High Performance Thin Layer Chromatographic (HPTLC) Analysis of Dan Shen (*Salvia miltiorrhiza*) Roots Grown in Different Regions of the World

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Abstract

Context: Comparative analysis of bioactive compounds in Dan Shen (*Salvia miltiorrhiza*) roots grown in China and the United States

Objective: Determine whether the roots of Dan Shen (*Salvia miltiorrhiza*) organically cultivated in the United States have a similar constituent profile to roots grown and imported from China.

Research Design and Methods: Samples of Dan Shen (*Salvia miltiorrhiza*) were collected from a variety of Asian sources. Using High Performance Thin Layer Chromatography (HPTLC), these were then compared to samples grown organically in the United States.

Results: The roots grown organically in the U.S. showed high levels of the bioactive tanshinones and salvianolic acid. In all cases, they compared favorably with the Asian samples both qualitatively and quantitatively. Both organically grown samples from the U.S. appeared to have equal or higher levels of these important compounds.

Conclusions: Dan Shen (*Salvia miltiorrhiza*) can be successfully grown outside of China with organic agricultural methods. The harvested roots appear to have high levels of known bioactive compounds. This has positive implications for the environment, both in the reduction of pesticide and synthetic fertilizer usage, and in reducing pressure on Chinese agricultural land due to increased demand for Chinese herbs worldwide.

Introduction

China's agricultural land is coming under increased pressure from development, industrialization, and the growing demand for Chinese herbs worldwide. There is also an increasing demand for locally grown organic Chinese herbs, for both environmental and quality reasons. By cultivating these herbs locally and organically, pressure can be relieved on China's farmland and wild plant populations. When organic cultivation methods are employed, no pesticides or chemical fertilizers will be put into the local environment.

It is also possible to cultivate some Chinese herbs that are endangered in the wild. This has already been done successfully in the U.S. with endangered local herbs like goldenseal. Wild populations are preserved, while the demand for the herb is met by sustainable organic cultivation.[1]

[1] AHPA goldenseal survey measures increased agricultural production. *HerbalGram*. Austin, TX: American Botanical Council and the Herb Research Foundation. Spring 1999. (46) p. 66-67.

There is a common belief that Chinese herbs are somehow different when they are grown outside of China. In reality, the important criteria for optimum plant growth are climate, latitude, and soil conditions, not national borders. This project collected samples of processed Chinese herbs from China, Taiwan, and Hong Kong and compared them to the same processed Chinese herbs grown organically in the United States. Comparisons will be made with both qualitative and quantitative methods.

Until recently, the only North American source for Chinese herbs has been suppliers who import them directly from China. For some of these herbs, there has been a significant amount of analytical chemistry conducted in laboratories in China, Japan, North America, and Europe. Since the above discussion is concerned with growing these herbs by organic methods in the United States, it is necessary to evaluate a sampling of these herbs that are grown by this means in order to compare them with the same herb grown in China. Traditionally, as well as currently, the method of evaluation has been organoleptic, performed by experienced herbalists who recognize the color, taste, smell and consistency of high quality herbs. While this remains an important means of assessing herb quality, it is still subjective.

High performance liquid chromatography (HPLC) and thin layer chromatography (HPTLC) are both universally accepted methods for evaluating the chemical composition of natural products. HPLC can give a more precise quantitative image of the constituents, while HPTLC can give a more qualitative image of the chemical profile, along with a general quantitative value. By employing HPTLC to evaluate the two sets of Chinese herbs, it will be possible to make an objective comparison between them. This study looked at HPTLC analyses of the samples. A future study will more precisely quantify the results with HPLC.

The herb selected for this study is Dan Shen (*Salvia miltiorrhiza*), a major medicinal agent in the traditional Chinese materia medica. In a 2005 study of pesticide residues in Chinese herbs purchased in the farming areas of China and the markets of Hong Kong, the Dan Shen samples contained residues of organochlorine pesticides, making it an ideal candidate for this research.[2] The red-colored roots of this Chinese sage species are employed in formulas that invigorate, cool, or nourish the blood, making it an important ingredient in prescriptions for cardiovascular, gynecological, inflammatory, and psycho-emotional disorders. Modern research has also found that compounds in Dan Shen have anti-cancer effects.

Traditional Energetics, Functions, and Indications for Dan Shen[3]

Botanical name: *Salvia miltiorrhiza*
Pharmaceutical name: Salviae miltiorrhizae Radix

[2] Leung KS, Chan K, Chan CL, Lu GH. Systematic evaluation of organochlorine pesticide residues in Chinese materia medica. *Phytother Res*. 2005 Jun;19(6):514-8.
[3] Bensky D, Clavey S, Stoger E. Chinese Herbal Medicine – Materia Medica, 3rd Edition. 2004; pp. 602-604.

Common name: Salvia root, Red Sage root
Properties: Bitter, Slightly Cold
Channels: Heart, Pericardium, Liver
Functions:

1. Invigorates the blood and dispels stasis
2. Clears heat and soothes irritability
3. Cools the blood and reduces abscesses
4. Nourishes the blood and calms the spirit

Indications:

1. A wide variety of blood stasis disorders in any part of the body. Most commonly used for problems affecting the lower abdomen, chest, or hypochondria. It is a very important herb in gynecology.
2. Especially useful for restlessness, irritability, palpitations, and insomnia due to heat entering the nutritive level. Can also be used in patterns of Heart and Kidney yin deficiency.
3. An adjunctive herb to treat sores or the early stages of breast abscess.
4. Palpitations and insomnia due to either heat entering the nutritive and blood levels or insufficient Heart blood.

Dosage: 6 - 15 grams
Cautions / Contraindications: Do not use in pregnancy

<u>Formulas containing Dan Shen</u>

1. Dan Shen Yin:[4]

Radix Salviae Miltiorrhizae (Dan Shen): 30 grams
Lignum Santali Albi (Tan Xiang): 4.5 grams
Fructus seu Semen et Pericarpium Amomi (Sha Ren): 4.5 grams

Actions: Invigorates the blood, dispels blood stasis, promotes the movement of qi, and alleviates pain.

Indications: Abdominal or epigastric pain which may radiate upward accompanied by signs and symptoms of blood stasis and qi stagnation.

2. Qing Ying Tang:[5]

Cornu Bubali (Shui Niu Jiao): 30-120 grams
Radix Scrophulariae Ningpoensis (Xuan Shen): 9 grams
Radix Rehmanniae Glutinosae (Sheng Di Huang): 15 grams
Tuber Ophiopogonis Japonici (Mai Men Dong): 9 grams
Flos Lonicerae Japonicae (Jin Yin Hua): 9 grams
Fructus Forsythiae Suspensae (Lian Qiao): 6 grams
Rhizoma Coptidis (Huang Lian): 4.5 grams
Herba Lophatheri Gracilis (Dan Zhu Ye): 3 grams
Radix Salviae Miltiorrhizae (Dan Shen): 6 grams

[4] Bensky D, Barolet R. Chinese Herbal Medicine – Formulas & Strategies. 1990; p.318.
[5] Bensky D, Barolet R. Chinese Herbal Medicine – Formulas & Strategies. 1990; p.75.

Actions: Clears the nutritive level, relieves fire toxin, drains heat, and nourishes the yin.

Indications: High fever that worsens at night, severe irritability and restlessness, a scarlet, dry tongue, and a thin, rapid pulse. Some patients are thirsty, some delirious, and some exhibit faint and indistinct erythema and purpura.

3. Huo Luo Xiao Ling Dan:[6]

Radix Angelicae Sinensis (Dang Gui): l5 grams
Radix Salviae Miltiorrhizae (Dan Shen): l5 grams
Gummi Olibanum (Ru Xiang): 15 grams
Myrrha (Mo Yao): l5 grams

Actions:
Invigorates the blood, dispels blood stasis, unblocks the collaterals, and alleviates pain.

Indications:
Pain in various locations such as the heart, stomach, abdomen, back, leg, or arm, bruising and swelling due to traumatic injury, rheumatic pain, fixed abdominal masses, internal or external ulceration, a dark tongue or one with static points, and a wiry pulse.

Bioactive Compounds in Dan Shen: Tanshinones and Salvianolic Acid

The most extensively studied compounds in Dan Shen are the various tanshinones and salvianolic acid. The chromatograms in this study will show the presence and concentration of these important constituents. However, it is the whole herb and all of its compounds that have therapeutic importance. According to the American Herbal Product Association's white paper on marker compounds, "It is rare that a single compound or group of compounds is solely responsible for the physiological effect of a botanical; rather, there are usually many types of compounds that are therapeutically relevant."[7] The presence or absence of the tanshinones and salvianolic acid are simply indicators of the potential quality and therapeutic efficacy of the samples, as well as a confirmation of their identity.

There have been numerous studies on the therapeutic effect of the tanshinones and salvianolic acid. Some examples are below.

Anti-cancer Effects
In a study of human non-small-cell lung cancer cells, Tanshinone I significantly inhibited the migration and invasion of the cells in vitro. It also reduced the growth of tumors and metastasis in immunodeficient mice.[8]

[6] Bensky D, Barolet R. Chinese Herbal Medicine – Formulas & Strategies. 1990; p.329.
[7] Eisner, S., et al. *Use of Marker Compounds in Manufacturing and Labeling Botanically Derived Dietary Supplements*. American Herbal Products Association. 2001.
[8] Lee CY, Sher HF, Chen HW, Liu CC, Chen CH, Lin CS, Yang PC, Tsay HS, Chen JJ. Anticancer effects of tanshinone I in human non-small cell lung cancer. *Mol Cancer Ther*. 2008 Nov;7(11):3527-38.

Tanshinone IIA was able to halt mitosis in the HeLa line of cancer cells. It was also able to cause the affected cells to enter apoptosis faster than the cancer drugs vincristine or taxol.[9]

An *in vitro* study demonstrated that Tanshinone IIA inhibited the proliferation of MDA-MB-231 human breast cancer cells.[10] A study in mice with human breast cancer also demonstrated that tanshinone IIA inhibited cell growth.[11]

Tanshinone I induced apoptosis in human breast cancer cells. It affected both the estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) cell lines.[12] Another study of Tanshinone I and MDA-MB-231 breast cancer cells found that it reduced their adhesion to healthy cells, inhibited the migration of the cancer cells through the extracellular matrix, reduced tumor mass, and decreased metastasis.[13]

In a study of the Colo-205 human colon cancer cells line, Tanshinone IIA reduced cell growth and induced apoptosis.[14]

Tanshinone IIA induced apoptosis in human hepatocellular carcinoma cells.[15,16,17] The same effect was found with a Dan Shen / Chi Shao extract.[18]

Tanshinone IIA induced apoptosis and inhibited cell adhesion and invasion effects in acute promyelocytic leukemia cells.[19, 20, 21, 22]

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[9] Zhou L, Chan WK, Xu N, Xiao K, Luo H, Luo KQ, Chang DC. Tanshinone IIA, an isolated compound from Salvia miltiorrhiza Bunge, induces apoptosis in HeLa cells through mitotic arrest. *Life Sci*. 2008 Sep 12;83(11-12):394-403.

[10] Su CC, Lin YH. Tanshinone IIA inhibits human breast cancer cells through increased Bax to Bcl-xL ratios. *Int J Mol Med*. 2008 Sep;22(3):357-61.

[11] Wang X, Wei Y, Yuan S, Liu G, Lu Y, Zhang J, Wang W. Potential anticancer activity of tanshinone IIA against human breast cancer. *Int J Cancer*. 2005 Sep 20;116(5):799-807.

[12] Nizamutdinova IT, Lee GW, Son KH, Jeon SJ, Kang SS, Kim YS, Lee JH, Seo HG, Chang KC, Kim HJ. Tanshinone I effectively induces apoptosis in estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) breast cancer cells. *Int J Oncol*. 2008 Sep;33(3):485-91.

[13] Nizamutdinova IT, Lee GW, Lee JS, Cho MK, Son KH, Jeon SJ, Kang SS, Kim YS, Lee JH, Seo HG, Chang KC, Kim HJ. Tanshinone I suppresses growth and invasion of human breast cancer cells, MDA-MB-231, through regulation of adhesion molecules. *Carcinogenesis*. 2008 Oct;29(10):1885-92.

[14] Su CC, Chen GW, Kang JC, Chan MH. Growth inhibition and apoptosis induction by tanshinone IIA in human colon adenocarcinoma cells. *Planta Med*. 2008 Sep;74(11):1357-62.

[15] Lee WY, Chiu LC, Yeung JH. Cytotoxicity of major tanshinones isolated from Danshen (Salvia miltiorrhiza) on HepG2 cells in relation to glutathione perturbation. *Food Chem Toxicol*. 2008 Jan;46(1):328-38.

[16] Yuan SL, Wei YQ, Wang XJ, Xiao F, Li SF, Zhang J. Growth inhibition and apoptosis induction of tanshinone II-A on human hepatocellular carcinoma cells.*World J Gastroenterol*. 2004 Jul 15;10(14):2024-8.

[17] Tang Z, Tang Y, Fu L. Growth inhibition and apoptosis induction in human hepatoma cells by tanshinone II A. *J Huazhong Univ Sci Technolog Med Sci*. 2003;23(2):166-8, 172.

[18] Hu S, Chen SM, Li XK, Qin R, Mei ZN. Antitumor effects of chi-shen extract from Salvia miltiorrhiza and Paeoniae radix on human hepatocellular carcinoma cells. *Acta Pharmacol Sin*. 2007 Aug;28(8):1215-23.

Tanshinone IIA induced apoptosis in human glioma cells.[23]

Anti-inflammatory Effects

Cryptotanshinone showed anti-inflammatory effects by inhibiting the migration of macrophages.[24]

Cryptotanshinone inhibits the cyclooxygenase-2 (Cox 2) enzyme, a major mediator of inflammation.[25]

Tanshinone I inhibited arachidonic acid metabolism, a mediator of the inflammatory response.[26]

Cardiovascular Effects

Tanshinone IIA was found to activate human cardiac potassium channels, which affect heartbeat frequency. This provides a possible mechanism for the use of Dan Shen in cardiac arrhythmias.[27]

Tanshinone IIA has also been found to inhibit human aortic smooth muscle cell migration, which is involved in the formation of arteriosclerotic plaques.[28]

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[19] Liu JJ, Lin DJ, Liu PQ, Huang M, Li XD, Huang RW. Induction of apoptosis and inhibition of cell adhesive and invasive effects by tanshinone IIA in acute promyelocytic leukemia cells in vitro. *J Biomed Sci*. 2006 Nov;13(6):813-23.

[20] Meng W, Yang Y, Deng C, Liu T, Jia Y. Study on the relationship between NB4 cell apoptosis induced by tanshinone IIA and the cell mitochondrial transmembrane potential. *Zhonghua Xue Ye Xue Za Zhi*. 2002 Jun;23(6):297-300.

[21] Sung HJ, Choi SM, Yoon Y, An KS. Tanshinone IIA, an ingredient of Salvia miltiorrhiza BUNGE, induces apoptosis in human leukemia cell lines through the activation of caspase-3. *Exp Mol Med*. 1999 Dec 31;31(4):174-8.

[22] Yoon Y, Kim YO, Jeon WK, Park HJ, Sung HJ. Tanshinone IIA isolated from Salvia miltiorrhiza BUNGE induced apoptosis in HL60 human premyelocytic leukemia cell line. *J Ethnopharmacol*. 1999 Dec 15;68(1-3):121-7.

[23] Wang J, Wang X, Jiang S, Yuan S, Lin P, Zhang J, Lu Y, Wang Q, Xiong Z, Wu Y, Ren J, Yang H. Growth inhibition and induction of apoptosis and differentiation of tanshinone IIA in human glioma cells. *J Neurooncol*. 2007 Mar;82(1):11-21.

[24] Chiou WF, Don MJ. Cryptotanshinone inhibits macrophage migration by impeding F-actin polymerization and filopodia extension. *Life Sci*. 2007 Jun 20;81(2):109-14.

[25] Jin DZ, Yin LL, Ji XQ, Zhu XZ. Cryptotanshinone inhibits cyclooxygenase-2 enzyme activity but not its expression. *Eur J Pharmacol*. 2006 Nov 7;549(1-3):166-72.

[26] Kim SY, Moon TC, Chang HW, Son KH, Kang SS, Kim HP. Effects of tanshinone I isolated from Salvia miltiorrhiza bunge on arachidonic acid metabolism and in vivo inflammatory responses.*Phytother Res*. 2002 Nov;16(7):616-20.

[27] Sun DD, Wang HC, Wang XB, Luo Y, Jin ZX, Li ZC, Li GR, Dong MQ. Tanshinone IIA: a new activator of human cardiac KCNQ1/KCNE1 (I(Ks)) potassium channels. *Eur J Pharmacol*. 2008 Aug 20;590(1-3):317-21.

[28] Jin UH, Suh SJ, Chang HW, Son JK, Lee SH, Son KH, Chang YC, Kim CH. Tanshinone IIA from Salvia miltiorrhiza BUNGE inhibits human aortic smooth muscle cell migration and MMP-9 activity through AKT signaling pathway. *J Cell Biochem*. 2008 May 1;104(1):15-26.

Tanshinone IIA significantly enhanced Nitric Oxide production in human vascular endothelial cells, a mechanism that controls vasodilation.[29] Cryptotanshinone also demonstrated this effect.[30]

Tanshinone IIA inhibited oxidative stress in human aortic smooth muscle cells.[31]

Salvianolic acid B inhibited plasminogen activator inhibitor type 1 production, which is involved in in the development of atherosclerosis.[32]

Tanshinone IIA inhibited adhesion in human blood platelets.[33]

Alzheimer's Disease

Salvianolic acid B inhibits the formation of Abeta fibrils, which are found in plaques in the brains of Alzheimer's patients. It also breaks down fibrils which have already formed.[34]

Antioxidant Effects

Salvianolic acid B demonstrated protective effects against oxidative damage in neural cells. It did this at lower concentrations than ginkgo biloba extract.[35]

Tanshinone IIA demonstrated protective effects against oxidative damage in human umbilical vein endothelial cells.[36]

Tanshinone IIA inhibited the oxidation of low density lipoprotein, the "bad" cholesterol.[37]

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[29] Huang KJ, Wang H, Xie WZ, Zhang HS. Investigation of the effect of tanshinone IIA on nitric oxide production in human vascular endothelial cells by fluorescence imaging. *Spectrochim Acta A Mol Biomol Spectrosc*. 2007 Dec 31;68(5):1180-6.

[30] Zhou Z, Wang SQ, Liu Y, Miao AD. Cryptotanshinone inhibits endothelin-1 expression and stimulates nitric oxide production in human vascular endothelial cells.*Biochim Biophys Acta*. 2006 Jan;1760(1):1-9.

[31] Zhang HS, Wang SQ. Nrf2 is involved in the effect of tanshinone IIA on intracellular redox status in human aortic smooth muscle cells. *Biochem Pharmacol*. 2007 May 1;73(9):1358-66.

[32] Zhou Z, Liu Y, Miao AD, Wang SQ. Salvianolic acid B attenuates plasminogen activator inhibitor type 1 production in TNF-alpha treated human umbilical vein endothelial cells. *J Cell Biochem*. 2005 Sep 1;96(1):109-16.

[33] Jiang KY, Ruan CG, Gu ZL, Zhou WY, Guo CY. Effects of tanshinone II-A sulfonate on adhesion molecule expression of endothelial cells and platelets in vitro. *Zhongguo Yao Li Xue Bao*. 1998 Jan;19(1):47-50.

[34] Durairajan SS, Yuan Q, Xie L, Chan WS, Kum WF, Koo I, Liu C, Song Y, Huang JD, Klein WL, Li M. Salvianolic acid B inhibits Abeta fibril formation and disaggregates preformed fibrils and protects against Abeta-induced cytotoxicty. *Neurochem* Int. 2008 Mar-Apr;52(4-5):741-50.

[35] Liu CS, Cheng Y, Hu JF, Zhang W, Chen NH, Zhang JT. Comparison of antioxidant activities between salvianolic acid B and Ginkgo biloba extract (EGb 761). *Acta Pharmacol Sin*. 2006 Sep;27(9):1137-45.

[36] Lin R, Wang WR, Liu JT, Yang GD, Han CJ. Protective effect of tanshinone IIA on human umbilical vein endothelial cell injured by hydrogen peroxide and its mechanism. *J Ethnopharmacol*. 2006 Nov 24;108(2):217-22.

Effects on Osteoporosis

Animal studies have previously shown that Dan Shen can affect the occurrence of osteoporosis in rats with their ovaries removed. To examine this effect, an extract of tanshinone I, tanshinone IIA, cryptotanshinone, and dihydrotanshinone was examined and found to inhibit the action of osteoclasts. However, this effect was much stronger when the tanshinones were administered along with a whole extract of Dan Shen, indicating that some other compounds in Dan Shen potentiate this effect.[38]

Materials and Methods[39]

Dan Shen samples were gathered, identified, and archived under the auspices of the American Herbal Pharmacopoeia Botanical Reference Standards program, and the HPTLC was carried out by CAMAG of Muttenz, Switzerland. The chemical reference standards were provided by Chromadex of Boulder, CO.

High Performance Thin Layer Chromatography (HPTLC) for the identification of *Salvia miltiorrhiza (Dan Shen)* root

For identification of *Salvia miltiorrhiza*, method A provides a fingerprint of different compounds, including tanshinones and salvianolic acid. For investigation of contents on individual tanshinones, method B was applied.

Sample preparation

Method A: 0.5 g of milled raw material is mixed with 5 mL of methanol, sonicated for 10 min, and centrifuged for 5 min at 5000 rpm. The supernatant is used as test solution.

Method B: 0.5 g of milled raw material is mixed with 5 mL of ethyl acetate, sonicated for 10 min, and centrifuged for 5 min at 5000 rpm. The supernatant is used as test solution.

Standard preparation (optional)

0.6 mg each of tanshinone I, tanshinone IIA, cryptotanshinone, and dihydrotanshinone are individually dissolved in 1.5 mL of ethyl acetate. 1 mg of salvianolic acid B is dissolved in 1.5 mL of methanol.

NOTE: As additional markers rutin and hyperoside may be used (not present in evaluated samples): 1 mg each of rutin and hyperoside is dissolved in 5 mL of methanol.

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[37] Niu XL, Ichimori K, Yang X, Hirota Y, Hoshiai K, Li M, Nakazawa H. Tanshinone II-A inhibits low density lipoprotein oxidation in vitro. *Free Radic Res*. 2000 Sep;33(3):305-12.

[38] Kim HK, Woo ER, Lee HW, Park HR, Kim HN, Jung YK, Choi JY, Chae SW, Kim HR, Chae HJ. The correlation of Salvia miltiorrhiza extract-induced regulation of osteoclastogenesis with the amount of components tanshinone I, tanshinone IIA, cryptotanshinone, and dihydrotanshinone. *Immunopharmacol Immunotoxicol*. 2008;30(2):347-64.

[39] American Herbal Pharmacopoeia. Monograph draft *Salvia miltiorrhiza*. (www.herbal-ahp.org)

Reagent preparation

Sulfuric acid reagent: 20 mL of sulfuric acid are dissolved in 200 mL of ice cooled methanol.

Plate preparation *(Method B)*

HPTLC plates 10x10 cm or 20x10 cm silica gel 60 F254 are impregnated with caffeine by immersion for 1 s in a solution of 40 g/L caffeine in dichloromethane, followed by drying at room temperature for 5 min, and heating at 80 °C for 5 min

Chromatographic conditions

Stationary phase:	*Method A*: HPTLC plates 10x10 cm or 20x10 cm silica gel 60 F254. *Method B*: HPTLC plates 10x10 cm or 20x10 cm silica gel 60 F254 caffeine impregnated
Mobile Phase:	*Method A*: toluene, dichloromethane, ethyl acetate, methanol, formic acid 4:6:8:1:4 *Method B*: toluene, ethyl acetate, acetic acid 95:5:1
Sample application:	5 µL *(method A)* or 10 µL *(method B)* of test solutions and 2 µL of standard solutions are applied each as 8 mm band with a minimum of 2 mm distance between bands. Application position should be 8 mm from lower edge of plate.
Development:	10x10 cm or 20x10 cm Twin Trough Chamber, saturated for 20 min with filter paper, 5 or 10 mL of developing solvent in each trough. Developing distance is 70 mm from lower edge of plate. Development of the plate should be performed at 33% relative humidity for optimum separation. Dry plate in a stream of cold air for 5 min.
Detection:	*Method A:* 1.) UV 254 nm 2.) Sulfuric acid reagent: The plate is immersed in sulfuric acid reagent for 1 s, then heated for 5 min at 100°C. Examination under white light. *Method B:* 1.) White light 2.) Sulfuric acid reagent: The plate is immersed in sulfuric acid reagent for 1 s, then heated for 5 min at 100°C. Examination under white light.
Results:	Compare to the chromatograms provided.

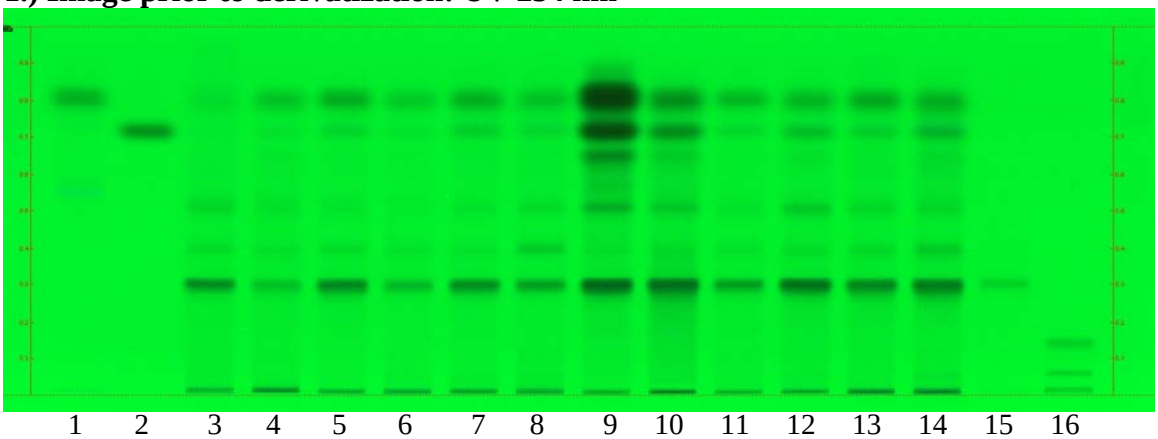
Note: The chromatograms were developed on HPTLC plates which allow for better separation, sharper zones, reduced development time, and require less solvent consumption than standard TLC plates. The method can also be run with standard TLC plates adjusting the sample volume as needed.

Plates 1 and 2: HPTLC of Salvia miltiorrhiza root

Lane 1	Tanshinone I, tanshinone IIA (with increasing Rf)	
Lane 2	Dihydrotanshinone, cryptotanshinone (with increasing Rf)	
Lane 3	Salvia miltiorrhiza root	Tablet product; expires 2011
Lane 4	Salvia miltiorrhiza root	Hong Kong herb market, 2002
Lane 5	Salvia miltiorrhiza root	Taiwan herb market, 2003
Lane 6	Salvia miltiorrhiza root	San Francisco Chinatown market, 2005
Lane 7	Salvia miltiorrhiza root	San Francisco Chinatown market, 2005
Lane 8	Salvia miltiorrhiza root	San Francisco Chinatown market, 2006
Lane 9	Salvia miltiorrhiza root	Freshly harvested and dried specimen from organic herb farm in Petaluma, California, 2007
Lane 10	Salvia miltiorrhiza root	Dried sample from organic herb farm in Petaluma, California, 2006
Lane 11	Salviae miltiorrhizae radix	Sample from analytical lab; source unknown
Lane 12	Salviae miltiorrhizae radix	Sample from analytical lab; source unknown
Lane 13	Salviae miltiorrhizae, Radix	Sample from analytical lab; source unknown
Lane 14	Salvia miltiorrhiza Bunge	Sample from analytical lab; source unknown
Lane 15	Salvianolic acid	
Lane 16	Rutin, hyperoside	

Plate 1
Method A - Identification of *Salvia miltiorrhiza* root

1.) Image prior to derivatization: UV 254 nm

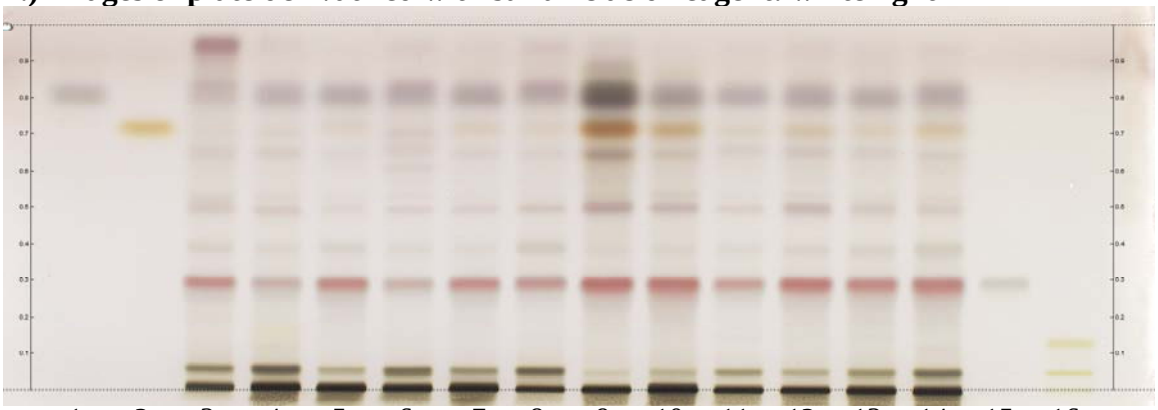


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Discussion of the chromatogram

The standards tanshinone I and tanshinone IIA are co-eluting (Lane 1, Rf=0.80), as are dihydrotanshinone and cryptotanshinone (Lane 2, Rf=0.72). The standards salvianolic acid (Lane 15, Rf=0.30), rutin (Lane 16, Rf=0.07), and hyperoside (Lane 16, Rf=0.15) show quenching zones. There is a quenching zone in all samples corresponding to salvianolic acid. Tanshinones I/IIA are detected in various amounts in all samples. Dihydrotanshinone/cryptotanshinone are detected in lower amounts in almost all samples. Rutin and hyperoside are not detected in any of the samples. All samples have two weak quenching zones (Rf=0.40 and 0.51) above the zone of salvianolic acid. Samples on lane 9 and 10 show an additional zone (Rf=0.64) below dihydrotanshinone/cryptotanshinone.

2.) Images of plate derivatized with sulfuric acid reagent: white light



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

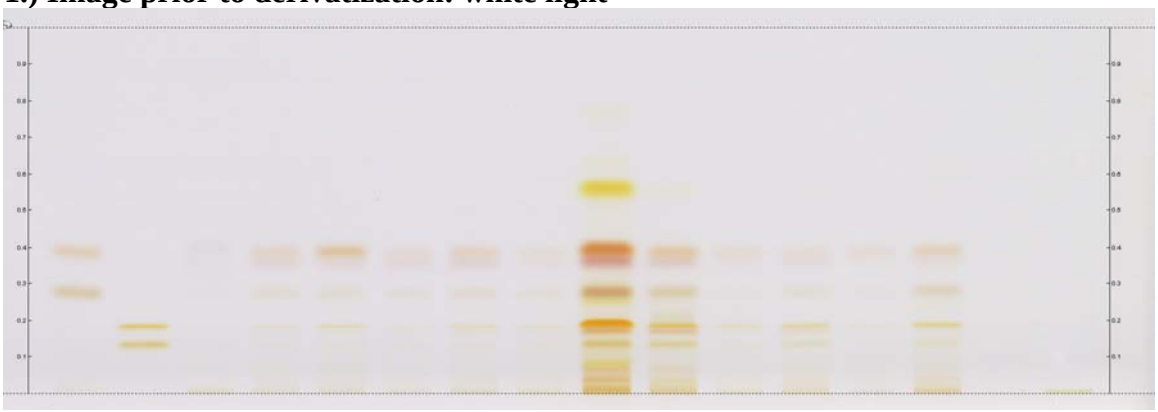
Discussion of the chromatogram

The standards tanshinone I/IIA show a broad grey zone (Lane 1, Rf=0.80). The standards dihydrotanshinone/cryptotanshinone (Lane 2, Rf=0.72) show a yellow zone. The standard salvianolic acid (Lane 15, Rf=0.30) shows a purple to red zone, rutin (Lane 16, Rf=0.07) and hyperoside (Lane 16, Rf=0.15) show yellow zones. There is an intense red zone in all samples corresponding to salvianolic acid. Tanshinones I/IIA are detected as broad grey zone in all samples. Dihydrotanshinone/cryptotanshinone is detected in different amounts

in almost all samples. All samples show a dark brown zone at the same Rf value as rutin. All samples also show an intense black zone at the application position. Several zones in different intensities are present in all samples between salvianolic acid and dihydrotanshinone/cryptotanshinone. One sample shows an intense brown zone (Lane 3, Rf=0.95) right below the solvent front.

Plate 2
Method B - Separation of tanshinones

1.) Image prior to derivatization: white light

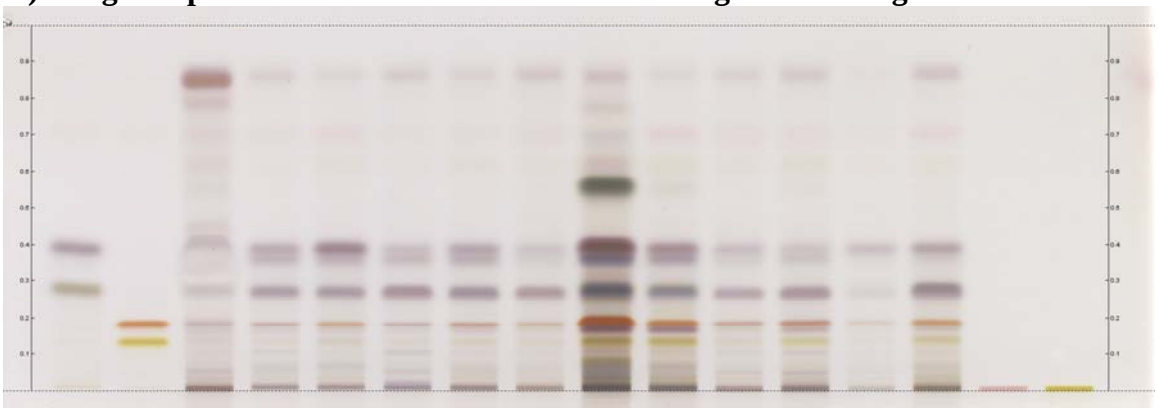


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Discussion of the chromatogram

The standards tanshinone I (Lane 1, Rf=0.27), tanshinone IIA (Lane 1, Rf=0.39), dihydrotanshinone (Lane 2, Rf= 0.13), and cryptotanshinone (Lane 2, Rf=0.18) show yellow to orange zones. Salvianolic acid, rutin and hyperoside are not eluting with this method. The samples show yellow and orange zones in different intensities corresponding in color and position to those of the individual tanshinones. Several samples show an additional red zone (Rf=0.35) just below tanshinone IIA. The sample on Lane 9 shows unusually intense zones and a yellow zone (Rf=0.55) which is detected in no other sample

2.) Images of plate derivatized with sulfuric acid reagent: white light



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Discussion of the chromatogram

The standards tanshinone I (Lane 1, Rf=0.27) is detected as a grey zone, tanshinone IIA (Lane 1, Rf=0.39) as a purple zone, dihydrotanshinone (Lane 2, Rf= 0.13) as a yellow zone, and cryptotanshinone (Lane 2, Rf=0.18) as a dark orange zone. Salvianolic acid, rutin and hyperoside are not eluting with this method. Some samples (Lanes 9, 10, 12, 14) show a distinct yellow zone corresponding to dihydrotanshinone. All samples show a narrow orange zone of different intensity corresponding to cryptotanshinone, a greyish purple zone corresponding to tanshinone I and a purple zone of different intensity corresponding to tanshinone IIA. Several samples show an additional weak purple zone (Rf=0.35) right below tanshinone IIA. The sample on Lane 9 shows an intense dark green zone (Rf=0.55) which is detected in no other sample. One sample shows an intense red zone (Lane 3, Rf=0.95) right below the solvent front.

Results

In the HPTLC analysis, Lanes 9 and 10 represent samples of Dan Shen roots obtained from Chinese Medicinal Herb Farm in Petaluma, California (http://www.chinesemedicinalherbfarm.com). They were grown with certified organic agricultural methods. Lane 9 represents roots harvested in 2007, and Lane 10 represents dried roots harvested in 2006. It can be seen that the bands representing the bioactive tanshinones and salvianolic acid are qualitatively similar to and quantitatively more dense than the samples collected from various herb suppliers (Lanes 3 – 8, 11 – 14). This indicates that the Dan Shen roots grown locally with organic methods met or exceeded the quality of Dan Shen roots purchased from typical suppliers of Chinese herbs. The sample from the most recent harvest showed the highest level of bioactive compounds, possibly due to its freshness, demonstrating another advantage of growing herbs locally. Future studies should use a larger number of domestic samples, and attempts should be made to acquire Chinese sample material from the most recent harvest.

Conclusions

This study has demonstrated that it is possible to grow high quality Chinese herbs in the West with certified organic agricultural methods. This has a number of positive implications:

Environmental Impact

By using “green” non-toxic environmentally friendly agriculture, the rapidly growing use of Chinese herbs will not adversely affect the environment by adding to the already high toxic load of pesticides and fertilizers. By adopting these methods, Chinese herb farms can help begin the task of restoring the health of the environment. Although organic farming is beginning to take hold in China, it has been actively practiced in the United States for over 30 years. Considering this, Chinese Medicinal Herb Farm has offered to train any interested Chinese farmers in these methods.

Another positive impact of this study is that it shows the plausibility of growing high quality herbs outside of China. With China's rapid industrial growth, farmland is at a premium. By growing some of their herbs outside China, they will be able to expand production to meet increasing demand, without the need of using valuable Chinese farmland.

Human Impact

It has long been known that agricultural pesticides and synthetic fertilizers have an adverse effect on human health. With cancer and environmentally caused illnesses rising worldwide, it is essential that exposure to these chemicals is reduced. The rise in the use of these toxic chemicals has increased exponentially since the end of the second World War. This was brought to the attention of the public in 1962 in the revolutionary book Silent Spring,[40] by Rachel Carson. This viewpoint was attacked vigorously by the chemical industry and by governmental agricultural agencies for decades. In recent years, with the evidence of the harmful effects of agricultural chemicals mounting, the organic agricultural movement has finally taken hold in the West. There are now national organic agricultural standards in the United States.[41] This movement is now in its early stages in China as well. Hopefully, this study will encourage more Chinese farmers to consider growing their herbs by organic methods.

Economic Impact

The availability of organically grown Chinese herbs should have little to no adverse financial impact on the Chinese herb market. Herbs grown by conventional methods are typically less expensive, and there will always be a large market for people who want the least expensive herbs. On the other hand, the demand for organically grown Chinese herbs is very high in the West, and many people are willing to pay a premium price for them, much as they do with organically grown food. There are many people who won't use Chinese herbs out of fear of exposure to pesticides and heavy metals, whether this is warranted or not. The availability of organic Chinese herbs will open up the market to these people, as well as to the large amount of people who only purchase organic produce and Western herbs. With the establishment of reliable organic certification agencies in China, these organic Chinese herbs could be grown in China as well as the West.

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[40] Carson, Rachel. Silent Spring. 1962.
[41] United States Department of Agriculture, National Organic Program: http://www.usda.gov/wps/portal/!ut/p/_s.7_0_A/7_0_1OB?navid=ORGANIC_CERTIFICATIO&parentnav=LAWS_REGS&navtype=RT