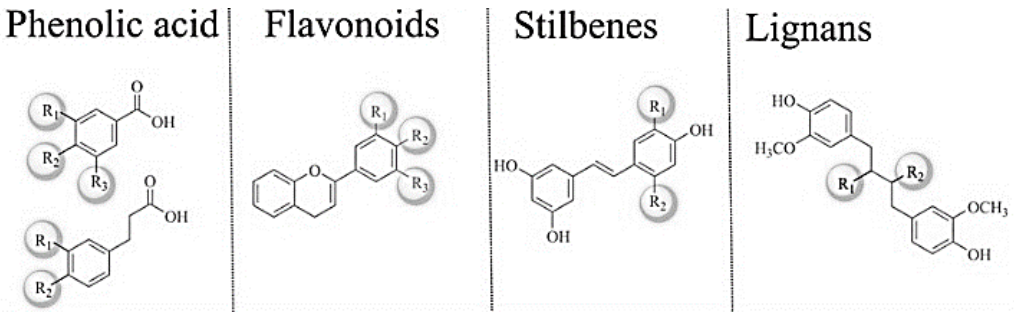

Figure (1): Classification of Polyphenols [16].

There are several methods for quantification of total polyphenols content (TPC), which based on colorimetric measurements. Total phenols can be measured using the Folin-Ciocalteu reaction and the results are typically expressed as Gallic acid equivalents [18].

The aim of this study is to determine the percentage of TPC present in the peels, seeds and juice of pomegranates grown in different Syrian regions, and compare the results with some previous studies on pomegranates.

Samples and Materials:

- Samples: Fresh pomegranate fruits (*Punica granatum L.*) from four region of Syria, and the table 1 showed their abbreviations used in this study.

- Methanol (CH_3OH), Molecular Weight (ML) 32.04, Purity (P) 99.7%, Chem Lab-Belgium.
- Sodium Carbonate (Na_2CO_3), (ML) 105.99, (P) 99.5%, Qualikems-India.
- Gallic acid ($C_7H_6O_5.H_2O$), (ML) 170.12, (P) 97.5%, Titan biotech-India.
- Folin–Ciocalteu reagent (FCR), Titan biotech-India.
- Distilled deionized (DI) water.

Table 1: The abbreviations of samples

Region	Fruit	Juice	Peels	Seeds
Aleppo	A*	A*J	A*P	A*S
Hama	B*	B*J	B*P	B*S
Daraa	C*	C*J	C*P	C*S
Raqqa	D*	D*J	D*P	D*S

Apparatus:

- Double-beam (UV-VIS) Spectrometer (Model 20-1650-01-0390) China.
- Ultrasonic bath (Model UG41204), HHS, China.
- Centrifuges, Hettich, Germany.
- Oven, (Model C310.0549) Germany.

Solution Preparation:

- Gallic acid standard (GAE): 500mg of Gallic acid was dissolved in a small amount of distilled water with slight heating, and then completed the volume to 100 ml to make a standard Solution (5000 mg\L). Standard calibration series of Gallic acid (concentration 50 -250 mg\L) were prepared.
- Sodium carbonate solution (20%): 10 g of sodium carbonate were dissolved in 50ml of distilled water.

Samples preparation:

- Preparation of pomegranate peels powder (ppp): mature pomegranate fruits were washed and cut manually to separate the seeds and peel. The rind (peels) was cut into small pieces using a sharp knife and dried in an oven at 60 ± 5 ºC for 6 hours. Then the TP of ppp were extracted according to the method of Duke et al. (2003) [19]. A known weight (0.5 g) of dried ppp was extracted with 25 ml methanol for 1 hour using ultrasonic bath.
- Preparation of pomegranate seeds powder: Pomegranate seeds were obtained by manual removing of the pericarp from the rest of the fruit, isolating the endocarp and subsequently

carefully splitting the tegmental pulp surrounding the seeds. Then were washed with water several times and dried in an oven at 60±5 ºC for 6 hours.

- (0.5 g) of pomegranate peel powder or pomegranate seed powder was extracted with 25 mL methanol for 1 hour by ultrasonic bath. Peel extracts for samples (A-C) were diluted with a ratio (1:60) and peel extracts for samples (B-D) were diluted with a ratio (1:30) with (Dl) water. For seed extract a centrifugation was applied to remove cloudiness from solution.
- Preparation of pomegranate juice: Cold pressed pomegranate juice was isolated from the seeds, filtered, and then diluted with a ratio of (1:10) for all samples.

Determination of TP:

Identification of TP in investigated pomegranate fruit was performed on (UV_VIS). The absorbance of 750 nm was assured by scanning Standard number (3) from calibration series in the interval (700-800) nm (showed in Figure 2), the wavelength corresponding to the maximum absorption was 750 nm, and hence it was used in this research.

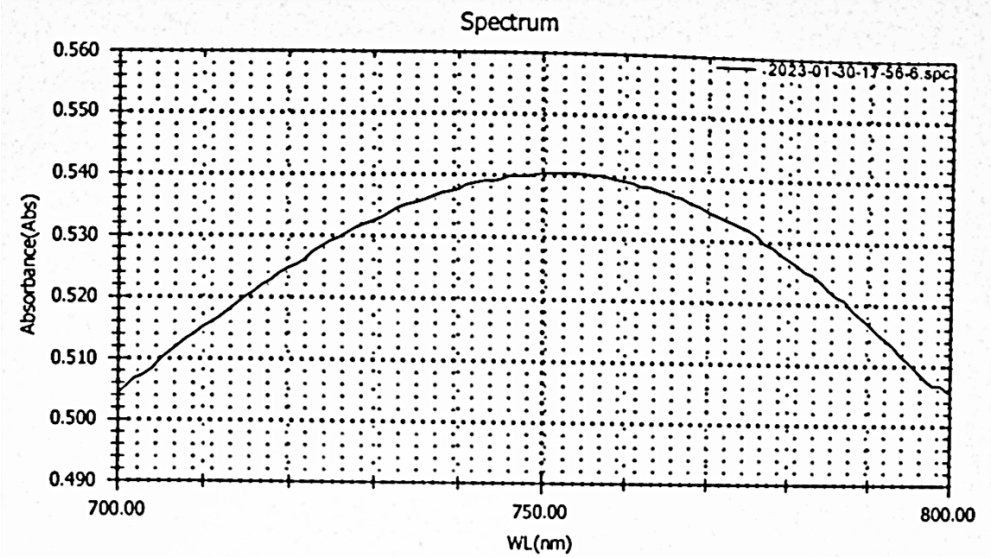

Figure (2): determining of the maximum absorbance wavelength by conducting spectroscopy of a standard series sample.

The measurement of TP content was conducted according to the modified Folin–Ciocalteu colorimetric method [20]. For each sample 200 µL of the previous prepared extract were moved in a test tube and 400 µL of (FCR) were added followed by 4 mL (DI) water. After 10 min standing 2 mL of 20% sodium carbonate solution were added. Each sample was allowed to stand for 60 min at room temperature and then measured at 750 nm using an UV/Vis spectrophotometer. Three replications for each sample were done (n=3).

Gallic acid was used as a standard and results were expressed as Gallic acid equivalents (GAE) per 100 g in a dry mass (DM). See Figure 3.

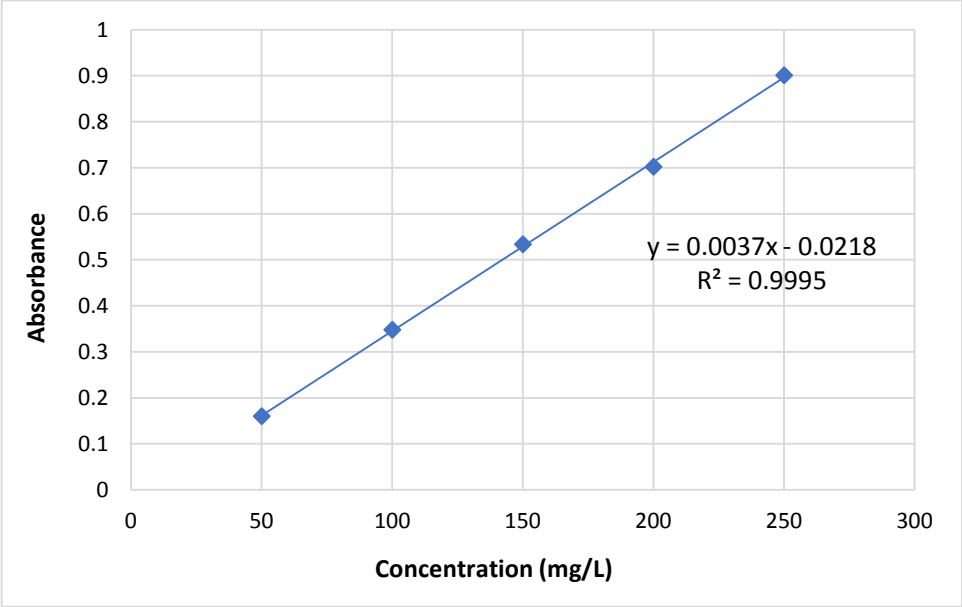

Figure (3): Gallic acid calibration series.

Result and Discussion:

The concentration of TP in the studied samples varied according to the geographical regions, as shown in Figure 4. The total concentration of TP in the samples of Aleppo, Raqqa, Hama and Daraa were (9084.4, 8545.67, 7823.83, 7808.26 mg/L) in descending order.

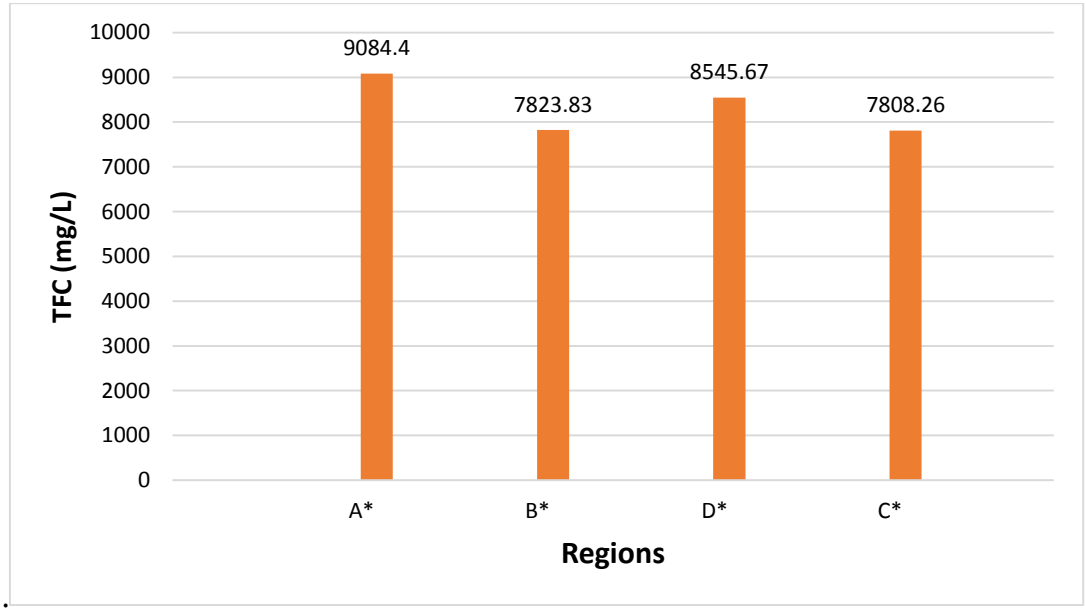

Figure (4): Total Polyphenols content in pomegranates from different Syrian regions. See abbreviations in table 1.

The reason for the differences in the concentration of polyphenols in pomegranates in the various Syrian regions is due to several climate factors. Temperature plays a major role, where hot and dry whether (such as in Aleppo and Raqqa, which locate in the north of Syria) increases the maturing of the fruit, improves quality and concentration of the chemical compounds in pomegranates. Fertile of soil also plays an important role, well-drained sedimentary soil is the best. Pomegranates can be grown in light sandy and clay soils, while excess moisture is an unfavourable factor for the growth and cultivation of pomegranates [21].

To assure the effect of climate on the concentration of TFC, the results of this study were compared to a Turkish study [22], where the TFC of Turkish pomegranates was in the range (5276.7– 2676.8 mg/L), which is lower than the total TFC in Syrian pomegranates.

Many studies of pomegranates showed that TFC varies between different parts of the plant. [22-23-24]. Figure (5) presents the TP content TFC in juice, peel and seed of the studied samples from four Syrian region.

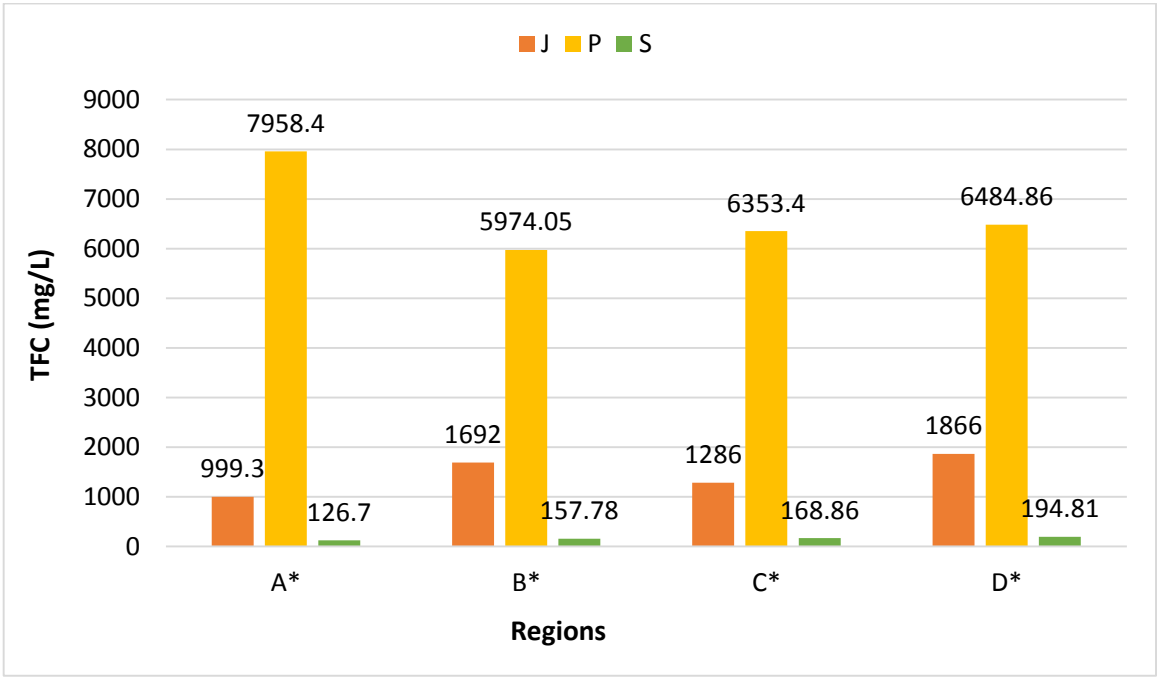

Figure (5): Total polyphenols content in juice, peel and seed from four Syrian region. See abbreviations in table 1.

It was observed that the highest Mean of total polyphenols content (TFC) in pomegranate samples was in peels (6692.67 mg/L), followed by juice (1460.8 mg/L), and the lowest TFC

was in seeds (162.03 mg/L), see Figure 5. In comparison to a previous study of Syrian pomegranate peel [21], the TFC in peels was (345.309 mg/L) much lower than in our study, due to the different climate and cultivation soil kind.

Furthermore, the results showed that TFC in juice was the highest in sample D*J (1866 mg/L) and the lowest in sample A*J (999.3 mg/L), while TFC was in samples B*J and C*J (1692 mg/L, 1286 mg/L) respectively. This content was also lower than what presented in another Syrian studies of pomegranates juice (2457.8 mg/L) [21], (2999.1 mg/L) [25] due to different cultivation regions and harvesting time and also using different extraction methods.

It was also observed that the highest TFC value in peel was in sample A*P (7958.4 mg/L), and the lowest in B*P (5974.05 mg/L), while in samples C*P and D*P were (6353.4 mg/L, 6484.86 mg/L) respectively. Thus, Syrian pomegranate peel is responsible for the majority of the total polyphenols content in pomegranate.

TFC in seeds was the highest value in sample D*S (194.81 mg/L) and lowest content in sample A*S (126.70 mg/L), followed by samples C*S and B*S (168.86 mg/L, 157.78 mg/L) respectively.

A similar result was reported by Gözlekçi et al [22], which found that peel usually contained higher amount of phenolic compounds (3547.8-1775.4 mg/L), While TFC in the juice between (1551.5-784.4 mg/L), While the seeds also had the lowest polyphenols content (177.4 -117 mg/L). Compared to this study, the concentrations in the peels were (7958.4-5974.05 mg/L), with juice (1866-999.3 mg/L) and by seeds (194.81-126.7 mg/L). TFC in Syrian pomegranate is higher than TFC in Turkish pomegranates due to the variation of environmental factors (soil, climate and rain) between Syria and Turkey.

The results in this study can be interpret better by using the soil map and the annual average rainfall in Syria. Figure 6 showed the annual average rainfall in Syria [26]. The three areas (Aleppo, Hama and Daraa) have almost the same annual average rainfall (250 mm), and Raqqa has less than (100 mm) but its location at Al-Fuat River has a great effect on TP in juice and seeds in comparison to other samples.

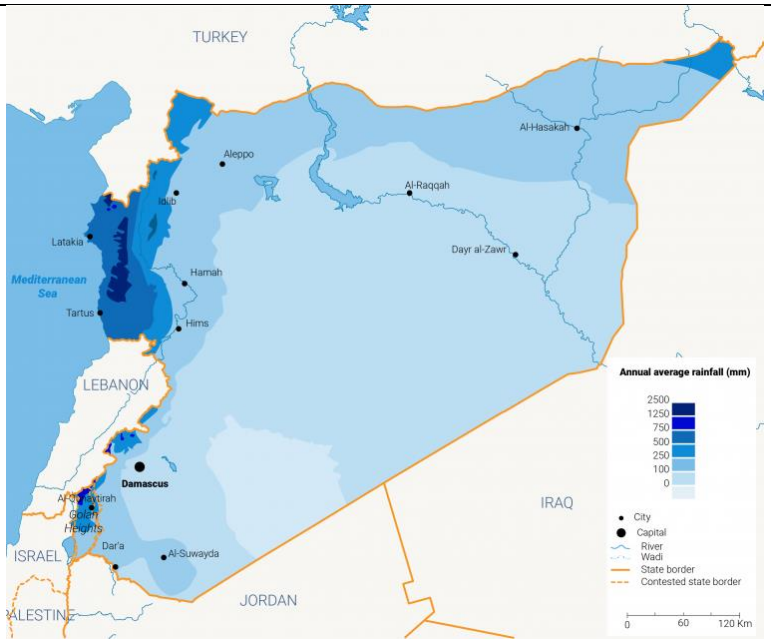

Figure 6: Annual average rainfall in Syria [26].

Figure 7 showed the soil map of Syria which performed by the Arab center for the studies of arid zones and dry lands (ACSAD) [27]. According to several Studies [27, 28, 29], the most common soils in the studied regions is presented in table (2). Grumusol soil which located in Aleppo and Daraa may be responsible for high TPC in Samples A* and C*. Whereas Cinnamonic soil seems to have no effect in our study. Moreover, Gypsiferous soil may be the responsible for the high peels TPC in Samples A* and D*.

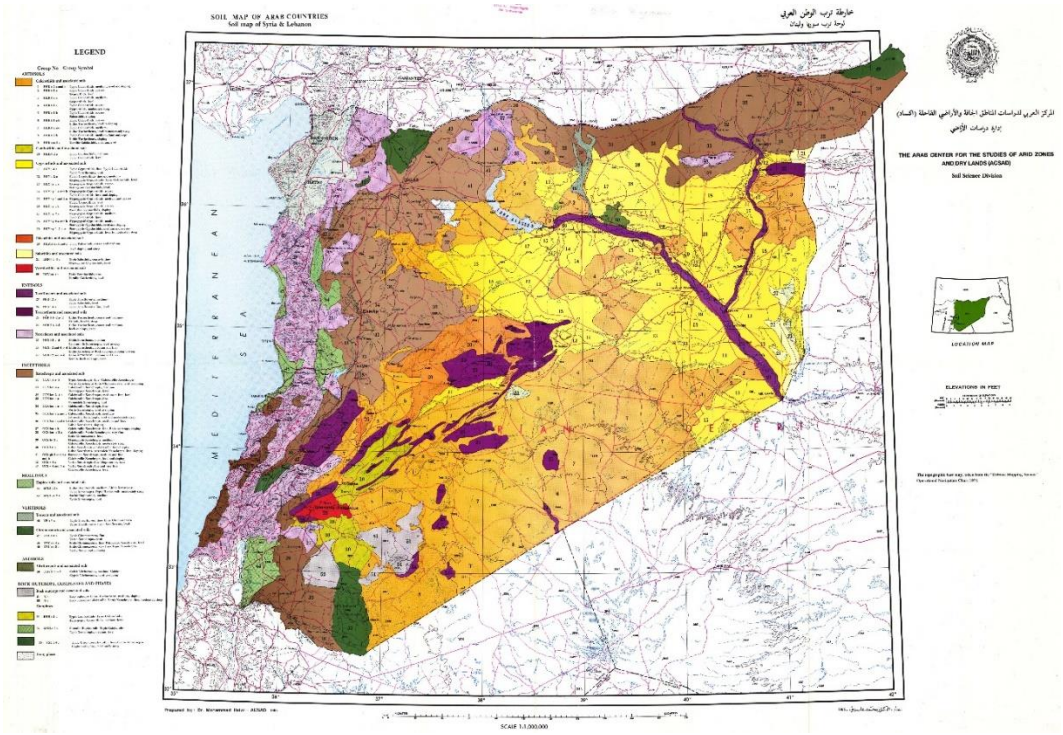

Figure 7: Soil Map of Syria & Lebanon. [27].

Table 2: Common Soils in the studied regions

City	Type of Soils
Aleppo	Cinnamonic, Gypsiferous and Grumusol
Hama	Cinnamonic, Grumusol
Daraa	Grumusol
Al-Rarqa	Gypsiferous, Cinnamonic

Summary of results:

The results showed that the total polyphenols content changes depending on regions and fruit parts. In all regions, the highest levels of total polyphenols content were obtained from the peel extracts, (5974.05 to 7958.4 mg/L). However, the total polyphenols content of pomegranate juice and seed extract ranged from 999.3 to 1866 mg/L and 126.7 to 194.81 mg/L, respectively. "Pomegranate of Aleppo" showed the highest amount of the total polyphenols content among the four regions tested.

Conclusion:

The results showed that the total polyphenols contents in pomegranate vary considerably from one cultivar to another. It was found that the total polyphenols in Aleppo pomegranates are the highest, while Daraa pomegranates are the lowest.

In addition, the concentration of polyphenols varied depending on different parts of the fruit. The results also showed that the peels contain the largest phenolic content, while the seeds have the lowest phenolic content. Syrian pomegranate peel was responsible for the majority of the total polyphenols content in pomegranate. Compared to Syrian studies, the results were close with slight differences, according to the region Geographical.

Moreover, the total concentration of polyphenols in the Syrian pomegranate (juice, peels, and seeds) was higher than that in the Turkish pomegranate according to Turkish studies about Turkish pomegranates, due to the different environmental conditions suitable for the growth and cultivation of pomegranates.

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