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Comparing Different Cultivars of Iranian Pomegranate by the Amount of Epicatechin and Epigallocatechin Gallate

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The pomegranate, *Punica granatum*, is cultivated in tropical and subtropical region. Pomegranates contain a polyphenols such as gallic acid, ellagitannins, gallotannins, chlorogenic acid, caffeic acid, ferulic acid, coumaric acids, and catechin and anthocyanins. The term catechin is also commonly used to refer to the related family of flavonoids and the subgroup flavan-3-ols. In this study we want to compare the amount of catechin in different cultivars of Iranian pomegranate.
Place and Duration of Study: Department of Horticulture and Department of Food science and technology, Khorasgan (Isfahan) Branch, Islamic Azad University, Isfahan, Iran, between march 2010 and November 2012.
Methodology: We gathered the fruit of Iranian pomegranate cultivars in order to make them dry in freeze dryer (w028, Germany). All of the freeze dryer samples were grained and became a soft powder before analysis. 0.5 g sample, mixed with 25 ml of 80 percent ethanol-water solution, was kept in an ultrasonic bath to extract catechins (M. G. Miguel,

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2010). Experiments showed that 80 percent of methanol -water gives the best result and this liquid is the best type. Five minutes of putting in the ultrasonic bath is equivalent to 1 hour of mechanical shaker. 2 ml of high Centrifuge for 5 minutes in a 14000 rpm round by the 0.45ml pourfilter and 20 ml of HPLC to liquid chromatography were injected (J. Dai, 2010). The liquid chromatography was equipped to 1050 HP pomp, 1100HP thermostat, injector750 Rheodyne and fluorescence remover HP 1100. Returnable faze was used in 30 degree centigrade temperature and in C18 column. 100*4/6mm 2i.d;3μm with pre column of c18 Altima were equipped. Washing the isocratic by liquid phase of 9% of acetonitrile in the 2% of Acid citric in a 11 m per minutes separation with the insulator in a EX=280nm of catechin, they were determined by value of catechins aggregation of plotting (μg/ml) with calibration graph were done and the calibration with the stock solution in the 40% methanol got ready.

Results: Results also showed that the highest amounts of Epigallocatechin gallate were existed in “Malas Saveh” and the lowest amounts of epicatechin were existed in “Alak Parand Saveh”.

Conclusion: Our data showed that Epicatechin and Epigallocatechin gallate are the most abundant catechin in pomegranate and they are a potent antioxidant that may have therapeutic application.

Keywords: Pomegranate; polyphenols; epicatechin; health effect.

1. INTRODUCTION

The pomegranate, *Punica granatum*, is an ancient, mystical, and highly distinctive fruit that belongs to the Punicacea family. Pomegranate is one of the commercially important fruit which is extensively cultivated in many tropical and subtropical regions such as Iran [1,2].

The pomegranate tree typically grows 12-16 feet, has many spiny branches, and can be extremely long lived, as evidenced by trees at Versailles, France, known to be over 200 years old. The leaves are glossy and lance shaped, and the bark of the tree turns gray as the tree ages. The flowers are large, red, white, or variegated and have a tubular calyx that eventually becomes the fruit. The ripe pomegranate fruit can be up to five inches wide with a deep red, leathery skin, is grenade shaped, and crowned by the pointed calyx. The fruit contains many seeds (arils) separated by white, membranous pericarp, and each is surrounded by small amounts of tart, red juice [1]. The edible part of the fruits contains acids, sugars, vitamins, polysaccharides, polyphenols and minerals, however, several factors may contribute to the chemical changes, including cultivars, environmental conditions, ripening, storage and postharvest treatments, which may affect fruit quality and health beneficial compounds [3].

The consumption of pomegranate has been associated with beneficial health effects, such as prevention of oxidation of both low and high density lipoprotein, blood pressure, inflammatory, atherosclerosis, prostate cancer, heart disease, and HIV-1. These beneficial effects have been attributed to the high levels of antioxidant activity due to the high content of polyphenols such as gallic acid, ellagitannins, gallotannins, chlorogenic acid, caffeic acid, ferulic acid, coumaric acids, and catechin and anthocyanins. The term catechin is also commonly used to refer to the related family of flavonoids and the subgroup [4,5]. Catechin possesses two benzene rings (called the A- and B-rings) and a dihydropyran heterocycle (the C-ring) with a hydroxyl group on carbon 3. The A ring is similar to a resorcinol moiety while the B ring is similar to a catechol moiety. There are two chiral centers on the molecule on carbons 2 and 3. Therefore, it has four diastereoisomers. Two of the isomers are in trans configuration and are called catechin and the other two are in cis configuration and are called epicatechin [6]. The most common catechin isomer is the (+)-catechin. The other stereoisomer is (-)-catechin or ent-catechin. The most common epicatechin isomer is (-)-epicatechin (also known under the names L-epicatechin, epicatechol), (-)-epicatechol, l-acacatechin, l-epicatechol, epi-catechin, 2,3-cis-epicatechin or (2R,3R)-(-)-epicatechin. Epigallocatechin gallate (EGCG), also known as epigallocatechin 3-gallate, is the ester of epigallocatechin and gallic acid, and is a type of catechin. Some research reported that pomegranate juice has greater antioxidant capacity than other fruit juices and beverages [2]. In the present study that was done in 2012, we want to study the amount of epicatechin in different cultivars of Iranian pomegranate.

2. MATERIALS AND METHODS

Different cultivars of pomegranate "Togh Garden" "Post Siyah Yazd" "Ghojaghe Qom" Malas Esfahan" "Aban mahi Esfahan" "Alak Parand Saveh" Malas Saveh" " Post siyah Esfahan" Shirin Post sefid Saveh" were prepared for this study.

We gathered the black and white cultivars in order to make them dry in freeze dryer (w028, Germany). Then all of the freeze dryer samples were grained and became a soft powder before analysis. 0.5 g sample, mixed with 25 ml of 80 percent ethanol-water solution, was kept in an ultrasonic bath to extract catechins [3]. Experiments showed that 80 percent of methanol-water gives the best result and this liquid is the best type. Five minutes of putting in the ultrasonic bath is equivalent to 1 hour of mechanical shaker.

2 ml of high Centrifuge for 5 minutes in a 14000 rpm round by the 0.45ml pour filter and 20 ml of HPLC to liquid chromatography were injected [7]. The liquid chromatography was equipped to 1050 HP pomp, 1100HP thermostat, injector750 Rheodyne and fluorescence remover HP 1100. Returnable faze was used in 30 degree centigrade temperature and in C18 column. 100*4/6mm 2i.d;3µm with pre column of c18 Altima were equipped. Washing the isocratic by liquid phase of 9% of acetonitrile in the 2% of Acid citric in a 11 m per minutes separation with the insulator in a EX=280nm of catechin, they were determined by value of catechins aggregation of plotting (µg/ml) with calibration graph being done and the calibration with the stock solution in the 40% methanol being ready [8,9]

3. RESULTS AND DISCUSSION

Analysis of data showed the different amount of epicatechin among the pomegranate cultivars. Results showed that the highest amounts of epicatechin were existed in "Aban mahi Esfahan" and the lowest amounts of epicatechin were existed in "Malas Esfahan" (Fig. 1).

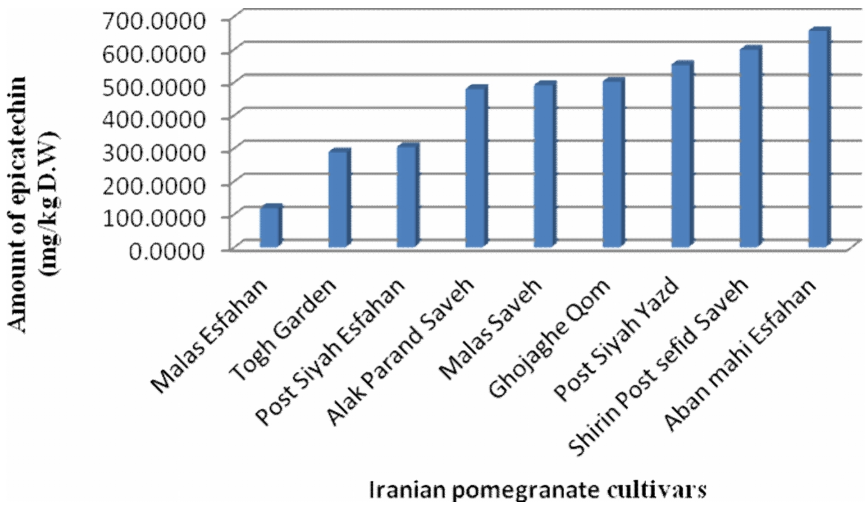

Fig. 1. Comparison between amounts of epicatechin in Iranian pomegranate cultivars

Analysis of data showed the different amount of epigallocatechin gallate among the pomegranate cultivars. Results also showed that the highest amounts of epigallocatechin gallate were existed in "Malas Saveh" and the lowest amounts of epigallocatechin gallate were existed in "Alak Parand Saveh" (Fig.2).

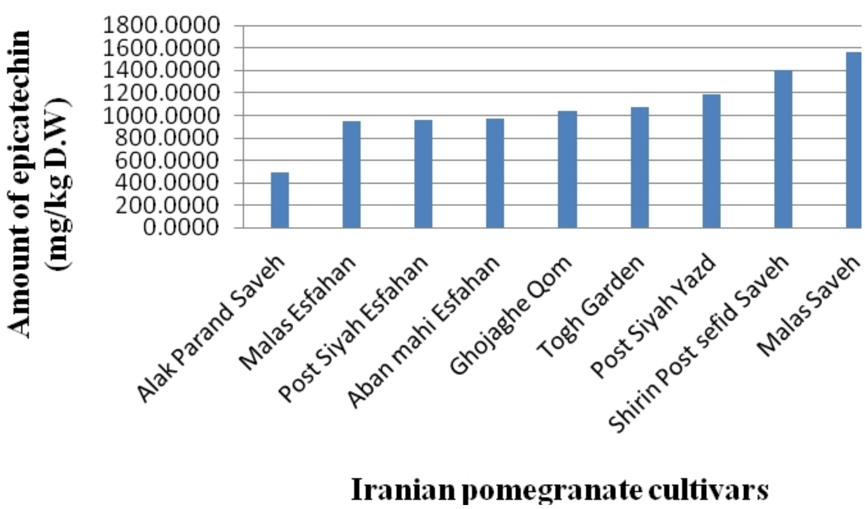

Fig. 2. Comparison between amounts of epigallocatechin gallate in Iranian pomegranate cultivars

Phenolics are compounds possessed one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Many of medicinal plants have a phenolics compounds [10]. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, *etc.*) and beverages (tea, coffee, beer, wine, *etc.*), and partially responsible for the overall organoleptic properties of plant foods. For example, phenolics contribute to the bitterness and astringency of fruit and fruit juices, because of the interaction between phenolics, mainly procyanidin, and the glycoprotein in saliva [11]. Many article are confirm exist of phenolic compounds in pomegranate fruit [12,13,14]. Anthocyanins, one of the six subgroups of a large group of plant polyphenol constituents known as flavonoids, are responsible for the orange, red, blue and purple colors of many fruits and vegetables such as apples, berries, beets and onions. It is known that phenolics are the most important compounds affecting flavor and color difference among white, pink and red wines; they react with oxygen and are critical to the preservation, maturation and aging of the wine. Plant phenolics include phenolics acids, flavonoids, tannins and the less common stilbenes and lignans [15]. Flavonoids are the most abundant polyphenols in our diets. The basic flavonoid structure is the flavan nucleus, containing 15 carbon atoms arranged in three rings (C6-C3-C6), which are labeled as A, B and C. Flavonoid are themselves divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the oxidation state of the central C ring. Their structural variation in each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation. Some of the most common flavonoids include quercetin, a flavonol abundant in onion, broccoli, and apple; catechin, a flavanol found in tea and several fruits; naringenin, the main flavanone in grapefruit; cyanidin-glycoside, an anthocyanin abundant in berry fruits (black currant, raspberry, blackberry, *etc.*); and daidzein, genistein and glycitein, the main isoflavones in soybean. Phenolic acids can be divided into two classes: derivatives of benzoic acid such as gallic acid, and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall. They are also referred to as proanthocyanidins because they are decomposed to anthocyanidins through acid-catalyzed oxidation reaction upon heating in acidic alcohol solutions. The structure diversity is a result of the variation in hydroxylation pattern, stereochemistry at the three chiral centers, and the location and type of interflavan linkage, as well as the degree and pattern of methoxylation, glycosylation and galloylation [7,16].

An *in vitro* assay using four separate testing methods demonstrated the pomegranate juice and its seed extracts have 2-3 times the antioxidant capacity of either red wine or green tea. Pomegranate extracts have been shown to scavenge free radicals and decrease macrophage oxidative stress and lipid peroxidation in animals and increase plasma antioxidant capacity in elderly humans. Studies in rats and mice confirm the antioxidant properties of a pomegranate by-product (PBP) extract made from whole fruit minus the juice, showing a 19-percent reduction in oxidative stress in mouse peritoneal macrophages (MPM), a 42-percent decrease in cellular lipid peroxide content, and a 53-percent increase in reduced glutathione levels. *In vitro* assay of a fermented pomegranate juice (FPJ) extract and a cold pressed seed oil (CPSO) extract found the antioxidant capacity of both are superior to red wine and similar to green tea extract [17]. Separate study in rats with CCl_4-induced liver damage demonstrated pre treatment with a pomegranate peel extract (PPE) enhanced or maintained the free-radical scavenging activity of the hepatic enzymes catalase, super oxide dismutase, and peroxidase, and resulted in 54-percent reduction of lipid peroxidation values compared to controls. Research in humans has shown a juice made from pomegranate pulp (PPJ) has superior antioxidant capacity to apple juice. Using the FRAP assay (ferric reducing/antioxidant power), found 250 mL PPJ daily for four weeks given to healthy elderly subjects increased plasma antioxidant capacity from 1.33 mmol to 1.46 mmol, while subjects consuming apple juice experienced no significant increase in antioxidant capacity [18].

4. CONCLUSION

Our data showed that Epicatechin and Epigallocatechin gallate are the most abundant catechin in pomegranate and they are a potent antioxidant that may have therapeutic application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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