

Bioaccessibility of Lead and Arsenic in Traditional Indian Medicines

Supporting Information

Iris Koch, Maeve Moriarty, Kim House, Jie Sui, William R. Cullen, Robert B. Saper, Kenneth J. Reimer

Description of Sample Ingredients

Details of sample ingredients are listed in Table SI-1.

Table SI-1

Details of sample ingredients for medicines tested in the present study.

ID	Product Name	RS ^a	Ingredients on Label
1	Mahasudarshan Specialty Tablets		<i>Swertia chirata, Emblicia officinalis, Terminalia belerica, Terminalia chebula, Picrorhiza kurroa, Tinspora cordifolia, Cyperus rotundus, Cedrum deodara, Plumbago zeylanica, Curcuma longo, Piper officinarum, Azadirachta indica, Holarrhena antidysenterica, Inula racemosa, Berberia aristata, Bambusa arundinacea, Embelia ribes, Vetiveria zizanoides, Solanum xanthocarpum, Aconitum hetrophyllum, Abies webbiana, Thalictum foliolosum, Sansvieria roxburghiana, Piper longum, Zingiber officale, Per nigrom, Santalum album, Carum copticum, Valeriana wallichii, Oldenlandia corymoboso, Sida cordifolia, Cinnamomum cassia, Syzgium aromaticum, Cinnamum tomala, Desmodium gangeticum, Ragonia arabica</i>
2	Shilajit Specialty Tablets	Y	Mineral pitch^b
3	Vital Lady	Y	Mineral pitch^b, iron oxide , asoka tree, lodh tree, red saunders, <i>Cyperus pertenuis</i> , mesua, eclipt, spiked ginger lily, turmeric, indian jalap, costus, long pepper, cinnamon, <i>Corallium rubrum</i> , bael tree, arani, oroxylum, fragrant padri tree, maly bush beech, sarivan, <i>Uraria picta</i> , blue wiss, heart-leaf sida
4	Worry Free	Y	Pearl , bacopa, dwarf morning glory, ashwagandha, jatamansi, greater galangal, licorice, indian tinospora
5	Yog-Raj Guggulu		<i>Balsamodendron mukul, Plumbago zeylanica, Piper longum, Ptychotis ajowan, Embelia ribes, Terminalia chebula, Terminalia belerica, Emblica officinalis, Cyperus rotundus, Piper nigrum, Elettaria cardamomum</i>
6	Commiphora mukul		Guggulu resin
7	Bacopa monniera		Brahmi leaf powder
8	Yogaraj Guggulu		<i>Balsamodendron mukul, Plumbago zeylanica, Piper longum, Ptychotis ajowan, Embelia ribes, Terminalia chebula, Terminalia belerica, Emblica officinalis, Cyperus rotundus, Piper nigrum, Elettaria cardamomum</i>
9	Ezi Slim		Neem chhal, punarnava, kalimarich, lasun, vidang, haritaki, bahera, amla, nagarmotha, pipal, sonith, safed chandan, sugandha bala, skandi, ushir, balamool, akanadi, ushir, balamool
10	Shilajeet Tablets	Y	Mineral pitch^b

ID	Product Name	RS ^a	Ingredients on Label
11	Mahayograj Guggulu	Y	Tin bhasma, silver bhasma, lead bhasma, iron bhasma, mica bhasma, oxidized iron bhasma, red sulphide of mercury, Zingiber officinale, Piper longum, Piper brachystachyum, Piper longum, Plumbago indica, Ferula asafetida, Trachyspermum roxburghianum, Brassica juncea (L.) Czern, Cuminum cyminum, Nigella sativa, Vitex agnus castus, Holarrhena pubescens, Cisampelos pariera, Embelia ribes, Piper chaba, Picrorhiza scrophulariiflora, Aconitum hetrophyllum, Clerodendrun serratu, Acorus calamus, Chonemorpha fragrans, Terminalia chebula, Terminalia bellirica, Phyllanthus embelica, guggulu resin
12	Brahmi Capsules		<i>Bacopa monniera</i>
13	Amoebica		Batsakadi quath, ghann sattwa, kutki churn
14	Arogyavardhini Bati	Y	Mineral pitch^b , arogyawardhini bati, kutki
15	Kanchanar Guggulu		Guggulu resin, ginger root, kanchanar herb, black pepper fruit, amalaki fruit, varuna bark, bibhitaki fruit, caramom seed, haritaki fruit, cinnamon bark, pippali fruit, bay leaf
16	Shilajit	Y	Mineral pitch^b
17	Lean Plus		Citrin, <i>Coleus forskohlii</i> , <i>Gymnema sylvestre</i> , bioperine
18	Neem Plus		Neem extract, gudduchi, bioperine
19	Prana: Breath of Life		Elecampane root, bayberry root bark, licorice root, marshmallow root, cinnamon bark, ginger root, cloves, ephedra, pippali
20	Bakuchi		<i>Cyamopsis psoralioides</i>
21	Brahmi		<i>Bacopa monniera</i>
22	Chairata		<i>Swertia chirata</i>
23	Cold Aid		<i>Piper nigrum</i> , <i>Carum copticum</i> , <i>Ipomoea turpethum</i> , <i>Cyperus officinale</i> , <i>Ocimum sanctum</i> , <i>Sitopladi churna</i> , root of <i>Pier longum</i> , <i>Eclipta alba</i>
24	Trifala Guggulu		Trifala, guggulu resin
25	Heart Plus		<i>Terminalia arjuna</i> , <i>Tinospora cordifolia</i> , <i>Emblica officinalis</i> , <i>Embelia ribes</i> , <i>Piper longum</i> , <i>Ipomea turpethum</i> , <i>Vitis vinifera</i> , <i>Ellattaria caardemom</i> , <i>Zingiber officinale</i> , <i>Iribulus terrestris</i> , <i>Foeniculum vulgare</i> , <i>Coriandrum sativum</i> , <i>Boerhaavia diffusa</i> , <i>Terminalia chebula</i> , dashmoola
26	Jatamansi		<i>Nardostachys jatamansi</i>
27	Kanta Kari		<i>Solanum xanthocarpum</i>
28	Lavan Bhaskar		Blend of 5 salts (e.g. sea salt, black salt, rock salt) , fennel, long pepper, long pepper root, black cumin, cinnamon leaf, <i>Mesua ferria</i> , rhubarb root, pomegranate seeds, cinnamon, cardamon
29	Licorice		<i>Glycyrrhiza glabra</i>
30	Mahasudarshan		<i>Swertia chirata</i> , <i>Picorrhiza kurrooa</i> , <i>Tinospora cordifolia</i>
31	Praval Pisti	Y	Corralium rubrum (coral)
32	Prostate Rejuv	Y	Asphaltum , <i>Tribulius terrestris</i> , <i>Boerhavia diffusa</i> , <i>Withania somnifera</i>

ID	Product Name	RS ^a	Ingredients on Label
33	Shilajit	Y	Mineral pitch^b
34	Sugar Fight		<i>Gymnema sylvestre, Azdirachta indica, Swertia chirata, Eugenia jambolana, Enicostemma littorale, Pterocarpus marsupium, Tinospora cordifolia, Curcuma longa, Emblica officinalis, Cassia auriculata</i>
35	Tagar		<i>Valeriana officinalis</i>
36	Yograj Guggulu		Rock salt , <i>Commiphora mukul, Piper longum, Ptyochotis ajowan, Carum carvi, Embilca ribes, celery seed, Cuminum cyminum, Cedrus deodara, Piper chaba, cardomom, Costus speciosus, Vanda spathulata/roxburghii, Tribulus terrestris, Coriandrum sativum, Terminalia chedbula, Terminalia beleric, Emblica officinalis, Cyperus rotundus, Zingiber officinalis, black pepper, long pepper, Cinnamomum cassia, Cymbopogon muricatus, Hordeum vulgare, Abies webbiana, Cinnamomum ciners</i>
37	Ayu-Arthri-Tone	Y	Zinc bhasma , rasna, punarnava, haridra, gokshur, ashwagandha
38	Ayu-Hemoridi-Tone	Y	Sunthi churna (<i>Zingiber officinale</i> powder), mirichurna (<i>Piper nigrum</i> powder), puimpli churna (<i>Piper longum</i> powder), daruhalad chuma (<i>Berberis aristata</i>), nishottar chuma (<i>Operculina turpethum</i>), tankanlahi shubhra (sodium bicarbonate), sajjikshar churna (sodium carbonate powder), nagkeshar churna (<i>Mesua ferrea</i> powder), guggul shuddha (<i>Balsamodendron mukul</i>), kumari ghanasar (<i>Aloe indica</i>), suran kanda (<i>Tacca aspara</i>)
39	Ayu-Leuko-Tone	Y	Tin bhasma, zinc bhasma, iron bhasma, calcium bhasma , shubhra, askand churna (<i>Withania somnifera</i> powder), shatauari churna (<i>Asparagus racemosus</i>), bhuikohala churna, triphala churna, ashok sal bharad (<i>Saraka indica</i>), lodhrasal bharad (<i>Symplocos racemosa</i>)
40	Ayu-Nephro-Tone	Y	Tin bhasma, zinc bhasma , yavakshar (potassium carbonate), uda, gandha biroja (<i>Pinus roxburghii</i>), punarnava churna (<i>Boerhaavia diffusa</i> powder), gokharu churna (<i>Tribulus terrestris</i> powder), shuddha guggul (<i>Balsamodendron mukul</i>)
41	AyurRelief™		<i>Elipta alba, Trichosanthes dioca, Elettaria cardamomum, Tinospora cordifolia, Cassia augustifolia, Achyranthes aspera, Cuminum cyminum, Caryophyllus aromaticus, Piper longum, Glycyrrhiza glabra, Asparagus racemosus, Emblica officinalis, Zingiber officinale, Terminalia belerica, Terminalia chebula</i>
42	GlucoRite™		<i>Aegle marmelos, Eugenia jambolana, Curcuma loga, Tinospora cordifolia, Casearia esculenta, Pterocarpus longum</i>

^aProducts were classified as *Rasa Shastra* (RS) medicines by an Indian-trained Ayurvedic physician (Y for yes). *Rasa Shastra* medicines are elaborately prepared compounds combining herbs with metals, minerals, and gems. Possible source of minerals are bold, and latin plant names are italicized.

^bMineral pitch (also known as Shilajit or Shilajeet) is obtained by scraping off the dried exudate of the plant *Asphaltum punjabinum* from rock.

Data Quality Assurance and Quality Control

Quality control tests as part of a quality assurance program are reported here for total arsenic and lead contents using an *aqua regia* digestion method with inductively coupled plasma (ICP)-mass spectrometry (MS), or ICP-optical emission spectrometry (OES) analysis, and for bioaccessible concentrations and percent bioaccessibility, for all samples. Quality control tests in each batch of experiments conducted consisted of procedural blanks, a control material, and a procedural

duplicate. Blanks were less than 1 mg.kg^{-1} bioaccessible arsenic and lead for all tests (instrument detection limits were $1 \text{ }\mu\text{g.L}^{-1}$ for both arsenic and lead by ICP-MS, $50 \text{ }\mu\text{g.L}^{-1}$ for arsenic and lead in aqua regia method blank by ICP-OES, and $100 \text{ }\mu\text{g.L}^{-1}$ for arsenic and lead in bioaccessibility extraction blanks by ICP-OES). The results for the control material included with the bioaccessibility tests, SRM 2710 (NIST) were within control limits established within our laboratory for all arsenic tests, and for all lead tests except one. For the lead batch where the control result was slightly low, the results were accepted because the lead value for the control was just outside the control limits (see Table SI-2). The results for the MESS-3 sample included in the analysis of total arsenic and lead were within control limits established by an accredited laboratory and used in our laboratory. Duplicate results indicated that sample heterogeneity, both in the bioaccessibility test and the test for total lead and arsenic content, was likely still a factor that might affect the precision of results, in spite of the preliminary steps taken of homogenizing the material (rather than analyzing individual pills).

One sample, Mahayograj Guggulu (11), was analyzed multiple times because initial results indicated extremely low bias in the total *aqua regia* digestion results. The sample was digested with increasing ratios of *aqua regia*:sample (ICP-OES was used for this analysis), and when ratios greater than 250 were used, constant concentrations of around $50,000 \text{ mg.kg}^{-1}$ (5 % by weight) were obtained. Hence the QC results for total concentrations were reported for this sample separately; all results were in control. The bioaccessibility test was conducted three times for this sample and a relative standard deviation for this sample was reported; repeatability was considered to be acceptable.

Table SI-2

Summary of results obtained from quality control tests included with the analysis of samples.

Test	As		Pb
Bioaccessibility	Gastric	Gastric + Intestinal	Gastric
Blanks (mg.kg ⁻¹)	<1, <1, <1, <1, <1	<1, <1, <1, <1, <1	<1, <1, <1, <1, <1
Duplicates (% RPD)	all nd	all nd	0.5, 51, 11, 35, 11
Sample 11 triplicates (% RSD)	19	9	4
SRM 2710 (% bioaccessibility) control range	24-57	23-49	53-86
SRM 2710 (% bioaccessibility)	40, 35, 38, 39, 37	37, 23, 44, 34, 32	64, 63, 64, 61, 51
Aqua Regia – ICP-MS	As		Pb
Blanks (mg.kg ⁻¹)	<0.05, <0.2, <0.2, <1		<0.05, <0.2, <0.2, <1
Duplicates (% RPD)	69, 11, 2.5, 34		33, 0.6, 23, 112
MESS-3 (control range, mg.kg ⁻¹)	14-19		14-20
MESS-3 (mg.kg ⁻¹)	16, 18, 16, 16		23 , 15, 16, 16
Aqua Regia – ICP-OES (sample 11)	As		Pb
Blank (mg.kg ⁻¹)	<1		<1
Replicates, n=5 (% RSD)	7		3
MESS-3 (control range, mg.kg ⁻¹)	14-19		14-20
MESS-3 (mg.kg ⁻¹)	15		14

For bioaccessibility testing, 5 QC tests were included with the 43 samples. For *aqua regia* – ICP-MS analysis for total arsenic and lead content, 4 QC tests were included with the 43 samples. Only gastric lead results were considered relevant. nd = not detectable, n/a = not applicable. RPD = relative percent difference ($[\text{difference between values}]/[\text{mean of values}] \times 100\%$); RSD = relative standard deviation ($[\text{standard deviation of 3 or more values}]/[\text{mean of values}] \times 100\%$).