Plant & Fungal Research (2018) 1(1): 38-42
http://dx.doi.org/10.29228/plantfungalres.6

December 2018

Composition and content of phenolic acids in fruit juice and flowers of *Punica granatum* L.

Aydan M. Zeynalova[1]
Eldar N. Novruzov
Institute of Botany, Azerbaijan National Academy of Sciences, Badamdar 40, Baku, AZ1004, Azerbaijan

Abstract: Pomegranate (*Punica granatum* L.) is an important source of bioactive compounds. These compounds are primarily phenolic or polyphenolic, flavonoids (catechins and isoflavones) and related compounds (phenolic acids and chalcones). The purpose of this study was to assess the quantitative and qualitative determination of phenolic acids in fruit juice and flowers of wild-growing pomegranate. For the first time the component composition and the content of phenolic acids in the juice of fruits and flowers of wild-type pomegranate growing on the territory of Galalti, Siyazan region of the Azerbaijan Republic was assessed by using high-performance liquid chromatography (HPLC). Chromatographic analysis was performed using Varian LC/MS (US Ser. No. 05755). Our results show that the samples of flowers and juice differ in both the qualitative and quantitative content of phenolic acids. The composition of the extract of flowers revealed the presence of caffeic, chlorogenic, cinnamic, ferulic, gallic, p-coumaric and sinapic acids. The same compounds were found in the fruit juice, with the exception of gallic acid. The main components in flowers were sinapic and ferulic acids, which constitute 98.5% of the total amount of phenolic acids, wheres in juice a prevalence of chlorogenic (43.4%) and sinapic (37.1%) acids were found. Most of the identified phenolic acids are well known to exhibit pharmaceutical properties, therefore it suggests that flowers and juice of wild-growing pomegranate might be exploit as a potential source of pharmaceutically active compounds and natural biopesticides.

Key Words: *bioactive compounds, high performance liquid chromatography, identification and quantitative determination*

Accepted for publication: 5 December 2018
[1]E-mail: aydan.zeynalova@gmail.com

INTRODUCTION

Phenolic compounds are one of the biggest and widely distributed groups of secondary metabolites in plants [Scalbert, Williamson, 2002]. Polyphenols are known for their pharmacological properties, such as antioxidants, anti-inflammatory, anti-mutagenic, anti-carcinogenic, and antimicrobial [Adams et al., 2006; Adiga et al., 2010]. Plant phenolics include phenolics acids, flavonoids, tannins and the less common stilbenes and lignans [D'Archivio et al., 2007; De la Rosa et al., 2010].

Phenolic acids (phenolcarboxylic acids) are phenols that include substances containing a phenolic ring and at least one organic carboxylic acid function. Depending on the carbon units of the lateral chain attached to the phenolic ring, the phenolic acids can be divided into C6-C3, C6-C2, and C6-C1 compounds, the most important being C6-C3 (derived from the hydroxycinnamic acid) and C6-C1 (compounds with a hydroxybenzoic structure). Although the basic skeleton remains the same, phenolic acids differ in the number and position of the hydroxyl groups on the aromatic ring.

Pomegranate (*Punica granatum* L.) is an important source of bioactive compounds. These compounds are primarily phenolic or polyphenolic, flavonoids (catechins and isoflavones) and related compounds (phenolic acids andchalcones) [Aviram et al., 2000]. According to Naveena et al. [2011], it is reported that pomegranate juice is effective for the prevention of atherosclerosis, oxidation of low-density lipoproteins, prostate cancer, platelet aggregation and various cardiovascular diseases. M.D. Sumner et al. [2005] reported that daily consumption of pomegranate juice for 3 months can reduce myocardial ischemia and improve the performance of patients with myocardial perfusion. It was found that pomegranate flower extract contains triterpenic acids such as oleanolic, ursolic and phenolic acids - gallic acid, which inhibited lipopolysaccharide-induced activation of nuclear factor kappa B in macrophages and could reduce cardiac fibrosis in diabetic fatty rats [Huang et al., 2005].

The purpose of this study was quantitative and qualitative determination of phenolic acids in fruit juice and flowers of wild-growing pomegranate to explore their possible use in food and medicine industries.

MATERIAL AND METHODS

Plant material. Fruit and flowers of the wild-growing *P. granatum* were collected in the territory of Galalti, Siyazan region of the Azerbaijan Republic. The flowers of pomegranate were collected at the beginning of July and the fruits at the end of September.

The fruits of pomegranates were divided and the outer leathery skin covering the seeds was removed manually. Arils were manually separated from the fruits and the juice was obtained by mechanical press, and stored at -80°C. The flowers were dried at 40°C and then crushed with a grinding mill.

Sample preparation. 38.8 ml of ethanol and 1.2 ml of HCl were added 10 g of to dried petals (at 40°C) and stored in a refrigerator at 4°C for 12 hours. The extract were then filtered through a Buchner funnel and dried in a rotary evaporator at 40°C under vacuum before passing through a C18 mini cartridge.

1 ml of HCl was added to freshly squeezed pomegranate juice (500 ml). Then juice was centrifuged and distilled in a rotary evaporator at 40°C under vacuum. A cold solution of 200 ml of ethanol and 1 ml of HCl was added to the remaining mass. Next, the juice before passing through the C18 mini cartridge was again dried in a rotary evaporator at 40°C under vacuum.

Mini-columns C18 contain chains of C18 bound on silicon hydroxide, retain hydrophobic organic compounds (for example, anthocyanins, phenols), while allowing matrix interferences such as sugars and acids to pass through to waste. Washing the retained pigments with ethyl acetate will further remove phenolic compounds and further washing with methanol will remove anthocyanins.

The ethyl acetate fractions were dried in a rotary evaporator and then dissolved in 80% ethanol. Thus, the samples for HPLC were ready for analysis.

Equipment. The HPLC method was used for identification and quantification of phenolic acids of juice and flowers of *P. granatum.* Chromatographic analysis was performed using Varian LC/MS (US Ser. No. 05755). The identification of the phenolic acids from the chromatograms of the plant extracts studied was carried out by comparing the retention time with the corresponding standards purchased from Sigma Aldrich. The best selectivity with the help of the photodiode array detector reached 280 nm for benzoic acids, 320 nm for hydroxycinnamic acids. Equation (1) is used to calculate the concentration of phenolic acids.

Polyphenols standards. Gallic acid monohydrate (5995-86-8), sinapic acid (530-59-6), p-coumaric acid (501-98-4), caffeic acid (331-39-5), cinnamic acid (140-10-3) and ferulic acid (537-98-4) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Mobile phases . The gradient consisted of two eluents: A-water/phosphoric acid (99:0.2) and B-acetonitrile/ water/phosphoric acid (50/49.8/0.2). The flow rate was 1.0 ml/min.

$$Y = \frac{\mathrm{C \times V}}{\mathrm{W}} \times \qquad (1)$$

Equation. C - concentration of phenolic compounds in the sample (mg); V - volume of total extract (ml); W - weight of the sample of the plant taken for analysis; x-dilution.

RESULTS AND DISCUSSION

The composition of the extract of flowers revealed the presence of caffeic, chlorogenic, cinnamic, ferulic, gallic, p-coumaric and sinapic phenolic acids is showed in Table. The same acids composition was found in the juice of pomegranate, with the exception of gallic acid.

Table. Concentration of phenolic acids injuice and petals of pomegranate.

Phenolic acids	Juice (mg/L)	Petals (mg/L)
Caffeic acid	1.92	0.95
Chlorogenic acid	7.66	1.43
Cinnamic acid	0.11	0.49
Ferulic acid	1.26	22.84
Gallic acid	-	1.76
p-coumaric	0.14	0.06
Sinapic acid	6.54	283.65
Total	17.63	311.18

As it can be seen from the table, the main component among hydroxycinnamic acid derivatives in pomegranate juice is chlorogenic acid (43.4%). In the sample of flowers, among the derivatives of hydroxycinnamic acid, synaptic acid prevails, as it accounts for 91.2% of the total amount of phenolic acids. Unlike flower extract, gallic acid was absent in pomegranate juice. In Fig. 1 and 2 are shown the HPLC chromatograms of phenolic acids in pomegranate juice and petals.

Most of the identified phenolic acids exhibit certain pharmaceutical properties. It is known that caffeic acid selectively blocks the biosynthesis of leukotrienes, components associated with immunoregulatory diseases, asthma and allergic reactions [Koshiharaet al., 1984].

Gallic acid is widely used in the pharmaceutical industry in the treatment of a number of diseases. Re-

search has established that gallic acid exhibits cytotoxicity against cancer cells without harming healthy cells. Gallic acid is also used to treat albuminuria and diabetes [Shahidi, Naczk, 2004].

One of the main components in our extract of pomegranate flowers was ferulic acid (22.84%). Ferulic acid has many physiological functions, including antioxidant, antimicrobial, anti-inflammatory, antithrombotic, and antitumor activities [Fujisawa et al., 2002; Harris et

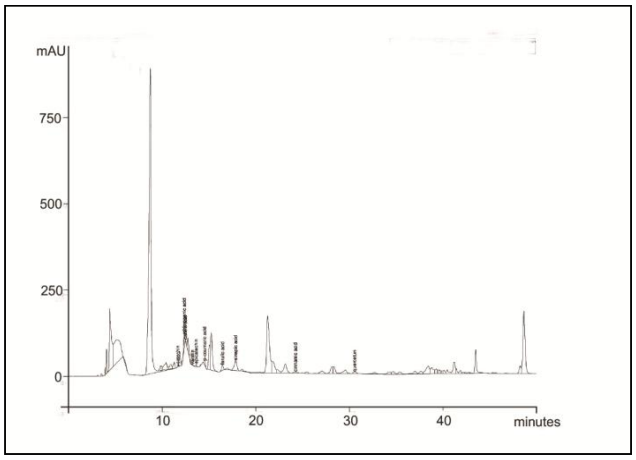

Figure 1. Chromatogram of phenolic acids in a sample of pomegranate fruit juice.

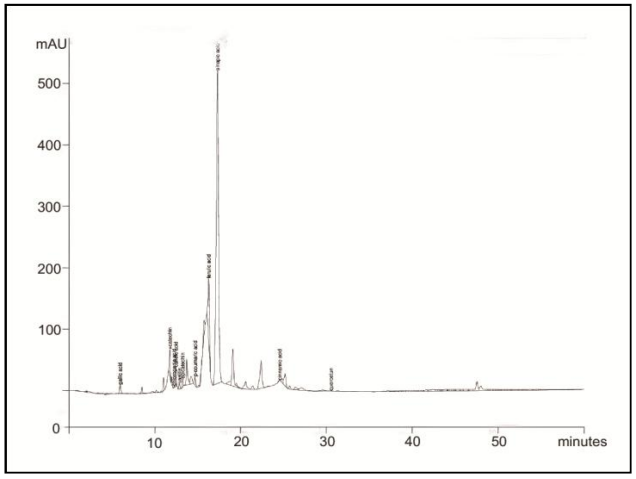

Figure 2. Chromatogram of phenolic acids in a sample of pomegranate flower petals.

al., 2007; Hosoda et al., 2002; Soobratteeet al., 2005]. Synapic acid, which is the dominant acid in the extract of pomegranate flowers (283.65 mg / l), has an antitoxic effect caused by arsenic in the liver tissue [Pari, Jalaludeen, 2011].

Pomegranate for many years enjoys the continuously increasing attention of researchers. This is largely due to its value for medicine as a source of anti-inflammatory, anti-sclerotic, anticancer and other drugs. Despite all the beneficial properties of this plant, wild pomegranate growing on the territory of Azerbaijan has not yet been objects of study for identify the component composition and pharmacological activity. Based on the results reported in this work it is possible to speculate that Azerbaijan wild-growing pomegranate may be a promising source of pharmaceutically active compounds and natural biopesticides.

REFERENCE

Adams S., Seeram P., Aggarwal B., Takada Y., Sand D., Heber D. (2006) Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signalling in colon cancer cells. *J Agri Food Chem*., 54: 980-985.

Adiga S., Trivedi P., Ravichandra V., Debashree D., Mehta F.(2010) Effect of *Punica granatum* peel extract on learning and memory in rats. *Asian Pac J Trop Med.,* 3(9): 687-690.

Aviram M., Dornfeld L., Rosenblat M., Volkova N., Kaplan M., Coleman R., Hayek T., Presser D., Fuhrman B. (2000) Pomegranate juice consumption reduces oxidative stress, atherogenic modification ToLdl and Platelet Aggregation. Studies in Human and in Atherosclerotic Apolipoprotein Deficient Mice. *Am. J. Clin.Nutr.* 71: 1062-1076.

Brett C., Waldron K. (1996) Cell wall architecture. In: Brett C., Waldron K.: Physiology and biochemistry of plant cell walls. *Chapman and Hall*, London, 4-57.

D'Archivio M., Filesi C., Di Benedetto R., Gargiulo R., Giovannini C., Masella R. (2007) Polyphenols, dietary sources and bioavailability. *Ann. Ist. Super. Sanita,* 43: 348-361.

De la Rosa L.A., Alvarez-Parrilla E., Gonzalez-Aguilar G.A. (2010) Fruit andvegetable phytochemicals-Chemistry, Nutritional Value, and Stability. 1st edn. *Wiley-Blackwell Publisher*: Oxford, UK, 1488 pp.

Fujisawa S., Atsumi T., Kadoma Y., Sakagami H. (2002) Antioxidant and prooxidant action of eugenol-related compounds and their cytotoxicity. *Toxicology,* 177: 39.

Harris C.S., Mo F., Migahed L., Chepelev L., Haddad P.S., Wright J.S., Willmore W.G., Arnason J.T., Bennett S.A.L (2007) Plant phenolics regulate neoplastic cell growth and survival: a quantitative structure-activity and biochemical analysis. *Can J Physiol Pharmacol*., 85(11): 1124-1238.

Hosoda A., Ozaki Y., Kashiwada A., Mutoh M., Waka-

bayashi K., Mizuno K., Nomura E., Taniguchi H. (2002) Syntheses of ferulic acid promoters and their suppressive effects on cyclooxygenase-2 promoter activity. *Bioorg Med Chem.,* 10: 1189.

Huang T.H.W., Yand Q., Harada M., Li G.Q., Yamahara J., Ronfogalis B.D., Li Y. (2005) Pomegranate flower extract diminishes cardiac fibrosis in Zucker diabetic fatty rats. *J Cardiovasc Pharmacol,* 46: 856-862.

Koshihara Y., Neichi T., Murota S.I., Fujimoto A.N., Tatsuno T. (1984) Caffeic acid is a selective inhibitor for leukotriene biosynthesis. *Biochim Biophys Acta.,* 792: 92.

Naveena B.M., Sen A.R., Vaitiyanathan S., Patil G., Kondatah N. (2010) Antioxidant potential of pomegranate juice incooked chicken patties. *J Muscle foods,* 21: 557-569.

Pari L., Jalaludeen M.A. (2011) Protective role of sinapic acid against arsenic: induced toxicity in rats. *Chem Biol Interact.*, 194 (1): 40-47

Scalbert A., Williamson G. (2002) Dietary intake and bioavailability of polyphenols. *Int J Nutr,* 130: 2073-2085.

Shahidi F., Naczk, M. (2004) Phenolics in food and nutraceuticals: sources, applications and health effects. Boca Raton: CRC Press, 575 p.

Soobrattee M.A., Neergheen V.S., Luximon-Ramma A., Aruoma O.I., Bahorun T. (2005) Phenolics as potential antioxidant potential mechanisms and actions. *Mutation Res.*, 579: 200-213.

Sumner M.D., Elliot-Eller M., Weidner G., Daubenmier J.J., Chew H.M., Marlin R., Raisin G.J., Ornish D. (2005) Perfusion in patients with coronary heart disease. *Am J Cardiol*, 96: 810-814.

Punica granatum L. növünün meyvə şirəsi və çiçəyində fenol turşularının tərkibi və miqdarı

Aydan M. Zeynalova,
Eldar N. Novruzov
AMEA Botanika İnstitutu, Badamdar şossesi 40, Bakı, AZ1004, Azərbaycan

Nar (*Punica granatum*) əhəmiyyətli bioloji aktiv maddələrin mənbəyi sayılır. Bu maddələr əsasən fenol və ya polifenol, flavanoid (katexin və izoflavon), o cümlədən fenol törəmələri tərkibli (fenol turşularıvə xalkon) birləşmələrdir. İşin əsas məqsədi yabanı narın meyvə şirəsi və çiçəklərinin kəmiyyət və keyfiyyət tərkibinin tədqiqidir. Azərbaycan Respublikasının Siyəzən rayonu Qalaaltı kəndində yabanı halda bitən narın meyvə şirəsi və çiçəyinin fenol turşularının kəmiyyət və keyfiyyət tərkibi ilk dəfə tərəfimizdən öyrənilmişdir. *P. granatum* növünün meyvə şirəsində və çiçəyində olan fenol turşularının identifikasiyası və miqdarının təyin olunmasında yüksək effektivli maye xromatoqrafiya metodu istifadə olunmuşdur. Xromatoqrafik analiz Varian LC/MS (US Ser. № 05755) vasitəsilə yerinə yetirilmişdir. Məlum olmuşdur ki, şirə və çiçək nümunələrinin tərkibindəki fenol turşuları həm keyfiyyət, həm də kəmiyyət tərkibi ilə bir-birindən fərqlənir. Çiçək ekstraktının tərkibində kofein, xlorogen, darçın, ferul, qal, p-kumarin və sinap fenol turşuları aşkar edilmişdir. Meyvə şirəsinin tərkibində qal turşusu istisna olmaqla, eyni fenol turşular aşkar olunmuşdur. Müəyyən edilmişdir ki, çiçəkdə olan fenol turşuları arasında sinap və ferul turşuları əsas komponentlər olub, ümumi fenol turşularının 98.5%-ni təşkil edir. Meyvə şirəsində isə xlorogen (43.4%) və sinap (37.1%) turşuları üstünlük təşkil edir. Təyin olunmuş əksər fenol turşuları farmakoloji xüsusiyyətlərə malikdir. Narın müxtəlif hissələrinin fenol turşularının tərkibinin öyrənilməsi göstərir ki,adı çəkilən növün nəyinki meyvə şirəsi eyni zamanda ləçəkləri də farmaseptik fəal birləşmələrin və biopestisidlərin təbii potensial mənbəyi hesab oluna bilər.

Açar sözlər: *bioloji aktiv komponentlər, yüksək effektli maye xromatoqrafiya, identifikasiyası və miqdarın təyini*

Состав и содержание фенольных кислот в соке плодов и цветках *Punica granatum* L.

Айдан М. Зейналова
Эльдар Н. Новрузов
Институт Ботаники, НАНА, Бадамдар 40, Баку, AZ1004, Азербайджан

Гранат (*Punica granatum*) является важным источником биологически активных соединений. Эти соединения в основном относятся к фенолам или полифенолам, флавоноидам (катехинам и изофлавонам) и родственным соединениям (фенольные кислоты и халконы). Цель данного исследования – количественное и качественное определение типов фенольных кислот в соке плодов и цветках дикорастущего граната. Впервые был изучен компонентный состав и количественное содержание фенольных кислот в соке плодов и цветках дикорастущего граната (*P. granatum*), произрастающего на территории села Галаалты Сиазаньского района Азербайджанской Республики. Для идентификации и количественного определения фенольных кислот использовали метод высокоэффективной жидкостной хроматографии. Хроматографический анализ выполняли с использованием Varian LC/MS (US Ser. № 05755). Установлено, что образцы цветков и сока граната различаются как по качественному составу, так и по количественному содержанию фенольных кислот. В составе экстракта цветков выявлено наличие кофейной, хлорогеновой, коричной, феруловой, галловой, п-кумаровой и синаповой фенольных кислот, в соке плодов обнаружены те же кислоты, за исключением галловой кислоты. Выявлено, что основными компонентами среди фенольных кислот цветка являются синаповая и феруловая кислоты, составляющие 98,5% от общего количества фенольных кислот, а сока - хлорогеновая (43,4%) и синаповая (37,1%) кислоты. Большинство идентифицированных фенольных кислот проявляют определенные фармацевтические свойства. Состав фенольных кислот различных частей граната позволяет считать, что в качестве потенциального источника фармацевтически активных соединений и природных биопестицидов можно использовать не только сок плодов, но и лепестки цветков данного растения.

Ключевые слова: *биоактивные компоненты, высокоэффективная жидкостная хроматография, идентификация и количественное определение*