SUPPLEMENTARY MATERIAL

Natural product extracts of the Canadian prairie plant, *Thermopsis rhombifolia*, have anti-cancer activity in phenotypic cell based assays

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Abstract

Many plant species within the terrestrial ecological zones of Canada have not yet been investigated for plant species with anti-cancer activity. We examined the scientific literature describing the endemic flora from the prairie ecological zone and selected the species, *Thermopsis rhombifolia*, locally known as the buffalo bean, for investigation of its anti-cancer potential. We tested it in cell based assays using phenotypic screens that feature some of the hallmarks of cancer. An ethanolic extract prepared from *T. rhombifolia* was cytotoxic to HT-29 (colon) and SH-SY5Y (brain) cancer cell lines, and showed little cytotoxicity to a normal human cell line (WI-38). In phenotypic assays, we identified activities in the extracts that target cell death, cell cycle and cell adhesion. These data highlight the anti-cancer potential of previously untested plants found in northern ecological zones, and the feasibility of using pertinent phenotypic assays to examine the anti-cancer potential of natural product extracts.

Keywords

Thermopsis rhombifolia (Fabaceae), Hallmarks of cancer, HT-29 cells, SH-SY5Y cells, WI-38 cells, Phenotypic screening

Figure Captions**Figure S1. The plant species *Thermopsis rhombifolia*.**

A. Image of *Thermopsis rhombifolia* in prairie habitat. Scale bar = 10 cm. B. Image of flowers and bean shaped legumes, which are present on plants in the spring season. Scale bar = 3 cm. C. Image of legumes, seeds and leaves. Scale bar = 2 cm.

Figure S2. DNA sequence of the *psbA-trnH* intergenic spacer and the ITS internal transcribed spacer region (bar code regions) of *T. rhombifolia*.

A. *T. rhombifolia psbA-trnH* intergenic spacer DNA was amplified by the polymerase chain reaction, sequenced, and compared to the DNA sequences of *T. lanceolata*, *T. alpine*, *T. smithiana*, and *T. chinensis*. DNA sequence alignments were prepared using NCBI software. Dashes indicate sequence gaps and asterisks indicate shared sequence identity in all species. Numbers indicate base positions.

B. *T. rhombifolia* ITS internal transcribed spacer region DNA was prepared and analysed as described in A.

Bar in A = 10 cm

Bar in B = 3 cm

Bar in C = 2 cm

A psbA-trnH intergenic spacer

lanceolata	ATAACTTCCCTCTAGACCTGGCTGCGGTCGAGGCTCCATCTCTAAATGGATAATATTTAG	60
alpina	ATAACTTCCCTCTAGACCTGGCTGCGGTCGAGGCTCCATCTCTAAATGGATAATATTTAG	60
smithiana	ATAACTTCCCTCTAGACCTGGCTGCGGTCGAGGCTCCATCTCTAAATGGATAATATTTAG	60
chinensis	ATAACTTCCCTCTAGACCTGGCTGCGGTCGAGGCTCCATCTCTAAATGGATAATATTTTG	60
rhombifolia	AAAACTTCCCACTAGACCTGGCTGCGGTCGAGGCTCCATCTCTAAATGGATAATATTTTG	60
* * * * *		
lanceolata	GTCTTAAAGTATACGAGTTTTTAAAAAGTAAAGGAGCAATAGAAAAAAGTCTGTTGCTCCT	120
alpina	GTCTTAAAGTATACGAGTTTTTAAAAAGTAAAGGAGCAACAGAGTTTTTCTATTGCTCCT	120
smithiana	GTCTTAAAGTATACGAGTTTTTAAAAAGTAAAGGAGCAACAGAGTTTTTCTATTGCTCCT	120
chinensis	GTCTTAAAGTATACGAGTTTTTAAAAAGTAAAGGAGCAATAGAAACACT-CTGTTGCTCCT	119
rhombifolia	GTCTTAAAGTATACGAGTTTTTAAAAAGTAAAGGAGCAACAGAGTGTTC-CTATTGCTCCT	119
* * * * *		
lanceolata	TTACTTTGACTTCTTTATTTATATTTTAAAGTCTTTGTTTTTATATTGCTTGCGAAGTC	180
alpina	TTACTTTTACTTCTTTATTTATATTTAGAAAGTCTTTATTTTTTATATTGCTTGCGAAGTC	180
smithiana	TTACTTTTACTTCTTTATTTATATTTAGAAAGTCTTTATTTTTTATATTGCTTGCGAAGTC	180
chinensis	TTACTTTTACTTCTTTA-----TTTGAAGTCTTTTTTTTACATTACTTGCGAAATC	172
rhombifolia	TTACTTTTATTTTAGA-----AGTCTTTTTTTTTTACATTACTTGCGAAATC	170
* * * * *		
lanceolata	ATATGTATTCCAAAAAGAGAAAAAAATGAATGTTTCCATTTCTGGTTTATTTTTCTTT	240
alpina	ATATGTATTCCAAAAAGAGA--AAAAAATGAATGTTTCCATTTCTGGTTTATTTTATCTT	238
smithiana	ATATGTATTCCAAAAAGAGA--AAAAAATGAATGTTTCCATTTCTGGTTTATTTTATCTT	238
chinensis	ATATGTATTCCAAAAACAT--AAAAAATGAATGTTTCCATTTTCTGGTTTATCTTATCTT	230
rhombifolia	ATATGTATTCCAAAAACATAAAAAAAATGAATGTTTCCATTTTCTGGTTTATCTTATCTT	230
* * * * *		
lanceolata	ATAAGAAAA--AAGAAATGATAACGAAAGGGTTGAAATTGAAGTCTAGGTAATTTTACA	297
alpina	ATAAGAAAA--AAGAAATGATAACGAAAGGGTTGAAATTGAAGTCTAGGTAATTTTACA	295
smithiana	ATAAGAAAA--AAGAAATGATAACGAAAGGGTTGAAATTGAAGTCTAGGTAATTTTACA	295
chinensis	ACAAGAAAAAAGTTGAATGATAATGAAAGGGTTGAAATTGAAGTCTGGGTAATTTTACA	290
rhombifolia	ACAAGAAAAAAGTTGAATGATAATGAAAGGGTTGAAATTGAAGTCTGGGTAATTTTACA	290
* * * * *		
lanceolata	TAGTAGTAGGGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTGTGAATC	349
alpina	TAGTAGTAGGGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTGTGAATC	349
smithiana	TAGTAGTAGGGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTGTGAATC	347
chinensis	TAGTA-TAGGGCGGATGTAGCCAAGTGGATCAAGGCAGT-----	329
rhombifolia	TAGTA-TAGGGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTTGAATC	341

lanceolata	87.87
alpina	90.77
smithiana	91.07
chinensis	93.27
rhombifolia	100.00

B ITS internal transcribed spacer region

smithiana	TCTGAGCCTAACAAAGCAGTGTGACCCCGCAATTTGTTTGACTACTCAGGGGTGGCTAGA	60
alpina	TCTGAGCCTAACAAAGCAGTGTGACCCCGCAATTTGTTTGACTACTCAGGGGTGGCTAGA	60
chinensis	TCAAAGCCTAACAATGCAGTGTGACCCCGCAATCTGTTTGACCATAAGGGGTGGCTAGA	60
montana	TCTGAGCCTAACAAATGCAGTGTGACCCCGCAATTTGTTTGACTACTAAGGGGTGGCTAGA	60
rhombofolia	TCAAAGCCTAACAAATGCAGTGTGACCCCGCAATTTGTTTGACTACTAAGGGGTGGCTAGA	60
	* * * * *	
smithiana	GGTGTCTGGCTCCTCGGTCCCCCTCTGTGTGCGAGGTGCCCTCCTGTGTGGGTCTCCT	120
alpina	GGTGTCTGGCACCTTTGGTCCCCCTCTGTGTGCGAGGTGCCCTCCTGTGTGGGTCTCCT	120
chinensis	GGTGTCTGGCACCTTCGGTCCCCCATTTGTGTGAGTAGAGCCCGCTCTTGTGTG-TGTCTCCT	119
montana	GGTGTCTGGCACCTTCGGTACCCCATTGTGTGAGAGGAGCCCGCTCTTGTGTG-TGTCTCCT	119
rhombofolia	GGTGTCTGGCACCTTCGGTACCCCATTGTGTGAGAGGAGCCCGCTCTTGTGTG-GTCTCCT	119
	* * * * *	
smithiana	CCTGGCCTAACAAAAAACCACCGCGCTCGAATGCGCCAAGGAAATCAAGATTGTCTAGTG	180
alpina	CCTGGCCTAACAAAAAACCACCGCGCGCAATGCGCCAAGGAAATCAAGATTGTCTAGTG	180
chinensis	CCTGGCCTTATAAAAAAACCACCGCGCGCAATGCGCCAAGGAAATCAAGATTGTCTAGTG	179
montana	CCTGGCCTTATAAAAAAACCACCGCGCGCAATGCGCCAAGGAAATCAAGATTGTCTAGTG	179
rhombofolia	CCTGGCCTTATAAAAAAACCACCGCGCGCAATGCGCCAAGGAAATCAAGATTGTCTAGTG	179
	* * * * *	
smithiana	CGCCCCCGTTGGCACCCGAGACGGTGTGCGTGTGCGGGCGCGTGTGTGACACACATATCCCA	240
alpina	CGCCCCAGTTGGCACCCGAGACGGTGTGCGTGTGCGGGCGCGTGTGTGACACACATATCCCA	240
chinensis	CGTCCCCGTTGGCACCCGAGACGGTGTGCGGTGTGCGGGTGGCGTGTGTGACACGATATCCCA	239
montana	CGTCCCCGTTGGCACCCGAGACGGTGTGCGGTGTGCGGGTGGCGTGTGTGACACGATATCCCA	239
rhombofolia	CGTCCCCGTTGGCACCCGAGACGGTGTGCGGTGTGCGGGTGGCGTGTGTGACACGATATCCCA	239
	* * * * *	
smithiana	AAGACTCTCGGCAACGGATATCTCGGCTCTTGATCGATGAAGAAGCTAGCGAAATGCGA	300
alpina	AAGACTCTCGGCAACGGATATCTCGGCTCTTGATCGATGAAGAAGCTAGCGAAATGCGA	300
chinensis	AAGACTCTCGGCAACGGATATCTCGGCTCTTGATCGATGAAGAAGCTAGCGAAATGCGA	299
montana	AAGACTCTCGGCAACGGATATCTCGGCTCTTGATCGATGAAGAAGCTAGCGAAATGCGA	299
rhombofolia	AAGACTCTCGGCAACGGATATCTCGGCTCTTGATCGATGAAGAAGCTAGCGAAATGCGA	299
	* * * * *	
smithiana	TACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCGTTGAACGCAAGTTGCGCCC	360
alpina	TACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCGTTGAACGCAAGTTGCGCCC	360
chinensis	TACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCGTTGAACGCAAGTTGCGCCC	359
montana	TACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCGTTGAACGCAAGTTGCGCCC	359
rhombofolia	TACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCGTTGAACGCAAGTTGCGCCC	359
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smithiana	GACGCCATCAGGTGAGGGGACGCGCTGCCTGGGTGTGCGACATCGTTGCCCCCAATGCCT	420
alpina	GACGCCATCAGGTGAGGGGACGCGCTGCCTGGGTGTGCGACATCGTTGCCCCCAATGCCT	420
chinensis	GAAGCCATCAGGCCGAGGGGACGCGCTGCCTGGGTGTGCGACATCGTTGCCCCCAATGCCT	419
montana	GAAGCCATCAGGCCGAGGGGACGCGCTGCCTGGGTGTGCGACATCGTTGCCCCCAATGCCT	419
rhombofolia	GAAGCCATCAGGCCGAGGGGACGCGCTGCCTGGGTGTGCGACATCGTTGCCCCCAATGCCT	419
	* * * * *	
smithiana	CAGCCTTGTGCTAGGCTTTGAGTGGGGCGAATGTTGGCTTCCCGGAGCAAGGTCTCACG	480
alpina	CAGCCTTGTGCTAGGCTTTGAGTGGGGCGAATGTTGGCTTCCCGGAGCAAGGTCTCACG	480
chinensis	CAGCCTTGTGCTAGGCTTCGAGCGGGGCGAGTGTGGCTTCCCGGAGCATATGTCTCGCG	479
montana	CAGCCTTGTGCTAGGCTTCGAGCGGGGCGAATGTTGGCTTCCCGGAGCATATGTCTCACG	479
rhombofolia	CAGCCTTGTGCTAGGCTTCGAGCGGGGCGAATGTTGGCTTCCCGGAGCATATGTCTCACG	479
	* * * * *	
smithiana	GTTGGTTGAAAATTGAGTCCGTGTTGGAGGGGCGCGCATGGATGGTGGTTGAGTAAAA	540
alpina	GTTGGTTGAAAATTGAGTCCGTGTTGGAGGGGCGCGCATGGATGGTGGTTGAGTAAAA	540
chinensis	GTTGGTTGAA-AATTGAGTCCGTGTTG-AGGTCGCGCGCATGGCTGGTGGTTGAGTAAAA	537
montana	GTTGGTTGAA-AATTGAGTCCGTGTTGGAGGGTCCCGCATGCATGGTGGTTGAGTAAAA	538
rhombofolia	GTTGGTTGAA-AATTGAGTCCGTGTTGGAGGGTCCCGCATGCATGGTGGTTGAGTAAAA	538
	* * * * *	
smithiana	GCTCGAGATCGATCGTGCCTGCTACTCGTGCCTGATTGGGACTTTGTGACCCATGGGCG	600
alpina	GCTCGAGATTGATTGTGCGCTGCTACTCGTGCCTGATTGGGACTTTGTGACCCATGGGCG	600
chinensis	GCTCGAGATCGATCGTGCCTGCTACTCGGATTGGGACTTGTGACCCATGAGCG	597
montana	GCTCGAGTTCGATCGTGCCTGCTACTCGGATTGGGACTTGTGACCCATGAGCG	598
rhombofolia	GCTCGAGTTCGATCGTGCCTGCTACTCGGATTGGGACTTGTGACCCATGAGCG	598
	* * * * *	
smithiana	TCCAGTTGGTGCCTATGAC-----	620
alpina	TCTTGTTCGTGCCTATGAC-----	620
chinensis	CCT-TTGTGGTTGCCATGAC-----	616
montana	TCT-TTGGGTGCCCATGACGGGAC	622
rhombofolia	TCT-TTGTGGTTGCCCATGACGGGAC	622
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1: smithiana 92.07
2: alpina 90.61
3: chinensis 98.05
4: montana 99.52
5: rhombifolia 100.00