

Extraction of Nutraceuticals from Milk Thistle

I. Hot Water Extraction

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Abstract

Milk thistle contains compounds that display hepatotoxic protection properties. We examined the batch extraction of silymarin compounds from milk thistle seed meal in 50, 70, 85, and 100°C water as a function of time. After 210 min of extraction at 100°C, the yield of taxifolin was 1.2 mg/g of seed, a 6.2-fold increase over the results obtained in a Soxhlet extraction with ethanol on pretreated (defatted) seeds. Similarly, the yield of silychristin was 5.0 mg/g of seed, a 3.8-fold increase. The yields of silybinin A and silybinin B were 1.8 and 3.3 mg/g of seed, respectively, or roughly 30% of the Soxhlet yield. The ratios of the extracted compounds, and particularly the ratios at long extraction times, showed that the more polar compounds (taxifolin and silychristin) were preferentially extracted at 85°C, while the less polar silybinin was favored at 100°C.

Index Entries: Milk thistle; extraction; water; silymarin; flavanolignans.

Introduction

Milk thistle (*Silybum marianum*) is an annual or a biennial plant native to the Mediterranean and North Africa. It grows wild throughout Europe, North Africa, the Americas, and Australia but can also be cultivated (1). The plants can reach a height of 10 ft with dark and shiny leaves, and purple to reddish flowers. Milk thistle has an indeterminate growth habit, resulting in staggered flowering and maturity (2). The seeds of the plant contain a group of flavanoid compounds commonly named silymarin (3).

The term *silymarin* usually encompasses the dihydroflavonol—taxifolin—and the flavanolignans—silybinin, isosilybinin, silydianin, and

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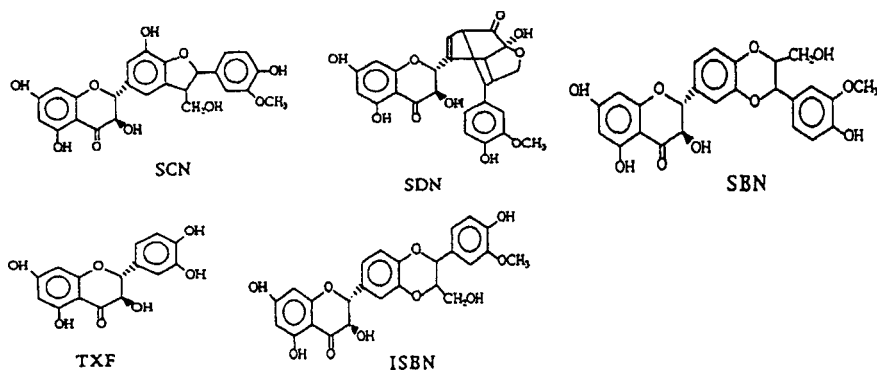


Fig. 1. Structures of silychristin (SCN), silydianin (SDN), silybinin (SBN), taxifolin (TXF), and isosilybinin (ISBN).

silychristin (Fig. 1). Some studies suggest that silybinin reduces the biliary cholesterol concentration (4). It has also been demonstrated that silybinin is useful in the intervention of hormone refractory human prostate cancer (5). Furthermore, the combination of silybinin and silychristin has been found helpful in decreasing the nephritic effects of chemical-induced injury (6).

The Deutsches Arzneibuch procedure for silymarin extraction is a two-step process in which seeds are first defatted in a Soxhlet extraction with petrol for 4 h, followed by a second Soxhlet extraction with methanol for 5 h (7). Using this procedure, Benthin et al. (8) reported silybinin yields of 11 mg of silybinin/g of seed. These investigators also extracted milk thistle using pressurized liquid extraction techniques, in which 12 mg of silybinin/g of seed was obtained. In extracting 0.4-mm particle-size milk thistle seed meal in a Soxhlet with petrol for 24 h, followed by an ethanol Soxhlet for 4 h, Wallace et al. (9) reported a silybinin yield of 16 mg/g of seed meal. The differences in the results obtained by Wallace et al. (9) and Benthin et al. (8) may not be significant, since the silybinin content of seed batches varies significantly (2).

Wallace et al. (9) reported the analysis of three off-the-shelf milk thistle products, of which only two products contained silymarin compounds. Inconsistency between herbal supplement label and product content is not uncommon. For example, an analysis of ephedra products (10) showed a broad range of ephedra alkaloid content, pointing most likely to manufacturing problems. The lack of consistency among products can be owing in part to the extraction step, where the desired molecules diffuse from the bulk herb to a solvent phase, usually ethanol, methanol, acetone, hexane, or petroleum ether. To increase the quality of products, the extraction step should be well characterized, in terms of both rates and appropriate solvents.

The use of hot liquid water as an extraction solvent has recently caught the attention of some researchers (11,12). Water is very useful in extracting polar compounds and may also be useful in extracting polar compounds from plant material without prior defatting. In increasing the water tem-

perature up to its subcritical temperature, a decrease in the dielectric constant is observed. For example, water at 250°C (523 K) displays a dielectric constant of 27, which is the realm of that of methanol (33) and ethanol (24). As a result, hot liquid (hot/liquid) water has solubility characteristics at increased temperature that are similar to ethanol and methanol. The solubilities of anthracene, pyrene, chrysene, perylene, and carbazole (13) and of *d*-limonene, carvone, eugenol, 1,8-cineole, and nerol (14) were determined in 289 and 498 K (hot/liquid) water, where increases were observed with temperature. Kubátová et al. (12) showed that the extraction of peppermint compounds using (hot/liquid) water at 175°C required 15 min, as compared to 4 h with hydrodistillation. The use of (hot/liquid) water as an extraction solvent shows promise as the search for milder and “greener” solvents is intensified.

This article presents results from the extraction of silymarin compounds from milk thistle seeds using (hot/liquid) water as the solvent, a first step in process characterization. Silymarin compounds, ranging from highly polar (taxifolin) to less polar (silybinin), were extracted in 50–100°C water over a period of 17 h. A maximum temperature of 100°C was employed in order to maintain liquid water at atmospheric pressure. Increased pressure experiments will be run in the future. In our study, we compared the compounds extracted, as the temperature increased, and the dielectric constant. We also examined the effect of water extraction temperature on extraction yield.

Materials and Methods

Extraction Experiments

Milk thistle seeds were purchased from Frontier Herbs (Norway, IA) and ground with a coffee grinder to an average particle diameter of 0.4 mm. Extraction experiments were conducted at 50, 70, 85, and 100°C using 2 g of seed (contained in a cheesecloth bag) in 200 mL of deionized water. Leaching at 100°C was carried out in a 500-mL glass round-bottomed flask, fitted with a condenser for total reflux. The flask was heated in an electric mantel, and water was used to condense the vapor. The leaching experiments at 50, 70, and 85°C were carried out in 500-mL bottles in a shaker water bath (Dubnoff Metabolic Shaking Incubator; Precision Scientific, Winchester, VA) set at 80 strokes/min. Although the process conditions were slightly different when operating at or below 100°C, the long diffusion times observed in the experiments helped minimize the small differences in the systems.

Samples of extraction water, as a “tea,” were taken in triplicate every 30 min, including time zero, using a 1-mL pipet. Since the solids were held in a permeable bag, they were not removed during sampling. Time zero was arbitrarily set as the time when the water started boiling (100°C), or when the temperature of the water in the bottles equilibrated with the set experimental temperature (50, 70, 85°C). Aliquots were placed in preweighed test

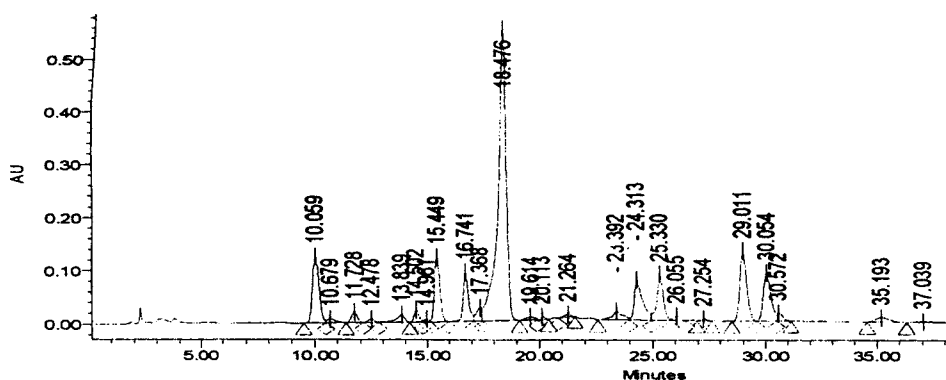


Fig. 2. Typical chromatogram of milk thistle seed extract. Retention times of taxifolin, silychristin, silydianin, silybinin A, and silybinin B were 10.059, 18.476, 21.264, 24.313, and 25.330 min, respectively. Note that this particular seed lot contained miniscule amounts of silydianin.

tubes and weighed to determine aliquot weight. Subsequently, the aliquots were evaporated to dryness in a SpeedVac (Savant, Holbrook, NY). To the dried sample, 1 mL of methanol was added to redissolve the residue. After vortexing and centrifuging (10g), the supernatant was filtered and analyzed, as described next.

Chemical Analysis

The silymarin concentrations were determined by high-performance liquid chromatography using a Waters system (Milford, MA) consisting of an Alliance 2690 separations module and a 996 Photodiode Array, controlled with Millennium³² chromatography software (9). Separation of the silymarin compounds was obtained using a Symmetry® (Waters) C₁₈ pre-column placed in series with a Symmetry (Waters) C₁₈ column (150 × 4.6 mm, 5 µm), both at 40°C. A 10-µL sample volume was injected. Solvent A was 20:80 methanol:water, while solvent B consisted of 80:20 methanol:water. The gradient program was initiated with 85:15 solvent A:solvent B flowing for 5 min, followed by a linear gradient of 45:55 solvent A:solvent B for 15 min. The proportions of 45:55 solvent A:solvent B were then held constant for 20 min and brought back to 85:15 solvent A:solvent B over 10 min. The flow rate was 0.75 µL/min, and the silymarin compounds were monitored at 290 nm. Peak identification was confirmed by mass spectrometry (Pharmalytics, Saskatoon, Saskatchewan, Canada). Calibration curves were prepared with silybinin from Sigma (St. Louis, MO), taxifolin from Extrasynthese (Lyon, France), and silychristin and silydianin from PhytoLab (Hamburg, Germany). No standard was available for isosilybinin, and thus this compound was excluded from the analysis. The silybinin standard obtained from Sigma contained two distinct peaks, which are further referred to as silybinin A (first peak) and silybinin B (second peak). A sample chromatogram from the extraction of milk thistle seeds is shown in Fig. 2.

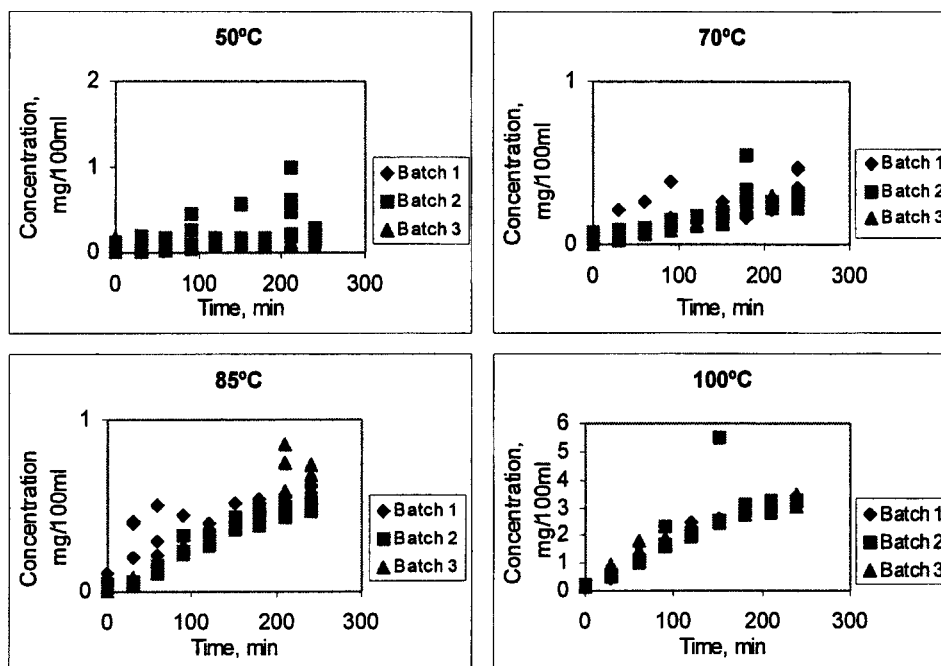


Fig. 3. Silybinin B concentration as a function of extraction time at different temperatures. Results show all the batches for all temperatures.

Results and Discussion

For all temperatures, three distinct experiments were conducted, of which three samples were taken per time point (total of nine samples per time point). Figure 3 demonstrates the reproducibility of the concentration-time data at each temperature by showing the silybinin B concentration in the extract water with time. The reproducibility of the data at each temperature was generally quite good, with only a few “outliers” observed in the data trend. In addition, the reproducibility of the data improved with increasing temperature as the concentration of the extracted compound increased.

Figure 4 shows typical results from the extraction of taxifolin, silychristin, silybinin A, and silybinin B, presented as the yield of each compound (mg/g of seed) as a function of extraction time and temperature. Each of the extracted compounds showed a consistent pattern of increasing yield with temperature and time as equilibrium was approached. For each of the silymarin compounds, extraction with 100°C water produced the highest yield and concentration of compounds. After 210 min of extraction at 100°C, the yield of taxifolin was 1.2 mg/g of seed, while the yields of silychristin, silybinin A, and silybinin B were 5.0, 1.8, and 3.3 mg/g of seed, respectively. Assuming that a 4 hr extraction of pretreated (defatted) seeds with ethanol gives the highest yield of compounds, the percentage yield of

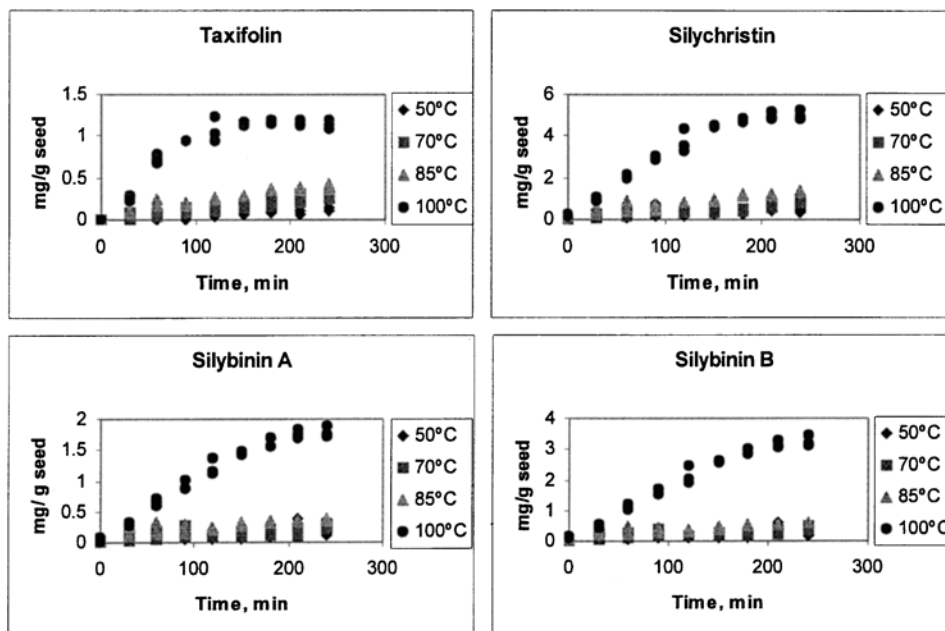


Fig. 4. Silymarin as a function of time at different temperatures. Results are based on the first experiment of each batch.

taxifolin after 210 min was 720%, while the percentage yields of silychristin, silybinin A, and silybinin B were 480, 30, and 33%, respectively. Note that the extraction yields of the polar compounds taxifolin and silychristin were significantly higher than the extraction yields in ethanol, indicating that water is a better solvent for extracting polar compounds from milk thistle. After 300 min of extraction, the yields of taxifolin, silychristin, silybinin A, and silybinin B were 0.92 (550% of Soxhlet results), 4.7 (440%), 1.8 (30%), and 3.4 (34%) mg/g of seed, respectively (data not shown). A slight decrease in the yield of taxifolin was observed after 150 min, perhaps indicating the onset of decomposition. Overall, water extraction at 100°C yielded about 65% of the amount of the total silymarins obtained in the two-step Soxhlet extraction (with defatting) performed by Wallace et al. (9).

The ratios of the concentrations of taxifolin, silychristin, and silybinin A to the concentration of silybinin B at 85 and 100°C as a function of time are shown in Fig. 5. These temperatures were chosen because the silymarin concentrations were not as large at temperatures below 85°C. As noted in Fig. 5A, at 85°C the ratio of taxifolin to silybinin B increased rapidly to 0.7 g/g and then held constant at that level. At 100°C, the ratio reached a maximum of 0.65 g/g and then gradually fell with time to 0.35 g/g. This reduction in the ratio at 100°C shows that the taxifolin concentration reached its maximum faster than silybinin B. A similar behavior for the ratio of silychristin to silybinin B is noted in Fig. 5B. At 85°C, the ratio

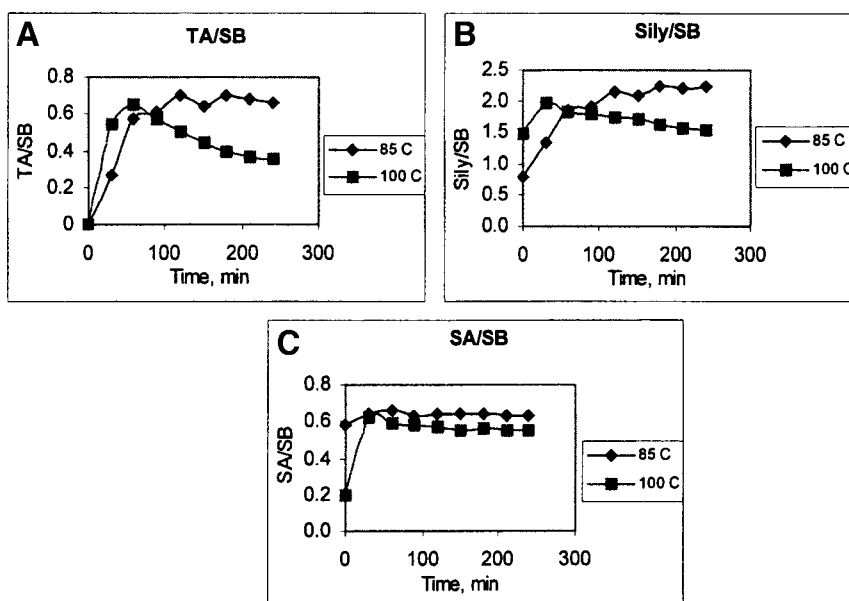


Fig. 5. Compound ratio as function of time for 85 and 100°C experiments. (A) Taxifolin to silybinin B ratio; (B) silychristin to silybinin B; ratio. (C) silybinin A to silybinin B ratio.

rapidly rose to just above 2.0 g/g and then gradually increased before leveling out at 2.2 g/g. At 100°C, the ratio increased to a maximum of 2.0 g/g and then gradually fell to 1.5 g/g. Figure 5C shows that, excluding an initial sharp increase, the ratio of silybinin A to silybinin B at 85°C was constant at 0.65 g/g. At 100°C, the ratio was constant at about 0.6 g/g, again excluding the initial period of sharp increase.

These ratios, and particularly the ratios at long extraction times, show that the more polar compounds (taxifolin and silychristin) are preferentially extracted at 85°C, while the less polar compounds (silybinin A and B) are more easily extracted at 100°C (see also the data in Table 1). The data reported by Wallace et al. (9) showed that the ratios of taxifolin to silybinin B, silychristin to silybinin B, and silybinin A to silybinin B were 0.02, 0.1, and 0.6, respectively. Thus, the ratios of extraction products using water at 100°C more closely resemble the Soxhlet extraction results than the water extractions at temperatures below 85°C. More dramatic differences in polar and nonpolar compound extraction with water are expected as the temperature of liquid water is further increased, thereby lowering the dielectric constant.

Although the yields of taxifolin, silychristin, silybinin A, and silybinin B using water were less than what is reported in ethanol (9), this technology shows promise because of the omission of the defatting step. An oil-removal step was found necessary in the extraction procedures proposed by Kahol et al. (15) and Benthin et al. (8). It is hoped that the

Table 1
Calculated Ratios of Compound/Silybinin B as Function of Temperature^a

Water temperature (°C)	Dielectric constant ϵ	Ratio of compound to SB ^b		
		Taxifolin/SB	Silychristin/SB	Silybinin A/SB
50	70	0.916	2.006	0.615
70	64	0.594	1.869	0.639
85	60	0.661	2.237	0.630
100	56	0.352	1.546	0.551

^aThese ratios were calculated at the last sampling point.

^bSB, silybinin B.

work of Wallace et al. (16) comparing the extraction of nondefatted and defatted milk thistle seed meal using ethanol as the solvent will shed more light on this subject matter.

Conclusions

Water is not only an interesting alternative solvent because of its low operating and disposal costs, but is also effective in extracting the more polar silymarin compounds from milk thistle seed. For each of the compounds, extraction with 100°C water gave the highest yield and concentration. After 210 min of extraction at 100°C, the yield of taxifolin was 1.2 mg/g of seed, a 6.2-fold increase over Soxhlet extraction of pretreated seeds with ethanol, while the yield of silychristin was 5.0 mg/g of seed, a 3.8-fold increase. The yields of silybinin A and silybinin B were 1.8 and 3.3 mg/g of seed, respectively, or roughly 30% of the yield in the Soxhlet extraction. The ratios of the extracted compounds, and particularly the ratios at long extraction times, showed that the more polar compounds (taxifolin and silychristin) were preferentially extracted at 85°C, while the less polar compounds (silybinin A and B) were favored at 100°C.

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