Value-added Products From Milk Thistle "Waste"

At present, only a small component of the milk thistle plant (silymarin from the seed) has market value. The seed contains significant qualities of oil - yet this oil is presently regarded as a waste product. The plant also produces a huge amount of biomass - another waste product unless alternate uses are discovered. Potential uses of the waste biomass are animal fodder or alternatively a starting point for biofuel - especially as the stems and leaves are rich in high energy latex compounds. These latex compounds may also have value as a feedstock in the manufacture of industrial materials … like rubber and latex. At present, there are no crops being grown for latex in Canada - but there is demand for latex as the starting point in the manufacture of rubber and other materials. The potential to extract products of potential use as fuel, food, fodder and medicine from a single easy to grow plant would appear to make milk thistle an ideal model for of a future bio-based economy.

This project sought to identify potential value in the "waste products" generated by the milk thistle crop.

1.0 Recovery of Value-added Products from Milk Thistle

1.1. Seed oil

The research literature indicates that milk thistle seed contains 12-26% fixed oils, depending on the genotype and the growing conditions. At present, these oils represent a waste product which is discarded following silymarin extraction from the seed.

This project evaluated the oil concentration, composition, total yield and potential value of the oils recovered from the newly developed milk thistle lines being commercialized by the SHSA.

Experimental design
- Seed source - SHSA lines generated in agronomy trials
  - Variables examined
    - - Total oil content
    - - Oil composition
    - - Impact of agronomic variables and site/growing season on oil yields

Milk Thistle seed samples covering the previously outlined range of variables (cultivar, site of production, year of production) were sent to Dr. Martin Reaney of the Dept. of Applied Microbiology and Food at the University of Saskatchewan for analysis.

Oil was extracted from small samples of milk thistle seed using a Goldfisch extraction apparatus and the oil composition was than tested via NMR.   A cold press was used to extract the oil from larger (1 kg) samples of a limited number of seed lots.

Averaged over the cultivars tested, seed oil content was lower in 2004 (20.2%), than in 2005 (25.2%), or 2006 (24.9%)(Table 1.1). The low oil content in 2004 likely reflects the immature stage of the crop when hit by an early killing frost. The 2005 and 2006 growing seasons were much more favorable, leading to higher yields of more mature seed with a higher seed oil content. Differences in seed coil content between cultivars were not consistent from year to year. Oil yields from cold press extraction were comparable to the yields obtained using the Goldfisch apparatus (Table 1.1).

Table 2.1.   Seed oil content for various milk thistle lines in 2004-2006.

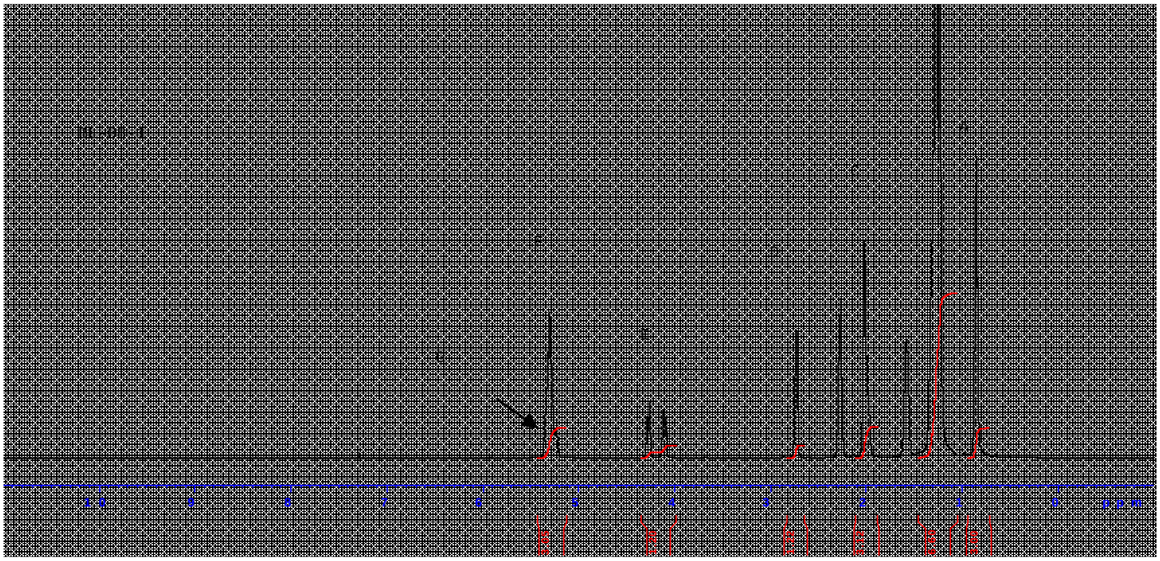
Sample ID	**% Oil Content**
Richter's (2004)	20.77
Austra Hort. Holland (2004)	18.44
Kalyx Holland (2004)	24.55
Bolier Holland (2004)	22.58
Cruz. E. Europe (2004)	17.3
Richter's (2005)	25.21
Austra Hort. Holland (2005)	25.01
Kalyx Holland (2005)	24.94
Bolier Holland (2005)	25.86
Austra Hort. (2006)	23.73
Bolier (2006)	26.18
Austra Hort. (2004) 1kg	20.53
Bolier Holland (2004) 1Kg	22.67
Kalyx Holland (2005) 1Kg)	26.81

NMR analysis of the oils showed no significant differences among the samples (data not shown). The fatty acid profile of all samples was essentially similar (Table 1.2).

Table 1.2 Fatty acid profile of the oil extracted from the seed harvested from several cultivars of milk thistle in 2004-2006.

							G=E/4	F1=F-G
	A	B	C	D	E	F	G	F1
Richter's 2004	3	16.605	3.12	1.22	1.2016	3.0485	0.3004	2.7481
Austra Hort. Holland 2004	3	16.819	3.0887	1.2034	1.2161	3.005	0.304025	2.700975
Kalyx Holland 2004	3	16.953	3.1906	1.253	1.2543	3.118	0.313575	2.804425
Bolier Holland 2004	3	16.64	3.0944	1.2187	1.2262	3.0275	0.30655	2.72095
Cruz E. 2004	3	16.8	3.0829	1.2015	1.2063	2.9927	0.301575	2.691125
Austra Hort. Holland 2005	3	17.195	3.1331	1.1815	1.2405	2.9865	0.310125	2.676375
Richter's 2005	3	17.129	3.0706	1.1557	1.202	2.9553	0.3005	2.6548
Kalyx Holland 2005	3	17.021	3.0466	1.1346	1.1892	2.9125	0.2973	2.6152
Austra Hort. 2006	3	16.733	3.0806	1.2159	1.2098	3.3093	0.30245	3.00685
Austra Hort. 2004 1Kg	3	16.664	3.0597	1.1389	1.1585	2.9165	0.289625	2.626875
Bolier 2006	3	17.409	3.0263	1.038	1.2072	2.8339	0.3018	2.5321
Bolier Holland 2005	3	17.279	2.9565	1.0042	1.1806	2.7318	0.29515	2.43665

Fig 1.1. NMR analysis of the oil profile typical of Milk Thistle.



The seed oil content and fatty acid profile of milk thistle oil, along with several of the most commercially important oils used in food production are summarized in Table 1.3.

Table 1.3. Seed oil content (%) and fatty acid profile (%) of oil extracted from Milk Thistle seed and several other commercially important oil crops.

	Seed oil content	Unsat/sat ratio	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Milk Thistle	**23**	**6:1**	**9**	**5**	**23**	**57**	
Canola	43	15:1	4	2	61	21	10
Sunflower	47	7:1	7	5	19	68	
Corn	4	7:1	11	2	24	57	
Soybean	18	6:1	11	4	24	57	7

The fatty acid profile of the oil extracted from milk thistle was comparable to the oil extracted from corn, sunflower and soybean. This suggests that the oil would likely have acceptable performance characteristics for use in food processing – assuming that it does not have any unusual/undesirable flavour characteristics. However, the fact that the oil composition is comparable to mainstream commercial products like corn and soybean oil means that it will be in direct market competition with these low cost alternatives.

Based on the seed yields and seed oil content seen in this study, oil production/unit area for milk thistle is well below that of the key oil producing crops (Table 1.4). The low oil yield/unit area, coupled with the fact the fatty acid profile is not unique suggests that growing milk thistle as an oil crop is not an attractive option. However the results do suggest the potential to use the milk thistle oil that is left over from silymarin extraction as another profit stream – much like the wine industry is capturing value (grape seed oil) from the residues left from their main processing objective.

Table 1.4. Oil production/unit area for milk thistle and other oil producing crops

	Seed Yield (#/a)	Seed Oil (%)	Oil Yield (#/a)
Milk Thistle	**1000**	**23**	**230**
Canola	1500	43	645
Sunflower	1100	47	517
Corn	6700	4	260
Soybean	2500	18	450

2.1 Identification/Recovery of other useful by-products from milk thistle

Milk thistle gets its name from the “milky” exudate that oozes from any cuts to the leaves, stems or roots. The chemical composition of the milk thistle exudate is not known - but closely related species have been identified as sources of latex. The fact that milk thistle produces a large biomass with minimal management or inputs, suggests its potential efficiency as a ‘bio-factory’. As the vast majority of the milk thistle plant is, at present, effectively a waste product - any alternate uses for this waste material would represent another potential profit source.

Test material grown in 2006 and 2007 by the University of Saskatchewan was made available to Dr. John Balsevitch, G. Bishop and L. Deibert – who are natural products biochemists employed by PBI/NRC, Saskatoon.

Leaves of milk thistle were harvested just past full flowering. The leaves were allowed to air dry and processed *via* grinding and followed by extraction with 60% methanol (aq) followed by 100% methanol. A 50 g sample of dried leaf material yielded after evaporation of the combined methanolic extracts about 15 g of residue.

The combined methanolic extract was analyzed by HPLC-MS and observed to contain two main flavonoid glycuronides tentatively identified as apigenin and luteolin 7-O-glucuronides (Fig 2.2.1) based on the spectral data and a published report of similar compounds being observed in the flowers.[1]

In the samples examined the putative “apigenin” was the major flavonoid (ca. 7:3 ratio).

Processing of the extract *via* partitioning between ethyl acetate/water and butanol/ water afforded three fractions – ethyl acetate soluble (1.1 g), butanol soluble (1.8 g) and water soluble (12 g). The ethyl acetate fraction was composed of lipophilic materials including pigments and triterpenes, the butanol fraction was composed largely of the flavonoid glucuronides, while the aqueous fraction contained polar materials believed to be unidentified salts and amino acids/proteins. Samples of relatively pure flavonoids could be obtained by further processing the flavonoid containing extracts on a reverse phase column using gradient water-methanol elution.

2.2.1 Flavonoids in Milk Thistle

In general, flavonoids are widespread in the plant kingdom but glycuronide derivatives are not the most common representatives – glycosides and aglycones are much more common. Flavonoids are mostly anti-oxidants, considered to possess nutraceutical qualities, and by and large, generally recognized as safe (GRAS status). Apigenin glucuronides have previously been isolated from alfalfa leaves[2] and galacturonides identified in milk thistle flowers[1].

Searching the patent literature afforded two[3,4] patents which describe utilization of flavonoid glycuronides. In one they were observed to promote improved solubility and uptake of various drugs; in the other they were used to prolong the life of natural anthocyanin pigments used as coloring agents.

1 R = H, Apigenin 7-O-ß-D Glucuronide, MW 446
2 R = OH,Luteolin 7-O-ß-D Glucuronide, MW 462

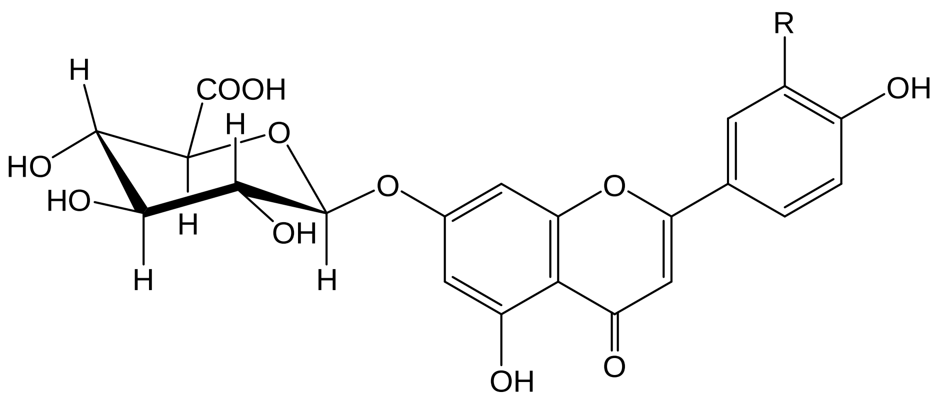


Fig. 2.2.1. Putative structures of major (1) and minor (2) flavonoids observed in air-dried mature *Silybum marianum* leaves obtained from field plants, University of Saskachewan 2006/07.

2.2.2 Non-Flavonoid Components

Examination of the hplc-ms data (see Appendix 2.1) led to the tentative identification of triterpenes having a molecular weight of 456, possibly ursolic and oleanolic acids. Previously reported[5] oxygenated triterpenes were not observed. The leaves are also considered a rich source of amino acids (both free and in proteins, (14%) as well as magnesium and potassium (source: http://www.ars-grin.gov/cgi-bin/duke/farmacy2.pl) and are considered edible on removal of the spiny thorns. The bulk of the extract was the water soluble material which, based on the above report, was likely a mixture of salts, amino acids, proteins, and possibly polysaccharides.

2.2.3 Bioassays/Reported Activities

The major flavonoid (MW 446) was evaluated in hemolysis and apoptosis assays. It was found not to be hemolytic in sheep red blood cells (HD_{50} >100 µM) and did not cause any increase in caspase 3/7 activity or mitochondrial pertabation in PC-3 human prostate cancer cells at 50 µM (Dual Sensor MitoCasp assay using flow cytometry). Both of these assays suggest that the flavonoids have low cytotoxicity. This is further corroborated by historical usage of the leaves as a tea:

"The flowers and leaves of the milk thistle plant can also be used in an infusion. A milk thistle hot tea is used to stimulate milk production in nursing mothers, as well as to treat digestive problems." (http://www.disability-resource.com/medical-health/alternative-medicine/herbal/antioxidant-rich-milk-thistle-herbal-remedy.php). The above results in combination with reports of historical usage suggest low toxicity of the contained flavonoids. Leaf extracts have been shown to extert anti-inflammatory properties in rats.[7]

2.2.4. Conclusion: Potential Utility of Aerial Parts of Plant

Since the aerial part of the plant represents quite a large amount of biomass, suitable processing could lead to quantities of triterpenes (ursolic and oleanolic acids), potentially useful in cosmetic formulations (based on published anti-inflammatory properties), apigenin/luteolin glucuronides potentially useful in nutra- and /or pharmaceutical applications as outlined in the cited patents, and protein/amino acids potentially useful for food/feed applications.

For commercial development, nutraceutical claims for the flavonoids would have to be established and identification of salts and amino acids present in the aqueous fraction would be required.

Finally, development of milk thistle without thorns would be interesting – perhaps the plant could then be developed as a nutritious salad crop for human use or as a forage crop. Allergy considerations may temper potential food uses.

Appendix: 2.1 HPLC-MS Data for Milk Thistle Leaf Extracts

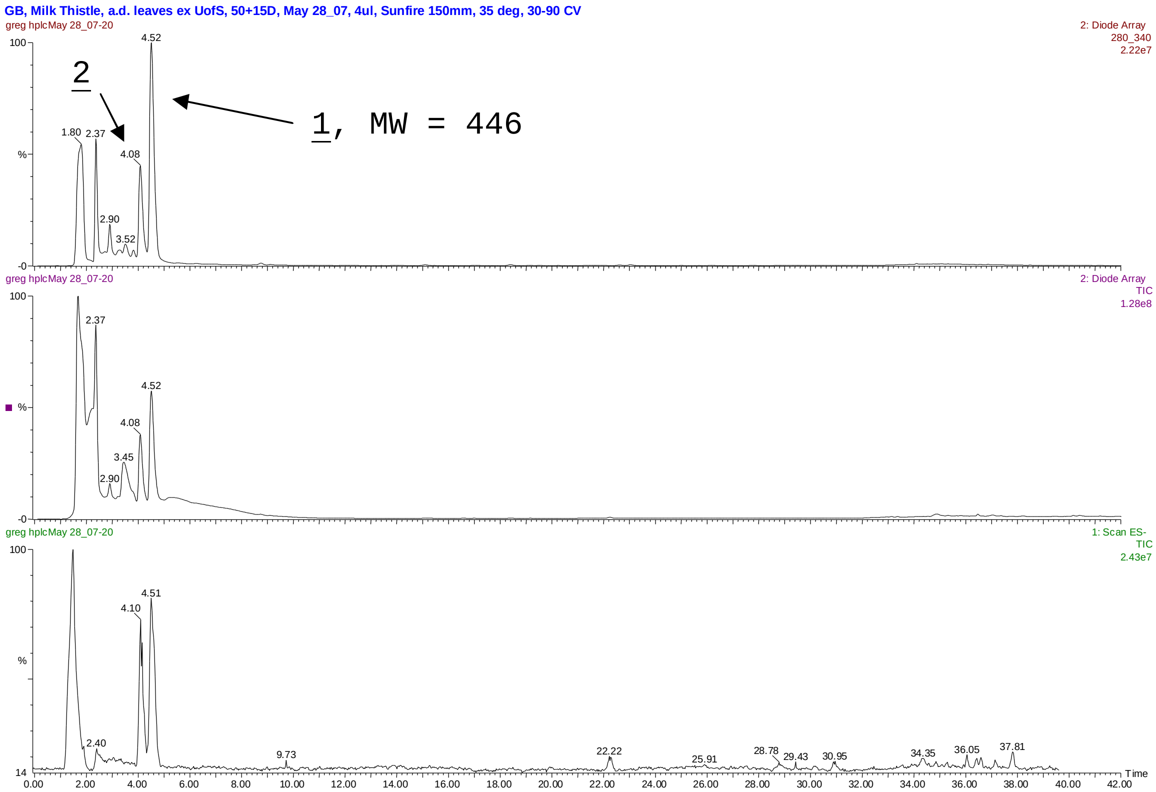


Fig. 2.2.1. HPLC profiles of extract obtained from air-dried mature leaves of milk thistle. Top: uv detection @ 280-340 nm; Middle: uv detection @ 200-400nm; Bottom: mass detection (*m/e* 100 – 1900).

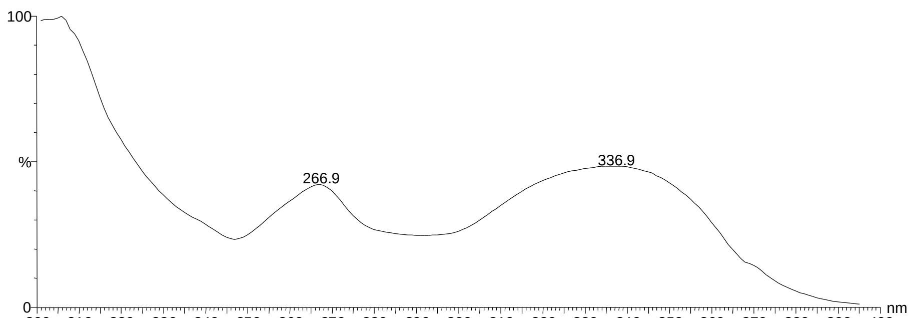


Fig 2.2.2. UV spectrum of compound 1 and 2.

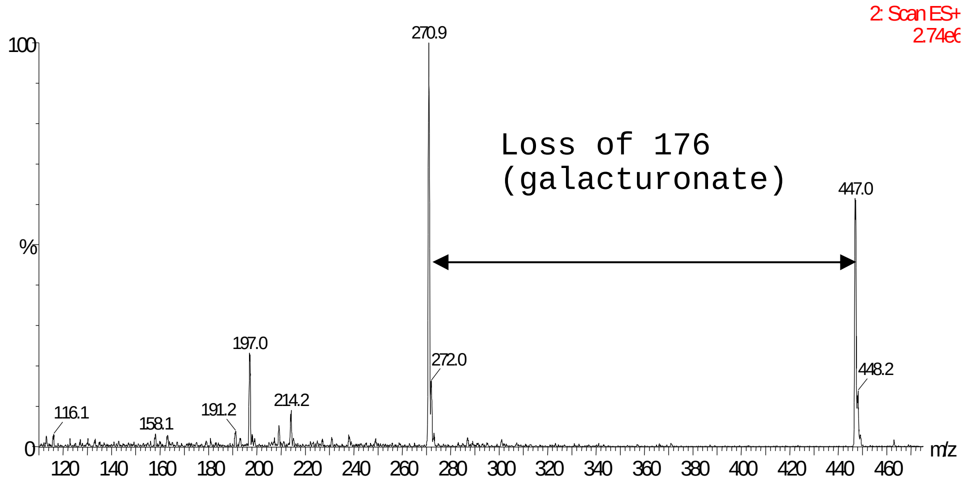


Fig. 2.2.3. Mass spectrum (electrospray, positive ion) of compound 1, showing loss of galacturonate (or glucuronate).

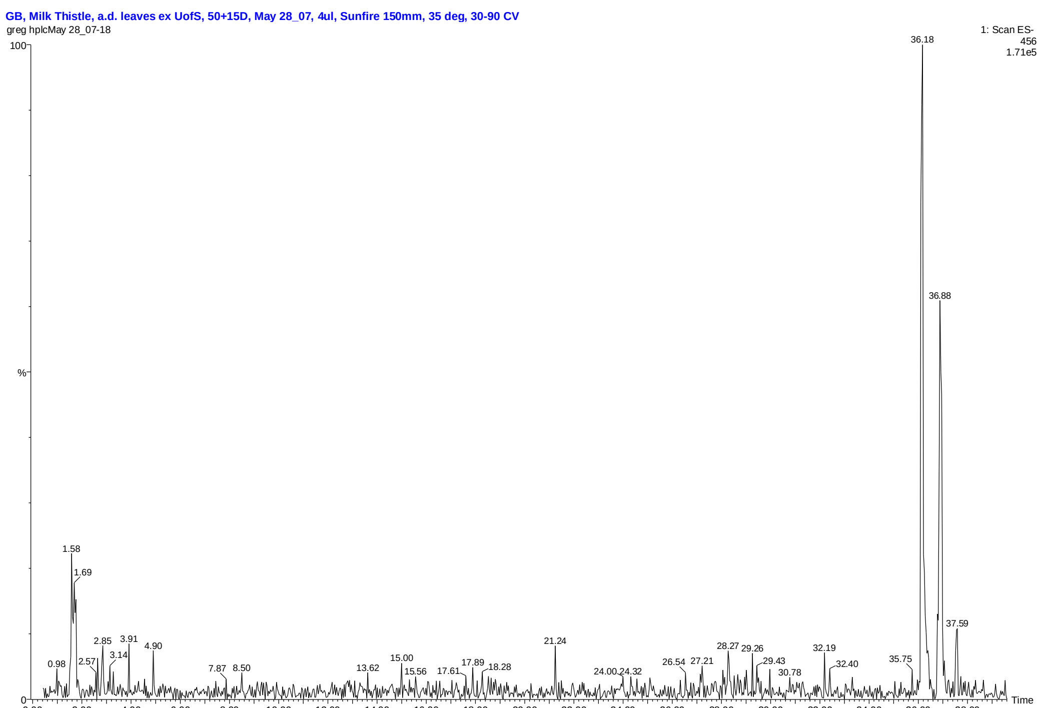


Fig. 2.2.4. Extracted ion (m/z 456) mass chromatogram of MT leaf extract. Peaks tentatively assigned as oleanolic and ursolic acids.