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BOTANICAL ADULTERANTS PREVENTION
BULLETIN

Adulteration of Pomegranate
Ingredients and Products

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Goal: This bulletin provides information on issues of adulteration of pomegranate (*Punica granatum*) juice and fruit extracts as an update to previous reports.[1,2] It may serve as guidance for quality control personnel, the international phytomedicine, botanical supplement and food/flavor industries, and the extended natural products community in general. It provides a summary of the scientific data and methods regarding the occurrence of adulteration, the market situation, and economic and safety consequences for the consumer and the industry.

Pomegranate *Punica granatum*
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1. General Information

1.1 Common name: Pomegranate

1.2 Other common names:[3]

Dutch: granaatappel
French: grenade, pomme de grenade (Quebec Province, Canada)
German: Granatapfel
Guatemalan: granad (Q'eqchi, Maya)
Indonesian: gangsalan
Italian: melograno, melogranato, pomo punica
Malay: dalima
Persian: anâr (انار)
Portuguese: fruit: romã; tree: romeira, romazeira
Samoan: limoni
Sanskrit: dalim or dadima
Spanish: granada
Thai: tab tim

1.3 Accepted Latin binomial: *Punica granatum* L.

1.4 Synonym: *Punica nana* L.

1.5 Botanical family: Lythraceae

Note: Pomegranate was previously classified in the botanical family Punicaceae, which has been combined with the family Lythraceae on the basis of genetic and morphological characteristics.[4]

1.6 Distribution: Pomegranate is believed to have originated in an area currently encompassing Iran, Afghanistan, Pakistan, and northern India, but it is now widely distributed and commercially grown in many subtropical and temperate areas of the world. Pomegranate was introduced to and cultivated throughout the Mediterranean region before recorded history, perhaps as long ago as 5000 BCE. Apparently, it was brought to China at least as early as the Han dynasty (between 206 BCE and 220 CE). It was introduced into Spanish America in the late 16th century and into what is now California by Spanish settlers in 1769.[3] Currently, it is widely cultivated throughout northern and tropical Africa, the Middle East and Caucasus regions of Asia, the Indian subcontinent, Central Asia, the drier parts of Southeast Asia, notably China, and parts of the Mediterranean Basin.[3,5] In the United States, it is also cultivated in parts of Arizona and the San Joaquin Valley in California.

Pomegranate has played a prominent role in Greek mythology, symbolism, and ceremonies, as well as numerous religious beliefs, including Buddhism, early Christianity, Hinduism, Islam, Judaism, and Zoroastrianism.[6,7]

1.7 Plant part and form: Pomegranate is a fruit-bearing deciduous shrub or small tree that grows between 5 and 10 m tall. All parts of the pomegranate plant (root, bark, leaves, flowers, fruit, and seeds) have been used in Ayurvedic medicine as an anti-parasitic agent and blood tonic to treat ulcers, canker sores, and diabetes.[7]

However, this Bulletin will focus solely on the fruit, fruit juice, and extract products.[8] The vast majority of pomegranate dietary supplements are reportedly manufactured from either dehydrated pomegranate juice (also known as juice concentrate, not an extract) or extracts made from pomegranate fruit and/or fruit parts by using a solvent (e.g., water, ethanol, methanol, or combinations thereof), often after the juice has been removed by mechanical pressing.[9,10] Extraction of the seeds (arils) provides an oil rich in fatty acids, notably the unique punicic acid,[11] which is of growing interest in the 'cosmeceutical' industry.[12]

1.8 General uses: In the past two decades, considerable research has been conducted on the potential health benefits of pomegranate fruit,[9,10,13] including its antitumor,[14-16] antidiabetic,[17] cardioprotective,[18-21] antioxidant,[22-23] antimicrobial, anti-Alzheimer's,[24] anti-inflammatory,[25] and antiviral properties.[26] More recent publications (2019-2020) have provided insight into the mechanism of action of pomegranate-derived materials on inflammatory processes,[27] diabetes,[28] and cardiometabolic factors.[29]

2. Market

2.1 Importance in the trade: According to the Agricultural and Processed Food Products Export Development Authority (APEDA, New Delhi, India),[30] an organization established by the Indian government to help facilitate trade of agricultural commodities, the value of the global pomegranate fruit market was estimated at US $8.2 billion in 2018. The food/beverage segment is the most important with respect to the overall sales, while the cosmetic and pharmaceutical markets together account for roughly one-third of sales.[12] APEDA lists consumption of pomegranate juice and interest in pomegranate ingredients for cosmetics as main drivers for the current sales increase. A 2014 estimate suggested that 150,000-200,000 metric tons of fresh pomegranates and 3.7 million gallons of pomegranate juice concentrate were sold annually. Pomegranate fruit-derived products are not among the most popular dietary supplements in the United States, ranking in positions 70-100 with regard to the sales in the years from 2014-2017, with the actual ranking depending on the market channel. These supplements are predominantly sold in the natural channel with annual sales of US $1.02-1.14M in this channel (T. Smith [American Botanical Council] e-mail to S. Gafner, September 2, 2015, September 3, 2015, and June 19, 2018. K. Kawa [SPINS] e-mail to S. Gafner, July 11, 2016). All the materials of commerce discussed in this review are derived from the fruit, whether from the seed, juice, or rind/husk/peel.

2.2 Sources: The fruit is harvested in the Northern Hemisphere between September and February, while the Southern Hemisphere has fruit harvests between March and May. India and Iran are the major producers of pomegranate, followed by China, Turkey, and the United States.[31,32] In 2017, India produced 2.8 million metric tons of pomegranate fruit, and over 1 million metric tons were grown in Iran. In comparison, Turkey produced 500,000 tons in 2017, and California (US) reached a peak production of 283,000 tons in 2013.[32]

2.3 Raw material forms: The fruit is the raw material, usually fresh, whole. Juice is pressed from the whole fruit, while extracts are generally prepared from the rind, arils, and fruit pulp, usually after juice has been pressed.

2.4 Market dynamics: A number of factors play a role with regard to the pricing for pomegranate fruits.[33] These include climatic conditions, such as heavy rains during pollination, which can greatly reduce the fruit set, and severe droughts that can also limit the fruit yield. A particularly important factor is the temperature, as very low temperatures in the pomegranate orchards can damage the trees. Reportedly, severe winter freezes in 2007 (–21°C for three consecutive days) and 2016 (–10°C for 48h in late autumn) damaged 36,931 ha and 35,000 ha, respectively, of pomegranate orchards in Iran. Also important are economic factors, such as competitive pressure from oversupply when harvests are above the expected volumes. Such impacts can be seen by the monthly price fluctuations of pomegranate fruit, as reflected in lower prices during the main harvesting months (September to January) in the northern hemisphere.

However, the average annual wholesale price of pomegranate fruit has been remarkably stable; from 2002-2011, the price fluctuated between only €2.50 and €3.50 per kilogram.[34] Prices for pomegranate extracts vary depending on the content in polyphenols, but generally are in

the range of US $40-200/kg (B. Darji [Verdure Sciences] email to S. Gafner, January 26, 2021). An informal investigation in January of 2021 into costs for bulk pomegranate extracts of retail and e-commerce company Alibaba (Hangzhou, China) provided pricing starting as low as US $10/kg. Pricing for ellagic acid (EA)-based extracts obtained from Chinese gallnuts generally ranged from US $5-100, depending on quantity and EA content. Chinese gallnuts are EA-rich excretions formed after insect attacks on young branches or twigs of several several *Rhus* spp. (Anacardiaceae), Aleppo oak (*Quercus infectoria*, Fagaceae), and other plants. The pricing data for EA-rich gallnut extracts are included here to indicate a likely explanation for the very low prices listed on the internet for some pomegranate extracts (see Section 3 below).

In addition, pomegranate bark extracts are also available in the international marketplace. While punicalin and punicalagin were originally isolated from pomegranate peel extracts and structures assigned by Mayer et al.,[35] they were subsequently found in pomegranate bark by Tanaka et al., who also revised the chemical structures.[36]

3. Adulteration

As with any agricultural commodity with a relative fixed supply, prices for pomegranate began to rise with increasing demand, driven by the rapid escalation of consumer interest in the health benefits of pomegranate over the past two decades. Faced with increased demand, a rather fixed or strained supply line, and resulting higher prices, unscrupulous suppliers and manufacturers have turned to various forms of adulteration. At least three different forms of adulteration have been reported in pomegranate products in the global marketplace: pomegranate juices diluted with water or containing juice(s) from other (lower-cost) fruits; pomegranate extracts spiked with additional exogenous EA or polyphenols; and products made mostly from unknown or unidentified source materials, with little-to-no pomegranate constituents.[1,2]

3.1 Known adulterants:

3.1.1 Juice

Historically, juices have been adulterated by dilution with water, often with added sugar, or less expensive fruit juices (e.g., apple, grape). The color of pomegranate juice, due to the anthocyanins present in the fruit, would be diluted by water or apple juice and, thus, adulteration with these ingredients is easily detected. However, there are also cases where anthocyanins isolated from sources that cost less than pomegranate have been added to pomegranate juice diluted with other juices or water. Adulteration with grape juice or exogenous anthocyanins would require more than just UV/Vis (ultraviolet-visible spectroscopy analysis) or optical density measurements for detection and confirmation. A variety of analytical methods can be deployed to compare the anthocyanin, procyanidin, EA, low molecular weight organic acid (e.g., tartaric, quinic, malic, and citric acids), sugar, and mineral profiles of test samples with authentic pomegranate juice.

3.1.2 Extracts

In contrast to pomegranate juice, adulteration of pomegranate extracts predominantly involves the addition of exogenous polyphenolic material, most commonly EA and/or anthocyanins. Pomegranate extracts that are not standardized to a particular marker (e.g., punicalins or punicalagins), but instead to a non-specific estimation of antioxidant capacity or total phenolics, are particularly vulnerable to the addition of inexpensive polyphenols, such as EA or tannins, to increase antioxidant activity, and/or to the addition of anthocyanins to adjust color. Hypothetically, a pomegranate-like material could be similarly constructed with procyanidins, anthocyanins, and EA, along with appropriate sugars and organic acids – all to resemble a pomegranate-derived extract, but this would likely be more expensive than a decent quality pomegranate extract. A search of the Internet revealed that EA is readily available from a number of sources, ranging from US $100-400/kg for EA sourced from pomegranate (*P. granatum*) bark to US $10-45/kg for EA from Chinese gallnuts.

3.2 Accidental or intentional adulteration: The nature of the adulterants found to date in pomegranate products indicates that all the adulterations discussed in this bulletin and previous reports by the ABC-AHP-NCNPR Botanical Adulterants Prevention Program (BAPP)[1,2] are intentional. A pomegranate juice product found to contain other fruit juice would be mislabeled at the very least, if that other juice component is not listed on the ingredients label, but the likelihood remains that such an oversight was intentional. Of course, if a juice product is labeled as a mixture of pomegranate juice and apple juice (or any other juice, e.g., cranberry, cherry, grape, peach, pear), then it is neither mislabeled nor adulterated, but rather a diluted pomegranate juice.

3.3 Sources of information supporting confirmation of adulteration: The evidence for adulteration of pomegranate juice and extract products is substantial[37-43] and has been reviewed in previous reports from BAPP.[1,2] In addition, two further studies, published since those earlier reports, have strengthened the argument that adulteration of pomegranate has been and remains a problem. In 2019, Mathon et al.[44] reported an ultrahigh-performance liquid chromatography mass spectrometry (UHPLC-MS) method to separate and quantify the punicalagins in pomegranate fruits from 14 cultivars. While the amounts varied somewhat among the cultivars, punicalagins A and B were present in all 14 cultivars examined. However, 20% of the commercial juice products evaluated contained no discernible punicalagins. In 2017, Cano-Lamadrid et al.[45] examined 11 juice and 11 extract product samples by HPLC-UV for punicalagin and EA content. They found no detectable punicalagins (A or B) or EA in 3 samples of each product category. Further, the content of all the analyte polyphenolics ranged widely in both product categories. While the authors used standard reference compounds in these analyses, they did not utilize reference juice or extract standards

for comparison. It is interesting to note that the authors identified the names of manufacturers of the juice and extract products examined in this study.

3.4. Frequency of occurrence: There appears to have been some improvement in the quality and authenticity of pomegranate juice and extract products since BAPP's first report on the subject in 2016.[1] At that time, the fail rates reported for both juices and extracts exceeded 35%, often by a significant amount. In the two more recent reports cited herein (see Sec 3.3), the fail rates were 20 and 27%, respectively. While this seems a marked improvement in the situation, it should be noted that only two relatively small studies have been reported recently, and a fail rate of 20% or higher is still unacceptable.

3.5 Analytical methods to detect adulteration: The analyses discussed in this section reflect analytical methods for pomegranate published after the release of the BAPP Laboratory Guidance Document on pomegranate.[2] These articles should be considered in conjunction with, and comparison to, those earlier methods. It should be noted that most of the articles discussed here focused on juice products.

An interesting pair of papers from Ghasempour et al[46,47] report the use of imprinted polymers, synthesized specifically to bind carmoisine (= azorubine), a synthetic diazo diaryl dye with a long history of use as a coloring agent in foods[46] and betanin, a red beet pigment also used in food coloring.[47] These molecularly imprinted polymers are formed around a template (the target molecule) in a mixture of a monomer, a cross-linking agent, a porogenic solvent, and an initiator. The template molecule can be stripped from the resulting resin by solvent extraction, after which the resin can then be used to extract the template molecule from a suspect juice. The authors demonstrated that molecularly imprinted polymers could be prepared and used to extract both betanin and carmoisine/azorubine from spiked juices. A search found no reference to betanin as a known adulterant of pomegranate juice, but there is a single literature report of the identification of carmoisine and ponceau 4R, another diazodiphenyl dye, in a pomegranate juice from Turkey.[48]

Dalmia employed direct sample analysis of juice samples on a time-of-flight mass spectrometer equipped with an ambient ionization capability. This system permits an analyst to skip sample preparation steps and analyze a sample, in this case juice, directly. The author utilized tartaric acid as a marker for adulteration with grape juice and malic acid as a marker for adulteration with apple juice.[49] In the same report, Dalmia demonstrated that the method could also be used to expose adulteration of olive oil.

Dasenaki et al.[50] reported a validated UHPLC-qTOF/MS method that applied metabolomic analyses of juices from two pomegranate, two apple, and seven grape varieties in order to develop metabolomic profiles of each sample and identify useful marker compounds for adulteration of pomegranate juice by the less expensive apple and grape juices. Sample preparation was simple; juices were prepared from the fruits, centrifuged, and filtered prior to injection onto the UHPLC column. Data analysis consisted of principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). Even though the identified marker compounds exhibited very different chemical fingerprints in the two varieties of pomegranate (Turkish Hicaz and Greek Ermioni), adulteration of both with grape or apple juice could be determined reliably to <1% (two marker compounds for apple juice, three for grape juice). The equipment and software involved are relatively expensive, but metabolomic approaches are increasingly being applied to adulteration problems.

Ghasemi et al.[51] reported a study of the volatile organic acids in pomegranate, sour red cherry, and red grape by solid phase microfiber extraction (SPME) and gas chromatography with a flame ionization detector (GC-FID). SPME-GC methodology is used widely in the flavor and fragrance industries and has considerable potential for application to adulteration and quality control, as suggested by this paper. However, the paper is not well written in English and is thus difficult to understand in a few instances, but the more relevant drawbacks are a reliance on hand-crafted SPME fibers, a GC column that yields poor peak shapes, and use of an FID detector instead of a mass spectrometer. Nonetheless, the paper points the way to development of a validated analytical method for focusing on volatile organic acids as a means to identify adulteration (or confirming the purity) of a given juice sample. A wide array of SPME fibers and GC columns are commercially available, and mass spectrometers are also commonplace detectors on GC systems.

Marchetti et al.[52] reported a non-targeted ^{1}H-NMR method, using nuclear Overhauser enhancement spectroscopy (NOESY), to determine the composition of juice mixtures by partial least squares regression analysis (PLS) of the resulting data sets using 6 different chemometric indicators. The researchers used four pure juices (orange, pineapple, apple, and pomegranate) and mixtures made from those. A feasibility study was conducted with the four pure juice samples and 26 different blends of the four (varying percentages of each juice in each blend). An external validation test set included 10 new blended samples, differing from the original 26 blends and five randomly selected blends from the original 26. The method successfully determined the relative concentrations (6.25-100%) of all four juices in these blends, supporting its potential as a method for detecting adulteration in complex, possibly undeclared mixtures. Sample preparation is straightforward — centrifuging solids out of the juice and adjusting the pH before preparing NMR samples. The method used a high field NMR (600 MHz), which is costly but widely available; data collection and processing are largely automated, but time consuming.

Hasanpour et al.[53] reported an NMR metabolomic profiling study of eight varieties of pomegranate from different regions of Iran, the major producer of pomegranate, in the

following regions: Bajestan and Kashmar (Khorasan Razavi province, eastern Iran), Tabas and Ferdows (South Khorasan province, eastern Iran), Taft, Meybod, Bahabad, Mehriz, and Ashkezar (Yazd, central Iran), Shiraz (southern Iran), Mazandaran (from Mazandaran, northern Iran), and Paveh (Kermanshah, western Iran). The ^{1}H-NMR data collected were analyzed by PCA and, ultimately, orthogonal partial least squares-discriminant analysis (OPLS-DA). While this study was not focused on, nor did it disclose, adulteration, it was included in this report because it revealed some striking differences in content of key metabolites used in adulteration studies. Especially notable was that the Mazandaran pomegranates grow wild in northern forests; they have similar, albeit the lowest, profiles of polyphenolic compounds, and the highest levels of citric and succinic acids, resulting in their tasting more sour than the other pomegranate samples. The five ecotypes from Yazd, along with Ferdows and Bajestan, exhibited the highest levels of anthocyanins and EA-containing polyphenolics. These results reinforce the importance of utilizing the appropriate reference materials for quality assurance and may be useful in selecting varieties for taste and beneficial effects.

Tang and Hatzakis employed some novel NMR supersequences to acquire multiple spectra (1D and 2D) simultaneously.[54] This allowed them to assess different approaches to targeted and untargeted metabolomic studies in a much shorter time. Their efforts were focused primarily on the organic acids, sugars, and some individual amino acids, although they did include identification of marker signals for punicalagins in their report. They found that different varieties had profiles that varied enough that they could be readily distinguished from one another. The information gathered on acids, sugars, and amino acids was sufficient to differentiate pomegranate juice and apple juice and, more importantly, a 1:1 mix of the two, suggesting that such NMR methodology could be used to detect adulteration of juices. Further development of this method with punicalins, punicalagins, and ellagic acid standards could be useful in detecting adulteration of pomegranate extract products.

A paper published by Girme et al.[55] in 2021 has addressed the issue of exogenous EA as an adulterant of pomegranate extracts. The researchers developed UFLC (ultrafast liquid chromatography) methods, with both UV and MS/MS detection systems, to identify and quantify both EA and marker compounds for adulterating species containing EA. Using those methods, they examined extracts of nine purported or likely sources of EA, before and after hydrolysis: *Camellia sinensis* (Theaceae) leaves, *Phyllanthus emblica* (syn. *Emblica officinalis*, Phyllanthaceae) fruits, *Quercus infectoria* (Fagaceae) galls or gallnuts, *Syzygium cumini* (Myrtaceae) fruits, *Terminalia* spp. (Combretaceae, bark – *T. arjuna*; fruit – *T. bellirica, T. chebula*), *Vitex negundo* (Lamiaceae) aerial parts, and *Vitis vinifera* (Vitaceae) seeds. They found low levels (<2.25% w/w) of free EA in all the species examined, but high levels of EA liberated upon hydrolysis in pomegranate (47.4%), oak galls (30.6%), and *Terminalia bellirica* and *T. chebula* (27.2% and 25.1%, respectively). All the others were less than 5.4%, with grape seed and green tea being negligible (<0.12%). Key takeaways from this study are (1) development of a rapid (14 min), sensitive assay for key markers for pomegranate and potentially adulterating species; (2) identification of catechin (galls) and chebulinic acid (*Terminalia* spp.) as markers for adulteration with EA-enriched exogenous extracts; and (3) flexibility in the analytical method to include other potential adulterating species (e.g., chestnut (*Castanea* spp., Fagaceae) or walnut (*Juglans* spp., Juglandaceae) shells/husks).

Finally, a 2020 publication by Brêtas et al.[56] describes a validated, miniaturized version of a classic spectrophotometric method for total polyphenols. The primary advantage of this method is that it is conducted in 96 well plates, allowing for the use of dramatically less reagent and solvent, as well as more rapid reading of the results. The disadvantage is that there is no specificity as to what is being analyzed, i.e., all polyphenolic compounds present in the extract are read as a composite sample, with no regard to exogenous substances and no emphasis on the perceived compounds of interest. Still, some manufacturers or marketers may find this a useful (and more economical) way to assess the antioxidant capacity of their product(s).

4. Genetic Studies of Pomegranate

While published reports of adulteration in pomegranate products have tapered off and development and application of analytical methods have remained rather steady since BAPP's previous reports,[1,2] research on the genetics of pomegranate has been rather vigorous over the past five years.

First, the entire genome of pomegranate has been determined and reported by two groups.[57,58] Qin et al.[57] sequenced the genome of the 'Dabenzi' cultivar from Anhui Province in China. They confirmed that the genera *Punica* and *Eucalyptus* are the most closely related of the 12 fully sequenced plants. They also established that the whole genome duplication event observed in *Eucalyptus* and estimated to have occurred about 110 million years ago (mid-Cretaceous) also occurred in *Punica*, meaning the two genera did not diverge until after that event. The group also identified genes involved in seed coat development and the biosynthesis of terpenoids, anthocyanins, and punicalagins, providing some fertile ground for research and breeding of pomegranate. Yuan et al.[58] sequenced and assembled the genome of the 'Taishanhong' cultivar, widely grown in China. The authors' data also supported the placement of the genus *Punica* in the family Lythraceae, and that the divergence of *Punica* and *Eucalyptus* occurred after the whole genome duplication event around 110 million years ago. Their data further suggested that this divergence occurred around 70 million years ago. The group focused their attention on the biosynthetic gene clusters associated with ellagitannin biosynthesis, finding similar genes that were expressed primarily in aril and peel, but were less active during fruit development. Perhaps the most significant finding in this study might be that the authors conducted sequence homology searches, but failed to identify any

genes encoding β-glucogallin O-galloyltransferase (GLUG) or galloyltransferase (GALT), key enzymes in known ellagitannin pathways. The authors hypothesized that either these genes have diverged so much in pomegranate that any discernible sequence homology is no longer recognized or alternative pathways have been developed in pomegranate to perform the transformations catalyzed by those enzymes. These two reports should stimulate a good deal of further research into the genetics of this ancient fruit.

Second, Yan et al.[59] determined and characterized the chloroplast genomes of three distinct cultivars of pomegranate: 'Nana' – a dwarf pomegranate with small, sour fruit and hard seeds; 'Tunisia' – a domesticated cultivar with sweet fruit and soft seeds; and 'Taishanhong' – an important cultivar in Chinese commerce (see above[52]), with delicious taste, a bright red peel, and hard seeds. Comparison of these sequences to those of two previously sequenced chloroplast genomes of Iranian pomegranates revealed significant similarity, i.e., low diversity. Thus, the chloroplast genome of pomegranate is not likely to be useful in distinguishing cultivars or for studies of genetic diversity in this genus. However, the authors also compared the chloroplast sequences of the five pomegranate cultivars with 80 other plants in the Myrtales. The resulting phylogenetic tree showed that the Lythraceae (*Punica* and 3 other represented genera) formed a single clade, with close associations with Onagraceae, Myrtaceae, and Melastomataceae, thus supporting the recent reassignment[4] of *Punica* (and the Punicaceae) to the Lythraceae.

Third, in a 2016 paper, Saminathan et al.[60] reported 10 miRNAs (micro RNAs) from the pomegranate cultivar 'Al-sirin-nar' that were differentially expressed at various stages of growth in leaves, flowers, and fruit. This report stimulated Patil and his colleagues[61] to subject the 17,439 microRNAs (miRNAs) reported by Qin[57] for the 'Dabenzi' cultivar to in silico analyses to exclude redundant sequences, resulting in 1922 unique, mature miRNAs and 1028 primary miRNAs. In turn, those miRNA sequences were examined for SSR (simple sequence repeat) markers; 897 markers were found and identified. The authors then used 15 distinct, representative pre-miRNA (precursor microRNA) SSRs to investigate the genetic relationships of 18 cultivars of pomegranate; three distinct clusters could be identified. Patil's team[62] continued this line of research, again using in silico analyses of the 'Dabenzi' cultivar's genome to identify a new set of 48,8353 Class I SSRs (>20 bp). Primer pairs were developed for 2850 of these SSRs, resulting in the identification of 82 polymorphic fragments, 13 of which were used to survey 46 pomegranate cultivars. That analysis separated the 46 cultivars into 4 clusters. At the same time, Patil's collaboration[63] used 21 recently identified[62] Class I SSRs to analyze 42 diverse pomegranate genotypes, a collection that included 16 Indian cultivars, 13 wild collections from various locations in India, and 13 samples labelled as "exotic cultivars" from sources outside of India (Afghanistan, Iran, Russia, Sri Lanka). The 42 genotypes were grouped in four clusters. Of note is the fact that the pomegranate samples from the wild exhibited the highest genetic diversity, indicating considerable promise for genetic improvement of commercial cultivars.

5. Possible Safety/Therapeutic Issues

No new safety concerns have arisen since the previous reports in this series.[1,2] The primary therapeutic concern remains the likely reduction of potential health benefits in an adulterated product.

The single report of carmoisine in pomegranate juice[46,48] is cause for some concern and warrants further investigation of pomegranate juice products from various sources and countries. Carmoisine is a member of the class of diazodiphenyl compounds, which are long-used colorant dyes now recognized as potential carcinogens. Members of this class have frequently been reported as adulterant colorants in turmeric (*Curcuma longa*, Zingiberaceae) and turmeric products.[65]

6. Conclusions

The number of reports of adulteration of pomegranate products has diminished somewhat since BAPP's previous reports.[1,2] While the percentage of reported adulterated products has declined, the rate of adulteration found in two more recent studies[44,45] (20 and 27% of tested products) remains unacceptable.

Ten recently published analytical methods were reviewed in this report. The methodologies included direct MS, UHPLC-MS, SPME-GC-FID, NMR, and a relatively new technique, imprinted polymers specifically designed to detect diazodiphenyl colorants or betanin (a red beet pigment). While all of these methods were developed for juice products, the NMR and imprinted polymer methods could also be adapted for use with extract products.

A 2021 publication[55] on the determination of adulteration by addition of exogenous EA provides a method and guidance for quality control/quality assurance of pomegranate extracts. The method described has the flexibility to be adapted to other potential sources of exogenous EA.

Research on the genetics of pomegranate is expanding rapidly, with the genome of two cultivars identified and numerous comparison studies illustrating the differences among cultivars and wild populations. Some of the key genes involved in the biosynthesis of the more prominent constituents of pomegranate have been identified, opening the door to genetic and breeding improvements.

The suggestion that diazodiphenyl colorants, well known as adulterants in the turmeric supply line,[64] might also be employed in coloring purported pomegranate products[46,48] means that manufacturers and marketers of pomegranate products should be vigilant for evidence of these adulterant compounds. *It is important to note that, beyond pomegranate, any naturally bright yellow, orange, or red materials in the botanical ingredient or supplement supply chain could be vulnerable to adulteration with this class of compounds.*

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Pomegranate *Punica granatum*
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REVISION SUMMARY

Version #, Author	Date Revised	Section Revised	List of Changes
Version 1, John H. Cardellina II, Stefan Gafner	n/a	n/a	n/a