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Batch Solvent Extraction of Flavanolignans from Milk Thistle (*Silybum marianum* L. Gaertner)

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Seeds of milk thistle (*Silybum marianum* L. Gaertner) contain silymarins and ca. 25% (w/w) of oil. A pretreatment step involving refluxing with petroleum ether is usually performed before extraction of the silymarins using organic solvents. This paper compares the extraction of whole and defatted milk thistle seeds in various solvents as a function of temperature. The extraction of whole seeds of milk thistle with water at 50, 70 and 85°C was also examined: the yield of silymarin increased with increasing water temperature. In most cases, ethanol at 60°C recovered the largest quantities of silymarins. However, boiling water proved to be an efficient extraction solvent for the more polar silymarins such as taxifolin and silychristin, even when using whole seeds. Extractions of defatted seed meal with boiling ethanol returned maximum yields of 0.62, 3.89, 4.04, and 6.86 mg/g defatted seed of taxifolin, silychristin, silybinin A and silybinin B, respectively. When extracting defatted seed meal with ethanol, yields of taxifolin, silybinin A and silybinin B were, respectively, 6.8-, 0.95-, 1.7- and 1.6-fold higher than when extracting whole seeds. When extracting with boiling water, the yields of silychristin, silybinin A, and silybinin B were 380, 47 and 50% higher for whole seeds compared with defatted seeds. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords: Extraction conditions; flavanolignan; silymarin; milk thistle; *Silybum marianum*.

INTRODUCTION

Milk thistle is an annual or biennial plant that is native to the Mediterranean and North Africa but has spread to other warm and dry climates in the Americas, Australia, and Europe. The plant can grow to heights of 10 feet, and its leaves are dark and shiny with white veins laced throughout (Hamid *et al*., 1983). Milk thistle has an indeterminate growth habitat, which results in staggered flowering and maturity (Carrier *et al*., 2002). The seeds of the plant contain the highest concentrations of flavanolignans, a class of compounds that display hepatoprotective properties. The flavanolignans, along with one dihydroflavanol, are collectively referred to as silymarin (Tittel and Wagner, 1978). Flavanolignans include silychristin, silydianin and the diastereomers silybinin A and silybinin B. Taxifolin, a dihydroquercitin, is a molecular precursor to the flavanolignans and is also included in the silymarin complex, as are the diastereomers isosilybinin A and isosilybinin B.

The therapeutic benefits of silymarins were demonstrated in several clinical studies, which showed the effects of silybinin on reducing biliary cholesterol levels (Duke, 1999), the ability of silybinin to intervene in hormone refractory human prostrate cancer (Zi and Agarwal, 1999), and the potential of silybinin combined with silychristin to improve the nephritic responses induced by chemical injury (Sonnenbichler *et al*., 1999). The silymarins most likely act as phase II enzyme inducers. Edwards *et al*. (2000) showed that silybinin increased glutathione levels in liver tissues of ethanol-fed rat pups, while decreasing levels of gamma glutamyl transpeptidase (GGTP). Hence, evidence demonstrating that the silymarins are potent compounds is accumulating.

In a previous study, two American off-the-shelf milk thistle products were extracted and analysed for their silymarin content (Wallace *et al*., 2003a). The results showed that the two brands contained variable amounts of silymarin ranging from 56.4 to 152 mg of silymarin/recommended daily dosage. Milk thistle products are not the only dietary supplements to vary in the quantities of active ingredients: De Los Reyes and Koda (2002) showed inconsistencies in the hyperforin and hypericin contents of St. John's Wort products, while *Ephedra* dietary supplements contained variable amounts of ephedrine (Gurley *et al*., 2000). The variability of active compounds in dietary supplements is alarming, especially when it is considered with respect to the spectrum of possible herb drug interactions reported by Gurley *et al*. (2002).

In order to reduce the pervasive inconsistencies reported with dietary supplements, a thorough understanding of the extraction step is necessary. This would provide valuable information to supplement manufacturers, while also providing information for compound identification and purification. For milk thistle, Benthin *et al*. (1999) suggested a two-step extraction process involving the defatting of the seed meal for 24 h with a non-polar solvent (such as petroleum ether) to remove the approximate 25% lipid content, followed by extracting the desired silymarins from the defatted seed meal with methanol. Although the use of milk thistle seed teas has been reported in folklore literature, the authors are not aware of any scientific reports pertaining to milk thistle seed extraction in water other than our own

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(Alvarez Barreto *et al*., 2003). Other investigations into the use of hot liquid water as an extraction solvent are emerging. Basile *et al*. (1998) described the superheated water extraction of savory and peppermint, while Kubátová *et al*. (2001) showed that the extraction of peppermint compounds using (hot/liquid) water at 175°C was performed in 15 min, as compared with 4 h with hydrodistillation.

The overall goal of this research programme is to extensively characterise the unit operation of extraction using silymarin recovery from milk thistle seed meal as a model system. This characterisation will yield valuable information necessary for standardising process manufacturing of dietary supplements. In addition, the results could be used in developing technology for the production of g quantities of single components necessary for nascent herb-drug interaction studies. The goal of this paper is to present results from a solvent survey for the extraction of silymarins from defatted and whole milk thistle seed meal.

EXPERIMENTAL

Extraction experiments. Milk thistle seeds were purchased from Frontier Herbs (Norway, IA, USA) and ground with a coffee grinder to an average diameter of 0.4 mm as in ASAE standard no. S319.1 (Wallace *et al*., 2003a). Whole seeds refer to ground seeds that were not previously extracted with petroleum ether: defatted seeds refer to whole ground seeds refluxed in a Soxhlet apparatus with petroleum ether for 24 h. All extraction experiments were performed with 2 g of ground seed. For all organic solvent extraction experiments, samples were taken in triplicate over a 10 h time period. Water extraction experiments were carried out over 17 h to minimise differences between extraction systems. Time zero was arbitrarily set as the time when the solvent boiled or when the solvent temperature equilibrated at the set experimental temperature.

The experiments were performed in triplicate for all temperatures and solvent conditions. As an example, each experimental condition was performed in three extraction vessels, and each was sampled in triplicate for every time point. Sampling consisted of taking an aliquot (1 mL) and determining its mass. For the non-boiling water experiments (50, 70 and 85°C), the aliquots were evaporated to dryness in a SpeedVac (Savant Instruments, Holbrook, NY, USA) instrument. For the 100°C water and organic solvent experiments, the aliquots were evaporated to dryness under a stream of nitrogen with low heat. To the dried residue, 1 mL of methanol was added, and the samples were mixed by vortex and centrifuged. The supernatant was filtered for HPLC analysis.

Water extraction experiments conducted at 50, 70 and 85°C were performed with 2 g of whole seed meal, contained in a cheesecloth bag, mixed with 200 mL of deionised water and placed in 500 mL bottles. The bottles were agitated in a shaking water bath (Precision Scientific, Winchester, VA, USA) set at 80 strokes/min.

For experiments with water at 100°C and with organics, 2 g of either whole or defatted milk thistle seed were placed in a cheesecloth bag, and mixed with 200 mL of organic solvent or water in a 500 mL round-bottom flask with boiling chips, or a 500 mL glass bottle with a cap to retard solvent evaporation. All boiling solvents (ethanol, methanol, acetonitrile, acetone, and water) were brought to, and held at, their respective boiling points with an electric heating mantle. Cold, liquid water was used to condense the vapour and to provide total reflux. It is important to note that the experiments performed with water at 50, 70 and 85°C were carried out in vessels different from those of the 100°C experiments. However, the 17 h extraction time used in water experiments minimised the differences in the extraction systems. All 60°C solvent extractions were carried out in a shaking water bath with the stroke speed set to 60 strokes/min. The calculated concentrations of silymarin for whole seed experiments were scaled to adjust for their 25% lipid content, and all values are reported in units of mg compound/g defatted seed.

Chemical analysis. The silymarin concentrations were determined by HPLC using a Waters system (Milford, MA, USA) composed of an Alliance 2690 separations module and a 996 photodiode array detector (PAD) controlled by Millennium^{32} chromatography software (Wallace *et al*., 2003a). Separation of the silymarin compounds was accomplished using a Waters Symmetry® C_{18} pre-column connected in series to a Waters Symmetry C_{18} column (150 × 4.6 mm i.d.; 5 μm), both maintained at 40°C. A sample volume of 10 μL was injected. The mobile phase consisted of methanol–water (20:80; solvent A) and methanol–water (80:20; solvent B). Elution commenced at 85:15 (A:B), was held isocratic for 5 min, followed by a linear gradient to 45:55 over 15 min; followed by 45:55 isocratic for 20 min, and returning to 85:15 over 10 min. The flow rate was 0.75 mL/min, and the silymarin compounds were monitored at 290 nm. Peak identification was confirmed by mass spectrometry (Wallace *et al*., 2003a). Calibration curves were prepared with silybinin and hesperetin from Sigma (St. Louis, MO, USA), taxifolin from Extrasynthese (Lyon, France) and silychristin and silydianin from PhytoLab (Hamburg, Germany).

When a solution of silymarin (Sigma) spiked with reference standards of taxifolin, silychristin, silydianin and silybinin was analysed by HPLC, it was observed [Fig. 1(a)] that the silydianin peak co-eluted with an unknown compound. Silydianin was, therefore, excluded from the analysis in some cases. No standard was available for the isosilybinin compounds, and thus these compounds were also excluded from the analyses. The silybinin standard (Sigma) contained two distinct peaks, which are hereafter referred to as silybinin A (first peak) and silybinin B (second peak). Mass spectrometry confirmed identical fragmentation patterns for these peaks, indicating the presence of one of four possible diasteromers.

RESULTS AND DISCUSSION

HPLC analysis

Figure 1(a) shows the chromatogram obtained after injection of 50 μL of a silymarin solution (1 mg/mL; Sigma) combined with 50 μL of a combination solution of taxifolin, silychristin, silydianin, silybinin and hesperetin

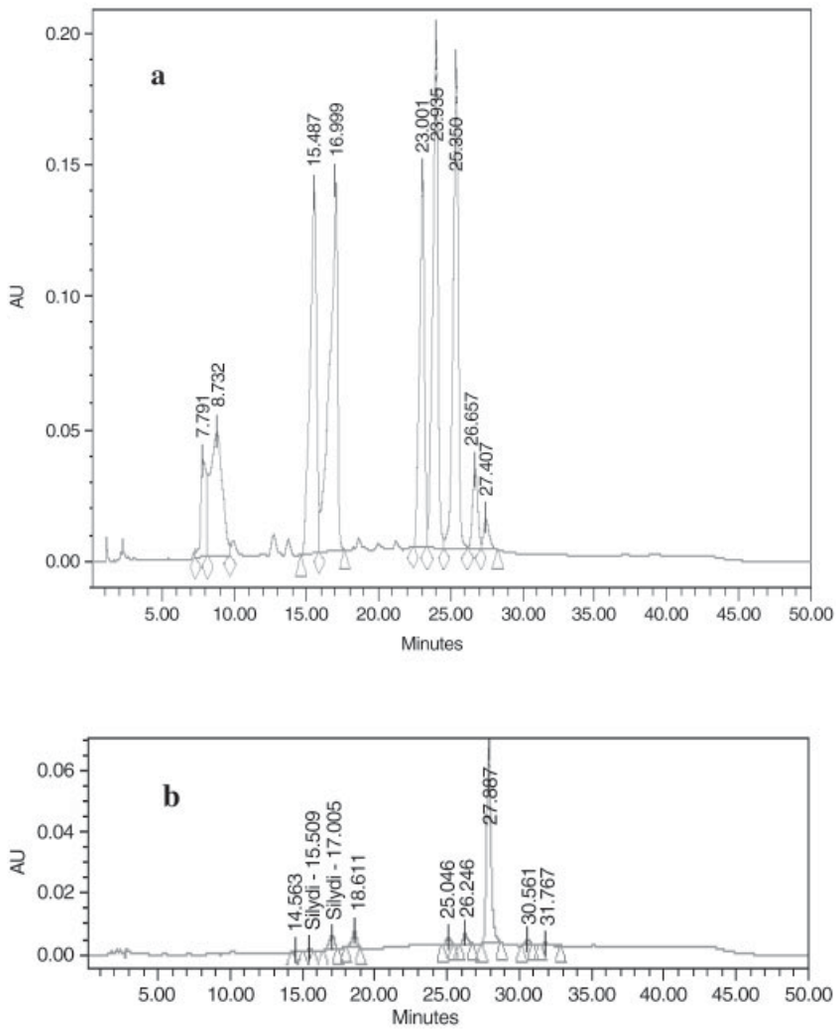

Figure 1. HPLC chromatograms of (a) a solution of silymarin (Sigma) spiked with hesperetin (retention time 25.4 min), taxifolin (8.7 min), silychristin (15.5 min), silydianin (16.9 min) silybinin A (23.0 min) and silybinin B (23.9 min), and (b) the extract of a product containing little silymarin spiked with the internal standard hesperetin (27.0 min). (For chromatographic protocol see Experimental section.)

(each 1 mg/mL). The peaks for taxifolin, silychristin, silybinin A, silybinin B and the internal standard hesperetin appeared at retention times 8.7, 15.5, 23.0, 24.0 and 25.4 min, respectively, with hesperetin eluting between the silybinin and the isosilybinin diastereomers. Linear calibration curves were generated for all five compounds over the concentration range 0.01–1.0 mg/mL and gave correlation coefficients ranging from 0.96 to 0.99 (data not shown). The HPLC method was tested on commercial milk thistle products: Fig. 1(b) shows the extraction of a product that contained little silymarin but the internal standard is clearly visible, thus confirming the value of hesperetin as internal standard.

Extractions with boiling solvents

Extraction of whole versus defatted seeds. The referenced procedure for the extraction of silymarin requires a two-stage process in which the seed meal is first defatted with an organic solvent (Benthin *et al.*, 1999). It was first desired to determine if this defatting step was essential as it is time-consuming (requires 24 h). The yields of taxifolin, silychristin, silybinin A and silybinin B as a function of extraction time, following extraction of whole and defatted seeds with boiling ethanol (79°C), are shown in Fig. 2(a–d) (yields presented as mg of product obtained/g defatted seed). The trend observed was a general increase in yield with time, followed by either a levelling off or a decrease in yield, perhaps due to compound degradation. For all of the compounds, defatted seeds produced higher concentrations of the silymarins than whole seeds extracted under similar conditions, corroborating the importance of using the two-step extraction process of Benthin *et al.* (1999). Extraction of defatted seed meal with boiling ethanol gave a maximum of 0.62, 3.89, 4.04 and 6.86 mg/g defatted seed for taxifolin, silychristin, silybinin A and silybinin B, respectively. Whole seeds extracted with boiling ethanol yielded 0.08, 2.0, 1.5 and 2.6 mg/g defatted seed for taxifolin, silychristin, silybinin A and silybinin B, respectively. When using defatted seed meal and ethanol, the yields of taxifolin, silybinin A and silybinin B were, respectively, 6.8-, 0.95-, 1.7- and 1.6-fold higher than when using whole seeds.

Water is attracting industrial attention as an extraction solvent and was thus tested for its ability to recover silymarin from whole and defatted milk thistle seeds.

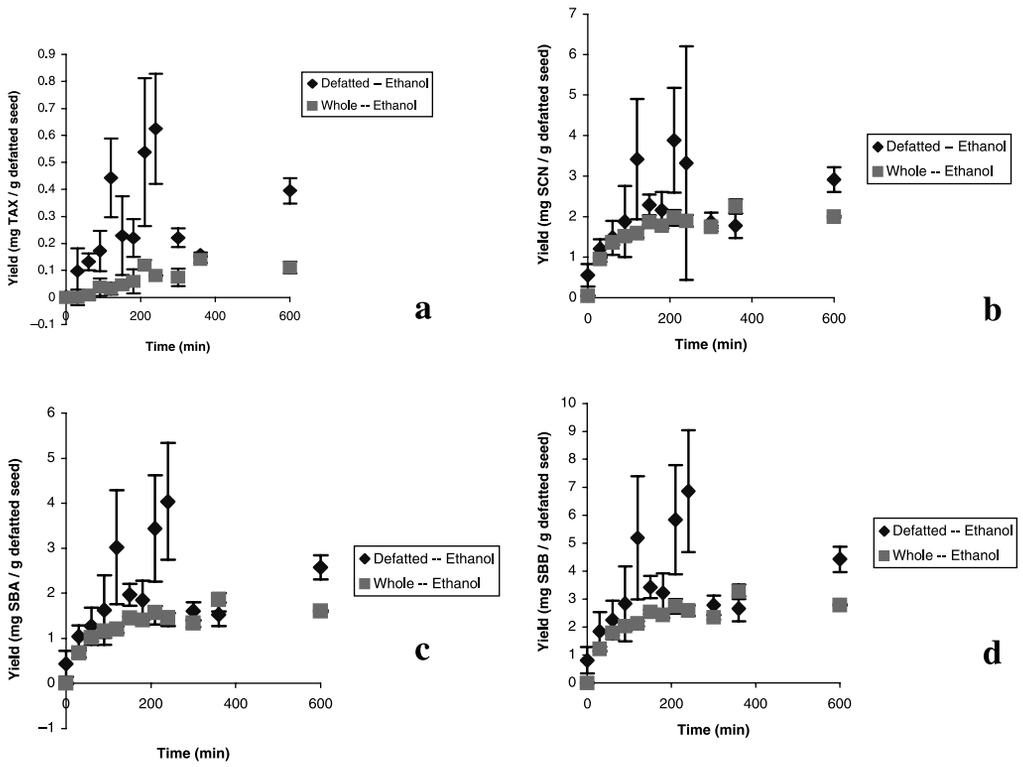

Figure 2. Recovery yields of (a) taxifolin (TAX), (b) silychristin (SCN), (c) silybinin A (SBA) and (d) silybinin B (SBB) from whole and defatted seeds of milk thistle extracted with ethanol at its normal boiling point (79°C).

Extraction experiments were carried out using boiling water (100°C) and yields determined as a function of time [Fig. 3(a–d)]. Surprisingly, extraction of whole seeds with water at 100°C gave higher yields of silymarin than were obtained from defatted seeds. Defatted seeds also showed some compound degradation arising from the heat applied by the boiling solvent and from the heat applied during post-extraction solvent evaporation. Interestingly, extraction curves measured using whole seeds did not show this same degradation and this may account for the higher yields obtained from whole seeds. The yields of taxifolin obtained from defatted seeds were comparable with, if not higher than, those obtained from whole seeds. The yields of silychristin, silybinin A, and

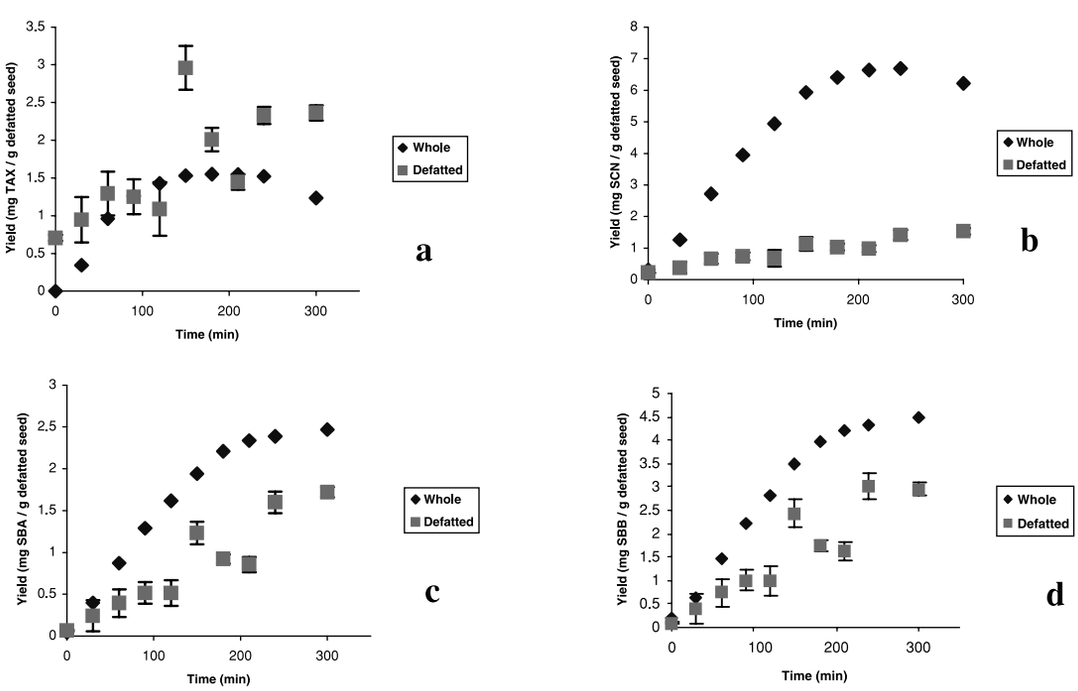

Figure 3. Recovery yields of (a) taxifolin (TAX), (b) silychristin (SCN), (c) silybinin A (SBA) and (d) silybinin B (SBB) from whole and defatted seeds of milk thistle extracted with water at its normal boiling point (100°C).

silybinin B from whole seeds were 6.7, 2.5 and 4.5 mg/g defatted seed, respectively. Yields of silychristin, silybinin A and silybinin B were 380, 47 and 50% higher from whole seeds extracted with boiling water than from defatted seeds. These results suggest that pre-treatment strategy and solvent choice are interdependent since the opposite trend was observed when extracting with ethanol.

Recoveries of silymarin using organic solvents. Previous studies have shown that ethanol or methanol can be employed to extract silymarin from defatted seed meal of milk thistle (Tittle and Wagner, 1978; Benthin *et al*., 1999). In order to determine the efficiency of various organic solvents for this extraction, a survey study was carried out with three additional solvents. The recoveries, as a function of extraction time, of taxifolin, silychristin, silybinin A and silybinin B from defatted seeds following extraction with boiling methanol (65°C), boiling acetonitrile (82°C) and boiling acetone (56°C) are presented in Fig. 4(a–d) and, for the sake of comparison, data from water and ethanol extraction are also included. For each of the solvents, the yield of silymarin increased as a function of time and reached a maximum at about 400 min of extraction. With the exception of taxifolin and silychristin, ethanol was the preferred extraction solvent. After ethanol, the decreasing order of preferred organic solvents for the extraction of silydianin and silybinin was methanol, acetonitrile and acetone. The maximum yields of taxifolin obtained from defatted seed following extraction with ethanol, methanol, acetonitrile and acetone were 0.6, 0.3, 0.2 and 0.1 mg/g defatted seed, respectively. Following extraction with water at 100°C, the maximum yield of taxifolin extracted was 1.5 mg/g defatted seed, this being 2.5 times greater than that obtained with ethanol. Silychristin was also preferentially extracted by boiling water with a maximum yield of 6.3 mg/g defatted seed: lower yields of 4.0, 2.1, 1.6 and 2.5 mg/g defatted seed were recorded for extraction with ethanol, methanol, acetonitrile, and acetone, respectively. The extraction of whole seeds of milk thistle with water at 100°C produced a ca. 70% increase in yield of silychristin yield compared with ethanol extraction. The highest yields of silybinin A were 4.0, 1.9, 1.7 and 2.0 mg/g defatted seed following extraction with ethanol, methanol, acetonitrile and acetone, respectively. In water at 100°C, the recovery of silybinin A reached 2.1 mg/g defatted seed, which is similar to those obtained with acetone, acetonitrile and methanol. Silybinin B yields with ethanol, methanol, acetonitrile and acetone were 7.0, 3.3, 3.3 and 2.9, respectively, but attained a maximum of 4.5 mg/g defatted seed with water at 100°C. Examination of all of the extraction yields shows that water extraction offers the highest potential for the more polar compounds, such as taxifolin and silychristin, while ethanol yields the highest recoveries of the silybinin diastereomers. When extracting the defatted milk thistle seeds with water or with ethanol, decreases in yields were observed after 180 min and above of extraction time. Interestingly, this trend was not observed when extracting with acetone, acetonitrile or methanol.

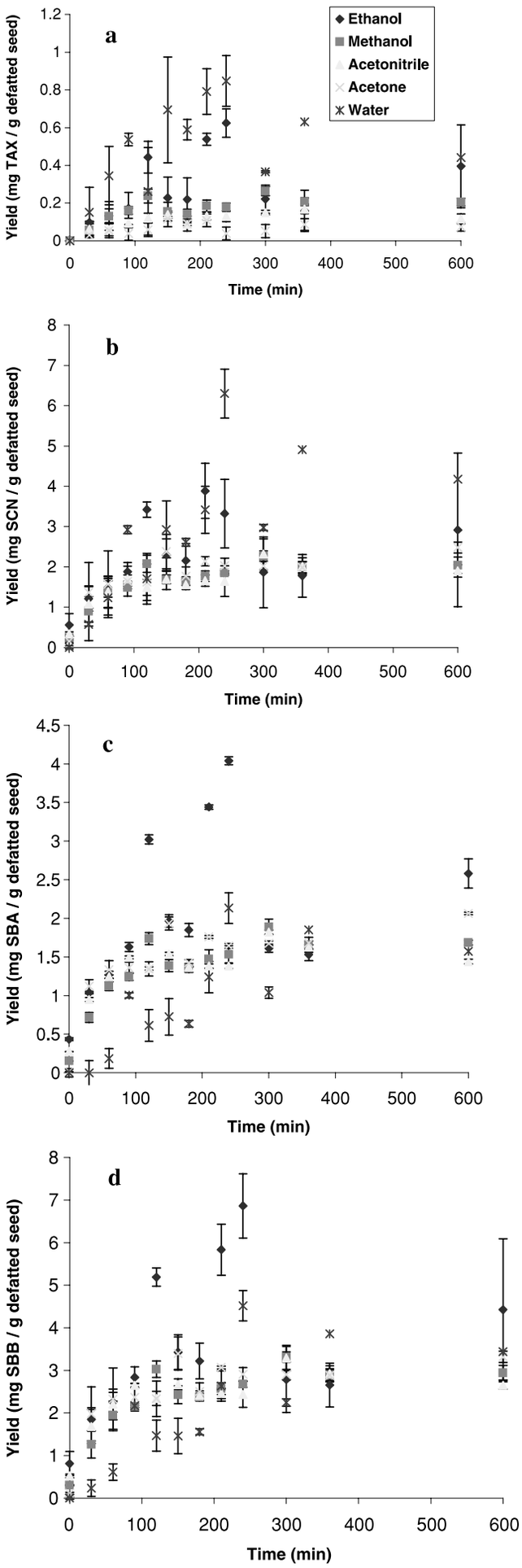

Figure 4. Recovery yields of (a) taxifolin (TAX), (b) silychristin (SCN), (c) silybinin A (SBA) and (d) silybinin B (SBB) from defatted seeds of milk thistle extracted with various solvents at their normal boiling points.

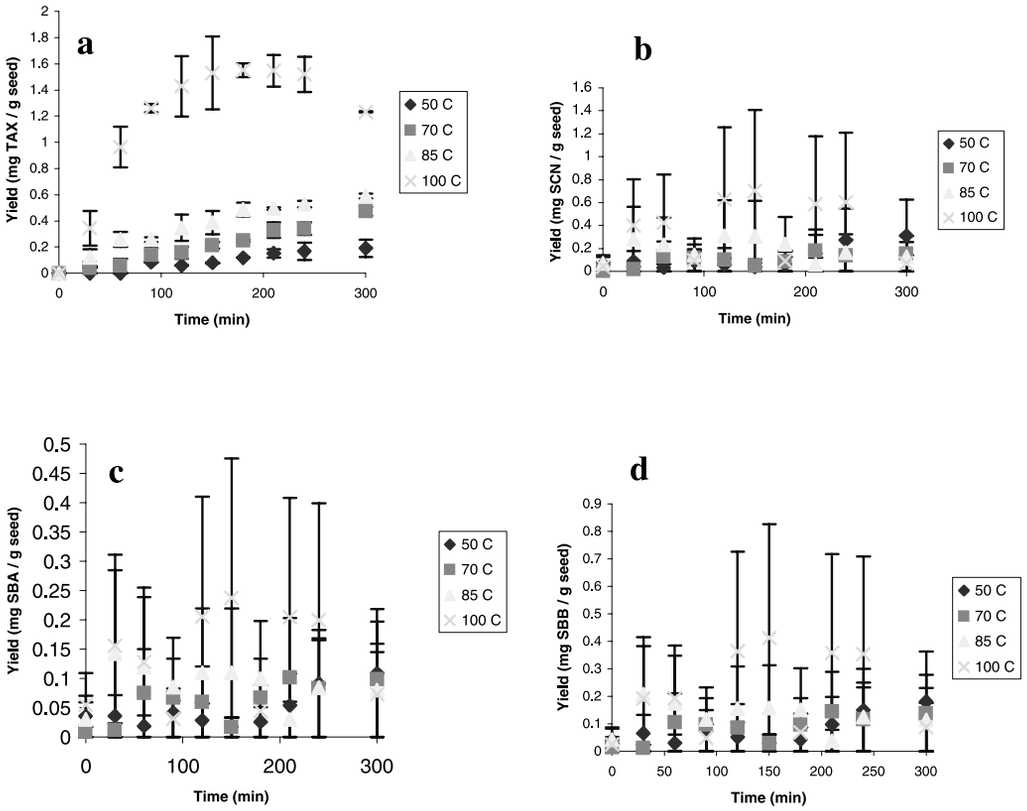

Figure 5. Recovery yields of (a) taxifolin (TAX), (b) silychristin (SCN), (c) silybinin A (SBA) and (d) silybinin B (SBB) from whole seeds of milk thistle extracted with water at different temperatures.

Extraction of silymarin with water at 50, 70, 85 and 100°C

In order to determine if water at temperatures lower than 100°C could be a useful extraction solvent for whole seeds, experiments were performed at 50, 70 and 85°C. The recoveries of taxifolin, silychristin and the silybinins as a function of time results are shown in Fig. 5(a–d). Of the four temperatures tested, water at 100°C yielded the highest levels of silymarin, while water at 50°C generated only about 25% of those yields. The levelling of yields as observed at 100°C did not occur at lower temperatures but instead a slow increase in yield with time was observed. The use of water was consistent with the findings of Basile *et al*. (1998) and Kubátová *et al*. (2001), which indicated that water could only be used as a solvent for some natural products, such as rosemary and peppermint. Work is currently underway to determine if higher water temperatures would improve extraction yields.

Extraction of defatted and whole seeds with solvents at 60°C

Extraction with water. Water extractions at 60°C produced relatively low recoveries from both whole and defatted seeds [Fig. 6(a–d)]. The yields of taxifolin and silychristin were highest with whole seeds [Fig. 6(a, b)], confirming the previous results of Alvarez *et al*. (2003). The silybinins were best recovered in water at 60°C from defatted seeds [Fig. 6(c, d)], whereas in boiling water whole seeds yielded slightly more of the silybinin diastereomers. From whole seeds extracted with water at 60°C, yields were approximately 1.9, 0.9, 0.7 and 1 mg/g defatted seed, respectively, for taxifolin, silychristin, silybinin A and silybinin B. Defatted seeds extracted with water at 60°C yielded 1.4, 0.6, 0.6 and 0.9 mg/g defatted seed, respectively, of taxifolin, silychristin, silybinin A and silybinin B. In boiling water, yields from whole seeds were 0.85, 6.3, 2.1 and 4.5 mg/g defatted seed for the mentioned extractants, respectively. Although boiling water showed good potential for the extraction of silymarin from milk thistle whole seed meal (Wallace *et al*., 2003b), water at sub-boiling temperatures did not prove to be an efficient choice of solvent compared with ethanol or methanol.

Extraction with methanol and ethanol. Figure 7(a, b) shows the yields of taxifolin obtained following extraction with ethanol and methanol, respectively, at 60°C. Substantially higher yields of all of the silymarins were obtained when extracting either whole or defatted milk thistle seed meal with these solvents at 60°C compared with extraction with water, boiling ethanol or boiling methanol. At an extraction time of 300 min, taxifolin recoveries in 60°C methanol were 4.0 and 3.4 mg/g defatted seed from whole and defatted seed meal, respectively [Fig. 7(a)]. Extractions with 60°C ethanol, also at 300 min, yielded 3.5 and 4.6 mg/g defatted seed, from whole and defatted seeds, respectively [Fig. 7(b)]. Interestingly, this maximum yield of 4.6 mg/g defatted seed of taxifolin is 6.4-times greater than the maximum value obtained with boiling ethanol. In 60°C methanol, the maximum yield of silychristin was reached at 600 min and was approximately 4.8 and 4.6 mg/g defatted seed for whole and defatted seed meal, respectively [Fig. 8(a)].

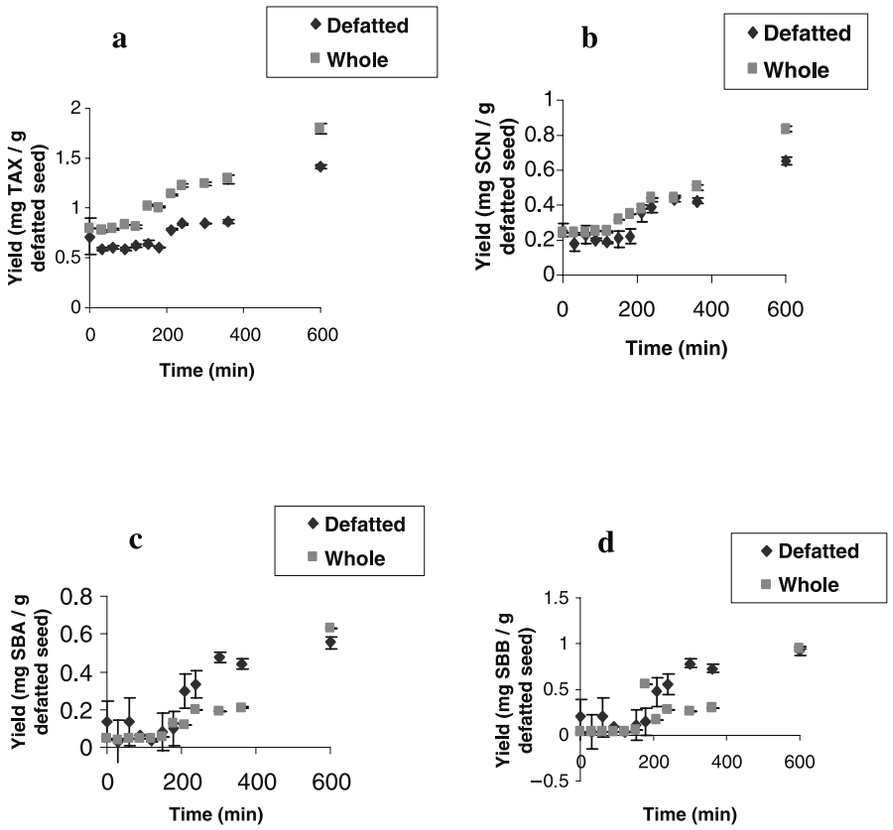

Figure 6. Recovery yields of (a) taxifolin (TAX), (b) silychristin (SCN), (c) silybinin A (SBA) and (d) silybinin B (SBB) from whole and defatted seeds of milk thistle extracted with water at 60°C.

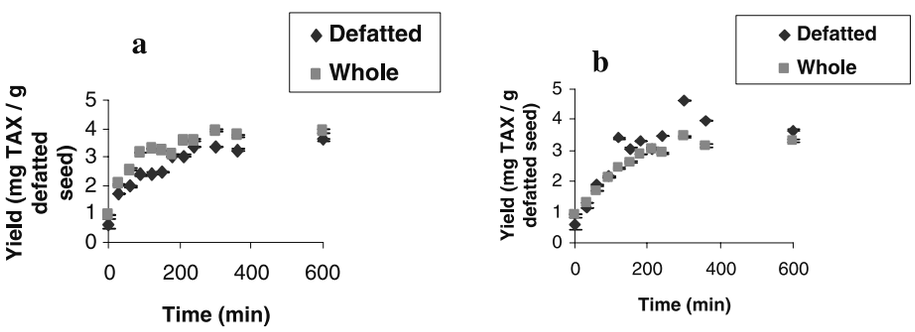

Figure 7. Recovery yields of taxifolin (TAX) from whole and defatted seeds of milk thistle extracted with (a) methanol at 60°C, and (b) ethanol at 60°C.

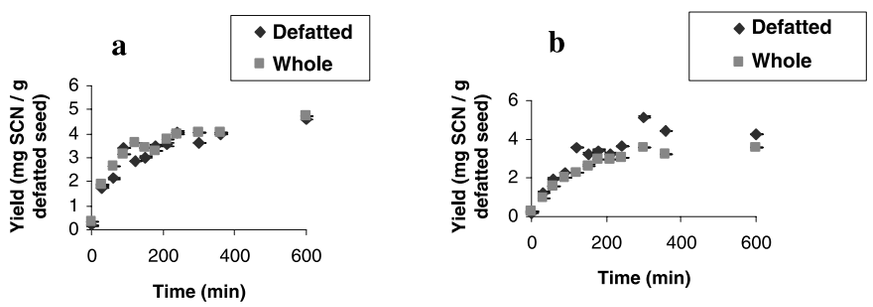

Figure 8. Recovery yields of silychristin (SCN) from whole and defatted seeds of milk thistle extracted with (a) methanol at 60°C, and (b) ethanol at 60°C.

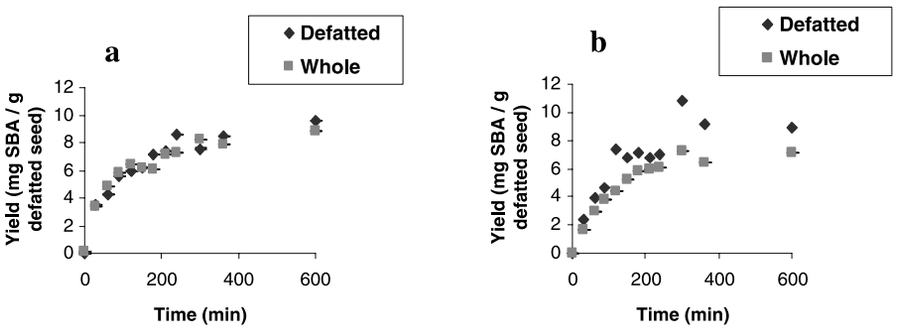

Figure 9. Recovery yields of silybinin A (SBA) from whole and defatted seeds of milk thistle extracted with (a) methanol at 60°C, and (b) ethanol at 60°C.

When extracting with ethanol [Fig. 8(b)], silychristin yields were highest at 300 min and reached 5.2 and 3.6 mg/g defatted seed from defatted and whole seeds, respectively. Silychristin could be extracted in very similar yields from all solvents, temperatures and seed types: an average value of 5.0 mg/g defatted seed was attained in most cases. These yields of silychristin are similar to those obtained following extraction of milk thistle whole seed meal with water at 100°C (approximately 5.0 vs. 6.7 mg, both per g defatted seed). Yields of silybinin A were also similar in whole and defatted seeds extracted with either methanol or ethanol [Fig. 9(a, b)]. In methanol, yields were highest after 600 min of extraction and reached a plateau at 9.6 mg of silybinin A/g of defatted seed. In ethanol, maximum yield of silybinin A occurred at 300 min and reached 11 mg/g defatted seeds. In experiments involving boiling solvents, the maximum level of silybinin A recovered was 4 mg/g defatted seed, which is 1.7-fold lower than the yield obtained in the 60°C experiments.

Silybinin B was recovered at the highest levels for both seed types [Fig. 10(a, b)] and reached a maximum of 16 mg/g defatted seed at an extraction time of 600 min in methanol, and 18.3 mg/g defatted seed in ethanol, from defatted seeds in both cases. Experiments with ethanol at sub-boiling temperatures gave results for silybinin A that were similar to those for silybinin B, i.e. solvent at 60°C produced a 1.7-fold increase in recovery compared with boiling ethanol. The recoveries of taxifolin and the silybinin diastereomers were significantly higher when extracting with ethanol or methanol at 60°C compared with yields obtained using boiling solvents. These data possibly provide an explanation for the lower silymarin recoveries with boiling solvents as extracting at lower temperatures probably reduces compound degradation. The curves of recovery concentration vs. time involving solvents at 60°C were smooth and recoveries generally increased as a function of time, whereas extractions with boiling solvents showed inconsistencies in concentrations as time progressed.

Compound ratios

A useful way to compare extraction data for differing solvents is by examining the ratios of compounds extracted. Table 1 presents the ratios of taxifolin to silybinin B, silychristin to silybinin B and silybinin A to silybinin B following extraction with boiling water and boiling ethanol with whole and defatted seeds, and for extractions with boiling methanol, acetonitrile and acetone with defatted seeds. All ratios were calculated as averages with data determined at the 240 min time point from three experiments, each with three replicates. The recovery ratios of taxifolin to silybinin B were highest when using defatted seeds with water at 100°C and attained a maximum value of 0.77 mg/mg (Table 1). Whole seeds extracted with boiling water also produced a high taxifolin to silybinin B ratio of 0.35. The best recovery ratio for silychristin to silybinin B was obtained when extracting whole seeds in water, reaching a value of 1.55 mg/mg, indicating that water is a more efficient extraction solvent for the more polar silymarins taxifolin and silychristin. The extraction of defatted seeds in methanol also resulted in a high silychristin to silybinin B ratio of 1.22 mg/mg. With the exception of defatted meal extracted in methanol, all extraction conditions yielded a similar silybinin A to silybinin B ratio (average 0.55 mg/mg). With methanol, the ratio of silybinin A to silybinin B was the highest at 1.00 mg/mg.

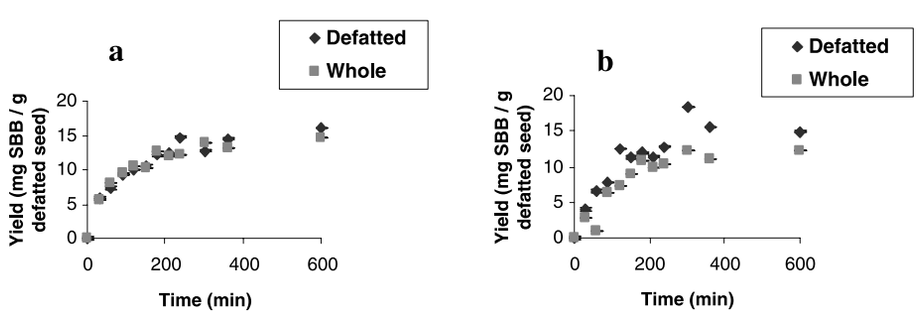

Figure 10. Recovery yields of silybinin B (SBB) from whole and defatted seeds of milk thistle extracted with (a) methanol at 60°C, and (b) ethanol at 60°C.

Table 1. The ratios of taxifolin, silychristin and silybinin A to silybinin B extracted following 240 min of extraction using the stated solvents at their normal boiling points

Solvent (seed meal)	Taxifolin/Silybinin B[a]	Silychristin/Silybinin B[a]	Silybinin A/Silybinin B[a]
Water (defatted)	0.77	0.46	0.53
Water (whole)	0.35	1.55	0.55
Ethanol (defatted)	0.09	0.60	0.58
Ethanol (whole)	0.04	0.73	0.56
Methanol (defatted)	0.06	1.22	1.00
Acetonitrile (defatted)	0.05	0.66	0.57
Acetone (defatted)	0.03	0.70	0.57

[a] Ratios calculated from the average of three experiments, each with three replicates ($n = 9$).

Depending on which silymarin compounds are targeted, both water and ethanol can be effective extraction solvents. Boiling water is more efficient for extracting the more polar silymarins, such as taxifolin and silychristin, than any of the organic solvents examined. If the targeted compounds are the silybinins, however, ethanol is the preferred extraction solvent providing silybinin yields of 2–2.5 times greater than those obtained with water. On the other hand, if it is desired to omit the defatting pre-treatment step for processing simplicity, boiling water is as efficient as ethanol for extracting the silybinins from ground whole seeds. Parallel studies aimed at characterising pre-treatment schemes and at examining the effect of temperature on extraction solvents are currently underway.

The differences in recoveries with boiling solvents compared with sub-boiling solvents were notable. Taxifolin yields were approximately 70% higher with ethanol at 60°C than with water at 100°C, which were the solvents of choice for this research and for research conducted with boiling solvents, respectively. Silychristin yields were nearly equal for extractions with 60°C ethanol, boiling ethanol and boiling water. The silybinin diastereomers were preferentially extracted with sub-boiling ethanol and methanol, yielding >60% more silybinin B in 60°C ethanol than in boiling ethanol. These findings suggest that the silymarins are extremely sensitive to heat. Operating at a temperature slightly below the boiling temperature of the organic solvent can increase extract concentrations greatly. The demonstration of heat sensitivity of the flavanolignans implies important consequences for post-extraction handling procedures. Processing milk thistle extracts under high-heat conditions may also destroy more than half of the silymarin content of a product.

The results of this study emphasise the importance of extraction studies for herbal medicines. Seed type and variability, solvent choices and critical temperatures can severely affect a milk thistle product. Water was a fair extraction solvent for milk thistle and was more efficient for targeting taxifolin or silychristin at its boiling temperature. Water, being cheap, non-toxic and easy to dispose of, is the ideal solvent for extracting products regarded as food supplements. Although recovery yields in the alcohols, especially at 60°C, were not markedly different between whole and defatted seed meal, it may be worthwhile to carry out industry-scale extractions with defatted seeds, as these extractions favour compounds that are presently the most sought after in medicine. The results of this study are consistent with the results of Basile *et al*. (1998) and Kubátová *et al*. (2001) in that water can be used as a solvent for natural products. Methanol and ethanol were shown to be preferred solvents for extracting the silybinin flavanolignans, and yielded higher quantities of these compounds when maintained at 60°C.

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