Antiviral Nutraceuticals from Pomegranate (Punica granatum) Juice

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CHAPTER 20

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INTRODUCTION

Pomegranate juice has been considered to have multiple health benefit for centuries [Jurenka 2008]. It is currently being marketed/promoted as an antioxidant [Khan et al. 2008] with disease-preventive, prophylactic, and therapeutic health benefits in aging [Afaq and Muktar 2006], prostate cancers [Siddiqui et al. 2004; Adhami and Muktar 2006, 2007; Bemi et al. 2006; Santilo and Lowe 2006; Bell and Hawthorne 2008; Syed et al. 2008] and cancers in general [Aggarwal and Shisodia 2004, 2006; Klass and Shin 2007; Syed et al. 2007; Heber 2008], cardiovascular health [Aviram et al. 2002], diabetes [Saxena and Vikram 2004; Katz et al. 2007; Li et al. 2008], dermatological conditions [Baumann 2007], and inflammation [Lansky and Neumann 2006]. Pomegranate (Punica granatum) juice from America was first shown by Neurath et al. [2005], to be the only fruit juice

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from a number of different fruit juices tested to possess an HIV-1 entry inhibitor activity; it was proposed as a candidate topical vaginal microbicide against all clades of HIV for prevention of HIV infection, which would afford a control to the females during a heterosexual encounter. This anti-HIV activity of pomegranate juice has yet to find itself to be a key agent in the proposed multipronged approach to target progression of HIV-infected person to AIDS (Figure 20.1). Considering the severe side effects of established anti-retrovirals and the predisposition to drug resistance, there is considerable scope for antiviral nutraceuticals from natural sources such as pomegranate juice to be used in the fight against HIV and AIDS. Subsequent studies described here have shown that the pomegranate juice has a far broader antiviral activity against enveloped viruses such as influenza, including the potentially pandemic H5N1 [Kotwal 2006], poxviruses, and herpes viruses; it can be also be ultrafiltered to separate the low-molecular-weight nutraceuticals termed enveloped virus neutralizing compounds (EVNCs) [Kotwal 2007].

**DISTRIBUTION OF POMEGRANATE CROPS**

Pomegranate trees grow in almost every continent. They may have originated in Arabia, and nowhere are they found in such abundance as in the markets and street corners as in Amman, Jordan, which can be described as the pomegranate capital of the world [G. J. Kotwal, unpublished observation]. These trees grow in the Mediterranean region in Europe. In the continent of Africa, the trees can be found in the southwestern-most tip of Africa in Cape Town, as well as in Egypt and Morocco and most likely across regions in Africa. The pomegranate tree is found in several parts of Asia and Australia and the warmer regions of North America, especially
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in California, Texas, and Florida. They were brought to North America from Spain [Welch 2002]. The antiviral activity from the pomegranate juice is quite comparable, whether it is derived tested from the commercially available POM, from California, or from homemade juice in Cape Town, South Africa [Kotwal, unpublished observation]. Pomegranate tree can grow as a highly branched, multi-stemmed tree ranging from 15 to 30 feet. If not harvested at the right time, a pomegranate tree without nets can be inhabited by birds, and pomegranate trees in the wild often get moldy and damaged. There is, therefore, a fairly narrow window during which pomegranate needs to be harvested. One needs to pluck the fruit from the trees one at a time and quickly separate from the damaged ones and store in a refrigerator. Generally, there is only one pomegranate crop per year. Pomegranate trees grow in places with lots of sunshine, range of acidic and alkaline pH, and in deep, well-drained clay soil. It can be propagated by planting seeds or cutting of a tree that bears good fruit.

**POMEGRANATE JUICE EXTRACTION**

After selection of undamaged or uninfected pomegranates, the pomegranates are processed by hand by first making a groove along the sides with a knife and then pulling out the calyx lobes of the pomegranate. Each pomegranate grain, or granatum, with its seeds is then removed and pooled in a clean beaker. Although each part of the pomegranate has different nutraceuticals, the process described here was developed to generate the pure juice. The contents of the beaker are then emptied in the inner compartment of a special mixer/grinder, which has a filter grid that retains the seeds and allows the juice to pass through during the grinding/juicing process. The filtered juice from the grinder is then sterile filtered and can be stored in a refrigerator. All testing for antiviral activity is then performed in a Biosafety Level 2 laminar flow hood if it is testing against laboratory strain of influenza, pox viruses, or herpes. Anti-HIV or hepatitis C virus, or testing against H5N1, is performed in a Biosafety Level 3 laminar flow hood. The sterile filtered pomegranate juice container has to be opened only in a sterile environment; if not, it can be easily contaminated with fungus and bacteria because it is rich in sugars and nutrients. Additional ultrafiltration is used to separate the larger than 3,000 Da proteins, for example, the lipid transfer proteins of the size 7–9 kDa from the EVNCs, which are less than 3,000 and most likely in the range of 500–1,000 Da. The EVNCs can be further purified by column chromatography, but the active ingredients remain a mixture that has potent and stable antiviral activity.

**POMEGRANATE COMPOSITION**

The pomegranate tree and its fruit can be considered as a bounty of unique compartmentalized nutraceuticals. As such, the composition can be considered within each of these anatomical areas (roots, stem/bark, leaves, flower, seeds, and...
juicy pulp) surrounding the seeds and the pericarp (peel and rind) and has been reviewed by Jurenka [2008]. The roots and the bark have a number of piperidine alkaloids and ellagitannins (punicalin, punicalagin). The flower has triterpenoids (maslinic acid, Asiatic acid), ursolic acid, and gallic acid. The pomegranate seeds are a powerful source of nutraceuticals with beneficial effects in cancer, lipidemia, and as antioxidants. The oils in the pomegranate seeds include elagic acid, fatty acids, predominantly linolenic acid (CLA, punicic acid), and phytosterols (beta-sitosterol, campesterol and stigmasterol) [Kaufmann and Wiesman 2007]. The pomegranate juicy pulp/Granatum, which is mostly water, is where we found the antiviral activity. We have not tested the other areas of the fruit or the tree and cannot at this time state whether there is any antiviral activity. The juice has a number of constituents, including red, brown, or pink color pigments such as anthocyanins, which can stain clothes, dishes, or sinks. The juice contains small molecules such as sugars (glucose), amino acids, iron and many other minerals, ascorbic acid, caffeic acid, ellagic acid, gallic acid, catechin, EGCG, quercitin, and rutin. Although it is not at this time known as to which of these compounds are responsible for the antiviral activity, one could speculate that it could be attributable to a mixture of compounds termed EVNCs and not any single or solo performer that can be associated with the antiviral activity of the pomegranate juice, and such EVNCs are not present in other fruit juices tested. Thus, ascorbic acid, iron, amino acids, and glucose could be considered not to influence the antiviral activity. It is possible that the other acids could, in concert, have a role in penetrating the viral lipid envelope and thus causing viral neutralization. In this context, it is noteworthy that Lanasky [2006] emphasizes that nutraceutical products, standardized to 40% ellagic acid, may be dangerous because the health benefits may be attributable to the synergy among the different pomegranate constituents and not restricted to the ellagic acid alone. Pomegranate juice also has fenhexamid [Hengel et al. 2003], but, because it is also present in juices from cranberry and blueberry, it is unlikely that the antiviral activity is attributable to fenhexamid, especially because neither blueberry nor cranberry was found to have antiviral activity. In addition to the smaller than 3,000 Da compounds, the pomegranate juice has two lipid transfer proteins (LTP1a and LTP1b) of a low molecular weight around 8 kDa [Zoccatelli et al. 2007]. These proteins are currently being investigated as elicitors of allergy and hence should be removed from the juice when considering any human antiviral trials. The peel/rind of the pomegranate has constituents similar to the juice with the exception of much greater quantities of flavonols, flavones, flavonones, and phenolic punicalagins.

**OBJECTIVES OF THE STUDY**

To determine whether the antiviral activity is specifically against all the clades of HIV or that it is also against other enveloped viruses, we have since tested the juice for antiviral activity against poxviruses, a number of influenza strains (including
H5N1), and herpes simplex viruses 1 and 2. Also, to determine whether the antiviral activity is found in pomegranate juice across the planet, we have tested and compared the antiviral activity from two different continents: North America and Africa.

**METHOD USED**

The overall design of a safe antiviral assay developed by us is illustrated in Figure 20.2. Essentially, we mixed a million virus particles of attenuated recombinant vaccinia virus, vGK5 [described previously by Kotwal et al. 1989], with varying amounts of the juice for 5 min to 1 h at 37°C and then obtained a virus titer on African Green Monkey cells called BSC-1 cells, with and without treatment. Similarly, for influenza strains, we quantitated the virus with and without treatment using an hemagglutinin inhibition assay as shown in Figure 20.3. To determine whether the pomegranate juice produced from a different continent can reproducibly have antiviral activity, we tested the pomegranate juice from the southwestern-most tip of Africa and compared it with the commercially available juice called POM. To identify the specific compounds and determine the structure of the bioactive antiviral compounds, we have separated the natural substance.
the juice containing only the less than 3,000 Da compounds and also tested it for antiviral activity.

RESULTS AND CONCLUSION

The pomegranate juice tested against vGK5 (Figure 20.4) as well as influenza strains and both the major herpes viruses had significant antiviral activity (Figure 20.5). Also, the clear pomegranate juice produced in South Africa had activity similar to that produced by a manufacturer in the United States. Besides possible benefits in preventing and treating cancers, heart diseases, diabetes, inflammatory diseases, and aging, pomegranate juice consumption in significant amounts or as the nutraceuticals termed
EVNCs could contribute to lowering viral infections attributable to influenza, hepatitis viruses, herpes viruses, and slowing down the progression of HIV-infected persons to AIDS. Growing pomegranate trees worldwide would contribute to sustain the growing demand of the pomegranate tree and to better health of people around the world.

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