JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

EFFECTIVE COMPOUNDS OF POMEGRANATE AND THEIR EFFECT ON ANIMAL CELLS

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ARTICLE INFO

Received 22. 10. 2013
Revised 28. 11. 2013
Accepted 8. 1. 2014
Published 1. 2. 2014

Review

OPEN ACCESS

ABSTRACT

This review describes possible effects of antioxidant compounds of pomegranate on animal cells. Pomegranate (*Punica granatum* L.) fruits are widely consumed. Pomegranate is one of the oldest known edible fruit. Spain is main producer in the Europe. Pomegranate contains bioactive polyphenols - punicalagin with molecular weight 1084. Part of punicalagin's molecule is ellagic acid. The both substances generate total antioxidant capacity of pomegranate. Punicalagin compounds present high antioxidant capacity - approximately 50%, ellagic acid as single molecule has 3% of antioxidant capacity. Punicalagin is molecule with high molecular weight and have to be hydrolised. Colonic microorganism metabolise yield of pomegranate (punigalagin or ellagic acid) to urolithin A and is detected in blood, urine or faeces. Extract from pomegranate can show anticarcinogenic effect, induction of cell - cycle arrest, apoptosis and proliferation. Extract from pomegranate has relieving effect on woman's menopausal symptoms, anxienty disorders, depression or attention deficit disorders. Ellagic acid introduces health benefits against cancer, cardiovascular diseases and other disease. It is possible, that compounds of pomegranates or their metabolites could have impact on different animal cells and regulate their intracellular mechanism.

Keywords: Antioxidant, apoptosis, ellagic acid, pomegranate, punicalagin

INTRODUCTION

Pomegranate - characteristic and effectson animal organism

Punica granatum belongs to the *Punicaceae* family and exists over 1000 cultivars (**Levin, 1994**). The pomegranate is a symbol of life, longevity, health etc. (**Mahdihassan, 1984**) and belongs among one of the oldest known edible fruit. It is cultivated in Iran, Afghanistan, India, Mediterranean countries and to some extent in the USA, China, Japan and Russia. Spain is the main European pomegranate producer and its production is mainly located in the province of Alicante (**Raisi *et al*., 2008; Vardin Fenercioglu, 2003; Calín Sánchez *et al*., 2010**). Pomegranate belongs to fruit, which is very rich on antioxidants (**Calín Sánchez *et al.,* 2010**) and contains bio-active compounds (**Calín Sánchez and Carbonell Barrachina, 2012**). For example among protective compounds belong antioxidants - hydrolysable tannins, that are compound abundant in some fruits and nuts, such as pomegranates, black raspberries, raspberries, strawberries, walnuts, and almonds (**Calín Sánchez and Carbonell Barrachina, 2012**). Hydrolysable tannins (antioxidants) are found in aril, rind and capillary membranes. Rinds and capillary membranes are richer source of antioxidant than arils. Among to antioxidants belong punicalagin and ellagic acid (**Calín Sánchez and Carbonell Barrachina, 2012**).
Edible part of pomegranate, 50% from total weight are arils and seed, but more than 50% of bioactive compounds (antioxidants) are described in husk or pulp membranes (antocyanidins, flavonoids, ellagitannins or minerals) (**Calín Sánchez and Carbonell Barrachina, 2012**). Pomegranate arils are composed of 85% water, 10% sugar (glucose, fructose and sucrose), 1,5% organic acid (citric acid, malic acid, fumaric acid etc.) and bioactive compounds - tannins or ellagitannins (punicalagin, punicalin, ellagic acid etc.) and flavonoids (catechin, quercetin etc.) (**Calín Sánchez and Carbonell Barrachina, 2012; Lansky and Newman, 2007**).Mineral compounds in pomegranate are in particularly potassium, nitrogen, phosphorum, magnesium or sodium. Pomegranate contains antioxidants in aril, rind and capillary membranes. (**Calín Sánchez and Carbonell Barrachina, 2012**).
Effect of pomegranate could have utilization in different medicine's ways, for example from India or Guatemala. Dried peels are decocted in water and used for problems - apthae, diarrhea or ulcers (**Lansky and Newman, 2007**). The pomegranate has a broad range of potentially therapeutic uses, including treatment and prevention of cancer, cardiovascular disease, Alzheimer's disease, inflammatory disease, oral and skin disease, obesity, erectile dysfunction and diarrhoea (**Calín Sánchez and CarbonellBarrachina, 2012**). Fatty acid from pomegranate play important role in prevention of cardiovascular disease. This fatty acid (linoleic, linolenic and arachidonic acids) can reduce HDL - cholesterol levels (**Calín Sánchez and Carbonell Barrachina, 2012**). Also compounds of pomegranate could stimulate serotonin and estrogens receptors (**Tyagi et al., 2012**). Extract from pomegranate has relieving effect on women's menopause symptoms, anxiety disorders, depression or attention deficit disorders (**Lee, 2013**).

Compounds of pomegranate – Punicalagins and Ellagic acid

Pomegranate contains more bioactive compounds, but ellagitannins like punicalagin and ellagic acid have the main antioxidant effect have (**Seeram et al., 2005**).

Punicalagins - characteristic, metabolism and effects

Pomegranate husk is rich on the hydrolysable tannins - mainly punicalagin, pedunculagin, punicalin (**Calín Sánchez and Carbonell Barrachina, 2012**). Punicalagins have two isomeric forms in pomegranate: α and β. They belong to group of tannins - polyphenolic compounds. Chemical name of punicalagin is 2.3-(S)- hexahydroxydiphenoyl-4.6-(S,S)-gallagyl-D-glucose (**Tyagi, 2012**). Punicalagin is connected gallagic acid with ellagic acid through molecule of glucose (**Cerdá *et al.*, 2003**). Punicalagins and ellagic acid are responsible for antioxidant activity and healthy benefits of pomegranates (**Tyagi, 2012**). Punicalagin is inducted in pericarp, husk or seeds. Extraction of punicalagin is 7.6% in water and 7.0% in alcohol. There are differences between amount of ellagic acid (0.2 % in water extraction and 0.4% in alcohol extraction) and punicalagins in pomegranates (**Tyagi, 2012**).

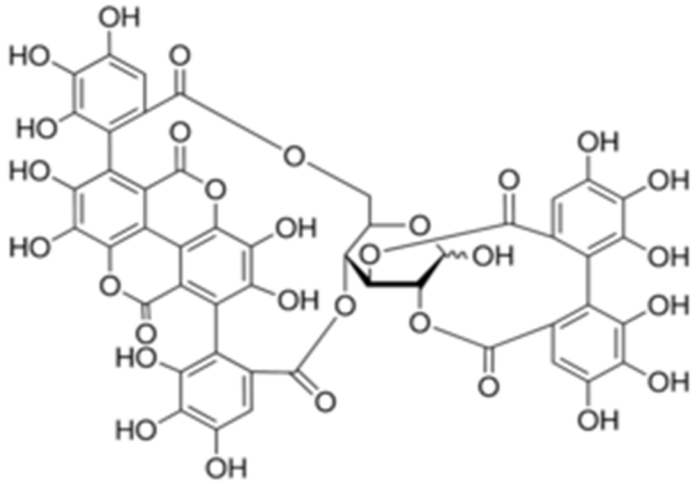

Figure 1 Punicalagin (**Sigma - Aldrich, 2013**)

ETs (ellagitannins) from different sources (walnuts, strawberry, pomegranate, oak-aged wine and raspberry) are not absorbed in humans but hydrolysed to yield EA (ellagic acid), which is further metabolised by the human colonic microflora to yield bioavailable 3.8-dihydroxy-6H-dibenzo[b, d]pyran-6-one (urolithin A) derivatives (**Cerdá *et al.*, 2004; Cerdá *et al.*, 2005**). These metabolites appear in blood, urine and faeces of human volunteers almost 24h after consumption of a single dose of ET (ellagitannin) - enriched meal, and complete clearance is not achieved after 56 h of the intake (**Cerdá *et al.*, 2005**). Little is known about the fate of ellagitannins in animals or humans and only low concentration of PC has been shown in plasma when a bolus dose was provided (**Cerdá *et al.*, 2003**). In general, these large molecules are not absorbed directly into the body (**Cerdá *et al.*, 2004**), and hydrolyzed in the intestinal tract over several hours leading to sustained blood levels of ellagic acid over 6h (**Seeram *et al.*, 2005**). Metabolite of punicalgins are presented ellagic acid, but in urine is inducted derivate - urolithin A (**Larrosa *et al.*, 2006**).
The punicalagin has been reported to be mainly responsible for the antioxidant capacity of pomegranate. It presents very high antioxidant capacity - approximately 50%, by contrast ellagic acid as single molecule has 3% of antioxidant capacity (**Aqila, 2012; Calín Sánchez and Carbonell Barrachina, 2012**). Punicalagin could inhibit proliferation of human tumor cells (oral, colon or prostate) (**Jemal *et al.*, 2011; Gil *et al.*, 2000**). Punicalagin or pomegranate's juice or extract have influenced proliferation and apoptosis of the cancer of breasts, prostate, colon, lung or skin (**Syed *et al.*, 2007**). Punicalagin and ellagic acid could regulation of cyclins A and B1, cell - cycle, arrest in S phase and induction of apoptosis via intrinsic pathway through Bcl- XL down - regulation with mitochondrial release of cytochrome c into the cytosol, activation of initiator caspase 9 and effectors caspase 3 by the colon cancer cells (**Larrosa *et al.*, 2005**).

Ellagic acid - characteristic, metabolism and effects

Ellagic acid belongs to pomegranate's ellagitannins and antioxidants. EA is polyphenolic compounds from plant materials (**Yuan-Chiang *et al.*, 2013**). EA is localised in a molecule of punicalagin. Its chemical name is 4,4′,5,5′,6,6′-hexahydroxydiphenic acid 2,6,2′,6′-dilactone (**Sigma - Aldrich, 2013**).

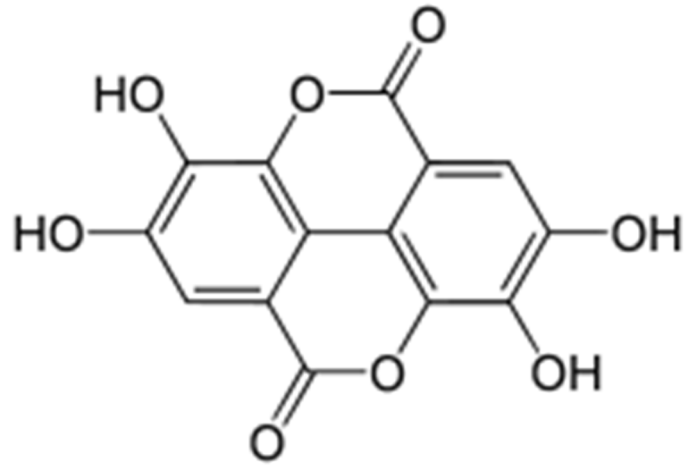

Figure 2 Ellagic acid (**Sigma - Aldrich, 2013**)

Content of ellagic acid in pomegranate is different in rind and arils. Amount of ellagic acid is from 2.49±0.1 mg.g^{-1} to 0.061±0.1 mg.g^{-1} (**Calín Sánchez and Carbonell Barrachina, 2012**), but a content of antioxidant is from 0.8 mg.g^{-1} to 1.8 mg.g^{-1} (**Lee, 2013**). Content of ellagic acid in pomegranate is different, because it may dependent on a harvest time, ripening of fruits and way of preparing a sample (**Lee, 2013**). Fresh fruits contain higher concentration antioxidants than treatment fruits - extract or dry samples (**Calín Sánchez and Carbonell Barrachina, 2012**).
The bioavailability of ellagic acid upon ingestion has been debated and reports have described that ellagic acid is metabolised by intestinal microorganisms to form urolithins that are less potent antioxidants but more lipophilic and, therefore, more readily absorbed over the intestinal mucosa into the circulation (**Cerdá *et al.*, 2004; Espín *et al.*, 2007**). It is well known to have a free radical scavenging activity and has been approved in Japan as an "existing food additive" for antioxidative purposes (**Yuan Chiang, *et al.*, 2013**).
Ellagic acid introduces health benefits against cancer, cardiovascular diseases and other disease (**Sun *et al.*, 2002**). EA can show *anticancer* effect, induction of cell cycle arrest, apoptosis and inhibition of tumor formation and growth in animals (**Seeram *et al.*, 2005**). Ellagic acid induced G0/G1 arrest through increase p53 levels and induced apoptosis through activation of caspase-3 activity in human bladder cancer (**Li *el al.*, 2005**). EA exhibits potent anticancer and *anticancer* activities towards breast, colorectal, oral, prostate (**Losso *et al.*, 2004**), pancreatic (**Yuan Chiang, *et al.*, 2013**), bladder (**Li *et al.*, 2005**), neuroblastoma (**Fjaeraa Nanberg, 2009**), melanoma (**Kim *et al.*, 2009**) and lymphoma cells (**Yuan Chiang *et al.*, 2013**). However, there is no evidence regarding the effect of EA on ovarian carcinoma (**Yuan Chiang et al., 2013**). Recent in vitro evidences revealed that 100μM EA represented little toxic effect on human normal cells (**Yuan Chiang *et al.*, 2013**).

CONCLUSION

This review describes possible effects of antioxidant compounds of pomegranate on animal cells. Punicalagin and ellagic acid as bioactive phytochemicals have proved influence on apoptosis and proliferation processes. More natural antioxidants have been shown to stimulate the expression of the tumor suppressor gene p53.Ithas have possible positive effect on treatment of variousdisease - cancer (prostate, breasts, colon, neuroblastoma cells), obesity, cardiovascular disease. *In vitro* studies are necessary for find out metabolism of ellagitannis and their effect to cell cycle. It is possible, that compounds of pomegranates or their metabolites could have impact on different animal cells and regulate their intracellular mechanism.

Acknowledgments: This work was financially supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects no. 1/0790/11, 1/0022/13, APVV-0304-12, and European Community under project no 26220220180: Building Research Centre „AgroBioTech".

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