ABC AHP NCNPR
Botanical Adulterants Prevention Program
BOTANICAL ADULTERANTS PREVENTION BULLETIN

Adulteration of Milk Thistle (*Silybum marianum*)

By Allison McCutcheon, PhD

American Botanical Council, Austin, TX 78723, USA

Correspondence: email

Citation (JAMA style): McCutcheon A. Adulteration of milk thistle (*Silybum marianum*). Botanical Adulterants Prevention Bulletin. Austin, TX: ABC-AHP-NCNPR Botanical Adulterants Prevention Program; 2020.

Keywords: Adulterant, adulteration, milk thistle, *Silybum marianum*, silymarin, silybin, silychristin, silydianin

Milk Thistle *Silybum marianum*
Photo ©2020 Steven Foster

Goal: The goal of this bulletin is to provide timely information on issues of adulteration of milk thistle (*Silybum marianum*, Asteraceae) fruit and its extracts to the international herbal industry and extended natural products community in general, by presenting data on the type and occurrence of adulteration, the market situation, and consequences for the consumer and the industry.

1. General Information

1.1 Common name: Milk thistle[1]

1.2 Other common names:

English:[1-3]	Mary's thistle, blessed milk thistle, spotted milk thistle,[2] bull thistle, gundagai thistle, holy thistle, lady's thistle, variegated artichoke, variegated milk thistle,[3] St. Mary's thistle
Arabic:[3,4]	shawk el-gamal (also written as shuk aljamal) (شوك الجمل), shuk al-halib (شوك الحليب), alsalabayn almurimi (السلبين المريمي)
Armenian:[5]	kat' ughtap'ush
Chinese:[5]	*nai ji* (奶蓟), shui fei ji (水飛薊)
Danish:[5,6]	marietidsel, mælk tidsel
Dutch:[6]	mariadistel
Finnish:[6]	maarianoh dake, hedelmä
French:[3,7,8]	chardon argenté, chardon Marie, lait de Notre Dame, silybe de Marie, chardon Notre Dame, épine blanche
German:[3,8]	Mariendistel, Frauendistel
Greek:	gaïdouránkatho gála (γαϊδουράγκαθο γάλα)
Hebrew:[5]	dilan mazui
Hindi:[5]	dugdh rom
Italian:[6,7]	cardo mariano, cardo di Maria
Japanese:[5]	ooazami (オオアザミ), mariaazami (マリアアザミ)
Norwegian:[6]	marietistel
Pharmacopoeial:[8]	fructus silybi Mariae, fructus cardui Mariae
Polish:[6]	ostropest plamisty
Portuguese:[3]	cardo-leiteiro
Russian:[3]	ostro-pestro (остро-пестро), rastoropša pyatnistaja (расторопша пятнистая)
Spanish:[3,7]	cardo de María, cardo lechero, cardo mariano, cardo asnal, cardo blanco, cardo santo, poma
Swedish:[3]	mariatistel

1.3 Accepted Latin binomial: *Silybum marianum* (L.) Gaertn.

1.4 Synonyms: *Carduus marianus* L., *Carthamus maculatus* (Scop.) Lam., *Cirsium maculatum* Scop., *Mariana mariana* (L.) Hill.,[8] *Silybum maculatum* (Scop.) Moench., *Silybum mariae* (Crantz) Grey, *Silybum pygmaeum* Cass.[9]

The synonyms *S. marianum* var. *marianum* and *S. marianum* var. *albiflorum* have been used to differentiate respectively, the commercial, purple-flowered variety from the white-flowered variety of milk thistle.

1.5 Botanical family: Asteraceae

1.6 Distribution range: Indigenous to North Africa, Asia Minor, southern Europe and southern Russian Federation; naturalized in North and South America, Australia, China, and Central Europe.[3,8]

1.7 Plant part: The plant part used is the dried mature fruit devoid of the pappus. Characteristic of the Asteraceae family, the one-ovule, achene-like fruit is called a cypsela (pl. cypselae). However, in common parlance (and in many scientific articles) the fruit is often referred to as a seed. To facilitate communication and maintain continuity with the literature, the generic terms fruit or seed are used in this document. The fruit is comprised of four parts: pericarp, integument, albumen (storage protein), and embryo (two large cotyledons with fat as storage material). Collectively, the albumen and embryo are described as the kernel, while the pericarp and integument may be called the seed coat or hull.[10-12]

1.8 Key constituents and markers: Silymarin, the main pharmacologically active fraction of milk thistle,[13-15] comprises 1.5–3.5% of the fruit by dry weight and 30–65% by high-performance liquid chromatography with ultraviolet detection (HPLC-UV),[16] or 65–80% by UV/Vis of the extract.[16-20] Silymarin is a complex mixture of 3-hydroxyflavonolignans (Figure 1), of which the primary components are silybin (syn. silibin, silibinin), silychristin (syn. silichristin), silydianin (syn. silidianin), isosilybin (syn. isosilibin), and the flavanonol taxifolin (syn. dihydroquercetin).[21-24] Silybin, isosilybin, and silychristin are each present as a pair of diastereoisomers. The seven flavonolignans and taxifolin are the primary compounds used to assess milk thistle quality. Silymarin is mainly found in the seed coat.[25,26]

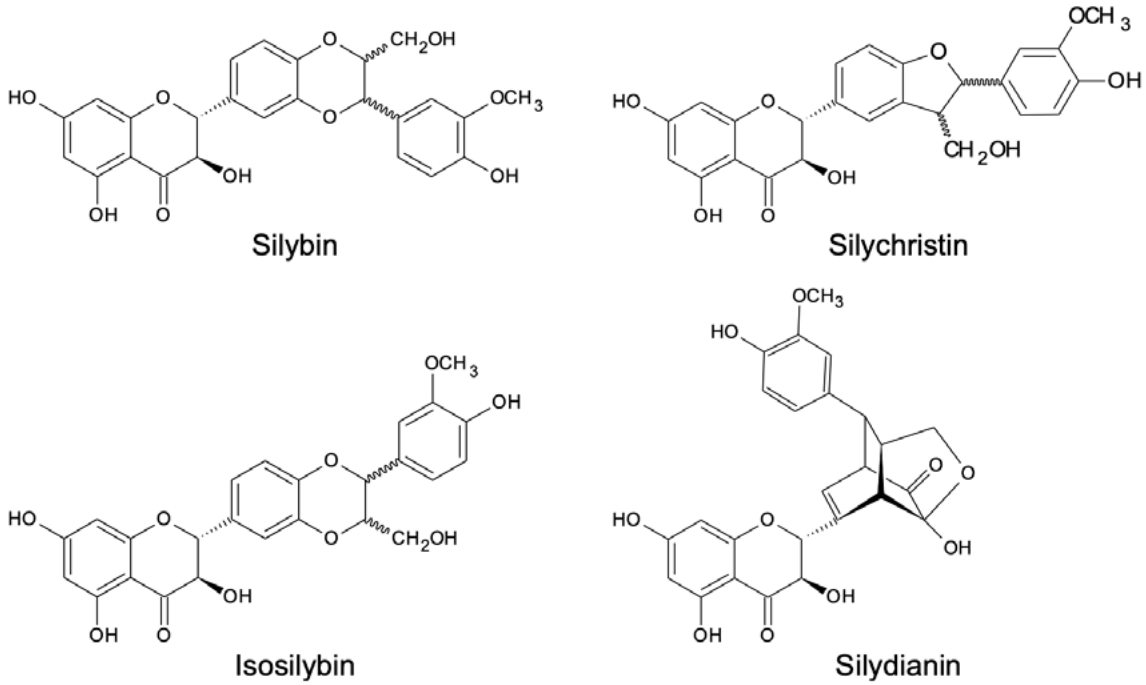
The most abundant component of silymarin, silybin (the mixture of silybins A and B), was the first constituent that was identified,[23,24] and it has been reported as the constituent with the highest bioactivity.[5,27,28] Additional minor flavonolignans have been identified, which together with a mixture of unknown flavonolignan oligomers and polymers constitute the remaining 20–35% of the silymarin fraction.[5,28]

The foregoing data pertain to purple-flowered milk thistle. In addition to the main silymarin constituents, a white-flowered milk thistle variety also contains the 3-deoxyflavonolignans silandrin, isosilandrin, silymonin, silyhermin, and neosilyhermin.[29-31]

The fruits also have a relatively high content of fatty oils (20–25%) that consist of fatty acids such as linoleic acid (60–66%), oleic acid (21%), palmitic acid (13%), phospholipids and vitamin E.[32-34]

1.9 General uses and forms: The principal medicinal product is the fruit extract standardized to contain a mini-

Figure 1: Principal flavonolignans in milk thistle fruit



mum of 45% silymarin by HPLC.[16] The European Pharmacopoeia specifies ethyl acetate, acetone, ethanol, methanol, and mixtures of water with acetone, ethanol, and/or methanol as appropriate solvents for extraction.[16] Standardized milk thistle extract has been used for supportive treatment of acute or chronic hepatitis and cirrhosis induced by alcohol, drugs, or toxins, treatment of dyspeptic complaints, and gallstones.[8,35] The dried extract is most commonly sold in the form of capsules or tablets. Traditional aqueous alcohol tinctures and extracts are also marketed but lack scientific data to support healthy liver claims. Silybin isolated from the silymarin extract is sold as a pharmaceutical treatment for toxic liver injury and hepatitis. The disodium salt of silybin C-2,3-dihydrogen succinate is approved for intravenous infusion in cases of acute death cap mushroom (*Amanita phalloides*, Amanitaceae) poisoning in some European countries, and approved for conditional use (physicians need to submit an application to the National Institutes of Health to gain access to it) for the same indication in the United States.[36]

The fruits (whole, pulverized, and powdered) are variously sold in bulk, tea bags, capsules, or tablets. A separate ingredient, the cold-pressed fruit oil, is sold in bulk as an edible oil and in capsules and soft gels as a dietary supplement.[37] The seed cake, flour, and aerial plant parts may be sold as food or fodder supplements.[5,37]

In addition to the medicinal uses, the photoprotective silymarin extract is also used as cosmeceutical ingredient.[37]

1.10 Production and processing: Knowledge of milk thistle production and processing is essential to understanding milk thistle adulteration. Milk thistle is cultivated as an annual commercial crop. Two stable chemotypes have been described: chemotype A with a high content of both silychristin and silybin and chemotype B with a high silydianin content.[38] A number of cultivars with a high silymarin content and other desirable agronomic characteristics have been developed, including Argintiu, Budakalaszi, Khoreslo, Babak Castle, Mirel, Silma, Silyb, and Szibilla.[39] In addition to genetic variables, silymarin content of the fruit can vary significantly (0.21–17.98% of the dry weight) depending on extrinsic factors such as geographical region, climate, growing, and harvesting conditions,[40-43] and production and processing methods.[5,37,44]

Primary processing involves pulverization, milling, or nano-grinding, followed by maceration or percolation of the fruit powder;[5,37,44] the particle size has been shown to affect silymarin extraction efficiency.[45] Silymarin is most commonly extracted from the whole pulverized fruits or from the seed cake (the residue which remains after the oil has been cold pressed).[46] However, silymarin may also be extracted from the other milk thistle plant parts as follows: roots (0.05%),[41] leaves (0.92%),[47] flowers (0.66 %), and fruit heads (10.67%).[41] There is a developing market for these by-products of milk thistle processing as food or fodder supplements, and for biodiesel production.[5,37]

Numerous methods for extracting silymarin have been described/patented.[38,40,41,45,47-60] Most of these methods are processes involving defatting, followed by silymarin extraction. After silymarin extraction, an additional defatting step using liquid-liquid extraction is often performed to further reduce the oil content.[61]

Defatting may be accomplished by cold pressing the oil from the fruit or solvent extraction. Cold pressing removes 60–65% of the oil. The cold pressed oil contains ~0.8–1.0 % silymarin and after further processing, it may be sold as an edible oil, dietary supplement, or biodiesel.[5,62] Another common process to remove the milk thistle seed oil is the extraction with hexane or petroleum ether, which may be followed by secondary defatting with di-isopropyl ether. On the industrial scale, defatting using solvents is associated with longer processing times, consumes relatively large amounts of organic solvents, and carries the risk of excessive residual solvent presence in the silymarin extract.[63] Alternative methods that preclude the need for defatting include hot water extraction, pressurized liquid extraction (PLE) with either acetone, ethyl acetate, or methanol, and mechanical separation. Hot water extraction offers a green alternative with low costs for solvent purchase and disposal; however, the high temperatures may degrade the silymarin.[58,59] PLE or accelerated solvent extraction (ASE) utilizes increased pressure to optimize extraction conditions by keeping the liquid solvent below its boiling point at higher temperatures, and thus increasing extraction efficiency and reducing costs for solvent purchase and disposal.[56] AbouZid et al. described a mechanical separation of the silymarin-bearing seed coat from the oil- and protein-rich kernel, which permits the extraction of the seed coat with an appropriate solvent to produce a silymarin extract with a high silymarin content.[63]

The European Pharmacopoeia (Ph. Eur.) specifies that silymarin extract is produced from the defatted fruit, using one or more of the following solvents: ethyl acetate; acetone or mixture of acetone and water; ethanol or mixture of ethanol and water; and methanol or mixture of methanol and water.[16] Several studies suggest that acetone extraction may be the most cost-effective and result in the least toxic solvent residues.[50,56,61] Isolated silymarin is an important ingredient for the herbal drug (pharmaceutical) market and the silybin-depleted marc which still contains some milk thistle flavonolignans reportedly is sometimes offered for sale as silymarin extract (or simply as milk thistle seed) to the dietary supplement industry.[64-66] The sale of products with little or no flavonolignans labeled as milk thistle seed, silymarin, or milk thistle extract is misleading and considered fraudulent. Most all scientific literature demonstrating the benefits of milk thistle fruit extract are based on products characterized to contain 30–65% (by HPLC) of the silymarin complex.[67]

By-products of milk thistle extraction, such as the seed flour (containing ~22% protein, 7% oil, and some residual silymarin), may be sold as a food and fodder supplement.[37] However, data on therapeutic benefits of milk thistle seed flour are lacking.

1.11 Other sources of silymarin/flavonolignans: The fruits of the related species, *S. eburneum*, have been reported to contain ~10% silymarin.[68] While there are

Table 1: Sales data for milk thistle dietary supplements in the United States from 2016-2019.[74-77]

Channel	2016		2017		2018		2019	
	Rank	Sales [US$]	Rank	Sales [US$]	Rank	Sales [US$]	Rank	Sales [US$]
Natural	6	9,968,995	7	9,960,892	8	10,419,926	10	10,010,699
Mainstream Multi-Outlet	16	17,077,481	17	16,799,553	20	16,596,226	23	16,244,188

limited data on the flavonolignan composition of this species, *S. eburneum* can be distinguished from milk thistle by the presence of high concentrations of isosilychristin.[69] Flavonolignans that differ in their chemical structure form silymarin compounds have been identified in members of the plant families Asteraceae, Berberidaceae, Chenopodiaceae, Flacourtiaceae, Fabaceae, Poaceae, and Scrophulariaceae.[22,28,70,71]

Silybin has been isolated from the dried whole plant of *Gentiana apiata* (Gentianaceae) collected in Shaanxi Province, China,[72] and from the fungal endophyte, *Aspergillus iizukae* (Trichocomaceae), isolated from the surface-sterilized leaves of milk thistle.[73]

2. Market

2.1 Importance in the trade: In 2018, milk thistle held the eighth position among the top-forty best selling herbal dietary supplements in the US natural channel with sales of $10,419,926, an increase of 3.5% compared to 2017.[74] However, retail sales fell slightly in 2019 to the tenth position in this channel to $10,010,699, a drop of 4.7% from 2018. Milk thistle dietary supplements ranked 23rd in the mainstream market retail channel in 2019, selling a measured total of $16,244,188.[75]

2.2 Market dynamics: Over the past three decades, sales have remained strong, with milk thistle consistently ranking among the forty top-selling herbal dietary supplements in the United States, particularly in the natural sales channel. However, when comparing the annual sales data from 2016–2019, the market has remained flat (Table 1). Both the type of milk thistle label claims and the content declared has broadened. Silymarin content claims range from 30–85% based on spectrophotometric ("UV/Vis") or high-performance liquid chromatography (HPLC) assessments. There is potential confusion with respect to levels of chemical standardization for silymarin and/or its constituents as different analytical methods produce lower or higher readings of constituents, respectively. Alternatively, some manufacturers specify silybin content (35–45%), some report flavonolignan content, and others do not make any marker content claims.

The scientific research on milk thistle over the past three decades also has had significant impacts on market dynamics, including advances in plant breeding and agricultural research which have led to the development of silymarin-enriched varietals and improved agronomic techniques to increase the silymarin content of milk thistle fruit; processing and extraction optimization (especially the differential extraction of only the seed coat and the resultant increases in silymarin yield and reduction in financial and environmental costs); and research and development of markets for milk thistle by-products.[5,37] The adaptation of these advances has allowed manufacturers to produce milk thistle extracts with higher silymarin contents at lower cost, and access new revenue streams by marketing milk thistle by-products.

Costs for bulk milk thistle extracts vary depending on the origin of the material. Authentic European milk thistle extract is sold as an ingredient in bulk quantities for a wholesale price of US $130-150/kg. (C. Bewicke [Ethical Naturals] email to S. Gafner, August 11, 2020; G. Ris [Indena] email to S. Gafner, August 14, 2020) Authentic milk thistle extracts from outside Europe complying with United States Pharmacopeia (USP) specifications for powdered milk thistle extract[20] are sold at the wholesale level for US $90-110 (Cal Bewicke email to S. Gafner, August 11, 2020). An informal review by S. Gafner of bulk prices for ingredients sold as containing milk thistle extracts labeled to contain 80% silymarin on retail and e-commerce company Alibaba (Hangzhou, China) showed costs being generally in the range of US $30-35/kg, although some suppliers claim to offer such extracts for as little as US $1/kg.

2.3 Supply sources: Teuscher et al. stated that milk thistle comes "from cultivated plants grown to a limited extent in northern Germany but mainly imported from Argentina, Austria, China, Hungary, Poland, Romania, and Venezuela."[35] Vereš and Týr reported that milk thistle is grown commercially as a medicinal plant in Europe, Egypt, China, and Argentina.[78]

Some manufacturers purchase milk thistle extracts rather than raw milk thistle fruit. As outlined in section 1.11, extraction methods significantly impact silymarin composition and content, as well as the cost of production. In addition to pharmacopeial 30–70% silymarin extracts of milk thistle fruit, milk thistle by-products are also known to be sold as "milk thistle extract." Products that do not contain the concentration and composition of silymarin as supported by the scientific literature may not deliver the liver-supportive benefits documented in the pharmacological and clinical research literature and expected by consumers and/or health professionals. Additionally, as noted, extracts prepared from by-products claiming to provide "silymarin" and not meeting the standard definition as established by pharmacopeial monographs and the scientific literature are considered to be adulterated.

There have also been significant shifts in the geographical origin of the commercial supply, and the analytical meth-

ods used to assess silymarin content. In the 1990s and early 2000s, Europe was the primary supplier of raw milk thistle and the analysis of silymarin content was largely based on spectrophotometry. Since then, the market has diversified with Asia now providing the majority of the international supply, and analytical methods have advanced considerably. However, concerns have been expressed that companies which market milk thistle products with label claims based only on spectrophotometric analysis may accidentally or intentionally be selling products that are silybin-depleted or contain <30% silymarin.[65,79]

3. Pharmacopeial Standards

The World Health Organization (WHO) and the USP specify the dried mature fruit contains not less than 1.5% silymarin, calculated as silybin.[8,18] The USP defines milk thistle extract as containing 90–110% of the claimed amount of silymarin, calculated as silybin, consisting of the sum of silychristin and silydianin (20–45%), silybin A and B (40–60%), and isosilybin A and B (10–20%).[20] The Ph. Eur. defines the dried standardized extract as containing "30–65% silymarin, expressed as silibinin, and corresponding to the sum of silychristin and silydianin (20–45% of silymarin), silybin A and B (40–60% of silymarin), and isosilybin A and B (10–20% of silymarin)."[16]

4. Adulteration

4.1 Known adulterants: Depleted *Silybum marianum* extracts, *Silybum eburneum*, and synthetic colorants. Globe thistle (*Echinops echinatus*, Asteraceae) reportedly has been confused with milk thistle in Pakistan due to the use of the same vernacular name "untkatara" for both milk and globe thistle in Hindi and Urdu,[80,81] but this appears to be a geographically limited issue.

4.2 Sources of information confirming adulteration

4.2.1 Chemical evidence: According to Langhammer,[82] it is difficult to discriminate between the species *S. marianum* and *S. eburneum* based on morphological characteristics and hence, wildcrafted material may contain the fruits of *S. eburneum*.

Numerous studies have found that commercial milk thistle products do not meet their label claims for silymarin content or pharmacopeial specifications for silymarin content.

Frommenwiler analyzed 31 milk thistle products which were acquired from the internet, local shops, and pharmacies in the United Kingdom (UK), by high-performance thin-layer chromatography (HPTLC).[83] Among these products, 10 had a Traditional Herbal Registration while the remaining 21 products were sold as food supplements. The 10 THR products, and seven food supplements were deemed to be of good quality; seven additional food supplements were consistent with the milk thistle fingerprint, except that these products were missing the zone for taxifolin. However, four food supplements contained a weak chromatographic fingerprint, and two did not show any of the characteristic milk thistle zones.

Fenclova et al. used a validated ultrahigh-performance liquid chromatography mass spectrometry (UHPLC-MS) method to assess the quality of 26 milk thistle supplements sold as capsules in the United States (n = 19) and Czech markets (n = 7). Of the 24 products with silymarin label claims, total silymarin (TS) content ranged from 35–125% of the declared content, with four products containing <50% of the declared content and four containing more than the declared content. The sum of flavonoid/flavonolignan content ranged from 5–393 mg/g of capsule content, the maximum recommended daily intake (RDI*, based on product label recommendations) ranged from 18–600 mg/day, and the sum of silybin A and B ranged from 36–66% of total silymarin (i.e., all of the products contained silybin).[84]

Fibigr et al. also used a validated UHPLC-UV method to evaluate the silymarin content of four teas and seven supplements sold in the Czech Republic. The total silymarin content of the teas ranged from 3.4 mg/g to 7.1 mg/g of tea. The supplements contained 39.5–77.6% of the claimed silymarin content; the two registered pharmaceutical preparations had the highest content (72.9% and 77.6% of the label claims). The seven supplement samples contained 42.9–59.4% silybin, calculated as the percentage of total silymarin.[85] While some of the food supplements had much lower than declared silymarin content, this may be partly due to different standardization methods rather than using spent milk thistle fruit.

Pendry et al. evaluated the silymarin content in 11 milk thistle tinctures purchased in the United UK using HPLC-UV. The tinctures varied in their extraction ratios (4.54–18.37 mg/mL w/v) and the percentage of alcohol (25–75%). Silymarin could not be detected in the seven samples extracted with 25% ethanol (regardless of the extraction ratio which ranged from 1:5 to 1:1), possibly due to the instability of flavonolignans in low alcohol tinctures.[86] Of the four samples with detectable amounts of silymarin, only one sample with an extraction ratio of 1:1 and an alcohol content of 70% provided an effective therapeutic dose and one sample did not exhibit the characteristic silymarin fingerprint, suggesting the sample was derived from an adulterant.[87]

Scientists at the University College of London (UCL) evaluated seven milk thistle products sold on the European market for a television show by the British Broadcasting Corporation (BBC) using high-performance thin-layer chromatography (HPTLC); six of the products were legally sold in the EU as food supplements with no silymarin label claims and one was a registered traditional herbal medicine (THM). Two of the supplements (36%) did not contain

* The RDIs for milk thistle issued by the European Medicines Agency[6] varies depending on the extraction solvent and the drug-extract ratio. However, the lowest RDI is generally above 200 mg. For comparison, the RDI for the most extensively clinically tested milk thistle product, Legalon® (Meda Pharma AB, Solna, Sweden) is standardized to 420 mg silymarin per day.

any milk thistle; one of these produced a faint fingerprint suggesting the presence of an unidentified synthetic adulterant. Another two supplements contained only low levels of milk thistle. Three products (one traditional herbal remedy and two food supplements) produced fingerprints comparable to the standard.[88] Further testing by UCL researchers showed that of 18 commercial supplements, products sold under a traditional herbal registration (THR) were of good quality, while 22% of food supplements were of poor quality with little or no detectable milk thistle.[89]

Eight of the 12 companies ConsumerLabs tested failed testing for silymarin content in commercial milk thistle products. Among milk thistle extract supplements claiming to provide 80% silymarin, amounts in five products actually ranged between 51.7–60.4% silymarin. Three other milk thistle supplements which were tested did not list their amount of silymarin but failed to meet minimum standards.[90] Some of the discrepancies observed are likely due to milk thistle supplement manufacturers using spectrophotometric methods for standardization, which, as noted above, produces a higher value for silymarin. ConsumerLab used a HPLC-UV method for the product analysis, which produces a reading of lower numerical value, and thus, perceptually of lesser chemical concentration.

Stranska and Hajslova used UHPLC-high resolution mass spectroscopy (HRMS) analysis to assess the silymarin content of milk thistle products sold in the EU. They determined that three out of seven milk thistle powdered extract products contained ≤ 50% of the declared silymarin content. Of the six milk thistle oil products assessed, three contained no detectable silymarin and three contained less than 15 mg/capsule; two of the milk thistle oils contained less than 30 mg/kg silymarin. Of the 10 milk thistle teas analyzed, five contained <30% silymarin and only two contained >60% silymarin.[91]

Anthony and Saleh assessed 45 commercial milk thistle products from the United States (24) and international markets (21) purchased in Cairo, Egypt, using HPLC-MS and HPTLC. Silymarin label claims ranged from 51.8 to 1358.0 mg/tablet. One product from the United States and three international products did not contain any silymarin, and 23 contained <30% silymarin (11 from the United States and 12 international). Four products from the United States and 17 international products showed non-silymarin bands at retention factors (Rfs) above silybin. Two US products stood out because of their relatively high concentration of silybin, representing 78% and 93% of the total silymarin, well above the 40–60% specified by the European and US pharmacopeias. It is not clear how such silybin-enriched extracts are obtained, but it could point to the addition of silybin made by chemical synthesis (a process frequently referred to as spiking), which was published as early as 2000.[92] Another explanation is the use of an extraction process that selectively enriches silybin.[93,94] Eight samples contained <100 mg/g, nine samples contained 100–200 mg/g, six contained 200–300mg/g, eight contained 300–400 mg/g, five samples contained 400–500 mg/g, and five samples contained >500 mg/g.[95]

Lee et al. evaluated the content of silychristin, silydianin, silybin A, silybin B, isosilybin A, and isosilybin B in seven commercial milk thistle extracts purchased in Philadelphia, PA using HPLC-MS. Six of the products were manufactured in the United States and one was manufactured in China.[96] Silydianin was the least abundant constituent, and the content of this constituent did not vary significantly among the samples. There was considerable heterogeneity among the samples in the content of silychristin, silybin A, silybin B, isosilybin A, isosilybin B, and total silymarin. Of the six US products, one had substantially higher levels of total silymarin, and two products had significantly lower contents of total silymarin, while the remaining three were intermediate and very similar in content. The Chinese extract had the lowest contents of total silymarin. Since the authors did not divulge the exact sample preparation procedure, only a relative comparison of silymarin contents among the products can be made. The relative content in silybin (39–46% of total flavonolignans) in the six US samples was relatively consistent, while the relative silybin content in the sample from China (33.8%) was a bit lower.[96]

Kvasnicka et al. used HPLC-UV to analyze the silymarin content of six commercial silymarin extracts purchased in the EU. They reported that the total silymarin content of the silymarin extracts ranged from 17.21–63.96%; among three different batches of the same product, the content ranged from 41.6–63.96%. In all of the samples, silybin made up between 40–60% of the total flavonolignans.[97]

Three additional publications detailed results from quantitative analysis of commercial milk thistle products using HPLC-UV.[98-100] In two publications, all of the analyzed products contained significantly less silymarin than the amount claimed on the label; however, the silybin content was between 40–60% of total flavonolignans.[98,99] In the third paper, Schulz reported that the nine commercial milk thistle products tested contained 58–116% of the silymarin label claim, when measured by HPLC-UV.[100] These findings can be attributed in most cases to the manufacturers' use of a spectrophotometric method for determining silymarin content (per section 4.7 below).

A consistent theme of all these published data on milk thistle quality is the market presence of products with lower than declared contents in silymarin. In addition, four papers provided evidence of milk thistle adulteration based on the absence of any of the characteristic silymarin signals, or additional peaks/zones from undeclared adulterants.[87,88,95,101] The identity of these adulterants was not determined in any of the four investigations.

Before the advent of official HPLC-UV methods for the flavonolignan quantification, most manufacturers relied on spectrophotometric methods. Some manufacturers use a direct UV-spectrophotometric method (absorbance at 288 nm; without derivatization) for quantitative estimation of silymarin in the extracts using silybin as the reference standard, while others use a formerly official method which calculates the total flavonolignan content based on the absorption of a milk thistle extract solution after a reaction with 2,4-dinitrophenylhydrazine in alkaline conditions at 490 nm,[102] or variations thereof. Many suppliers still use

spectrophotometric methods for standardization.[66] Fraudsters have reportedly taken advantage of the lack of specificity of UV/Vis tests by adding undisclosed colorants to milk thistle extracts in order to provide an appearance of compliance with standardization requirements.[103]

4.2.2 Other evidence of adulteration: There is ample evidence (see section 4.2.1) of commercial products containing lower amounts of silymarin. While such data do not provide direct evidence for milk thistle adulteration, as it is possible that a manufacturer uses low-quality starting material, leading to an extract with low amounts of flavonolignans, there is consensus from industry experts that these low-quality products are most often due to intentional silymarin depletion of milk thistle fruit, constituting the fraudulent sale of a low-quality product to unsuspecting buyers.[64-66,104]

4.3 Accidental or intentional adulteration: The sale of products that do not meet their silymarin label claims could be attributed to the use of the spectrophotometric method to determine silymarin content; however, the sale of products that do not contain any detectable silymarin (and in some cases, contain foreign substances) cannot be accidental unless the manufacturer(s) did not have adequate quality assurance programs. The sale of depleted milk thistle fruit extracts is done on purpose, since such extracts can be sold at a lower price and thus provide a competitive advantage to fraudulent suppliers and manufacturers.[64-66] Regarding other forms of adulteration, as most of the commercial milk thistle supply is produced under cultivation, the risk of accidental adulteration is very low; however, the misidentification of other thistle species may occur when milk thistle fruits are collected in the wild.[80]

4.4 Frequency of occurrence: The milk thistle monograph in Wichtl (2016) stated that adulteration "practically never occurs," and in the milk thistle fruit supply chain that may largely remain true.[35] However, as summarized in section 4.2 the evidence accumulated since 1994 suggests that 30–50% of commercial products that are labeled to contain milk thistle *extract* do not meet their label claims for silymarin content. The occurrence of products that did not contain silymarin ranged from 0–28.6% and the incidence of products with evidence of an adulterant ranged from 0–46%, demonstrating that the trade of substandard extracts as an ingredient is extensive.

4.5 Possible safety/therapeutic issues: While the sale of milk thistle products with sub-therapeutic levels of silymarin pose no immediate safety concerns, the therapeutic impacts are substantial, considering that consumers who use

Milk Thistle *Silybum marianum*
Photo ©2020 Steven Foster

milk thistle may have serious liver disease or liver dysfunction and may not obtain the benefits expected. Additionally, the presence of unidentified adulterants may potentially pose safety issues. As Spink and Moyer point out, the act of adulteration, although carried out with economic or financial motivation, can have an effect that can lead unintentionally to a public health concern.[105]

4.6 Analytical methods to detect adulteration: For whole fruit, botanical identity may be confirmed by a qualified analyst based on organoleptic and macroanatomical characteristics. The identity of powdered material may be determined using a combination of organoleptic, microscopic, and chemical techniques. Both the Ph. Eur. and USP specify HPTLC methods for the identification of milk thistle fruits,[18,19] powdered milk thistle fruit,[106] and powdered extracts.[16,20] Langhammer reported a simple chemical test with sulfuric acid to differentiate *S. marianum* and *S. eburneum*.[82] but the sensitivity to identify mixed batches may be lacking.

There are numerous chemical techniques that can be used to authenticate milk thistle extracts, including TLC,[107] HPTLC,[20] HPLC-UV,[44,85,86,108] HPLC-MS,[84,86,96,109,110] mid- and near-infrared spectroscopy (MIR and NIR),[111,112] nuclear magnetic resonance (NMR),[108] and various combinations of these methods. Quantitation of silymarin in a given extract is complicated by the fact that part of what is considered to be silymarin is a mixture of flavonolignans. Though they differ slightly, the Ph. Eur. and USP provide official HPLC-UV methods for authentication of milk thistle extracts and quantification of silymarin content. Silymarin depletion and silybin spiking can be determined by measuring the individual flavonolignan concentrations, e.g., using HPLC-UV. However, other forms of adulteration such as the presence of dyes may not be detected unless the methods have been specifically developed to do so.[113,114] Genetic approaches, such as random amplified polymorphic DNA (RAPD) analysis,[115-117] have focused mainly on the distinction among milk thistle genotypes, but such methods could be used as well to differentiate milk thistle and closely related species. Since the same DNA is found in all parts of a specific plant species, DNA-based identification methods cannot distinguish between genuine and depleted milk thistle extracts.

4.7 Perspectives: A number of hypotheses have been put forth to explain the frequent occurrence of milk thistle products containing <30% silymarin, including silybin depletion, the use of spectrophotometry to quantify silymarin content, milk thistle by-products, dyes, and excipients. Silybin depletion is a reasonable explanation, because silybin is easily isolated from silymarin extract by precipitation, isolated silybin has a much higher market value than silymarin, and the silybin-depleted marc might be sold inappropriately as silymarin extract. However, none of the studies described in section 4.2.1 reported evidence supporting this hypothesis (i.e., all of the products that contained silymarin also contained silybin in appropriate proportions to the other marker constituents). Another explanation is the sale of silymarin as a whole to the pharmaceutical industry, while the leftover, post-extract milk thistle fruit mass (marc) might be re-extracted and sold to the dietary supplement and cosmetic industries.

When the label in commercial products claims 80% silymarin, it is indicative that the number is based on analysis by UV/Vis spectrophotometry and not HPLC-UV. The high incidence of milk thistle products containing <30% silymarin reported in the published literature has also been attributed to the use of spectrophotometry to analyze silymarin content. The 2,4-dinitrophenylhydrazine method assesses the content of all ketones/flavones in milk thistle samples, not only the flavonolignans that constitute the silymarin complex. Hence, this technique typically overestimates silymarin content.[65,79,118] Direct analysis at 288 nm usually provides even higher "silymarin" contents as many compounds absorb at this wavelength and thus interfere with the assay. In addition, spectrophotometric analysis may not detect other forms of adulteration, such as the presence of milk thistle by-products, other herbs with high flavone/flavonolignan content, or dyes. For these reasons, HPLC-UV analysis superseded spectrophotometry for the authentication of milk thistle extracts.

The substitution/addition of non-functional plant parts and the deliberate mislabeling of these products is one of the oldest forms of adulteration.[113] The entry of milk thistle by-products into the commercial supply chain, together with the increase in the complexity of supply networks, has created an environment where these inexpensive, "near-identical" milk thistle materials that may pass both spectrophotometric and cursory chromatographic analyses can effectively be sold as " milk thistle extracts."[119] The presence of milk thistle by-products in commercial milk thistle preparations may also account for the appearance of HPTLC bands that do not occur with authentic fruit extracts.

The increasing incidence of unauthorized colorants/dyes in herbs and spices has been ranked as a serious hazard.[120] In the absence of analytical methods specifically designed for their detection, this form of adulteration may go undetected,[101,114,121-123] especially when combined with milk thistle by-products.

A largely unexplored explanation of finding such a large array of substandard products is the presence of large amounts of excipients in finished milk thistle products. Silymarin has poor oral bioavailability and a number of novel formulations specifically designed to enhance bioavailability have been developed.[124-135] However, the impact of these excipients/processes on the efficiency of silymarin extraction in the gastrointestinal tract has not been investigated.

Considering the issue from a geographical perspective, China has become a leading supplier of milk thistle and several authors have reported that "Chinese" milk thistle extracts contain <30% silymarin. However, this constitutes weak evidence when the low number of samples that have been analyzed (<10) are considered in relation to the high

volume of milk thistle that China produces. More rigorous research with representative sampling is needed before this postulate can be given credence.

Unfortunately, most of the investigators reporting the occurrence of milk thistle products containing <30% silymarin did not conduct in-depth analyses of the actual chemical composition of the suspect products. In the absence of further evidence, it is unclear which of these hypotheses may be correct; however, considering the current domination of HPLC analysis in the industry, it strains credibility to accept that the recently reported deficiencies may be blamed on spectrophotometric overestimation of silymarin content.

Further, none of these postulates can explain the alarmingly high incidence of milk thistle products that do not contain any silymarin.

5. Conclusions

Regardless of the underlying cause, the relatively high incidence of materials labeled as milk thistle that contain little or no silymarin is clearly an ongoing problem, a problem that is legally defined within some countries as food fraud.[104,136,137] Lack of adequate therapeutic benefit in patients believing that they can utilize milk thistle for serious liver disease, and loss of consumer confidence due to products that do not meet their label claims are serious concerns. The sale of products labeled as containing milk thistle but lacking the claimed ingredient and containing unidentified adulterants instead potentially may also pose a health concern. However, it is a risk which may be prevented with appropriate supply chain qualification and adequate quality control, and quality assurance protocols.

6. References

1. McGuffin M, Kartesz JT, Leung AY, Tucker AO. *American Herbal Products Association's Herbs of Commerce.* 2nd ed. Silver Spring, MD: American Herbal Products Association; 2000.
2. *Silybum marianum* (L.) Gaertn. Integrated Taxonomic Information System (ITIS) online database; 2020. https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=38413#null. Accessed June 5, 2020.
3. *Silybum marianum* (L.) Gaertn. USDA, Agricultural Research Service, National Plant Germplasm System. Germplasm Resources Information Network (GRIN-Taxonomy); 2020. https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?id=33952. Accessed June 5, 2020.
4. يميريم نيبلس. Wikimedia Foundation, Inc. https://ar.wikipedia.org/wiki/%D8%B3%D9%84%D8%A8%D9%8A%D9%86_%D9%85%D8%B1%D9%8A%D9%85%D9%8A. Accessed July 16, 2020.
5. Chambers CS, Holečková V, Petrásková L, et al. The silymarin composition… and why does it matter??? *Food Res Int.* 2017;100:339-353.
6. European Union herbal monograph on *Silybum marianum* (L.) Gaertn., fructus. In: London UK, ed. European Medicines Agency Committee on Herbal Medicinal Products (HMPC): European Medicines Agency; 2018.
7. Invasive Species Compendium. *Silybum marianum*. Centre for Agriculture and Bioscience International (CABI). https://www.cabi.org/isc/datasheet/50304. Accessed June 5, 2020.
8. Fructus Silybi mariae. *WHO Monographs on Selected Medicinal Plants.* Vol 2. Geneva, Switzerland: World Health Organization; 2002:300-316.
9. *Silybum marianum* (L.) Gaertn. The Plant List. Version 1.1; 2013. http://www.theplantlist.org/tpl1.1/record/gcc-114114. Accessed June 5, 2020.
10. Cappelletti EM, Caniato R. Silymarin localization in the fruit and seed of *Silybum marianum* L. Gaertn. *Herba Hungarica.* 1984;23:53-66.
11. Stoiljković Z, Petrović S, Ilić B. Examination of localization of silymarin and fatty oil in *Silybum marianum* (L.) Gaertn. fruit. *Chem Ind Chem Eng Q.* 2007;13:55-59.
12. Kaczmarek F, Mrugasiewicz K, Lutomski J, Gorecki P, Scigacz M, Kotlarek B, Inventors. Sposob wytwarzania z nasion ostropestu plamistego (*Silybum marianum* (L.) Gaertn.) koncentratu o duż ej zawartos´ci sylimaryny. [Method of manufacturing a concentrate with a high content of silymarin from milk thistle (*Silybum marianum* (L.) Gaertn.) seeds.]. 1978.
13. Wagner H, Hörhammer L, Seitz M. Chemical evaluation of a silymarin-containing flavonoid concentrate from *Silybum marianum* (L.) Gaertn. [German]. *Arzneimittel-Forsch.* 1968;18(6):696-698.
14. Wagner H, Hörhammer L, Münster R. On the chemistry of silymarin (silybin), the active principle of the fruits from *Silybum marianum* (L.) Gaertn. (syn. *Carduus marianus* L.) [German]. *Arzneimittel-Forsch.* 1968;18(6):688-696.
15. Wagner H, Diesel P, Seitz M. Zur Chemie und Analytik von Silymarin aus *Silybum marianum* Gaerth [German]. *Arzneimittel-Forsch.* 1974;24:466-470.
16. Silybi mariani extractum siccum raffinatum et normatum. *European Pharmacopoeia (Ph. Eur. 10.0)*. Strasbourg, France: European Directorate for the Quality of Medicines and Health Care; 2020:1501-1502.
17. Morazzoni P, Bombardelli E. *Silybum marianum* (*Carduus marianus*). *Fitoterapia.* 1995;66:3-42.
18. United States Pharmacopeia. Milk thistle. *USP43-NF38*. Rockville, MD: United States Pharmacopeial Convention; 2020.
19. Silybi mariani fructus. *European Pharmacopoeia (Ph. Eur. 10.0).* Strasbourg, France: European Directorate for the Quality of Medicines and Health Care; 2020:1499-1501.
20. United States Pharmacopeia. Powdered milk thistle extract. *USP 43-NF 38*. Rockville, MD: United States Pharmacopeial Convention; 2020.
21. Kim NC, Graf TN, Sparacino CM, Wani MC, Wall ME. Complete isolation and characterization of silybins and isosilybins from milk thistle (*Silybum marianum*). *Org Biomol Chem.* 2003;1(10):1684-1689.
22. Kaloga M. Isosilychristin, ein neues Flayonolignan aus *Silybum marianum* L. Gaertn. / Isosilychristin, a New Flavonolignan from *Silybum marianum* L.Gaertn [German]. *Z Naturforsch B.* 1981;36(2).
23. Pelter A, Hänsel R. Struktur des Silybins: I. Abbauversuche [German]. *Chemische Berichte.* 1975;108(3):790-802.
24. Pelter A, Hänsel R. The structure of silybin (silybum substance E6), the first flavonolignan. *Tetrahedron Lett.* 1968;9(25):2911-2916.
25. Giuliani C, Tani C, Maleci Bini L, Fico G, Colombo R, Martinelli T. Localization of phenolic compounds in the fruits of *Silybum marianum* characterized by different silymarin chemotype and altered colour. *Fitoterapia.* 2018;130:210-218.
26. Hlangothia D, Abdel-Rahman FH, NguyÅn To, Anthony K, Saleh MA. Distribution of silymarin in the fruit of *Silybum marianum* L. *Pharm Anal Acta.* 2016;7:1-4.
27. Biedermann D, Vavříková E, Cvak L, Křen V. Chemistry of silybin. *Nat Prod Rep.* 2014;31(9):1138-1157.
28. Csupor D, Csorba A, Hohmann J. Recent advances in the analysis of flavonolignans of *Silybum marianum*. *J Pharm Biomed Anal.* 2016;130:301-317.
29. Szilágyi I, Tétényi P, Antus S, et al. Struktur von Silandrin und Silymonin, zwei neuen Flavanolignanen aus einer weißblühenden *Silybum marianum* Varietät [German]. *Planta Med.* 1981;43(2):121-127.
30. Samu Z, Nyiredy S, Baitz-Gács E, et al. Structure elucidation and antioxidant activity of (-)-isosilandrin isolated from *Silybum marianum* L. *Chem Biodiversity.* 2004;1(11):1668-1677.
31. Nyiredy S, Samu Z, Szücs Z, Gulácsi K, Kurtán T, Antus S. New insight into the biosynthesis of flavanolignans in the white-flowered

variant of *Silybum marianum*. *J Chromatogr Sci.* 2008;46(2):93-96.
32. Harrabi S, Romdhane H, Daassa M, Fellah H. Fatty acid and triacylglycerol compositions of milk thistle seeds growing wild in Tunisia (*Silybum marianum* L.). *Acta Aliment Hung.* 2015;44:304-310.
33. Hadolin M, Skerget M, Knez Z, Bauman D. High pressure extraction of vitamin E-rich oil from *Silybum marianum*. *Food Chem.* 2001;74(3):355-364.
34. Yin QF, Wang SH, Nan JX, Li SY. The fatty acid compositions of *Silybum marianum* by GC–MS. *J Yanbian Univ (Nat Sci).* 1998;24:26-28.
35. Teuscher E, Willuhn G, Loew D. Silybi mariani fructus. In: Blaschek W, ed. *Wichtl - Teedrogen und Phytopharmaka*. Stuttgart, Germany: Wissenschaftliche Verlagsgesellschaft mbH; 2016:612-615.
36. Goetz G. Milk thistle extract combats mushroom poisoning. *Food Safety News [online]* 2011.
37. Andrzejewska J, Martinelli T, Sadowska K. Silybum marianum: non-medical exploitation of the species. *Ann Appl Biol.* 2015;167(3):285-297.
38. Martinelli T, Whittaker A, Benedettelli S, Carboni A, Andrzejewska J. The study of flavonolignan association patterns in fruits of diverging *Silybum marianum* (L.) Gaertn. chemotypes provides new insights into the silymarin biosynthetic pathway. *Phytochemistry.* 2017;144:9-18.
39. Ali A, Anestis K, Reza S. Breeding objectives and selection criteria for milk thistle [*Silybum marianum* (L.) Gaertn.] improvement. *Notulae Botanicae Horti Agrobo.* 2013;41(2):340-347.
40. Poppe L, Petersen M. Variation in the flavonolignan composition of fruits from different Silybum marianum chemotypes and suspension cultures derived therefrom. *Phytochemistry.* 2016;131:68-75.
41. Martin RJ, Lauren DR, Smith WA, Jensen DJ, Deo B, Douglas JA. Factors influencing silymarin content and composition in variegated thistle (*Silybum marianum*). *NZ J Crop Hort Sci.* 2006;34:239-245.
42. Karkanis A, Bilalis D, Efthimiadou A. Cultivation of milk thistle (*Silybum marianum* L. Gaertn.), a medicinal weed. *Ind Crops Prod.* 2011;34(1):825-830.
43. Radjabian T, Huseini HF. Anti-hyperlipidemic and anti-atherosclerotic activities of silymarins from cultivated and wild plants of *Silybum marianum* L. with different content of flavonolignans. *Iran J Pharm Therapeut.* 2010;9(2):63-67.
44. AbouZid SF, Chen S-N, Pauli GF. Silymarin content in *Silybum marianum* populations growing in Egypt. *Ind Crops Prod.* 2016;83:729-737.
45. Saleh IA, Kamal SA, Shams KA, Abdel-Azim NS, Aboutabl EA, Hammouda FM. Effect of particle size on total extraction yield and silymarin content of *Silybum marianum* L. seeds. *Res J Pharm Biol Chem Sci.* 2015;6(2):803-809.
46. El Sherif F, Khattab S, Ibrahim AK, Ahmed SA. Improved silymarin content in elicited multiple shoot cultures of *Silybum marianum* L. *Physiol Mol Biol Plants : an international journal of functional plant biology.* 2013;19(1):127-136.
47. Omar AA, Hadad GM, Badr JM. First detailed quantification of silymarin components in the leaves of *Silybum marianum* cultivated in Egypt during different growth stages. *Acta Chromatograph.* 2012;24:463-474.
48. Saleh IA, Vinatoru M, Mason TJ, Abdel-Azim NS, Aboutabl EA, Hammouda FM. Ultrasonic-assisted extraction and conventional extraction of silymarin from *Silybum marianum* seeds; A comparison. *Res J Pharm Biol Chem Sci.* 2015;6(2):709-717.
49. Elwekeel A, El-Fishawy AM, AbouZid SF. Silymarin content in *Silybum marianum* fruits at different maturity stages. *J Med Plant Res.* 2013;7:1665-1669.
50. Leko V, Inventor; Leko, V., assignee. Method for isolation of silymarin from *Silybum marianum* seeds. 2008.
51. Carrier DJ, Crowe T, Sokhansanj S, Wahab J, Barl B. Milk thistle, *Silybum marianum* (L.) Gaertn., flower head development and associated marker compound profile. *J Herbs Spices Med Plants.* 2003;10(1):65-74.
52. Subramaniam S, Vaughn K, Carrier DJ, Clausen EC. Pretreatment of milk thistle seed to increase the silymarin yield: an alternative to petroleum ether defatting. *Bioresour Technol.* 2008;99(7):2501-2506.
53. Gupta GK RS, Rao, PR. Isolation of antihepatotoxic agents from seeds of *Silybum marianum*. *Res Ind.* 1982;27:37-42.
54. Andrzejewska J, Sadowska K, Mielcarek S. Effect of sowing date and rate on the yield and flavonolignan content of the fruits of milk thistle (*Silybum marianum* L. Gaertn.) grown on light soil in a moderate climate. *Ind Crops Prod.* 2011;33(2):462-468.
55. Kahol AP, Singh KL, Tandon S, Kumar S, Inventors; Council of Scientific and Industrial Research (CSIR), assignee. Process for isolation of hepatoprotective agent silymarin from the seeds of the plant *Silybum marianum*. 2001.
56. Wianowska D, Wiśniewski M. Simplified procedure of silymarin extraction from *Silybum marianum* L. Gaertner. *J Chromatogr Sci.* 2015;53(2):366-372.
57. Wallace S, Carrier DJ, Beitle RR, Clausen EC, Griffis CL. HPLC-UV and LC-MS-MS characterization of silymarin in milk thistle seeds and corresponding products. *J Nutraceut Funct Med Foods.* 2003;4(2):37-48.
58. Duan L, Carrier DJ, Clausen EC. Silymarin extraction from milk thistle using hot water. Paper presented at: Proceedings of the Twenty-Fifth Symposium on Biotechnology for Fuels and Chemicals.2004; Breckenridge, CO.
59. Barreto JF, Wallace SN, Carrier DJ, Clausen EC. Extraction of nutraceuticals from milk thistle: I. Hot water extraction. *Appl Biochem Biotechnol.* 2003;105 -108:881-889.
60. Keshavarz Afshar R, Chaichi MR, Ansari Jovini M, Jahanzad E, Hashemi M. Accumulation of silymarin in milk thistle seeds under drought stress. *Planta.* 2015;242(3):539-543.
61. Intelmann D, Karlseder A, Inventors; Bionorica SE, assignee. Milk thistle extract of fruit shells of *Silybum marianum*, process of manufacture and use. 2015.
62. Szczucinska A, Lipkowski AW, Baranowska B, Walisiewicz-Niedbalska W, Rożycki K, Maciuszczak-Kotlarek H. Utylizacja odpadu nasion ostropestu plamistego. I. Olej z ostropestu plamistego jako antyutleniacz / Utilisation of milk thistle seed waste. Part I. Milk thistle oil as antioxidant [Polish]. *Ro´sliny Oleiste - Oilseed Crops.* 2003;25:717-724.
63. AbouZid SF, Chen S-N, McAlpine JB, Friesen JB, Pauli GF. *Silybum marianum* pericarp yields enhanced silymarin products. *Fitoterapia.* 2016;112:136-143.
64. Depletion. *Adulteration of Natural Extracts and Quality Challenges*. East Windsor, NJ: Sabinsa; 2018:12.
65. Gorman R. Adulteration...an ingredient checklist. *Nutraceuticals now [online]*. Inverness, Scotland: Johnson-Johnson Publishing; 2016.
66. Anonymous. Not all milk thistle extracts are made equal: Analytical methods, USP standards, and adulteration. *TherapeuticFocus [online].* 2018;5(1).
67. Assessment report on *Silybum marianum* (L.) Gaertn., fructus. London, United Kingdom: European Medicines Agency Committee on Herbal Medicinal Products (HMPC); 2016:1-79.
68. Halbach G, Winkler W. Notizen: Flavonoide Inhaltsstoffe in den Früchten von *Silybum eburneum* / Flavonoids in the fruits of the genus *Silybum eburneum* [German]. *Z Naturforsch B.* 1971;26(9):971-972.
69. Adzet T, Iglesias J, Martinez F. Flavonolignans in the fruits of *Silybum* genus taxa: a chromatographic and mass spectrometric survey. *Plant Med Phytother.* 1993;26(2):117-129.
70. Vue B, Chen QH. The potential of flavonolignans in prostate cancer management. *Curr Med Chem.* 2016;23(34):3925-3950.
71. Begum SA, Sahai M, Ray AB. Non-conventional lignans: Coumarinolignans, flavonolignans, and stilbenolignans. In: Kinghorn AD, Falk H, Kobayashi J, eds. *Fortschritte der Chemie organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products, Vol. 93*. Vienna, Austria: Springer Vienna; 2010:1-70.
72. Zhou L, Li XK, Miao F, et al. Further studies on the chemical constituents of Chinese folk medicine *Gentiana apiata* N.E. Br. *Journal of Asian Natural Products Research.* 2009;11(4):345-351.
73. El-Elimat T, Raja HA, Graf TN, Faeth SH, Cech NB, Oberlies NH. Flavonolignans from *Aspergillus iizukae*, a fungal endophyte of milk thistle (*Silybum marianum*). *J Nat Prod.* 2014;77(2):193-199.
74. Smith T, Gillespie M, Eckl V, Knepper J, Reynolds CM. Herbal supplement sales in US increase by 9.4% in 2018. *HerbalGram.* 2019;123:62-73.
75. Smith T, May G, Eckl V, Reynolds CM. US sales of herbal supplements increase by 8.6% in 2019. *HerbalGram.* 2020;127:54-67.
76. Smith T, Kawa K, Eckl V, Morton C, Stredney R. Herbal supplement sales in US increase 8.5% in 2017, topping $8 billion. *HerbalGram.* 2018;119:62-71.

77. Smith T, Kawa K, Eckl V. Herbal supplement sales in US increase 7.7% in 2016. *HerbalGram.* 2017;115:56-65.
78. Vereš T, Týr Š. Milk thistle (*Silybum marianum* (L.) Gaertn.) as a weed in sustainable crop rotation. *Res J Agric Sci.* 2012;44:118-122.
79. Smoller N. Milk thistle: Quality update. Vol 2020. Woodstock, NY: Woodstock Vitamins Blog (formerly Village Vitality); 2015.
80. Ahmad M, Khan MA, Hasan A, Zafar M, Sultana S. Chemotaxonomic standardization of herbal drugs milk thistle and globe thistle. *Asian J Chem.* 2008;6(20):4443-4459.
81. Plant Details for a *Echinops echinatus* ROXB. Institute of Trans-Disciplinary Health Sciences & Technology (TDU) and Foundation for Revitalisation of Local Health Traditions (FRLHT); 2020. http://envis.frlht.org/plantdetails/6abcf64dea9e5749f127dcd581bd4944/23f1315ef624d31a7138e3fce39a90b4. Accessed August 24, 2020.
82. Langhammer L. Anatomie und Histochemie der Früchte von *Silybum*. *Planta Med.* 1969;17(3):268-275.
83. Frommenwiler DA, Sharaf MHM, Reich E. The truth behind herbal products: how HPTLC can help herbal industry detect adulteration? *Planta Med.* 2019;85(18):ISL EA-06.
84. Fenclova M, Novakova A, Viktorova J, et al. Poor chemical and microbiological quality of the commercial milk thistle-based dietary supplements may account for their reported unsatisfactory and non-reproducible clinical outcomes. *Sci Rep.* 2019;9(1):11118.
85. Fibigr J, Šatínský D, Solich P. A new approach to the rapid separation of isomeric compounds in a *Silybum marianum* extract using UHPLC core-shell column with F5 stationary phase. *J Pharm Biomed Anal.* 2017;134:203-213.
86. Bilia AR, Bergonzi MC, Gallori S, Mazzi G, Vincieri FF. Stability of the constituents of calendula, milk-thistle and passionflower tinctures by LC-DAD and LC-MS. *J Pharm Biomed Anal.* 2002;30(3):613-624.
87. Pendry BA, Kemp V, Hughes MJ, et al. Silymarin content in *Silybum marianum* extracts as a biomarker for the quality of commercial tinctures. *J Herbal Med.* 2017;10:31-36.
88. Trust me – I'm a doctor. Do herbal supplements contain what they say on the label? . British Broadcasting Corporation (BBC) Two; 2015. http://www.bbc.co.uk/programmes/articles/4hX30rMYkMv9YjMTH38MY6/do-herbal-supplements-contain-what-they-say-on-the-label. Accessed September 23, 2019.
89. Booker A, Heinrich M. Value chains of botanicals and herbal medicinal products: A European perspective. *HerbalGram.* 2016;112:40-45.
90. Milk thistle and liver formula supplements review. ConsumerLab.com; 2019. https://www.consumerlab.com/reviews/milk_thistle_and_liver_supplements/milkthistle/. Accessed June 16, 2020.
91. Stranska M, Hajslova J. Herbal based dietary supplements and other food products: Assessment of quality and chemical safety. Institutional Cooperation of UCT Prague + UiT Tromsø; 2016; Tromsø, Norway.
92. Gu W, Chen X, Pan X, Chan ASC, Yang T-K. First enantioselective syntheses of (2R,3R)- and (2S,3S)-3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl-1,4-benzodioxan-6-carbaldehyde. *Tetrahedron: Asymmetry.* 2000;11(13):2801-2807.
93. Tan C, Xu X, Shang Y, Fu X, Xia G, Yang H. A novel approach for the efficient extraction of silybin from milk thistle fruits. *Pharmacogn Mag.* 2014;10(40):536-540.
94. De Iasi G, Feola M, Di Manzano CM, Inventors; Istituto Biochimico Italiano Giovanni Lorenzini S.P.A., assignee. An oral pharmaceutical formulation comprising silybin. 2017.
95. Anthony K, Saleh MA. Chemical profiling and antioxidant activity of commercial milk thistle food supplements. *J Chem Pharm Res.* 2012;4(10):4440-4450.
96. Lee JI, Narayan M, Barrett JS. Analysis and comparison of active constituents in commercial standardized silymarin extracts by liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr B.* 2007;845(1):95-103.
97. Kvasnička F, Bíba B, Ševčík R, Voldřich M, Krátká J. Analysis of the active components of silymarin. *J Chromatogr A.* 2003;990(1-2):239-245.
98. Eklund L, Simon JP, Ballenger J. High performance liquid chromatography of flavonolignans in commercial milk thistle supplements. *BIOS.* 2009;80(4):164-169.
99. Liu H, Du Z, Yuan Q. A novel rapid method for simultaneous determination of eight active compounds in silymarin using a reversed-phase UPLC-UV detector. *J Chromatogr B.* 2009;877(32):4159-4163.
100. Schulz H-U, Schürer M, Krumbiegel G, Wächter W, Weyhenmeyer R, Seidel G. Untersuchungen zum Freisetzungsverhalten und zur Bioäquivalenz von Silymarin-Präparaten. *Arzneimittel-Forsch.* 1995;45:61-64.
101. Frommenwiler DA, Reich E, Sudberg S, Sharaf MHM, Bzhelyansky A, Lucas B. St. John's wort versus counterfeit St. John's wort: An HPTLC study. *J AOAC Int.* 2016;99(5):1204-1212.

Milk thistle (*Silybum marianum*) fruits
Photo ©2020 Steven Foster

102. Monographie über Mariendinstelfrüchte. *Deutsches Arzneibuch 10 (DAB 10), Band 3*. Stuttgart, Germany & Frankfurt, Germany: Deutscher Apotheker Verlag & Govi Verlag; 1993.
103. Gafner S. Botanical ingredient adulteration – how some suppliers attempt to fool commonly used analytical techniques. 30th International Horticultural Congress; 2018; Istanbul, Turkey.
104. Chapter 9 - Federal Food, Drug, and Cosmetic Act, Subchapter IV - Food, Section 342 - Adulterated food. *United States Code, 2006 Edition, Supplement 5, Title 21 - Food and Drugs*. Washington, DC: U.S. Government Publishing Office; 2006.
105. Spink J, Moyer DC. Understanding and combating food fraud. *Food Tech.* 2013;67(1):30-35.
106. United States Pharmacopeia. Powdered milk thistle. *USP43-NF38*. Rockville, MD: United States Pharmacopeial Convention; 2020.
107. Wagner H, Bladt S. *Plant Drug Analysis: A Thin Layer Chromatography Atlas.* Berlin, Germany: Springer 2009.
108. Cheilari A, Sturm S, Intelmann D, Seger C, Stuppner H. Head-to-head comparison of ultra-high-performance liquid chromatography with diode array detection versus quantitative nuclear magnetic resonance for the quantitative analysis of the silymarin complex in *Silybum marianum* Fruit Extracts. *J Agric Food Chem.* 2016;64(7):1618-1626.
109. Graf TN, Cech NB, Polyak SJ, Oberlies NH. A validated UHPLC-tandem mass spectrometry method for quantitative analysis of flavonolignans in milk thistle (*Silybum marianum*) extracts. *J Pharm Biomed Anal.* 2016;126:26-33.
110. Shibano M, Lin A-S, Itokawa H, Lee K-H. Separation and characterization of active flavonolignans of *Silybum marianum* by liquid chromatography connected with hybrid ion-trap and time-of-flight mass apectrometry (LC–MS/IT-TOF). *J Nat Prod.* 2007;70(9):1424-1428.
111. Zavoi S, Fetea F, Ranga F, Pop RM, Baciu A, Socaciu C. Comparative fingerprint and extraction yield of medicinal herb phenolics with hepatoprotective potential, as determined by UV-Vis and FT-MIR spectroscopy. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca.* 2011;39(2).
112. Vágnerová L, Bradáčová M, Pluháčková H. The determination of contained compounds in milk thistle [*Silybum marianum* L. (Gaertn.)] by the means of FT-NIR. *MendelNet.* 2016;23:168-172.
113. Galvin-King P, Haughey SA, Elliott CT. Herb and spice fraud; the drivers, challenges and detection. *Food Control.* 2018;88:85-97.
114. Bessaire T, Savoy MC, Mujahid C, Tarres A, Mottier P. A new high-throughput screening method to determine multiple dyes in herbs and spices. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2019;36(6):836-850.
115. Ražná K, Hlavačková L, Bežo M, et al. Application of the RAPD and miRNA markers in the genotyping of *Silybum marianum* (L.) Gaertn. *Acta Fytotechnica et Zootechnica* (online). 2015;18(4):83-89.
116. AbouZid S. Authentication of *Silybum marianum* varieties using RAPD analysis. *Plant Tissue Cult Biotechnol.* 2014;24(1):57-63.
117. Hamouda M. Molecular analysis of genetic diversity in population of *Silybum marianum* (L.) Gaertn in Egypt. *J Genet Eng Biotechnol.* 2019;17(1):12.
118. AbouZid S. Silymarin, natural flavonolignans from milk thistle In: Rao V, ed. *Phytochemicals: A Global Perspective of Their Role in Nutrition and Health*. Rijeka, Croatia: InTech; 2012:255-272.
119. Eliot C. The new phenomenon of criminal fraud in the food supply chain. *NSF Int Rep.* 2014:1-187.
120. van Asselt ED, Banach JL, van der Fels-Klerx HJ. Prioritization of chemical hazards in spices and herbs for European monitoring programs. *Food Control.* 2018;83:7-17.
121. Dixit S, Khanna SK, Das M. A simple 2-directional high-performance thin-layer chromatographic method for the simultaneous determination of curcumin, metanil yellow, and Sudan dyes in turmeric, chili, and curry powders. *J AOAC Int.* 2008;91(6):1387-1396.
122. Petrakis EA, Cagliani LR, Tarantilis PA, Polissiou MG, Consonni R. Sudan dyes in adulterated saffron (*Crocus sativus* L.): Identification and quantification by ^{1}H NMR. *Food Chem.* 2017;217:418-424.
123. Oplatowska-Stachowiak M, Elliott CT. Food colors: Existing and emerging food safety concerns. *Crit Rev Food Sci Nutr.* 2017;57(3):524-548.
124. Voinovich D, Perissutti B, Magarotto L, Ceschia D, Guiotto P, Bilia AR. Solid state mechanochemical simultaneous activation of the constituents of the *Silybum marianum* phytocomplex with cross-linked polymers. *J Pharm Sci.* 2009;98(1):215-228.
125. Voinovich D, Perissutti B, Grassi M, Passerini N, Bigotto A. Solid state mechanochemical activation of *Silybum marianum* dry extract with betacyclodextrins: Characterization and bioavailability of the coground systems. *J Pharm Sci.* 2009;98(11):4119-4129.
126. Liang J, Liu Y, Liu J, et al. Chitosan-functionalized lipid-polymer hybrid nanoparticles for oral delivery of silymarin and enhanced lipid-lowering effect in NAFLD. *J Nanobiotechnol.* 2018;16(1):64.
127. Javed S, Kohli K, Ali M. Reassessing bioavailability of silymarin. *Alt Med Rev.* 2011;16(3):239-249.
128. Tung N-T, Tran C-S, Nguyen H-A, et al. Formulation and biopharmaceutical evaluation of supersaturatable self-nanoemulsifying drug delivery systems containing silymarin. *Int J Pharmaceut.* 2019;555:63-76.
129. Nasr SS, Nasra MMA, Hazzah HA, Abdallah OY. Mesoporous silica nanoparticles, a safe option for silymarin delivery: preparation, characterization, and in vivo evaluation. *Drug Deliv Transl Res.* 2019;9(5):968-979.
130. Méndez-Sánchez N, Dibildox-Martinez M, Sosa-Noguera J, Sánchez-Medal R, Flores-Murrieta FJ. Superior silybin bioavailability of silybin–phosphatidylcholine complex in oily-medium soft-gel capsules versus conventional silymarin tablets in healthy volunteers. *BMC Pharmacol Toxicol.* 2019;20(1):5.
131. Yousaf AM, Malik UR, Shahzad Y, Mahmood T, Hussain T. Silymarin-laden PVP-PEG polymeric composite for enhanced aqueous solubility and dissolution rate: Preparation and in vitro characterization. *J Pharm Anal.* 2019;9(1):34-39.
132. Ibrahim AH, Rosqvist E, Smått J-H, et al. Formulation and optimization of lyophilized nanosuspension tablets to improve the physicochemical properties and provide immediate release of silymarin. *Int J Pharmaceut.* 2019;563:217-227.
133. Theodosiou E, Purchartová K, Stamatis H, Kolisis F, Křen V. Bioavailability of silymarin flavonolignans: Drug formulations and biotransformation. *Phytochem Rev.* 2014;13(1):1-18.
134. Kosina P, Kren V, Gebhardt R, Grambal F, Ulrichová J, Walterová D. Antioxidant properties of silybin glycosides. *Phytother Res.* 2002;16 Suppl 1:S33-39.
135. Barzaghi N, Crema F, Gatti G, Pifferi G, Perucca E. Pharmacokinetic studies on IdB 1016, a silybin- phosphatidylcholine complex, in healthy human subjects. *Eur J Drug Metab Pharmacokinet.* 1990;15(4):333-338.
136. Food Chemical Codex. Appendix XVII: Food fraud mitigation guidance. *FCC 10*. Rockville, MD: United States Pharmacopeial Convention; 2016.
137. Morin J-F, Lees M. Definition of food fraud and food authenticity. In: Morin J-F, Lees M, eds. *FoodIntegrity Handbook*. La Chapelle sur Erdre, France: Eurofins Analytics France; 2018:XIII-XVII.

REVISION SUMMARY

Version # , Author,	Date Revised	Section Revised	List of Changes
Version 1, A. McCutcheon	N/A	N/A	None