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Pomegranate Products Laboratory Guidance Document

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Pomegranate *Punica granata*
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1. Purpose

Pomegranate has rapidly become one of the most popular 'healthy' fruits, with an array of extracts appearing in the botanical dietary supplement markets and a plethora of juice products in the beverage industry. There is considerable evidence that both product categories have been subjected to adulteration with various undeclared, lower-cost exogenous ingredients.[1] Therefore, this Laboratory Guidance Document presents a review of the analytical technologies used to determine whether pomegranate juice or extract products are adulterated and to identify the adulterants involved.

2. Scope

The analytical challenge arising from adulteration of pomegranate products is complex because different products are adulterated in different ways. Pomegranate juice has been found to be diluted by a variety of lower-cost, more readily-available juices, and colorants may be added to adjust the color to approximate true pomegranate juice more closely. Pomegranate extract products have been adulterated by addition of exogenous ellagic acid (EA) or made entirely from unknown or unidentified source materials, with little-to-no pomegranate constituents, but significant amounts of EA present. The methods discussed in this guidance document were developed for either juice or extract products, but may not be applicable to other pomegranate food products (e.g., yogurt or jelly) or medicinal products derived from pomegranate plant parts other than the fruit (e.g., leaves).

The evaluation of a specific analytical method or methods in this Laboratory Guidance Document for testing pomegranate materials does not reduce or remove the responsibility of laboratory personnel to demonstrate adequate method performance in their own laboratory using accepted protocols outlined in various domestic (in the United States) or international legal and/or regulatory documents, e.g., the 21 CFR Part 111 (Dietary Supplement GMPs, in the US Code of Federal Regulations) and Part 117 (Food Safety Modernization Act Final Rulemaking for Current Good Manufacturing Practice and Hazard Analysis and Risk-Based Preventive Controls for Human Food, in the US Code of Federal Regulations), and by AOAC International, International Standards Organization (ISO), World Health Organization (WHO), and the International Council on Harmonisation (ICH).

3. Common and Scientific Names

3.1 Common name: Pomegranate

3.2 Other common names

French: grenade, pomme de grenade (Quebec Province, Canada)
Spanish: granada
Italian: melograno
German: Granatapfel
Dutch: granaatappel
Persian: anâr (انار)
Sanskrit: dalim or dadima

3.3 Accepted Latin binomial: *Punica granatum* L.

3.4 Synonyms: *Punica nana* L.

3.5 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.[2]

4. Botanical Description

Punica granatum is a fruit-bearing deciduous shrub or small tree in the family Lythraceae that grows between five and 10 m (16-30 feet) tall. The pomegranate tree enjoys considerable longevity, with some specimens in France reported to have survived two centuries. On multiple, spiny branches, the deciduous leaves are opposite or in whorls of five or six, short-stemmed, oblong-lanceolate, leathery, and 1-10 cm (0.4-4 in) long. Showy red, white, or variegated flowers are found on the branch tips, singly or in clusters of up to five flowers. Nearly round, but crowned at the base by the prominent calyx, the pomegranate fruit has a tough, leathery skin or rind, and is basically yellow overlaid with light or deep pink or rich red. The interior is separated by membranous walls and white spongy tissue into compartments packed with transparent sacs filled with tart, flavorful, fleshy, juicy pulp (the aril). In each sac, there is one white or red, angular, soft or hard seed. The arils represent about 52% of the weight of the whole fruit.[3]

Pomegranate is believed to have originated in an area encompassing what are now Iran, Afghanistan, Pakistan, and northern India. Pomegranate has played a prominent role in Greek mythology, symbolism and ceremonies, as well as numerous other religious beliefs, including Buddhism, early Christianity, Hinduism, Islam, Judaism and Zoroastrianism.[3-5]

All parts of the pomegranate plant (root, bark, leaves, flowers, fruit, and seeds) have been used in Ayurvedic medicine in India for various health conditions that range from an anti-parasitic agent and blood tonic to treatments for ulcers, canker sores, and diabetes.[5] More recently, the antitumor, antidiabetic, cardioprotective, antioxidant, antimicrobial, anti-Alzheimer's, anti-inflammatory, and antiviral properties of preparations of the pomegranate fruit have received attention.[6-12]

5. Identification and Distinction Using Macroanatomical Characteristics

Fruit, a globose berry, 5–13 cm in diameter, with a leathery rind enclosing numerous seeds (arils), angular/wedge shaped, variously colored—yellowish green, white, reddish brown, or occasionally blackish purple.[13,14]

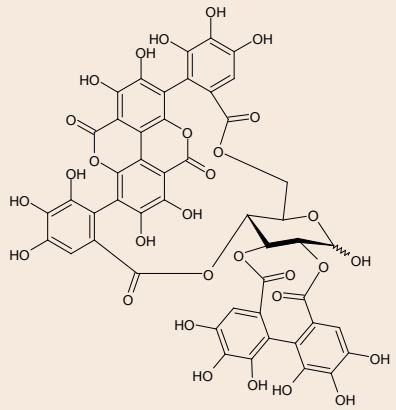
6. Identification and Distinction Using Microanatomical Characteristics

Thorough descriptions of the microscopic characteristics of pomegranate fruit have been published by the World Health Organization (WHO) and *The Ayurvedic Pharmacopoeia of India*.[13,14] A 2015 publication provides detailed information on the microscopical analysis of pomegranate fruit, along with color images of the important microanatomical features of pomegranate fruit.[15] While microscopic analysis will provide information about the authenticity and purity of cut or powdered crude pomegranate fruit, this approach is not suitable when the typical characteristics are absent, i.e., in juices or extracts. However, microscopy is useful in the analysis of juice powders to ensure that they have the characteristic features of juice powders processed in the manner declared; such analyses can reveal added bulking agents (diluents, e.g., maltodextrin) that may not be declared.

7. Genetic Identification and Distinction

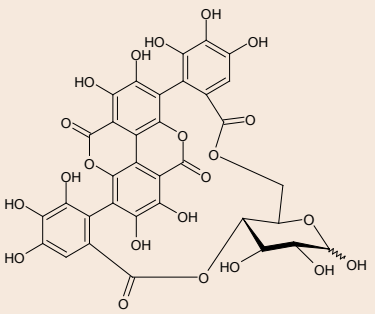
A 2016 study demonstrated the use of a DNA-based method, SCAR (Sequence Characterized Amplified Regions) analysis, to detect as low as 1% adulteration of pomegranate juice by ten other botanical sources of anthocyanins that have been reported or might be used as adulterants of pomegranate products. The SCAR marker selected as a positive control for pomegranate, designated $ScPg_{231}$, correctly identified eleven different accessions of *P. grana-*

Figure 1: Major polyphenols in peel and mesocarp of pomegranate fruit, according to Fischer et al.[26]



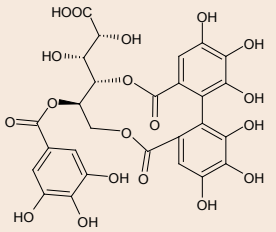
Punicalagins (1)

The term punicalagins describes a mixture of two diastereomers, punicalagin A and B

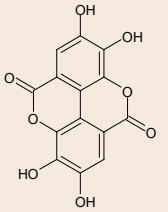


Punicalins (2)

The term punicalins describes a mixture of two diastereomers, punicalin A and B



Lagerstannin C (3)



Ellagic acid (4)

tum; moreover, it also identified pomegranate in four product mixtures – two herbal teas (2% and 20% pomegranate, respectively), a jam containing pomegranate, lemon, agave and pectin, and a juice mix containing 3.5% pomegranate juice concentrate. These results indicate that relatively short-length SCAR markers may be highly useful for identifying components with partially degraded DNA.[16]

Another report[17] expanded upon earlier studies[18-20] of another DNA-based method, RAPD (Random Amplified Polymorphic DNA), and demonstrated an ability to distinguish and identify 47 Chinese cultivars of *P. granatum* by application of RAPD. Screening of 60 primers revealed 11 primers that gave reproducible polymorphic band patterns. While previous RAPD investigations of pomegranate[18-20] used a cluster analysis approach to parse the data, the results from the primers employed in this study could be used to generate a cluster identification diagram, wherein the first primer selected divided the group of 47 cultivars into (+) or (-) subgroups (i.e., presence or absence of the marker band, respectively); in turn, each primer was then used to further divide the remaining groups in the same manner until all 47 cultivars were separated into distinct entities, based on polymorphic band patterns. This technique would have its best application in verifying the identity and cultivar of raw material supplies, rather than identifying adulteration in finished products, although the appearance of unexpected polymorphic band patterns would likely be an indication of substitution (different cultivar than expected or stipulated) or adulteration with other fruit. There are some well-known limitations to the RAPD methodology, in particular low reproducibility and difficulties in the interpretation of the results.[21]

8. Chemical Identification and Distinction

8.1 Chemistry of *Punica granatum* and potential adulterants

The chemistry of pomegranate is dominated by phenolic compounds of differing complexity, from benzoic acid (simple) to anthocyanins (complex) to gallotannins and ellagitannins (very complex).[7] The secondary metabolites of pomegranate are far from fully identified; a recent investigation of the outer skin, inner skin, and divider membranes revealed a total of 79 phenolic compounds, thirty of which had not been reported previously, including proanthocyanidins.[22]

Lignans were reported for the first time from pomegranate in 2009, adding to the family of phenolics found in this species.[23] Other chemical classes have also recently been reported from pomegranate, including triterpenes,[24], while the seed oil of pomegranate was found to contain phytosterols and an unusual fatty acid profile dominated by punicic acid, an omega-5 linolenic acid isomer with the carbon-carbon double bonds at positions 9, 11, and 13.[25] Figure 1 illustrates the more abundant polyphenolics in pomegranate (punicalagins [a mixture of isomers comprised of punicalagin A and B], **1**; punicalins, **2**; lagerstannin C, **3**; and ellagic acid, **4**).

Three different forms of adulteration have been detected in pomegranate products in the global marketplace: (a) addition of juices from other (lower cost) fruits to pomegranate juice; (b) spiking of pomegranate extracts with additional EA or polyphenols; and (c) products made mostly from unknown or unidentified source materials, with little-to-no pomegranate constituents. EA is the principal chemical adulterant of extracts, although its true source may be unknown (EA can be produced by chemical synthesis and/or extraction from other natural sources), while various sugars, organic acids, amino acids, and polyphenols can serve as markers of adulteration by other fruit juices.

8.2 Laboratory methods

Table 1 lists different methods used to analyze commercial pomegranate juice products for adulteration and considers the key advantages and disadvantages of each technique, while Table 2 provides a similar comparative analysis of analytical methods for products made from pomegranate extracts.

8.2.1 Juice

Comments: A quick look at the contents of Table 1 illustrates that some form of HPLC-UV analysis, with or without mass spectrometry and/or fluorescence detection, is the primary means of determining the polyphenol content of pomegranate juice (ellagitannins, EA, and gallotannins). This HPLC-UV approach can also highlight the presence of polyphenolics that should not be present in authentic pomegranate juice (e.g., polyphenolics from grape or cranberry juice).

The challenge with juices is determining what adulterant juices or additives (e.g., sugars, colorants) are present. There is a variety of options available to researchers/analytical groups, but the most useful of these appear to be analysis of sugar content (notably glucose, sucrose, and fructose) and organic acid content (citric, isocitric, malic, tartaric, quinic). There are cases where mineral content or amino acid profile might be instrumental in identifying a particular adulterant juice. $^{12}C/^{13}C$ ratios can be used to identify cases where synthetic or exogenous sugars have been added to a juice product.

The Krueger Food Laboratory analysis of >500 juices/juice products using ten different validated analytical methods, plus application of iterative statistical analysis of the resulting data, gave very robust profiles of the chemical content of pomegranate juice and likely or potential adulterant juices.[33] This report can provide very useful guidance or insight for the selection of an analytical strategy.

The 2017 report by Brighenti et al.[37] provides a HPLC-UV-ESI-MS^2 method, validated and compliant with ICH guidelines, for juice and extracts of peel. The authors identified 31 peaks in the juice chromatogram as phenolic compounds, and 51 in the chromatograms of mesocarp and exocarp extracts; oddly, two major peaks in the juice chromatogram (monitored at 268 nm) were not identified, even tentatively. The method described employs a fused core HPLC column, a relatively new column technology not reported in many analyses of botanicals, but the authors found that it gave better resolution with less solvent

consumption than either C_{18} or pentafluoro-phenyl phase columns. Thus, it seems amenable to rapid adoption for use in quality assurance and adulteration detection.

Not listed in Table 1, but of likely interest to manufacturers and marketers of pomegranate products, are some recent and current studies, by collaborating teams from Bruker BioSpin GmbH and SGF International e.V., reporting the development of NMR methodologies for the quality control of fruit juices.[38,39] This work was focused primarily on apple juice as the lead example and led, a few years later, to a published validation study on quantitative analysis of multiple components.[40] The technique requires a highly shielded 400 MHz NMR magnet with a flow injection system, permitting a high throughput of samples. A Bruker application note indicates that pomegranate juice can be qualitatively and quantitatively evaluated by this methodology.[41]

8.2.2 Extract

Comments: Somewhat surprisingly, the Botanical Adulterants Prevention Program retrieved only two detailed published analyses of extract-containing products for evidence of adulteration, but the results in both investigations were so strikingly similar that the evidence for widespread adulteration of pomegranate supplements is considered quite strong. Zhang et al.[42] analyzed 27 commercially available pomegranate extracts and found that only five

Table 1. Comparison of different analytical approaches to determine adulterants in pomegranate juice

Reference	Sample Set	Method(s)	Analyte(s)	Pro	Contra
Zhang (2009a)[27]	45 commercial juices from 23 manufacturers	HPLC-UV	anthocyanins, ellagitannins	standard equipment in many laboratories; analytes have strong UV chromophores; some reference compounds available	some equipment is moderately costly
		HPLC-RI	sugars	reference compounds available	RI detectors may not be available in all laboratories
		HPLC-UV (PDA)	organic acids – citric, isocitric, tartaric, malic	standard equipment in many laboratories; reference compounds available	multistep sample preparation
		ninhydrin -VIS	proline	simple, inexpensive; reference compound available	
		MS	^{13}C enrichment of sugars	best method to identify exogenous sugars	some equipment is moderately costly
		flame photometry	potassium	simple sample preparation/ procedure	equipment not found in all labs
Ehling (2011)[28]	6 samples freshly prepared juice; 10 commercial juice samples	HPLC-MS/MS with stable isotope dilution	organic acids – citric, isocitric, tartaric, malic, quinic	unambiguous determination of organic acids at low mg/L levels; reference compounds available	equipment is expensive
Nuncio-Jauregui (2014)[29]	pomegranate, grape, peach juice	HPLC-UV (PDA)[a]	organic acids – citric, tartaric, malic	standard equipment in many laboratories; reference compounds available	some equipment is moderately costly
		HPLC-RI[b]	sugars – glucose, sucrose, fructose	reference compounds available	RI detectors may not be available in all laboratories
		AA[c]	minerals – Na,K,Ca,Mg,Fe,Zn,Cu,Mn	reference materials available; simple, inexpensive	equipment not found in all labs
		ninhydrin-VIS	proline	simple, inexpensive; reference compound available	
		HS/SPME[d]-GC	volatile flavor/aroma compounds	standard equipment in some laboratories; reference compounds available	technique not commonly used in supplement industry
Tezcan (2013)[30]	pomegranate, apple juice	chiral MEKC-LIF[e]	amino acids	reference compounds available	equipment not found in all labs

of them contained significant amounts of the pomegranate-specific ellagitannins (punicalagins and punicalins). They found that 17 of the samples contained mostly EA; the remaining five extracts contained little or no ellagitannins or EA (and little antioxidant activity). Madrigal-Carballo et al.[43] analyzed 19 commercially available pomegranate extracts and reported that only seven produced polyphenolic profiles indicative of pomegranate, while 13 of the extracts (including one of the seven with a pomegranate profile) contained EA levels exceeding that expected from arils and rind. Of the latter group, six had little or no pomegranate ellagitannin content.

Both groups used HPLC-UV for their analyses, since all the analytes of interest have strong UV chromophores. Either method could be readily adapted for use in a company or commercial analytical laboratory. Validation of any method to be employed and the use of reference standards to verify peak identities is highly recommended. If a mass spectrometer is available for use in the analytical method, its use could both confirm the identity of known, anticipated compounds and help to identify any unexpected or unknown peaks observed in the HPLC chromatograms.

The paper by Fischer et al.[26] is included in Table 2 because it is a thorough analysis of a variety of juices and extracts by HPLC-UV-MS, providing evidence for the presence of a total of 48 phenolic compounds across the samples analyzed. This method has the potential to be developed and validated for use in verifying product identity and quality, while at the same time exposing any adulteration.

The paper by Li et al.[44] provides a useful HPLC-UV fingerprint of extracts of pomegranate peel, showing consistency of 10 collections from four orchards in China. This

Continued Table 1. Comparison of different analytical approaches to determine adulterants in pomegranate juice

Borges (2013)[31]	6 juices marketed as pure pomegranate; 20 pomegranate/other juice blends; 10 other juices	HPLC-PDA-MS/MS	polyphenols	reference compounds available	equipment is expensive
Borges (2010)[32]	4 juices, 1 wine	HPLC-PDA-MS-FD[f]	polyphenols	reference compounds available	equipment is expensive
Krueger Food Laboratories (2012)[33]	>500 juice samples	10 different ana-lytical methods, plus iterative statistical analysis	sugars, organic acids, polyphenols	reference compounds available; large sample set; can detect many forms of adulteration	10 analytical procedures; statistical analyses required
Vardin (2008)[34]	pomegranate and grape juice concentrate	FTIR[g], with chemometrics	fruity esters, acids -absorptions in carbonyl range (1780-1685 cm^{-1})	non-destructive method inexpensive	may not work for all juice adulterants
El Darra (2017)[35]	pomegranate and date juice concentrate	ATR[h]-FTIR	fruity esters, acids -absorptions in carbonyl range (1780-1685 cm^{-1})	non-destructive method, inexpensive	effective for date juice concentrate, but may not work for all juice adulterants
		HPLC-PDA	anthocyanins	standard equipment in many laboratories; reference compounds available	moderately expensive equipment
Gómez-Caravaca (2013)[36]	pomegranate juice	HPLC-PDA-ESI/qTOF/MS	13 anthocyanins and 14 other phenolic compounds	thorough analysis of large number of phenolics; some reference compounds available	equipment is expensive, not found in all laboratories
Brighenti (2017)[37]	pomegranate juice and extract	HPLC-DAD-ESI/MS	punicalagins A and B, ellagic acid, ellagic acid hexoside, ellagic acid deoxyhexoside, ellagic acid pentoside, cyanidin 3-*O*-glucoside, cyanidin 3,5-*O*-diglycoside	method validated, compliant with ICH[i]; some reference standards available	equipment is moderately expensive

[a] Photodiode array detection
[b] Refractive index detection
[c] Atomic absorption spectroscopy
[d] Head space – solid phase microextraction
[e] Micellar electrokinetic chromatography-laser induced fluorescence detection
[f] Fluorescence detection
[g] Fourier transform infrared spectroscopy
[h] Attenuated total reflectance
[i] International Conference on Harmonisation (of Technical Requirements for the Registration of Pharmaceuticals)

method has the potential to be validated for use in quality control of such extracts.

The recent report by Brighenti et al.[37] is discussed in the comments following Table 1 (Section 8.2.1); those comments are also applicable here.

Not listed in the table is an NMR study by a group led by Larive, who examined the ^{1}H-NMR spectra of a mixture of punicalagins A and B at various pHs.[45] The chemical shifts (position of the NMR signals) of the aromatic protons were found to be very sensitive to pH. The aromatic region of the NMR spectra of these compounds is not as signal-rich as the carbohydrate region, suggesting that this approach could conceivably be developed as a method of detection of adulteration or decomposition of extracts and products derived from extracts.

9. Conclusion

There is a growing body of data indicating that pomegranate juice and extract products are frequently adulterated. Possibly driven by supply/demand issues and/or economic incentives, such fraudulent products deprive consumers of the health benefits of pomegranate.

Various analytical methods are reviewed in this guidance document, with the seemingly most broadly applicable and fit-for-purpose of those highlighted for the benefit of readers.

Based on the available evidence, none of the known adulterants, whether they are other fruit juices or exogenous substances, represent an apparent safety concern to consumers.

10. References

1. Cardellina JH, II, Blumenthal M. Adulteration of pomegranate products — A review of the evidence. *HerbalGram.* 2016;112:62-69.
2. Graham SA, Hall J, Sytsma K, Shi S-H. Phylogenetic analysis of the Lythraceae based on four gene regions and morphology. *Int J Plant Sci.* 2005;166(6):995-1017.
3. *Morton JF.* Pomegranate, *Punica granatum* L. *In: Morton JF. Fruits of Warm Climates. 1987; 352–355. Purdue New Crops Profile website.* Available at: https://www.hort.purdue.edu/newcrop/morton/pomegranate.html. Accessed May 23, 2017.
4. Bhandari PR. Pomegranate *(Punica granatum* L*)*. Ancient seeds for modern cure? Review of potential therapeutic applications. *Int J Nutr Pharmacol Neurol Dis.* 2012;2:171-184.
5. Ruis AR. Pomegranate and the mediation of balance in early medicine. *Gastronomica.* 2015;15:22-33.
6. Miguel MG, Neves MA, Antunus MD. Pomegranate (*Punica granatum* L.): A medicinal plant with myriad biological

Table 2. Comparison of different analytical approaches to determine adulterants in pomegranate extracts and extract-based products

Reference	Method(s)	Analytes	Pro	Contra
Zhang (2009b)[42]	HPLC-PDA	punicalagins, punicalins, ellagitannins	standard equipment in most laboratories; reference standards available	equipment is moderately expensive; multiple analyses involved
	TEAC[a], GAE[b] and EAE[c]	total polyphenols		not particularly useful for adulteration analyses
Madrigal-Carballo (2009)[43]	HPLC-PDA	punicalagins, punicalins, ellagitannins, gallotannins	standard equipment in most laboratories; reference standards available	equipment is moderately expensive
Fischer (2011)[26]	HPLC-DAD–ESI/MS^n	anthocyanins, gallotannins, ellagitannins, gallagyl esters, hydroxybenzoic acids, hydroxycinnamic acids, dihydroflavonol	thorough analysis delineating 48 phenolic constituents; some reference standards available	equipment is rather expensive, not available in all laboratories
Li (2015)[44]	HPLC-UV	punicalagins A and B, ellagic acid, gallic acid	standard equipment in most laboratories; some reference standards available	reference standards not available for all peaks characteristic of HPLC fingerprint
Brighenti (2017)[37]	HPLC-DAD-ESI/MS	punicalagins A and B, ellagic acid, ellagic acid hexoside, ellagic acid deoxyhexoside, ellagic acid pentoside, cyanidin 3-*O*-glucoside, cyanidin 3,5-*O*-diglycoside	method validated, compliant with ICH[d]; some reference standards available	equipment is moderately expensive

[a] Trolox equivalent antioxidant capacity
[b] Gallic acid equivalent
[c] Ellagic acid equivalent
[d] International Conference on Harmonisation (of Technical Requirements for the Registration of Pharmaceuticals)

properties – A short review. *J Med Plants Res.* 2010;4:2836-2847.
7. Jasuja ND, Saxena R, Chandra S, Sharma R. Pharmacological characterization and beneficial uses of *Punica granatum*. *Asian J Plant Sci.* 2012;6:251-267.
8. Katz SR, Newman RA, Lansky EP. *Punica granatum*: Heuristic treatment for diabetes mellitus. *J Med Food*. 2007;10:213-217.
9. Jurenka J. Therapeutic applications of pomegranate (*Punica granatum* L.): A review. *Alt Med Rev.* 2008;13:128-144.
10. Yuan T, Ma H, Liu W, Niesen DB, Shah N, Crews R, Rose KN, Vattem DA, Seeram NP. Pomegranate's neuroprotective effects against Alzheimer's disease are mediated by urolithins, its ellagitannin-gut microbial derived metabolites. *ACS Chem Neurosci.* 2016;7:26–33.
11. BenSaad LA, Kim KH, Quah CC, Kim WR, Shahimi M. Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A&B isolated from *Punica granatum*. *BMC Compl Alt Med.* 2017;17:47-56.
12. Liu C, Cai D, Zhang L, Tang W, Yan R, Guo H, Chen X. Identification of hydrolysable tannins (punicalagin, punicalin and geraniin) as novel inhibitors of hepatitis B virus circularly closed DNA. *Antiviral Res.* 2016;134:97-107.
13. *WHO Monographs on Selected Medicinal Plants*. Volume 4. World Health Organization, Geneva, 2009; 118.
14. *The Ayurvedic Pharmacopoeia of India*. Part I, Volume II. New Delhi, India: Ministry of Health and Family Welfare, Government of India. 1999: 32.
15. Gohil KM, Prajapati PK, Harisha CR. Detailed micromorphological and pharmacognostic evaluation of Dadima fruit (*Punica granatum*). *Am J PharmTech Res*. 2015;5:237-244.
16. Marieschi M, Torelli A, Beghé D, Bruni R. Authentication of *Punica granatum* L.: Development of SCAR markers for the detection of 10 fruits potentially used in economically motivated adulteration. *Food Chem*. 2016;202:438-444.
17. Zhang YP, Tan HH, Cao SY, Wang XC, Yang G, Fang JG. A novel strategy for identification of 47 pomegranate (*Punica granatum*) cultivars using RAPD markers. *Genetics Mol Res*. 2012;11:3032-3041.
18. Sarkhosh A, Zamani Z, Fatahi R, Ebadi A. RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. *Sci Horticult.* 2006;111:24-29.
19. Masoud S, Saneghi A, Shahreiyari ZH, Noormohammadi Z, Farahanei F, Tabatabaei-Ardakanei SZ. RAPD and cytogenetic study of some pomegranate (*Punica granatum* L.) cultivars. *Caryologia* 2008;61:68-73.
20. Hasnaoui N, Messaoud M, Jemni C, Mokhtar T. Molecular polymorphisms in Tunisian pomegranate (*Punica granatum* L.) as revealed by RAPD fingerprints. *Diversity* 2010;2:107-114.
21. National Center for Biotechnology Information. Random Amplified Polymorphic DNA (RAPD). Available at: https://www.ncbi.nlm.nih.gov/probe/docs/techrapd/. Accessed January 25, 2018.
22. Ambigaipalan P, de Camargo AC, Shahidi F. Phenolic compounds of pomegranate byproducts (outer skin, mesocarp, divider membrane) and their antioxidant activities. *J Agric Food Chem.* 2016;64:6584-6604.
23. Bonzanini F, Bruni R, Palla G, Serlataite N, Caligiani A. Identification and distribution of lignans in *Punica granatum* L. fruit endocarp, pulp, seeds, wood knots and commercial

Pomegranate *Punica granata*
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juices by GC-MS. *Food Chem.* 2009;117:745-749.
24. Jiang HZ, Ma QY, Fan HJ, Liang WJ, Huang SZ, Dai HF, Wang PC, Ma XF, Zhao YX. Fatty acid synthase inhibitors isolated from *Punica granatum* L. *J Braz Chem Soc*. 2012;23:889-893.
25. Kaufman M, Wiesman Z. Pomegranate oil analysis with emphasis on MALDI-TOF/MS triacylglycerol fingerprinting. *J Agric Food Chem.* 2007;55:10405–10413.
26. Fischer UA, Carle R, Kammerer DR. Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MS^n. *Food Chem*. 2011;127:807–821.
27. Zhang Y, Krueger D, Durst R, Lee R. Wang D, Seeram N, Heber D. International multidimensional authenticity specification (IMAS) algorithm for detection of commercial pomegranate juice adulteration. *J Agric Food Chem.* 2009;57:2550-2557.
28. Ehling S, Cole S. Analysis of organic acids in fruit juices by liquid chromatography-mass spectrometry: An enhanced tool for authenticity testing. *J Agric Food Chem*. 2011;59:2229–2234.
29. Nuncio-Jauregui N, Calin-Sanchez A, Hernandez F, Carbonell-Barrachina AA. Pomegranate juice adulteration by addition of grape or peach juices. *J Sci Food Agr.* 2014;94:646-655.
30. Tezcan F, Uzaşçi S, Uyar G, Oztekin N, Erim FB. Determination of amino acids in pomegranate juices and fingerprint for adulteration with apple juices. *Food Chem*. 2013;141:1187-1191.
31. Borges G, Mullen W, Crozier A. Comparison of the polyphenolic composition and antioxidant activity of European commercial fruit juices. *Food Funct.* 2010;1:73–83.
32. Borges G, Crozier A. HPLC-PDA-MS fingerprinting to assess the authenticity of pomegranate beverages. *Food Chem.* 2012;135:1863-1867.
33. Krueger DA. Composition of pomegranate juice. *J AOAC Int.* 2012; 95:163-168. See also: Krueger Food Laboratories, *Fruit juice authenticity analysis*. Available at: http://www.kfl.com/pom.html, accessed 12 September 2017.
34. Vardin H, Yay A, Ozen B, Mauer L. Authentication of pomegranate juice concentrate using FTIR spectroscopy and chemometrics. *Food Chem.* 2008;108:742-748.
35. El Darra N, Rajha HA, Saleh FN, Al-Oweini R, Maroun R, Louka N. Food fraud detection in commercial pomegranate molasses syrups by UV–VIS spectroscopy, ATR-FTIR spectroscopy and HPLC methods. *Food Cont.* 2017;78:132-137.
36. Gómez-Caravaca AM, Verardo V, Toselli M, Segura-Carretero A, Fernández-Gutiérrez A, Caboni MF. Determination of the major phenolic compounds in pomegranate juices by HPLC–DAD–ESI-MS. *J Agric Food Chem.* 2013;61:5328-5337.
37. Brighenti V, Groothuis SF, Prencipe FP, Amir R, Benvenuti S, Pellati F. Metabolite fingerprinting of *Punica granatum* L. (pomegranate) polyphenols by means of high-performance liquid chromatography with diode array and electrospray ionization-mass spectrometry detection. *J Chromatogr A.* 2017;1480:20-31.
38. Spraul M, Schütz B, Humpfer E, Mörtter M, Schäfer H, Koswig S, Rinke P. Mixture analysis by NMR as applied to fruit juice quality control. *Magn Res Chem.* 2009;47:S130-S137.
39. Spraul M, Schütz B, Rinke P, Koswig S, Humpfer E, Schäfer H, Mörtter M, Fang F, Minoja A. NMR-based multi parametric quality control of fruit juices: SGF profiling. *Nutrients.* 2009;1:148-155.
40. Monakhova YB, Schütz B, Schäfer H, Spraul M, Kuballa T, Hahn H, Lachenmeier DW. Validation studies for multicomponent quantitative NMR analysis: the example of apple fruit juice. *Accred Qual Assur.* 2014;19:17-29.
41. NMR (Nuclear Magnetic Resonance) screening of juices, pulps and purees now significantly enhanced through increased quantification parameters and wider coverage, providing reliable targeted and non-targeted multi-marker analysis. Bruker website. Available at: https://www.bruker.com/news-records/single-view/article/bruker-announces-next-generation-juicescreener-30.html. Accessed March 14, 2018.
42. Zhang Y, Wang D, Lee RP, Henning SM, Heber D. Absence of pomegranate ellagitannins in the majority of commercial pomegranate extracts: implications for standardization and quality control. *J Agric Food Chem.* 2009; 57:7395-7400.
43. Madrigal-Carballo S, Rodriguez G, Krueger CG, Dreher M, Reed JD. Pomegranate (*Punica granatum*) supplements: Authenticity, antioxidant and polyphenol composition. *J Funct Food.* 2009;324-329.
44. Li J, He X, Li, Zhao W, Liu L, Kong X. Chemical fingerprint and quantitative analysis for quality control of polyphenols extracted from pomegranate peel by HPLC. *Food Chem.* 2015;176:7-11.
45. Kraszni M, Marosi A, Larive CK. NMR assignments and the acid–base characterization of the pomegranate ellagitannin punicalagin in the acidic pH-range. *Anal Bioanal Chem*. 2013;405:5807-5816.