

Supplementary data:

Chalcone synthase genes from milk thistle (*Silybum marianum*): isolation and expression analysis

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Table 1. Primers used in molecular cloning and expression profiling of the *SmCHS* genes.

Primer name	Purpose	Sequence
Degenerate Forward Primer1 (F1)	PCR	GYGHTAYCAAGCBGATTATCC
Degenerate Forward Primer2 (F2)	PCR	CCMAAATCMAARATCACCCACC
Degenerate Reverse Primer1 (R1)	PCR	ACCCANTCYAAMCCTTCWCCG
Degenerate Reverse Primer2 (R2)	PCR	GYTTRARCTCMACCTGRTCCAG
<i>SmCHS1</i> Forward Primer 1 (<i>CHS1F1</i>)	RACE	TTCTCGTGTCTTGTG
<i>SmCHS1</i> Reverse Primer 1 (<i>CHS1R1</i>)	RACE	GTTCTTTGAAATCAAC
<i>SmCHS1</i> Forward Primer 2 (<i>CHS1F2</i>)	RACE	AGGGTTCTCGTGTCTTGTG
<i>SmCHS1</i> Reverse Primer 2 (<i>CHS1R2</i>)	RACE	CCTCTGAGTCTGGTAAGATGG
<i>SmCHS1</i> Forward Primer 3 (<i>CHS1F3</i>)	RACE	CCTGACTTGAAGACAGAGAGG
<i>SmCHS1</i> Reverse Primer 3 (<i>CHS1R3</i>)	RACE	GGAAAGTAACCGCTGTGATC
<i>SmCHS2</i> Forward Primer 1(<i>CHS2F1</i>)	RACE	TTGTTTGCTCAGAAAG
<i>SmCHS2</i> Reverse Primer 1 (<i>CHS2R1</i>)	RACE	AGTAAGTTCCAATCAC
<i>SmCHS2</i> Forward Primer 2 (<i>CHS2F2</i>)	RACE	ATGTACCAACAAGGGTGCTCG
<i>SmCHS2</i> Reverse Primer 2(<i>CHS2R2</i>)	RACE	CCTCCTCCCTCAAGTGTAAC
<i>SmCHS2</i> Forward Primer 3 (<i>CHS2F3</i>)	RACE	GGTTCAGACCCTGAGTTCTCC
<i>SmCHS2</i> Reverse Primer 3(<i>CHS2R3</i>)	RACE	ACGAGTGAGTCAAGATGGTTC
<i>SmCHS1</i> Forward Primer (<i>CHS1F</i>)	Real-time PCR	CTTGATTCCCTCGTTGGTCAG
<i>SmCHS1</i> Reverse Primer (<i>CHS1R</i>)	Real-time PCR	CCATCTTACCAGACTCAGAG
<i>SmCHS2</i> Forward Primer (<i>CHS2F</i>)	Real-time PCR	TCTCTCCACAATCAAACGCC
<i>SmCHS2</i> Reverse Primer (<i>CHS2R</i>)	Real-time PCR	GAACCATCTTGACTCACTCGT
18s rRNA Forward	Real-time PCR	ATGATAACTCGACGGATCGC
18s rRNA Reverse	Real-time PCR	CTTGGATGTGGTAGCCGTTT

Table 2. Numbers of covered nucleotide (a), amino acid (b), and similarity per cent between three CHSs of milk thistle and other Asteraceae family species.

(A)							
CHS gene	Accession numbers	<i>SmCHS1 (F1R2)</i>		<i>SmCHS2 (F2R1)</i>		<i>SmCHS3 (Silybummarianum)</i>	
		Numbers of covered (bp)	Similarity(%)	Numbers of covered (bp)	Similarity (%)	Numbers of covered (bp)	Similarity (%)
<i>SmCHS1</i> (861 bp)	JN182805						
<i>SmCHS2</i> (747 bp)	JN182807	592	73				
<i>SmCHS3</i> (1406 bp)	JN182806	861	98	747	73		
<i>Gerbera hybrida1</i> (1381 bp)	Z38098	861	79	747	73	All	76
<i>Gerbera hybrida2</i> (1428 bp)	Z38096	861	83	747	71	All	80
<i>Callistephus chinensis</i> (1419 bp)	Z67988	861	84	747	72	All	81
<i>Chrysanthemum xmorifolium</i> (1416 bp)	DQ521272	861	83	747	72	All	80
<i>C. indicum</i> (828 bp)	AF511463	667	79	747	72	837	79
<i>C. vestitum</i> (828 bp)	AF511473	667	79	747	72	837	79
<i>C. lavandulifolium</i> (828 bp)	AF511465	667	79	747	72	837	79
<i>C. nankingense</i> (828 bp)	AF511469	667	78	747	71	837	78
<i>Helianthus annuus</i> (1336 bp)	DQ503694	848	70	747	62	1311	64
<i>Petasites fragrans</i> (515 bp)	EF128591	515	83	515	69	515	83
(B)							
CHS protein	Accession numbers	<i>SmCHS1 (F1R2)</i>		<i>SmCHS2 (F2R1)</i>		<i>SmCHS3 (Silybummarianum)</i>	
		Numbers of covered AA	Similarity (%)	Numbers of covered AA	Similarity (%)	Numbers of covered AA	Similarity (%)
<i>SmCHS1</i> (286 aa)	AFK65633						
<i>SmCHS2</i> (249 aa)	AFK65635	197	70				
<i>SmCHS3</i> (412 aa)	AFK65634	286	98	249	71		
<i>Gerberahybrida1</i> (403 aa)	CAA86220	286	90	249	71	All	83
<i>Gerbera hybrida2</i> (398 aa)	CAA86218	286	90	249	71	All	86
<i>Callistephuschinensis</i> (398 aa)	CAA91930	286	94	249	72	All	88
<i>Chrysanthemum xmorifolium</i> (397 aa)	ABF69124	286	93	249	73	All	88
<i>C. indicum</i> (276 aa)	AAM46964	222	90	249	70	279	89
<i>C. vestitum</i> (276 aa)	AAM46974	222	91	249	70	279	90
<i>C. lavandulifolium</i> (276 aa)	AAM46966	222	91	249	69	279	89
<i>C. nankingense</i> (276 aa)	AAM46970	222	90	249	69	279	89
<i>Helianthus annuus</i> (368 aa)	ABG35655	283	83	249	66	368	73
<i>Petasites fragrans</i> (171 aa)	ABO42664	171	94	171	71	171	93

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Gerbera_hybrida.chsmr_gchs1      ATGGCGTCCCTC-----CGTTGACATGAAGGCGATCAGAGATGCTCA
Chrysanthemum_x_morifolium.chm   ATGGCTTCCTT-----AACTGACATGCGGCCATTAGAGAGGCTCA
Callistephus_chinensis.chsmr     ATGGCTTCCAC-----AATCGATATTGCGGCTATCAGAGAAGCCCA
Gerbera_hybrida.chsmr            ATGGCCACCTCTCCGGCAGTCATCGACGTCGAGACAATCAGAAAAGCGCA
*****  **                    ** *  * * * * * * * * * * *

Gerbera_hybrida.chsmr_gchs1      ACGTGCAGAAGGTCCGGCGACCATTCTTGCCATCGGAAGTCAACTCCGG
Chrysanthemum_x_morifolium.chm   ACGGGCTCAAGGTCCAGCTACCATTCTAGCGATCGGCACAGCAACTCCGG
Callistephus_chinensis.chsmr     GCGGCGACAAGGTCCAGCTACCATTCTCGGATTGGCACTGCCACCCCGT
Gerbera_hybrida.chsmr            AAGGGCAGAAGGTCCGGCCACCATTCTCGCCATCGGCACCCGCCACTCCGG
*          ***** ** ***** ** * * * * * * * * * *

Gerbera_hybrida.chsmr_gchs1      CGAATTGCGTCTATCAAGCGGATTATCCCATTACTATTTTCGGATCACC
Chrysanthemum_x_morifolium.chm   CTAATTGTGTATACCAAGCTGATTATCCCATTACTATTTTCGGATCACT
Callistephus_chinensis.chsmr     CTAAGTGTGTCTATCAAGCTGATTATCCTGATTACTATTTTCGCATCACC
Gerbera_hybrida.chsmr            CCAACTGCGTTTATCAAGCGGATTATCCGACTACTATTTTCGTGTCC
* * * * * Y**H**Y**B***** * * ***** * * * * *
                          ChSDegF1 Primer                                 Ex-In
                                                                              ↓

Gerbera_hybrida.chsmr_gchs1      AAGAGTGAACACATGGTGGATCTCAAAGAGAAATTCAGCGCATGTCTGA
Chrysanthemum_x_morifolium.chm   AAAAGTGAACACATGGTGGATCTTAAAGAGAAATTCAGCGCATGTGCGA
Callistephus_chinensis.chsmr     AAAAGCGAACACATGGTCGATCTCAAAGAAAAATTCAGCGCATGTGCGA
Gerbera_hybrida.chsmr            GAGAGCGAACATATGGTGGATCTCAAAGAAAAATTCAACGCATGTGTGA
* * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Gerbera_hybrida.chsmr_gchs1      CAAGTCGATGATAAGGAAACGTTACATGCACATCAGAGGAGTATCTTA
Chrysanthemum_x_morifolium.chm   CAAGTCTATGATACGAAAACGATACATGCACCTCACGGAGGAGTATCTTA
Callistephus_chinensis.chsmr     CAAATCTATGATAAGAAAGCGATACATGCACCTCACAGAAGAATATCTAA
Gerbera_hybrida.chsmr            TAAATCAATGATAAGAAAACGATACATGCATATCACTGAAGAATTTTGA
* * * * * * * * * * * * * * * * * * * * * * * * * * *

Gerbera_hybrida.chsmr_gchs1      AACAAAACCTAACATGTGCGGTACATGGCGCCGTCGCTCGACGTCGGG
Chrysanthemum_x_morifolium.chm   AAGAGAACCCAAAGCCTTTGTGAGTACATGGCTCCGTCCCTTGATGCTCGC
Callistephus_chinensis.chsmr     AAGAGAACCCAGCCTTTGCGAGTACATGGCTCCATCACTGGATGCACGT
Gerbera_hybrida.chsmr            AAGAAAACCTAGTATGTGCAAGTTCATGGCGCCATCGTTGGATGCCCGG
* * * * * * * * * * * * * * * * * * * * * * * * * * *

Gerbera_hybrida.chsmr_gchs1      CAAGACCTGGTCGTGTCGAAGTCCCAAAGCTCGGCAAGGAAGCCGCCAT
Chrysanthemum_x_morifolium.chm   CAGGATGTGGTGGTGGTGGAGGTACCAAAGCTTGGAAAAGAACCCGCAAC
Callistephus_chinensis.chsmr     CAAGACTGTCGTGGTGGTGGAGTCCCAAAGCTGGGCAAGAAAGCCGCAAC
Gerbera_hybrida.chsmr            CAGGACTTGGTGGTGGTGGAGTCCCAAAGCTCGGAAAAGAACGTCGAAC
* * * * * * * * * * * * * * * * * * * * * * * * * * *

Gerbera_hybrida.chsmr_gchs1      GAAAGCCATCAAAGAATGGGGCCACCCAAATCCAAGATCACCCACCTCA
Chrysanthemum_x_morifolium.chm   AAAAGCTATTAAGAATGGGGACAACCCAAATCAAAAATCACCCACCTAA
Callistephus_chinensis.chsmr     CAAAGCTATTAAGAATGGGGCCAACCCAAATCAAAGATCACCCACCTCA
Gerbera_hybrida.chsmr            TAAGGCCATCAAAGAATGGGGGTTTCCCAAATCAAAGATCACCCACC TCG
* * * * * * * * * * * * * * * * * M * * * * * R * * * * * * *
                          ChSDegF2 Primer

Gerbera_hybrida.chsmr_gchs1      TCTTCTGCACCACCTCCGGCGTTCGACATGCCCGGGCGCGACTACCAGCTC
Chrysanthemum_x_morifolium.chm   TCTTCTGCACCACATCTGGTGTAGACATGCCCGGGGCTGATTACCAACTC
Callistephus_chinensis.chsmr     TCTTTTGCACCTACCTCCGGGTCGACATGCCTGGTGTGATTCACGACTC
Gerbera_hybrida.chsmr            TCTTCTGCACCACCTCAGGAGTTGACATGCCCGGAGCTGACTACCAGCTC
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Gerbera_hybrida.chsmr_gchs1      ACCAACTCCTCGGTCTCCGGCCATCCGTCAAACGCTTCATGATGTACCA
Chrysanthemum_x_morifolium.chm   ACCAACTCCTCGGCCTCCGCCCTTCAGTCAAACGTTTTATGATGTACCA
Callistephus_chinensis.chsmr     ACCAAGCTTCTCGGCCTGCGCCCTTCGGTCAAACGTTTTATGATGTACCA
Gerbera_hybrida.chsmr            ACCAAGCTCCTCGGACTTCGTCCTTCAGTCAAGCGTTCATGATGTACCA
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Gerbera_hybrida.chsmr_gchs1      ACAAGGCTGTCTCGCCGGCGGCACGGTTCTCCGGCTAGCCAAAGATCTCG
Chrysanthemum_x_morifolium.chm   ACAAGGTGCTTTGCAGGTGGGACGGTTCTTCGTCTAGCAAAGACCTCG
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Figure 1. continues

Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	ACAAGGGTGTTTTGCCGGGGGTACGGTTCCTCGTCTAGCCAAAGACCTTG ACAAGGTGTGTGCTGGTGGCACGGTCCCTCGTCTTGCCAAGGACCTTG ***** ** ** * ** * ** * ** * ** * ** * ** * ** * ** *
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	CGGAGAACAATAAAGGCGCTAGGGTCTTGTGGTGTGCTCCGAGATCACG CAGAAAACAACAAGGATGCACGTGTCTAGTTGTTTGTCCGAGATTACT CGGAGAACAACAAGGGTGTCTCGTGTCTTGTGTGTGCTCCGAGATCACT CTGAGAACAACAAGGGTGTCTCGTGTCTTGTGTTGCTCTGAGATCACT * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** *
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	GCGGTGACTTTCGAGGACCTAATGACACCCACCTTGATTCCCTCGTCGG GCAGTCACATTCGGTGGTCTAATGACACTCACCTTGATTCACTCGTTGG GCGGTACAGTTTCGTTGGCCCTAATGATACTCATCTTGACTCACTTGTGGG GCTGTCACTTCGGTGGCCCTAATGAGGGCCACCTTGACTCCTTAGTCGG ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** *
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	ACAGGCCTTGTTCGGCGACGGGGCTGCGGGGTGATCGTGGGTTCCGATC TCAAGCTTTGTTTCGGGATGGAGCTGCGGCTGTCAATGTTGGTTCAGATC TCAAGCTTTGTTTCGGGATGGGGCCCGTGCAGTCACTCGTGGGTGCTGACC GCAATCTCTTTGGGGACGGAGCTGCAGCAGTGATCATCGGTTTCAGACC * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** *
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	CCGACTTGACGACGGAGCGGCCGTTGTTTGAATGGTTTCCGCCGCTCAG CAGACTTGACAAAGGAGCGTCCGTTGTTTCGAGATGATATCTGTCTCAA CTGACTTGACGACCGAGCGGCCCTTGTGTTGAGATGATCTCTGCAGCTCAA CTGACTTGTCACTAGAGCGTCCATTTGTTGAGATGTTATCTGCAGCACAG * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** *
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	ACGATCTTGCCGGACTCCGAGGGAGCCATTGATGGACACTTGAGGGAAGT ACTATCTTACCAGACTCGGAGGGAGCAATCGATGGACACTTGAGGGGAAGT ACAATCTTGCCCGAATCCGAAGGAGCTATTGATGGGCACTTGAGGGGAAGT ACAATCTTGCCGATTTCTGAAGGGCCATCGACGGGCACCTGAAGGAGGT ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** *
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	AGGGTTGACGTTTTCATTTACTCAAAGACGTGCCTGGGTTGATATCGAAGA CGGGTAAACATTTCTCTCTCAAAGACGTACCGGGTTGATCTCCAAGA TGGGCTTACATTTCTCTTAAAGATGTCCCTGGGTTGATCTCAAAGA TGGACTCACGTTCCACCTACTCAAGGACGTCCCTGCATTGATTGCGAAGA * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** *
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	ACATAGAGAAAGCTTTAACGACGGCGTTTTCTCCGTTGGGTATCAACGAC ACATAGAGAAGGCATTGACACAAGCCTTTTCTCCATTAGGTATAAGTGAC ATATAGAGAAGGCATTGACACAAGCCTTTTCTCCATTAGGTATAACTGAC ATATAGAGAAGGCCTTGATACAAGCTTTTTCTCCTTTGAATATCAATGAC * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** *
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	TGGAACTCGATATTCTGGATAGCACATCCCGAGGTCCGGCGATACTGGA TGGAACTCGATCTTTTGGATCGCTCACCTGGTGGTCCAGCTATTCTGGA TGGAACTCGATCTTTTGGATCGCACATCCAGGAGGTCCAGCGATACTGGA TGGAACTCGATCTTTTGGATAGCTCATCTGGTGGTCCAGCAATACTGGA ***** ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * GACCT
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	CCAGGTGAGCTCAAGCTAGGGTTGAAGGAGGAGAAGCTTAGAGCTACTA CCAGGTTGAGCTTAAAGCTCGGTCTCAAGGAGGAGAAGATGAGAGCCACTA CCAGGTGAGCTCAAGCTCGGTCTCAAGGAGGAGAAAATGAGAGCGACAC TCAGGTGAGGTTCAAACTTGGCCTCAGAGAGGAGAAAATAAGGGCCTCGA ***** ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * RGTCAMCTCRARTTYG ChSDegR2 Primer
Gerbera_hybrida.chsmr_gchs1 Chrysanthemum_x_morifolium.chm Callistephus_chinensis.chsmr Gerbera_hybrida.chsmr	GACATGTTTAAAGCGAGTACGGTAACATGTCAAGTCTTGTGTGTTGTTT GACACGTTCTTAGTGAGTATGGAAACATGTCAAGTCTTGTGTTTGTTC GACATGTGCTTAGTGAGTATGGAAACATGTCCAGTCTTGTGTGTTGTTT GACATGTAAGTCAAGTGAATATGGTAATATGTCCAGCGCCTGTGTGTTGTTT ***** ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * ** *

Figure 1. *continues*

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Gerbera_hybrida.chsmr_gchs1      ATTATCGACGAAATGAGAAAGAAGTCGTCCGAGAACGGCGCCGGCACCAC
Chrysanthemum_x_morifolium.chm  ATTATTGATGAAATGAGGAAGAAGTCGGCTGAGGAAGGTGCAGCCACAAC
Callistephus_chinensis.chsmr    ATCATTGATGAGATGAGGAAGAAGTCGGCCGAGGATGGCGCTGCGACCAC
Gerbera_hybrida.chsmr           ATACTCGATGAGATGAGGAAGAAGTCAATCAAAGATGGAAAGACCACCAC
                                  ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * **
Gerbera_hybrida.chsmr_gchs1      CGGAGAAGGTTTGGAGTGGGGTGTTCGTGTTGGGTTTGGGCCTGGGTGTA
Chrysanthemum_x_morifolium.chm  CGGTGAAGGTTTAGATTGGGGTGTTTTATTCGGGTTCGGTCTGGTTTGA
Callistephus_chinensis.chsmr    CGGTGAAGGTTAGACTGGGGTGTTCCTTTGGGTTCCGGTCTGGTTTGA
Gerbera_hybrida.chsmr           CCGTGAAGGTTTAGAATGGGTTGTTTGTGTTGGGTTCCGACCGGGTTTGA
                                  *** ** * ** * ** * ** * ** * ** * ** * ** * ** * **
                                  GCCWCTTCCMAAYCTNACCCCA
                                  ChSDegR1 Primer
Gerbera_hybrida.chsmr_gchs1      CGGTGGAGACGGTGGTTCCTTCACAGTGTCCAACCACCGTGACGGTTGCC
Chrysanthemum_x_morifolium.chm  CGGTGAAACCGTGGTCTCCACAGCCTCCAACCACCTATATCGGTTGCA
Callistephus_chinensis.chsmr    CAGTGGAGACAGTGGTTCCTTCATAGCCTCCAACCACAATGGCGATTGCC
Gerbera_hybrida.chsmr           CAGTTGAGACTGTGGTTCCTCCACAGCCTCCCCGCTACTATTTAGTTGCC
                                  ● ** * ** * ** * ** * ** * ** * ** * ** * ** * ** * **

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Figure 1. Sequence alignment of *CHS* genes from four *Asteraceae* species; degenerate primers which were designed based on the conserved regions are highlighted by green colour. Intron position which was located at the conserved site is highlighted by blue colour (Ex-In).

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SmCHS2 -----
SmCHS3 -----
SmCHS1 GTGTATACCAAGCCGATTATCCAGATTACTATTTTCGGATCACCAACAGTGAACACATGG

SmCHS2 -----
SmCHS3 -----
SmCHS1 TCGATCTCAAAGAGAAATTCAAGCGCATGTGTATGTATTCACACTTTACATATATATTTA

SmCHS2 -----
SmCHS3 -----AAAGCACAACGGGCACAAGGTCCGGCCACCATTCTC
SmCHS1 GCATTCACAATATTGTTTTCAAAAATTATATTATATATATACAAACAAATTTAATAATAA

SmCHS2 -----
SmCHS3 GCCATCGGCACTGCCACTCCTGCAAATGTATCTATCAAGCCGATTATCCAGATTACTAT
SmCHS1 GAAAAAGTGTATCAAAGTTATTTAACTATTATTATACTTTAAAATAAATCAGTAGAATT

SmCHS2 -----
SmCHS3 TTTCGGATCACCAACAGTGAACACATGGTTGATCTTAAAGAGAAATTCAAGCGCATGTGT
SmCHS1 AATGGTAGGGTTTTGTTAATTTATATATATTTTACATGAACTGAATGAAATTTGCAGGT

SmCHS2 -----
SmCHS3 GACAAGTCTATGATAAGAAAGCGATACATGCACATCACAGAGGAGTTTCTAAAAGAAAAC
SmCHS1 GACAAGTCTATGATAAGAAAGCGATACATGCACATCACAGAGGAGTTTCTAAAAGAAAAC

SmCHS2 -----
SmCHS3 CCAAACATGTGCGAGTACATGGCTCCATCCCTTGACGCCCGTCAGGATGTGGTGGTCGTC
SmCHS1 CCAAACATGTGCGAATACATGGCTCCATCCCTTGACGCCCGTCAGGATGTGGTGGTCGTC

SmCHS2 -----
SmCHS3 GAAGTCCCCAAGCTCGGTAAAGAAGCAGCCACAAAAGCCATCAAAGAATGGGGAAACCCG
SmCHS1 GAAGTCCCCAAGCTCGGTAAAGAAGCAGCCACAAAAGCCATCAAAGAATGGGGAAACCCG
**

SmCHS2 AAATCAAAGATCACCCACCTCATCTTTTGCACCACCTCTGGAATTGACATGCCCGGTGCC
SmCHS3 AAATCAAAGATCACCTCATCTCATCGTCTGCACCACCTCCGGTGTGACATGCCTGGTGCC
SmCHS1 AAATCAAAGATCACCTCATCTCATCGTCTGCACCACCTCCGGTGTGACATGCCTGGTGCC
***** ** * ***** * ***** ** * ***** *****

SmCHS2 -----
SmCHS3 GACTATCAACTTGTCAAGATTCTTGGTCTCTCTCCCAATCAAACGGCTCATGATGTAC
SmCHS1 GACTACCAGATCACCAAGCTCCTTGGCCTCCGCCCTTCTGTCAAACGATTCATGATGTAC
***** ** * ***** * ***** ** * ***** *****

SmCHS2 -----
SmCHS3 CAACAAGGGTGCTCGGCCGAGCCATGGTCCTTCGTCTCGCCAAAGACCTTGCTGAAAAC
SmCHS1 CAACAAGGGTGTTTTGCAGGAGGCACGGTTCTCCGTCTCGCTAAGGACATTGCGGAAAAC
***** ** * ***** * ***** ** * ***** *****

SmCHS2 -----
SmCHS3 AATAAAGGTTACGTATCTTGTGTTTGGCTCAGAAAGCAATGCAATCATGTTTCGTGGA
SmCHS1 AACAAGGGTTCTCGTGTCTTGTGGTTTGGCTCAGAGATCACAGCGGTTACTTTCCGTGGA
** ** ***** ** ***** ***** ** ** * ** *****

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SmCHS2 Forward Primer

Figure 2. *continues*



Figure 2. Multiple nucleotide sequence alignment of *CHS* gene family members from milk thistle obtained by Clustal *W* program. Real-time PCR primers are highlighted by green colour.

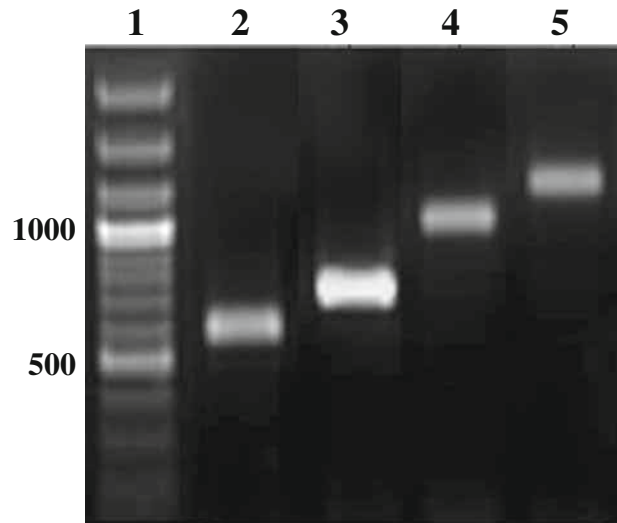


Figure 3. Gel electrophoresis of *CHS* gene PCR products by four degenerate primers Lane 1, a 100 bp plus molecular marker; lane 2, amplified fragments by F2R2; lane 3, amplified fragments by F2R1; lane 4, amplified fragments by F1R2; lane 5, amplified fragments by F1R1.

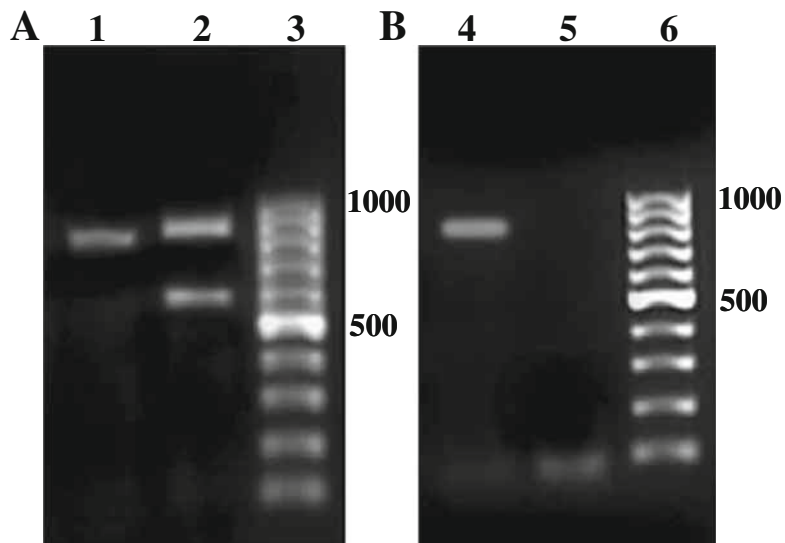


Figure 4. Gel electrophoresis of the cDNA fragments obtained by RACE. (a) 3'RACE: lane 1, amplified fragments by CHS1F2 and AUAP primers from *CHS1*; lane 2, amplified fragments by CHS2F2 and AUAP primers from *CHS2*; lane 3, DNA 100-bp molecular marker. (b) 5'RACE: lane 4, amplified fragment by CHS1R2 and AAP primers from *CHS1*; lane 5, amplified fragments by CHS2R2 and AAP primers in *CHS2*; lane 6, DNA 100-bp ladder.

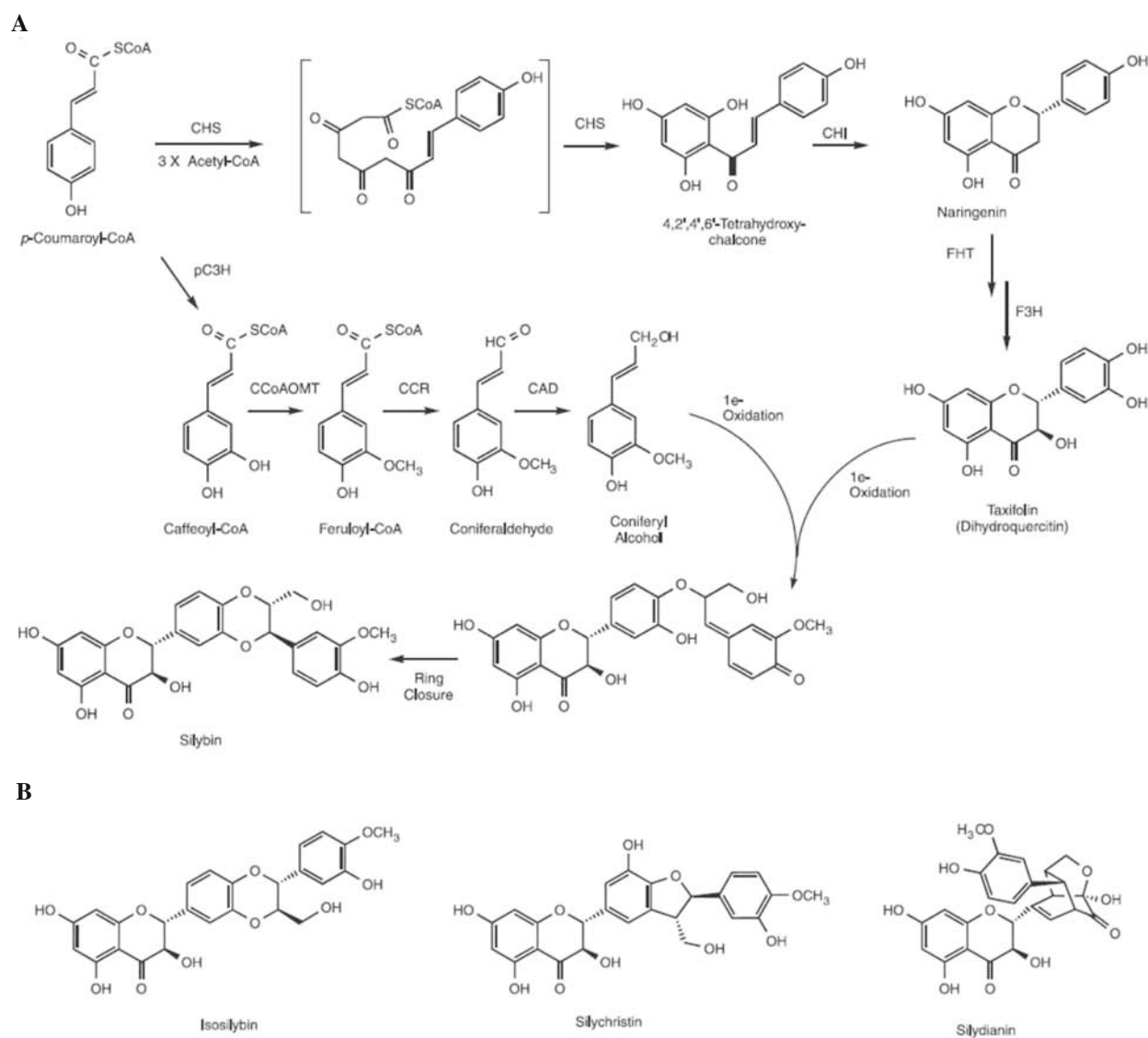


Figure 5. The biosynthetic pathway for some silymarin constituents. (A) The biosynthesis of flavanolignins involves the convergence of products from two phenylpropanoid branch pathways, monolignol and flavonoid biosynthesis. The monolignol component, coniferyl alcohol, derives from the two-step side-chain reduction of feruloyl-CoA, itself formed from the hydroxylation and O-methylation of p-coumaroyl-CoA. The flavonoid component, taxifolin (dihydroxyquercetin), derives from the two-step hydroxylation of naringenin, the precursor to most flavonoids. Naringenin is derived from the chain extension of p-coumaroyl-CoA with three acetyl-CoA units, followed by folding, and initial cyclization; all catalyzed by a single enzyme, chalcone synthase (CHS). Further ring closure catalyzed by chalcone isomerase (CHI) yields naringenin. Coupling follows a free-radical mediated process. (B) Alternative coupling between coniferyl alcohol and taxifolin, and further modifications, yields structurally diverse flavanolignans. Enzymes: 4CL, 4-coumaroyl-CoA ligase; pC3H, p-coumaroyl-CoA-3-hydroxylase; CCoAOMT, caffeoyl-CoA O-methyltransferase; CCR hydroxycinnamoyl-CoA oxidoreductase; CAD, cinnamyl alcohol dehydrogenase; FHT, flavanone-3-hydroxylase; F3H, flavonoid-3'-hydroxylase (Derived from Bernards 2010).

References

Bernards M. A. 2010 Plant natural products: a primer. *Can. J. Zool.* **88**, 601–604.