Angelica sinensis: Analytical investigations and ethnobotanical field study of a Chinese medicinal plant

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Cover picture: Purchased *Angelica sinensis* root

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Abstract

The roots of *Angelica sinensis* (当归: dāng guī) belong to the most commonly used Chinese medicines and have been used in China for almost 2000 years. *A. sinensis* roots are traditionally used to tonify and activate the blood, regulate menstruation and moisten the intestines. According to Chinese medicine the root can be divided into three distinct parts each having different therapeutic effects. Recently, evaluation of the pharmacological effects and quality assessment of *A. sinensis* roots have gained a lot of attention. Ferulic acid and Z-ligustilide are considered to be the biologically active compounds most often associated with the pharmacological activities and are used as marker compounds for the quality assessment of *A. sinensis* roots. Quantification of these marker compounds is usually carried out using GC or HPLC. However, quantification can also be carried out by HPTLC, but this has not been done in *A. sinensis* roots. In this study, by means of HPTLC chromatographic fingerprints are generated and ferulic acid and Z-ligustilide are quantified. In addition, using semi-structured interviews during fieldwork in Gansu and Yunnan the value chain of *A. sinensis* is examined. In this study I show that ferulic acid and Z-ligustilide contents vary depending on area of cultivation, processing, and which part of the roots is used. Higher amounts of both ferulic acid and Z-ligustilide are found in roots cultivated in Gansu compared to Yunnan. Sulfur-smoking and slicing of the roots significantly reduce both marker compounds. In addition, significant differences are found in the comparison of the three distinct parts head, body and tail. However, with the naked eye no obvious differences in color, intensity, amount and order of zones are seen on the HPTLC fingerprints. The semi-structured interviews provide an overview of the complexity of the value chain and help to develop an accurate picture of the cultivation as well as the trade of *A. sinensis*. The cultivation plays an important role in poor families in remote rural areas and helps to increase and diversify their income. Better understanding of the value chain and integration of all participants could improve cultivation and trading conditions of *A. sinensis.* HPTLC has turned out to be a valid and powerful tool not only for the identification of *A. sinensis*, but also for quantitative measurements. The analytical findings made in this study are in large part consistent with previous results using different analytical techniques. Nevertheless, the quality of *A. sinensis* roots is not assessed adequately by exclusively using the present evaluation paradigm for single chemical compounds.

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1. Introduction

1.1 Traditional Chinese medicine

Natural compounds extracted from plants have been used in all medicinal traditions to treat many different diseases (Da-Yuan et al. 1996). Medicinal knowledge about herbs, plants, flowers, leaves or roots can be traced back over centuries from Sumerians to ancient Egyptians, Muslim, Indian or Chinese medicines (Teiten et al. 2013). Initially, knowledge about the use of specific plants was passed on orally and was later on recorded in so called herbals (Leonti et al. 2010; Balunas and Kinghorn 2005). Traditional medicine involving natural remedies is still the primary form of treatment available to many people in developing countries (Da-Yuan et al. 1996). In China, a lot of empirical knowledge on the use of medicinal herbs has accumulated over centuries. Most of the classical writings such as the "Yellow Emperor's Inner Classic" (黄帝内經: *Huáng dì nèi jīng*) or the "Divine Husbandman's Classic of Materia Medica" (神农本草经: *Shén nóng běn cǎo jīng*) were written in the third century A.D. and date back to a long tradition in China (Bensky 2014). A nationwide study performed in the 1990's has shown that of the flora of China with approximately 30'000 higher plant species, no less than 7'294 species have been used medicinally (Qin and Xu 1998). This richness of medicinal plants is the main source of Chinese medicine (CM). Chinese medicine, or Traditional Chinese medicine (TCM)[1], comprises a wide array of medicinal practices sharing common concepts which have been developed in China and is considered to be one of the oldest healing systems (Tang et al. 2008). According to the World Health Organization the origin of CM dates back to 1000 years B.C. (Nestler et al. 2010). Most of the principles of CM differ from those of Western medicine. So does CM not only focus on the disease defined by specific pathological changes, but rather concentrates on the overall state of the body instead (Jiang 2005).

In Chinese medicine acupuncture, massage, moxibustion, food therapy, various kinds of physical exercises such as Qigong and herbal medicine are applied (Tang et al. 2008; Nestler et al. 2010). Unlike Western medicine, which generally addresses diseases with a single chemical compound relieving a single symptom, CM is characterized by its emphasis on supporting the self-healing capabilities of the body in order to maintain or restore balance

[1] The literal translation of the Chinese term 中医 (zhōngyī), which is applied by the People's Republic of China, is "Chinese medicine". The term "Traditional Chinese medicine" is mainly employed by scholars and practitioners of Chinese Medicine in the western world, but is not widely used in China. It was created in the mid-1950s for the use in foreign-language publications (Scheid 2002; 3). Throughout this thesis the term "Chinese medicine" is used.

(Wang et al. 2009). In Chinese medicine a prescription usually consists of several herbs (multi-ingredient formulas) and hence the effects of the remedies result from synergistic effects of various plants and their compounds (Da-Yuan et al. 1996). In clinical practice a unique diagnostic procedure including among others the examination of the tongue and the pulse is used. By means of this diagnosis a personalized mixture of up to 20 herbs is obtained that typically is taken as an aqueous decoction (Wang et al. 2009). This medical approach is thought to be suitable for the disease prevention and for the treatment of chronic diseases (Yuan and Lin 2000). Furthermore, Chinese people regard traditional medicine as being consistent with their own culture and believe that it plays a crucial role in helping the Chinese nation to flourish[2] (Da-Yuan et al. 1996).

1.2 *Angelica sinensis* in Chinese medicine

Species belonging to the Apiaceae family are widely used in various traditional medical systems and also play an indispensable role in Chinese medicine (Zschocke et al. 1998). The use of *Angelica sinensis* (Oliv.) Diels roots, in Chinese called 当归[3] (dāng guī), can be traced back almost 2'000 years to the "Divine Husbandman's Classic of Materia Medica" and is one of the most commonly used traditional medicines (Ai et al. 2013; Wei et al. 2008). The therapeutic actions that are historically attributed to *dang gui* in Chinese medicine are strengthening and invigorating the blood, tonifying the blood, regulating menses, moistening (unblock) the intestines and relieving pain (Liu et al. 2014; Upton 2003). It has been used for centuries primarily to invigorate blood circulation for the treatment of anemia, hypertension, chronic bronchitis, rheumatism and gynecological diseases such as menstrual disorders (Lay et al. 2013; Zhang et al. 2012). As a matter of fact, *dang gui* is one of the main herbs for regulating blood in women and is for this reason also referred to as "female ginseng" (Zhang et al. 2012). Nowadays, more than 70 formulas containing *A. sinensis* are recorded in the Chinese pharmacopoeia (Zhao et al. 2013). According to the Chinese pharmacopoeia (2010, Volume 1), *dang gui* can be used against a multitude of diseases such as blood deficiency, menstrual irregularities, amenorrhea or dysmenorrhea and many more (see Table 1). Furthermore, as per the theory of CM, *dang gui* can be divided into three distinct parts, namely head, body, and tail. These three functional parts are reported to have different

[2] Chinese medicine is inevitably linked to China's project of social transformation. In fact the state redefined the function of Chinese medicine several times after 1949 (Scheid 2002; 65).
[3] The pharmaceutical name is *Angelicae sinensis* radix.

therapeutic effects, whereby the head is mainly used to stop bleeding, the body to nourish the blood and the tail to quicken the blood (Wei et al. 2008). Besides its medical use, *dang gui* is also used as a health food product for women's care in Europe and America and constitutes one of the main exports of traditional Chinese medicine and health food (Ai et al. 2013; Zheng et al. 2015). In Asia *dang gui* is also used in cosmetics, which are said to have anti-aging effects (Zhang et al. 2012).

1.3 Recent pharmacological studies of *dang gui*

Recent chemical and pharmacological investigations support the power of *Angelica sinensis* roots (see Table 1). These studies suggest, that *dang gui* possesses anti-cancer as well as anti-oxidant activities and helps preventing both cardiovascular and cerebrovascular diseases (Hou et al. 2004; Lay et al. 2013). In addition, studies of various extracts or compounds purified from *dang gui* have been proven to increase myocardial blood flow and to be effective in the treatment of diabetes mellitus (Ai et al. 2013; Zhang et al. 2006). A traditional herbal decoction containing *dang gui* (当归补血汤: Dāngguī bǔxuètāng) is even proposed to be a potential and safe herbal agent for Alzheimer's disease (Zhang et al. 2009). Of the more than 70 isolated and identified compounds from *dang gui*, ferulic acid, Z-ligustilide and other phthalides are considered to be the biologically active compounds and most often associated with the pharmacological activities (Lao et al. 2004; Zhao et al. 2003). In addition, ferulic acid and Z-ligustilide are commonly used as marker components for the quality assessment of *dang gui* (Wei et al. 2008; Zhao et al. 2003; Yi et al. 2009).

Table 1 Effects and indications according to Chinese medicine and modern research. Use categories after Dal Cero et al. (accepted).

	According to Chinese medicine[4]	According to modern pharmacological studies
Effects:	**Tonic** - Strengthens and invigorates the blood, tonifies the blood **Gynecological** - Regulates the menses **Cardiovascular** - Activates blood circulation **Respiratory** - Disperses cold **Gastrointestinal** - Moistens (unblock) the intestines and relaxes the bowels **Nervous system** - Alleviates pain	**Cardiovascular** - Increases myocardial blood flow (Ai et al. 2013; Zhang et al. 2006) - Improves blood fluidity and inhibits platelet aggregation (Chen et al. 2013; Ou and Kwok 2004) **Other** - Anti-cancer as well as anti-oxidant activities (Hou et al. 2004; Lay et al. 2013) - Anti-inflammatory effects (Lay et al. 2013)
Symptoms/ Indications:	**Gynecological** - Menstrual disorders like irregular menstruation, amenorrhea and dysmenorrhea **Cardiovascular** - Blood deficiency with symptoms such as dizziness and palpitations **Gastrointestinal** - Constipation - Abdominal pain **Respiratory** - Chronic bronchitis **Skeletomuscular** - Rheumatism **Dermatological** - Sores, abscesses	**Cardiovascular** - Cardiovascular and cerebrovascular diseases (Hou et al. 2004; Lay et al. 2013) **Other** - Treatment of diabetes mellitus (potentially) (Ai et al. 2013; Zhang et al. 2006) - Alzheimer's disease (potentially) (Chen et al. 2013; Zhang et al. 2009) - Memory deficits (Cheng et al. 2011; Li et al. 2015) - Prevention of cancer (Shang et al. 2003) - Prevention of cerebral infections (Chen et al. 2013)

[4] According to Chinese pharmacopoeia (2010, Volume 1), Liu et al. (2014) and Upton (2003).

1.4 Ferulic acid and Z-ligustilide

Ferulic acid, a caffeic acid derivative, is an ubiquitous phenolic compound in plant tissues (see Fig.1; Mancuso and Santangelo 2014; Zhao and Moghadasian 2008). Many staple foods such as grain bran, whole grain foods, citrus fruits, banana, coffee, orange juice or broccoli as well as Chinese medicinal herbs like *Angelica sinensis* (Oliv.) Diels, *Cimicifuga racemosa* (L.) Nutt. and *Ligusticum wallichii* Franch. are sources of ferulic acid (Mancuso and Santangelo 2014). Since its discovery as an active component in Chinese medical herbs, ferulic acid has attracted much attention in the study of Chinese medicine (Ou and Kwok 2004). Ferulic acid has been found to have antioxidant, anti-inflammatory, analgesic and antithrombotic physiological properties (Vilian and Chen 2015). Thus, ferulic acid may be beneficial in the prevention and treatment of diseases which are linked to oxidative stress, including Alzheimer’s disease and atherosclerosis (Zhao and Moghadasian 2008).

Z-ligustilide is a volatile essential oil that is found to be the main active component of many Apiaceae medicinal plants (see Fig.1; Cheng et al. 2011). It makes up 45%-65% of the essential oil present in *dang gui* and is related to its fragrance (Upton 2003; Yi et al. 2009). Recent studies revealed that Z-ligustilide has significant anti-asthmatic, spasmolytic and neuroprotective activities and reduces oxidative stress in brain tissue (Dietz and Bolton 2011; Zhao et al. 2003). Therefore Z-ligustilide might be developed as a new medicine for the treatment of age-related conditions such as memory deficits (Li et al. 2015).

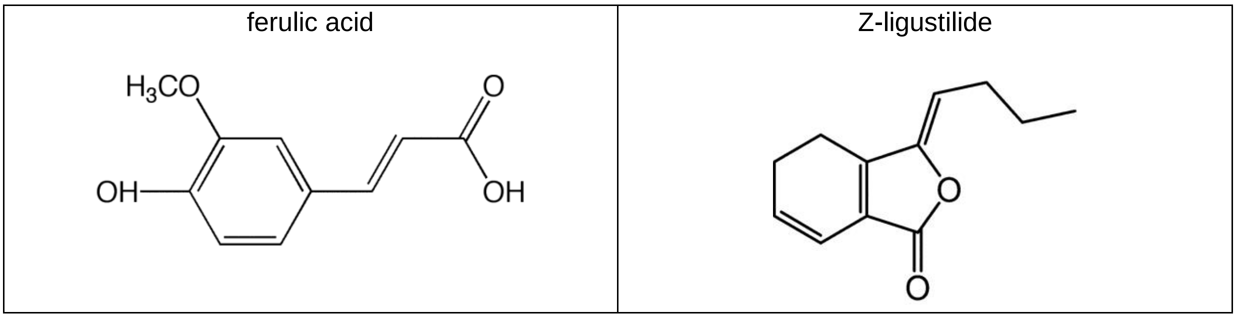

Fig.1 Ferulic acid and Z-ligustilide are associated with the pharmacological activities of *dang gui*.
(source: http://ricebranak.com/product/natural-ferulic-acid, http://jpet.aspetjournals.org/content/337/3/663/F1.large.jpg)

1.5 Value chain and quality control of *Angelica sinensis*

The demand for natural medicinal products is still increasing in western countries and plays an ever more important role in modern medicine (Zschocke et al. 1998). Unlike conventional synthetically produced pharmaceutical products, herbal medicinal products can vary in chemical composition and properties, as chemical constituents of plants are influenced by many factors such as growing location (climate, soil conditions), harvesting time and processing methods (Zhao et al. 2013; Joshi et al. 2004). Another major issue is safety, a common concern in all kind of herbal medicines, which is among others related to contamination, adulteration and improper processing of the raw material (Wang et al. 2009). Therefore, correct identification and quality assurance of the raw material is an inevitable prerequisite to ensure reproducible quality of herbal medicines (Joshi et al. 2004).

1.5.1 Botany and cultivation of *Angelica sinensis*

Angelica sinensis

Angelica sinensis (Oliv.) Diels is a herbaceous perennial endemic to China and belongs to the family Apiaceae (Zhang et al. 2012). *Angelica sinensis* grows from 90cm to 120cm tall, has a hollow stem and flowers are in umbels (see Fig.2; Foster and Yue 1992; 65-73). Its root is yellowish-brown, cylindrical in shape and approximately 15cm to 25cm long, branching into 2-10 primary branch roots at the lower end (Upton 2003). *Angelica sinensis* is a stenotopic species mainly confined to the transition zone between the Loess Plateau and the eastern rim of the Qinghai-Tibetan Plateau and grows best at an altitude of about 1’800 to 3’000m within cool and moist habitats (Lin et al. 2008; Zhang et al. 2012). Because of overharvesting for medicinal use, the wild plant is endangered in China (Zhang et al. 2012) .

Fig.2 Schematic representation of *Angelica sinensis.*
(source: http://acupunctureandphytotherapy.com/angelica-sinensis-dang-gui-chinese-materia-medica/)

Cultivation areas of Angelica sinensis

According to a production status investigation in 1998, *dang gui* has been cultivated in several provinces of China, namely Qinghai (青海: Qīnghǎi), Gansu (甘肃: Gānsù), Sichuan (四川: Sìchuān) and Yunnan (云南: Yúnnán). Traditionally only Gansu and Yunnan are considered to be genuine cultivation areas. In Gansu, the cultivation of *dang gui* mainly takes place in the south (see Fig.3 and 4) and accounts for 90% of the total Chinese *dang gui* production (Zhao et al. 2003). Among the cultivation areas in Gansu, Min County (岷县: Mínxiàn) has the largest yield nowadays producing more than 6’000 tons of *dang gui*, which makes up 70% of the nation-wide production and 80% of the exported *dang gui*. For this reason *dang gui* cultivated in the Min County has a good reputation throughout China and is sometimes even referred to as *min gui* (岷归: Mínguī). In addition, *dang gui* cultivated in Gansu is thought to be of the best quality and is regarded as an authentic and superior medicinal material (道地药材: Dàodì yàocái)[5] (Zhao et al. 2012). The production areas in Gansu largely consist of cold

[5] The term 道地药材 refers to a unique concept in CM that has been widely recognized in the Chinese pharmaceutical industry. It is defined as high-quality medicinal material that is produced in specific geographic regions with distinct natural conditions and ecological environment. Such medical material is considered to surpass the quality and therapeutic effects of the same medical material produced in other regions. 道地药材 is translated as “authentic medicinal material”, “genuine medicinal material” or “geo-authentic medicinal material” (Zhao et al. 2012; Zheng et al. 2015).

and humid mountainous regions with height above sea level more than 2000m and harsh production conditions (DYMYJFC 2012). Over the years the cultivation of medicinal plants is the main, sometimes the only economical resource of local farmers. For this reason, the production of *dang gui* in Gansu played an important role in the local economic development and still occupies a strong position in the agricultural production (DYMYJFC 2012). Many places, including Yunnan, introduced *dang gui* from Gansu. The cultivation has a long tradition in Yunnan and dates back to the 19th century, where according to records *dang gui* was first introduced to the Lanping County during the Qing dynasty around 1815-1850 (DGBS 2009). Yunnan *dang gui* is well known not only within but also outside of Yunnan and is also called *yun gui* (云归: Yúnguī). Furthermore, *dang gui* from Yunnan is also considered as 道地药材, a genuine medical herb (Zheng et al. 2015). In Yunnan the cultivation of *dang gui* is restricted to two geographically separated regions located in the northwest and in the east of the province (see Fig.5). By now Yunnan has the second largest production of *dang gui* after Gansu (YYMZJC 2013).

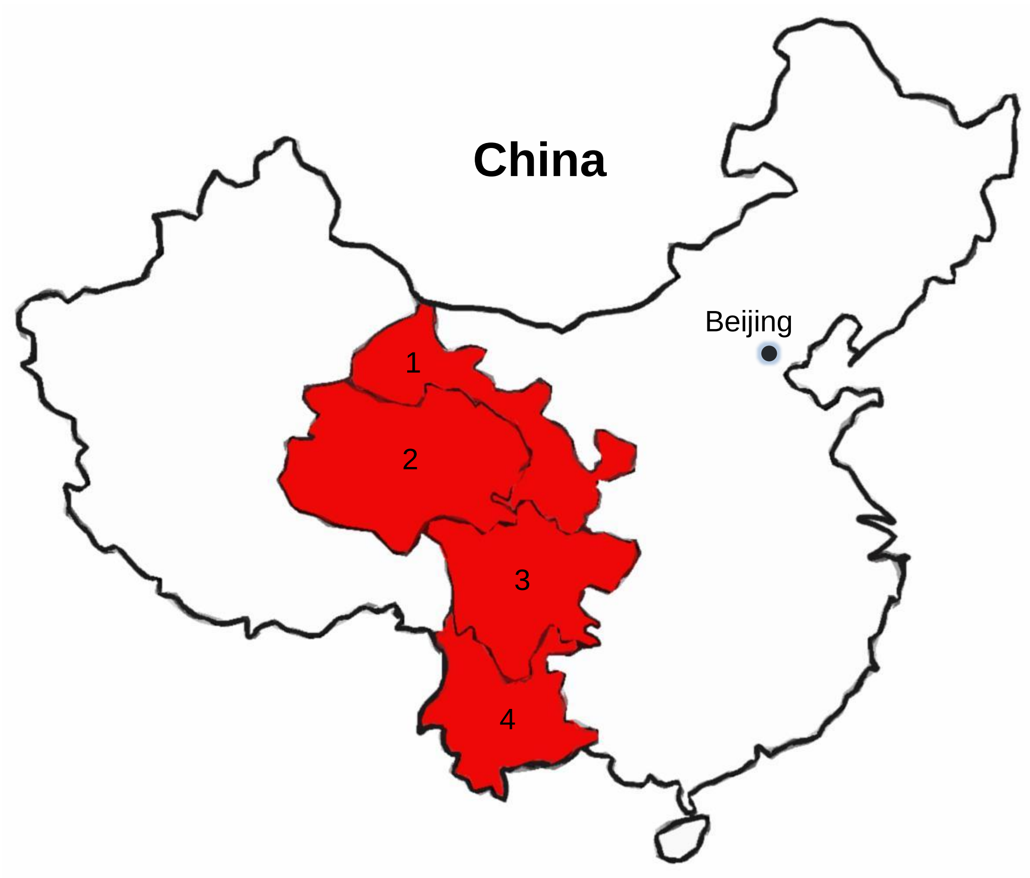

Fig.3 Provinces where *Angelica sinensis* is cultivated: 1 Qinghai, 2 Gansu, 3 Sichuan, 4 Yunnan.

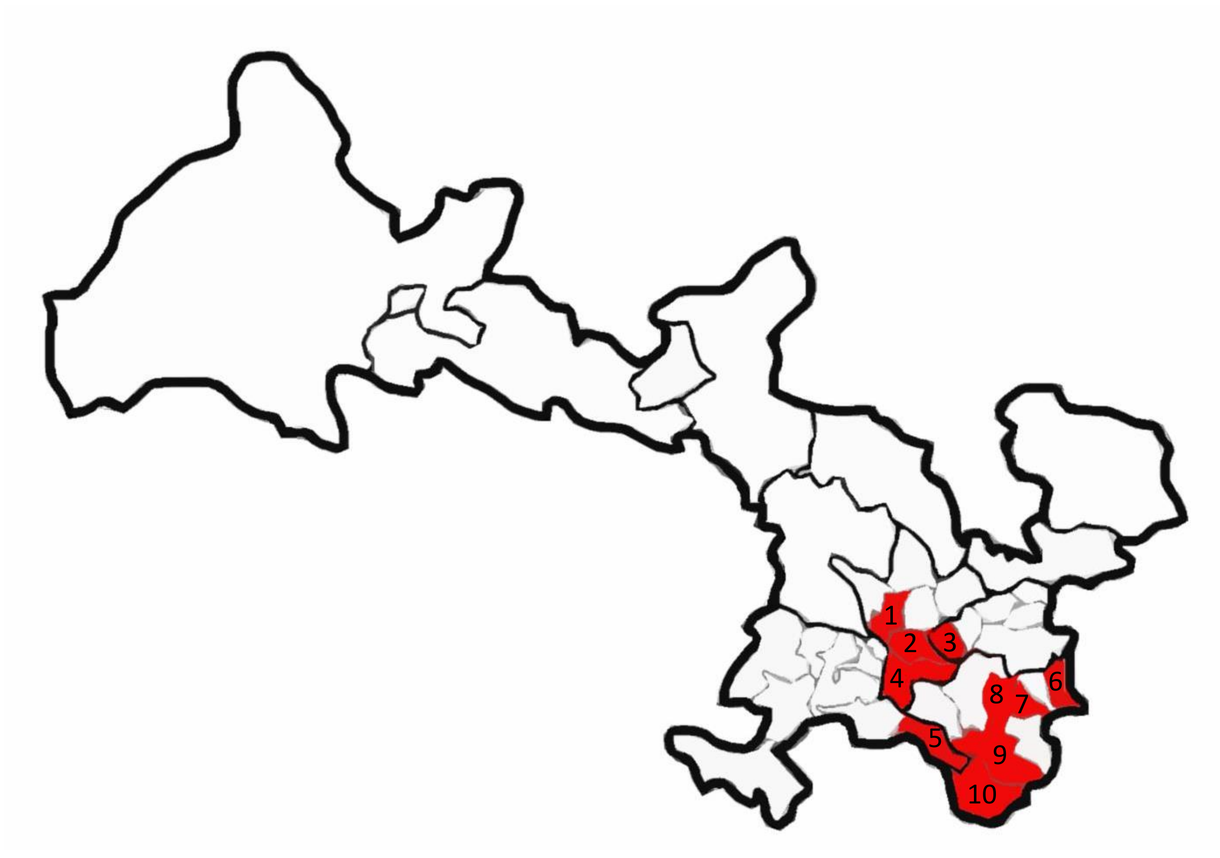

Fig.4 Cultivation areas in Gansu.

Table 2 Cultivation areas in Gansu (DYMYJFC 2012; 15-16). Numbers in table refer to numbers on the map above.

English name	Chinese Name	Nr.
Weiyuan County	渭源县 (Wèiyuán Xiàn)	1
Zhang County	漳县 (Zhāngxiàn)	2
Wushan County	武山县 (Wǔshān Xiàn)	3
Min County	岷县 (Mínxiàn)	4
Zhugqu County	舟曲县 (Zhōuqū Xiàn)	5
Liangdang County	两当县 (Liǎngdāng Xiàn)	6
Cheng County	成县 (Chéngxiàn)	7
Xihe County	西和县 (Xīhé Xiàn)	8
Wudu District	武都区 (Wǔdū Qū)	9
Wen County	文县 (Wénxiàn)	10

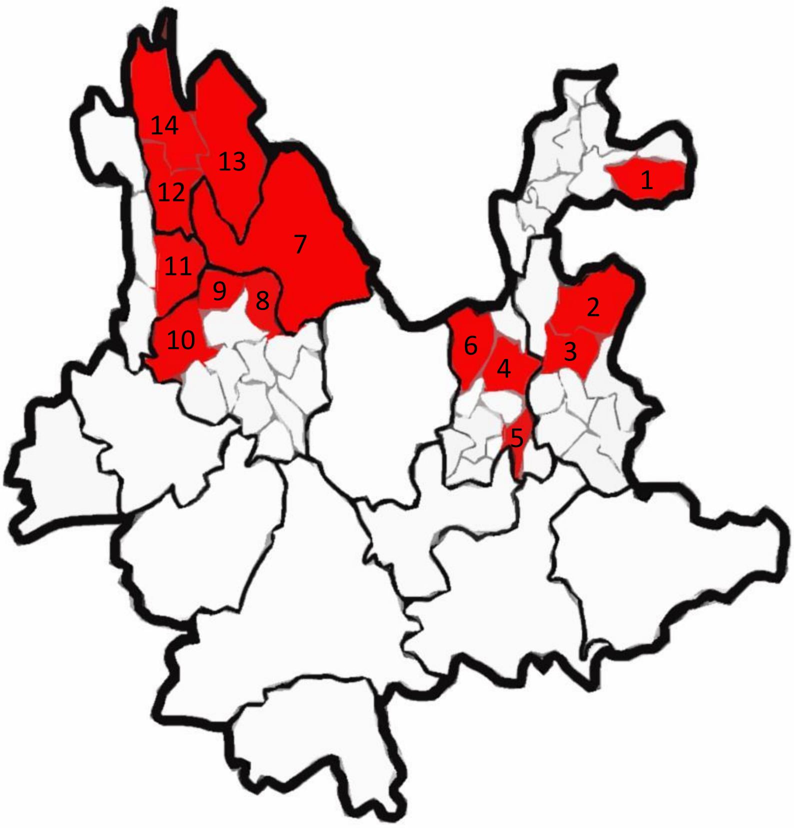

Fig.5 Cultivation areas in Yunnan.

Table 3 Cultivation areas in Yunnan (DYMYJFC 2012; 15-16). Numbers in table refer to numbers on the map above.

English name	Chinese name	Nr.
Zhenxiong County	镇雄县 (Zhènxióng Xiàn)	1
Xuanwei City	宣威市 (Xuānwēi Shì)	2
Zhanyi County	沾益县 (Zhānyì Xiàn)	3
Xundian Hui and Yi Autonomous County	寻甸回族彝族自治县 (Xúndiàn Huízú Yízú Zìzhìxiàn)	4
Yiliang County	彝良县 (Yíliáng Xiàn)	5
Luquan Yi and Miao Autonomous County	禄劝彝族苗族自治县 (Lùquàn Yízú Miáozú Zìzhìxiàn)	6
Lijiang City	丽江市 (Lìjiāng Shì)	7
Heqing County	鹤庆县 (Hèqìng Xiàn)	8
Jianchuan County	剑川县 (Jiāngchuān Xiàn)	9
Yunlong County	云龙县 (Yúnlóng Xiàn)	10
Lanping Bai and Pumi Autonomous County	兰坪白族普米族自治县 (Lánpíng Báizú Pǔmǐzú Zìzhìxiàn)	11
Weixi Lisu Autonomous County	维西傈僳族自治县 (Wéixī Lìsùzú Zìzhìxiàn)	12
Shangri-La City	香格里拉市 (Xiānggélǐlā Shì)	13
Deqin County	德钦县 (Déqīn Xiàn)	14

Cultivation practice and processing

Dang gui is typically grown at an altitude of 1500 to 3000m above sea level in cold and shaded locations, with deep, rich, sandy soil (Foster and Yue 1992; 65-73). *Dang gui* is propagated from seeds and the cultivation typically has a 3-year cycle (Lin et al. 2008). Its cultivation starts by sowing seeds for raising seedlings in June or July of the first year. Young plants are dug up in the autumn of the first year, over-wintered in a root cellar and then replanted early in the following spring (Foster and Yue 1992; 65-73). Unless expressly grown for producing seeds, inflorescence buds are pinched back throughout the growth of the plant in order to prevent the plants from diverting resources away through flower production. The roots are harvested at the end of the second or third year, whereas seeds are harvested from 3-

year-old plants (Upton 2003). In Yunnan, *dang gui* is sometimes sown in January and already harvested in December of the same year (Zhang et al. 2012).

After the roots are dug out, the dirt is shaken off, rootlets are removed and the roots are placed in the shade for several days to partially dry (Foster and Yue 1992; Zschocke et al. 2001). Then they are tied into bundles and smoke-dried by a fire made with wet wood (Upton 2003). *Dang gui* roots may be sulfured, which mainly acts as an insecticide, using sulfur dioxide gas by burning sulfur powder (see Fig.6). This gas is usually mixed with wood smoke during the drying process or burned separately after the smoke-drying (Foster and Yue 1992). Smoke drying is usually completed within 1 to 3 months. In Yunnan, instead of smoke-drying, roots are dried in the sun and frequently turned to prevent hardening of the surface. Roots that are supposed to be sliced are covered with a moist cloth or washed, which allows the roots to absorb enough moisture to facilitate slicing. Slicing is done when the roots are fresh (see Fig.6) prior to drying and the root slices are dried afterwards (Foster and Yue 1992; 65-73). There are additional processing methods applied in Chinese medicine that are thought to modify the action and the therapeutic use of *dang gui*. These are stir-frying with wine, stir-frying with earth or stir-frying and carbonizing, just to name a few (Upton 2003).

1.5.2 High performance thin layer chromatography in quality control

High performance thin layer chromatography (HPTLC) is a valuable tool for different aspects of quality assessment in herbal products (Vaykole et al. 2013). HPTLC is an analytical technique based on thin layer chromatography, but with enhancements compared to the basic method. These include automation of different steps and TLC plates with finer particle size, which offers better resolution and allows more accurate quantitative measurements (Reich and Schibli 2007; Attimarad et al. 2011). Chromatographic fingerprints by HPTLC can be obtained comparatively fast at low cost and thus meet the need of a modern quality control method (Joshi et al. 2004; Reich and Schibli 2007; 135). Furthermore, this analytical technique has proven to be a rapid method for quality evaluation, identification and determination of morphologically similar Apicaceae drugs (Zschocke et al. 1998). Chromatographic fingerprinting is regarded as a comprehensive qualitative approach for the purpose of identification as well as quality evaluation and assurance of traditional Chinese medicines (Yi et al. 2009; Zhao et al. 2013). In addition, thin layer chromatography is the method of choice in the Pharmacopoeia of the people’s republic of China (2012) for the identification of *dang gui*. Recent analytical studies focus on the quantification of the marker components ferulic acid and Z-ligustilide applying gas chromatography coupled with mass spectrometry or HPLC (Wei et al. 2008; Wedge et al. 2009; Zhao et al. 2003). However, the quantification of these two components can also be carried out by HPTLC, but this has not been done in *dang gui* (Vaykole et al. 2013).

Fig.6 Cultivation and processing of *Angelica sinensis* in Min County **(A)** Typical farmer's house **(B)** Field with *A. sinensis* in the second year of the cultivation cycle **(C)** Small rootlets are removed and the roots are stacked for smoke-drying **(D)** After the smoke-drying, roots are sulfured in plastic tents **(E), (F)** Slicing is done when the roots are fresh prior to drying and the slices are dried in the sun afterwards.

1.6 Aim of the study and research questions

In this Master's project ethnobotanical as well as chemical aspects of the Chinese medicinal plant *Angelica sinensis* are investigated. The following research questions are sought to be answered:

I. Where is *Angelica sinensis* currently cultivated and how is *A. sinensis* cultivated and processed?

II. What do participants of the value chain know about *A. sinensis* roots?

III. Do *A. sinensis* roots cultivated in Gansu and Yunnan result in distinct chromatographic fingerprints? Are there differences concerning the abundance of ferulic acid and Z-ligustilide?

IV. Has processing such as slicing and sulfuring an impact on the concentration of ferulic acid and Z-ligustilide?

V. How is the root size linked to the content of ferulic acid and Z-ligustilide in *A. sinensis* roots?

2. Material and methods

2.1 Study sites

Yunnan

Yunnan is a province of China located in the far southwest of the country (see Fig.4 and 7). This region features a great variety of landscapes ranging from subtropical rain forests to alpine landscapes, coupled with an extremely high biodiversity (Xu and Wilkes 2004). Yunnan has recently been included in the Indo-Burma biodiversity hotspot (Song et al. 2015). It is estimated that more than 18'000 plant species are present in Yunnan, including many rare and endemic species (Song et al. 2015). Yunnan is unique not only in respect of landscapes and biodiversity, but also regarding ethnic variety (Reinhardt and Sternfeld 2006). A large number of ethnic groups with distinct languages and customs inhabit this province and account for one third of the total population (Staub et al 2011, references therein). These are among others Bai (白族: Báizú), Dai (傣族: Dǎizú), Hani (哈尼族: Hānízú), Lisu (傈僳族: Lìsùzú) and Naxi (納西族: Nàxīzú), just to mention a few. Many wild and cultivated plants contribute to food security and health care on the one hand, and are of cultural significance on the other hand. How the different traditional lifestyles and customs of these ethnic minorities also find expression in the use and importance of medicinal herbs is a current object of interest (Weckerle et al. 2006).

Gansu

Gansu is a province located at the juncture of the Qinghai-Xizang Plateau, Loess Plateau and the Mongolia-Xinjiang Plateau in the northwest of China (see Fig.4 and 7). High mountains, river valleys, plains, deserts, grasslands and forests make up the diverse landscape of Gansu (Sun et al. 2004). The climate is semi-arid to arid continental climate, which means high temperatures during summer and low temperatures in winter (Zhang and McBean 2014). Gansu is one of the driest regions of China and faces, due to increased population pressures and intensive grazing coupled with the vulnerable arid environment, a number of ecological problems. These are widespread soil erosion and deterioration of grasslands (Tiangang et al. 2004). In fact, approximately 90% of the area in Gansu is affected by soil erosion and only 10% is covered by forests (Liu 2015).

2.2 Fieldwork

The fieldwork for this project took place in Yunnan and Gansu from the end of July to the end of December 2014. Before visiting the places of production of *dang gui*, background information was gathered by means of informal interviews at the market for Chinese medicine in Kunming called 新螺蛳湾中药批发市场 (Xīnluósīwān zhōngyào pīfā shìchǎng) located in Kunming, the capital of Yunnan. This gave us a first insight into the business of *dang gui* and provided information on the current places of production. Furthermore, we got a vague idea of what might be of importance concerning the quality and processing of *dang gui* and the semi-structured interview guide was adjusted accordingly. Based on this information and on the recommendations of my Chinese translator Chen Yulin, Gansu was chosen to be our first destination. In Gansu, the two villages 会川镇 (Huìchuānzhèn) and 甫子山村 (Fǔzǐshāncūn), which are famous for the cultivation of *dang gui*, were visited (see Fig.7). After the stay in Gansu, which only lasted three weeks (see constraints), we continued our fieldwork in Yunnan and spent the rest of the time in this province. As there was only little information about the cultivation in Yunnan available for us, the itinerary heavily relied on verbal information of informants and was perpetually adjusted. In Yunnan fieldwork mainly took part in the northwest and the east (see Fig.7).

All semi-structured interviews were conducted in Chinese with the help of the Chinese translator Chen Yulin (interview guides in Appendix III). Altogether, 118 interviews with participants of the value chain were done over a period of five months. The most detailed interviews were the ones with farmers (n=22), most of which were middle-aged (mean=42yr, SD=11yr) and male (n=19). The vast majority of the farmers was Han Chinese (n=15) and only a small portion belonged to the ethnic minorities Bai (n=5) or Lisu (n=2). The duration of each of these semi-structured interviews varied between 30 and 60 minutes. Interview partners were exclusively chosen by snowball sampling (Bernard 2011; 156-186). In most cases the interviewed farmers were willing to dig out one *Angelica sinensis* plant, which was then vouchered and stored in the plant press. After the interviews with farmers, short semi-structured interviews with dealers, Chinese medicine doctors and consumers were conducted in Kunming during November and December 2014 (see Appendix III). These interviews usually lasted only 5 to 15 minutes, depending on the cooperativeness and time availability of the interviewees. Interviews conducted with dealers took place at the market for Chinese medicine 新螺蛳湾中药批发市场 in Kunming. This is a recently opened market in the south of Kunming stretching over three levels where only Chinese medicines are sold (see Fig.13). In total 28 interviews with dealers (16 men, 12 women), without gathering information about age, were conducted. The interviews with CM doctors (n=14, 12 men, 2 women) were conducted in doctor's offices (診所: Zhěnsuǒ) scattered all over Kunming. Some doctor's offices were shared among CM and Western medicine practitioners, while in most cases CM practitioners had their own doctor's office. Again, no data about age of the interviewees was gathered. For the interviews with dealers and CM doctors, a convenience sampling attempt was chosen. In case of the interviews with consumers a quota sampling was done, as sex of the interviewees was taken into consideration (Bernard 2011; 144-147). These interviews were conducted within the botanical garden of the Kunming Institute of Botany with a total of 54 people (27 women, 27 men, average age=42yr, SD=17yr).

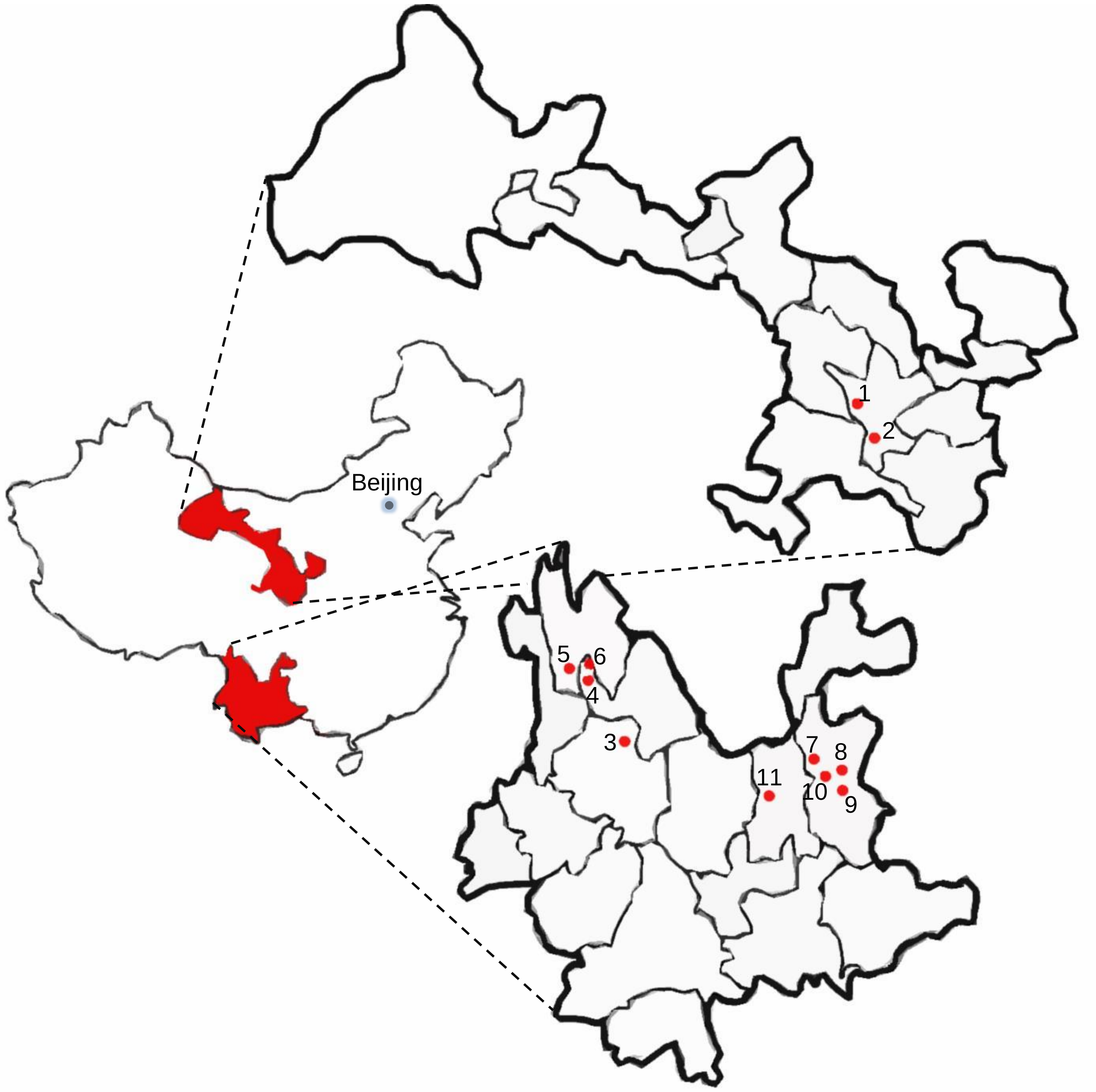

Fig.7 Interviews were carried out in Gansu and in northwest and east Yunnan.

Table 4 Places where interviews took place in Gansu. Numbers in the table refer to the map shown above.

	Location	Prefecture level	Inter-views	Date	Nr.
Gansu	会川镇 (Huìchuānzhèn)	Dingxi City (定西市: Dìngxī Shì)	4	10.08.2014-11.08.2014	1
	甫子山村 (Fǔzǐshāncūn)	Dingxi (定西: Dìngxī)	1	15.08.2014	2
Yunnan	马厂 (Mǎchǎng)	Dali Bai Autonomous Prefecture (大理白族自治州: Dàlǐ Báizú Zìzhìzhōu)	5	23.08.2014-24.08.2014	3
	拉美荣 (Lāměiróng)	Lijiang City (丽江市: Lìjiāng Shì)	1	09.09.2014	4
	腊八底 (Làbādǐ)	Diqing Tibetan Autonomous Prefecture (迪庆藏族自治州: Díqìng Zàngzú Zìzhìzhōu)	3	25.09.2014	5
	塔城 (Tǎchéng)	Lijiang City (丽江市: Lìjiāng Shì)	1	27.09.2014	6
	播乐 (Bōlè)	Qujing City (曲靖市: Qǔjìng Shì)	2	08.10.2014	7
	炎方 (Yánfāng)	Qujing City (曲靖市: Qǔjìng Shì)	2	09.10.2014	8
	菱角 (Língjiǎo)	Qujing City (曲靖市: Qǔjìng Shì)	2	10.10.2014-11.10.2014	9
	白水 (Báishuǐ)	Qujing City (曲靖市: Qǔjìng Shì)	1	13.10.2014	10
	昆明 (Kūnmíng)	Kunming City (昆明市: Kūnmíng Shì)	96	17.10.2014-22.12.2014	11

Counterpart

Fieldwork was organized in collaboration with Prof. 杨永平 (Yáng Yǒngpíng) of the Kunming Institute of Botany (昆明植物研究所: Kūnmíng Zhíwù Yánjiūsuǒ), Chinese Academy of Sciences, by whom I obtained a research permission for fieldwork and export permission for plants. Research was conducted in agreement with the Convention on Biological Diversity (CBD), including the Bonn guidelines on Access and Benefit Sharing (ABS).

Constraints

We left Gansu after three weeks, which in retrospect was too early. There are several reasons for this. First, in 龙潭村 (Lóngtáncūn) the local police urged us to stay in the most expensive hotel for safety reasons and therefore a long stay would not have been affordable. Second, we got into trouble with a boss we barely could get rid of at a marketplace in 龙潭村 (Lóngtáncūn), where all the processing and the trading of *dang gui* takes place. Third, we did not expect that it will be so difficult to find cultivation areas of *dang gui* in Yunnan. In Yunnan, there was almost no information about the cultivation areas available to us before the fieldwork and for this reason we sometimes had to rely solely on verbal information given by people asked on the street or in restaurants. Unfortunately, this information often turned out to be wrong and led to many utterly unsuccessful bus rides to remote villages, where no *dang gui* is cultivated at all. Furthermore, many cultivation areas in Yunnan are located in barely accessible regions high up in the mountains. Therefore a trip to these places was too costly and time-consuming.

2.3 Plant material

All plant material was collected during field work in Gansu and Yunnan between July and December 2014. Most of the samples were purchased at local markets and only a few plants were dug out from the field for the preparation of herbarium specimens or received as a gift from the farmers. We always asked the respective dealers about the place of cultivation and selling price without having bargained. Altogether, more than 200 samples were collected and stored in plastic bags (see table 5 and Fig.13). These consist of whole roots (全归: Quánguī; see Fig.8), only the sometimes already peeled and washed upper part of the root (归头: Guītóu; see Fig.9) and sliced roots (归片: Guīpiàn; see Fig.10). It is noteworthy that nowadays the combined head and body are sold as head, but in former times there was a clear distinction between head and body (Upton 2003). In the analytical part of this work head does not refer to the combination of head and body, but to the distinct uppermost part of the root. Some of the roots purchased are smoked by sulphur, while most of the roots are unsulfured ones (according to verbal statements of salesmen). If several *Angelica sinensis* roots were collected or purchased at once from the same dealer, they were treated as one sample (samples 1-44). The voucher specimens (n=4 from Gansu, n=16 from Yunnan) were deposited at the herbarium of the Institute of Systematic Botany (Z), University of Zürich. The nomenclature follows *The Plant List* (2013, Version 1.1) and *Flora of China* (1994 to present).

Fig.8 Whole root of *Angelica sinensis* called 全归 (Quánguī) in Chinese.

Fig.9 The combined head and body are nowadays commonly sold as head (归头: Guītóu). In former times only the uppermost part of the root was referred to as head.

Fig.10 Sliced *dang gui* (归片: Guīpiàn).

Table 5 Overview of all collected samples cultivated in Gansu and Yunnan differentiating between whole roots, head and slices. Head refers to the combination of head and body.

Area of cultivation	**Whole**	**Head**	**Sliced roots** (bags)
Gansu	101	5	10
Yunnan	79	14	0

2.4 HPTLC analysis

The HPTLC analysis was carried out in the lab of the headquarters of the CAMAG AG situated in Muttenz, Switzerland. For all experiments roots were first treated as single unit and hence processed as whole root and only later on samples belonging to the same sample were pooled or selected roots cut into pieces. All samples were processed in the following way. First, the roots were chopped and then grinded for 2.5 min at 14’200rpm to a finely granulated powder and then passed through a sieve of 355µm pore size. The unsieved remains were stored and later processed analogous to the sieved samples. 1g of each sample was mixed with 4mL of methanol and shaken for 10 min at 200rpm. Thereafter, the samples were centrifuged at 14’200rpm for 5 min and the supernatant was transferred into vials. Isoimperatorin, imperatorin, osthole, Z-ligustilide and ferulic acid (PhytoLab GmbH & Co. KG) were used as reference substances. 1mg of each isoimperatorin, imperatorin, osthole and ferulic acid were dissolved in 1ml of methanol and 10µl of Z-ligustilide were dissolved in 1ml of methanol. By means of the automatic TLC sampler (ATS4, CAMAG) 4µl of isoimperatorin, imperatorin, osthole, Z-ligustilide and 2µl of ferulic acid were applied on the plate. Of all the sample solutions 4µl were applied. The plates were subsequently developed in the automatic developing chamber (ADC2, CAMAG), whereas the chamber was saturated, the developing distance set to 70mm from lower edge and the relative humidity set to 33% ($MgCl_2$). A mixture of toluene, ethyl acetate and acetic acid at a ratio of 90:10:1 (v/v/v) was used as developing solvent. After development the plate was dipped into a sulphuric acid solution (10% sulphuric acid in methanol, CAMAG Chromatogram Immersion Device III; speed: 3, time: 0) and heated at 100°C for 5 min. For the purpose of documentation, pictures of the plates were taken prior to derivatization under white RT light (R=remission, T=transmission), UV 254nm and 366nm and white RT after derivatization, respectively. To examine the differences among parts within the root, 20 selected roots (sample 44) were divided into three parts head, body and tail (归头: Guītóu, 归身: Guīshēn, 归尾: Guīwěi) according to verbal instructions given by Chinese doctors and analyzed separately. In addition, to see how ferulic acid and Z-ligustilide are distributed along the layers of the root a preliminary analysis was done of roots separated into outer and inner part. As the roots were of rubber-like consistency they were cut only in two pieces and the separation is not premised on root physiological properties. In order to assess the reproducibility of the method itself three beforehand grinded samples were extracted three times and each extract applied three times on three different plates. The relative standard deviation was then calculated for each sample within plate. To determine loss on drying, which was carried out following the instruction of the European Pharmacopoeia (2014, 8th edition, volume 2; 1753), 12 representative samples were selected. Thereafter, 1g of each grinded sample was heated at 105°C in an oven for 2h. For the semi-quantitative assessment, a single level calibration of each standard, ferulic acid and Z-ligustilide were prepared in the following concentration: 1mg/ml of ferulic acid and 10 µl/1ml. The profiles of the samples and the standards were generated from the images using the visionCATS software (CAMAG, version 2.0) under 366nm before derivatization (see Fig.11). The areas of the peaks of the references and the samples were then used for quantification (see Fig.12). To ensure comparability of the amounts of ferulic acid and Z-ligustilide of samples between plates, a single level calibration was conducted for each plate separately.

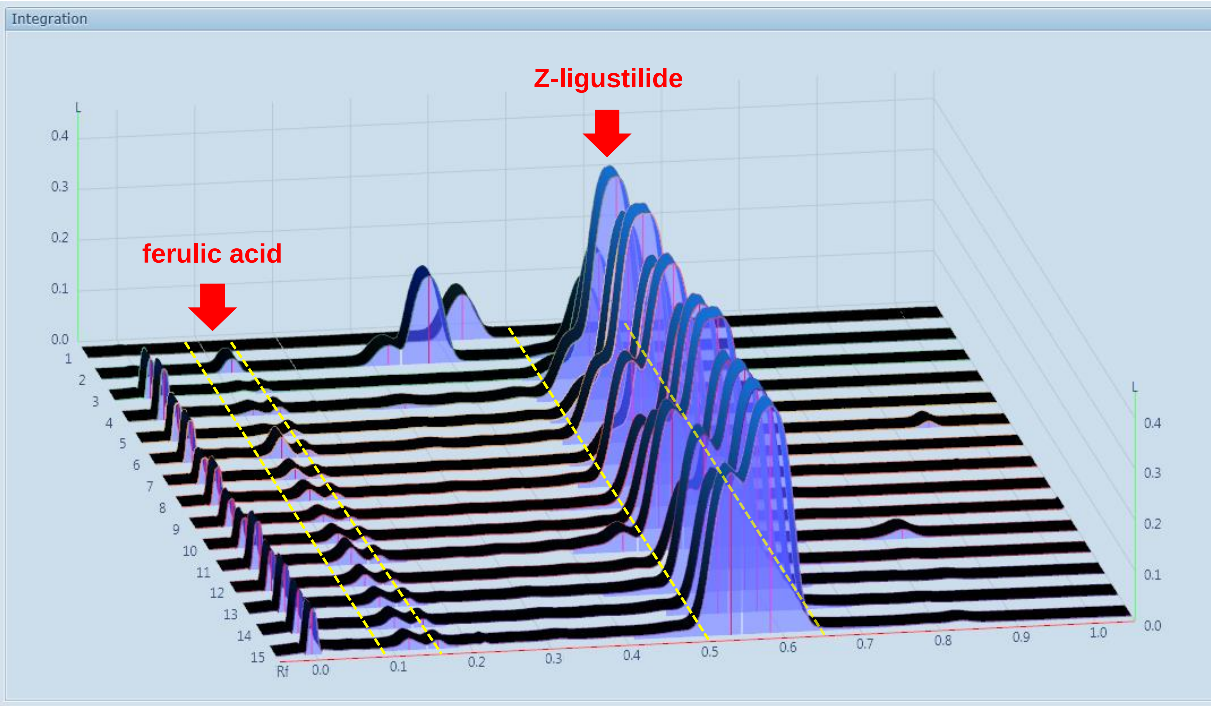

Fig.11 Profiles of the standards (lane 1-2) and the samples (lane 3-15) were generated from the images under 366nm before derivatization.

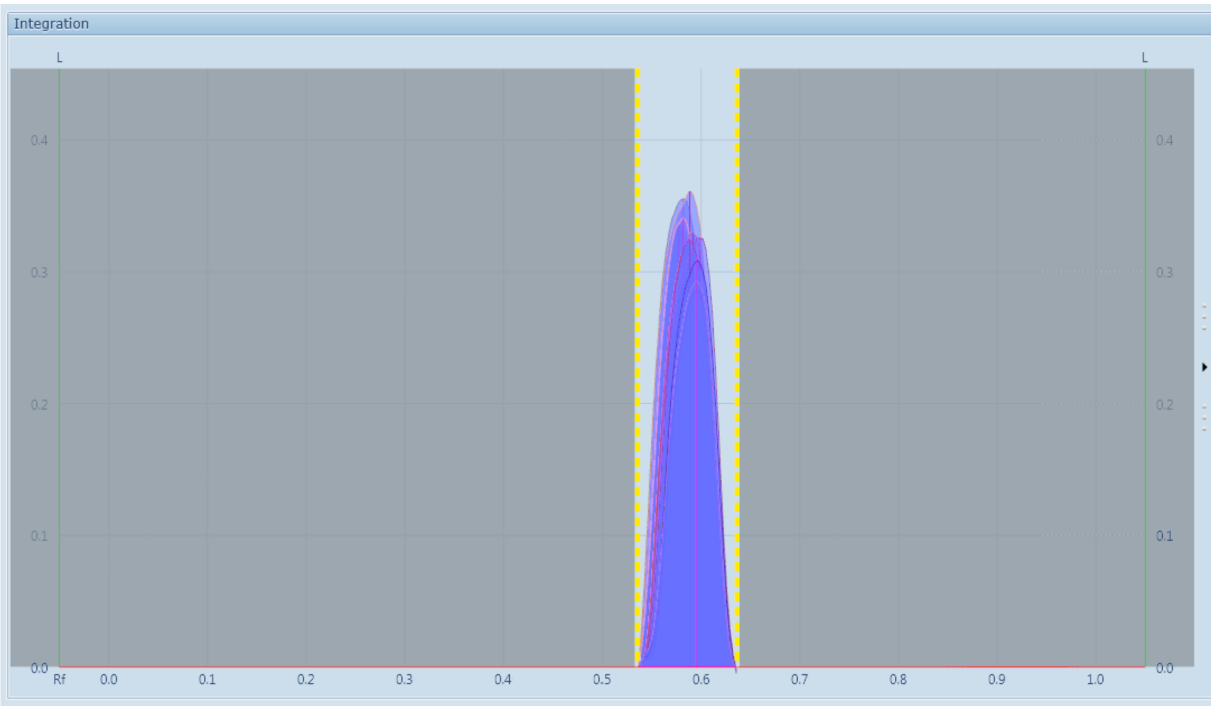

Fig.12 The peak areas were then integrated and used to calculate the amounts of ferulic acid and Z-ligustilide.

2.5 Statistical tools and tests

Statistical analyses were performed using the Statistical Package for Social Sciences (IBM SPSS Statistics 22, Chicago). Assumption of normality was verified by a Kolmogorov-Smirnov test ($n<2000$). The data was then tested for homogeneity of variances using a Levine’s test. For detection of differences of ferulic acid and Z-ligustilide levels between parts of the root an one-way analysis of variance (ANOVA) was performed followed by a Duncan's new multiple range test (MRT; as the group variances are homogenous) to determine pairwise differences among parts of the root. For all other statistical examinations an unpaired t-test was done. Unless stated otherwise, differences were considered significant at $p<0.05$. Graphs were created using GraphPad Prism 6 (6.05 trial).

Fig.13 Trading of *dang gui* in Kunming and in Min County **(A)** Informal interviews with dealers at the market for Chinese medicine in Kunming **(B)** This recently opened market stretches over three levels and only Chinese medicines are sold **(C)** Farmers bring their harvest to the village's marketplace in order to sell *dang gui* to local dealers **(D)** Fresh roots tied into bundles **(E)** During fieldwork purchased roots were stored in plastic bags.

3. Results

3.1 Fieldwork data

Cultivation and processing of Angelica sinensis

Throughout the cultivations areas the field area per household where *Angelica sinensis* is cultivated ranges from 335 to 12’000m^2 (n=16, mean=2492m^2, SD=3198m^2). There were striking differences found between the three distinct cultivation areas (p=0.004). In Gansu, the field size averaged 3996m^2 (n=5, SD=2175m^2), whereas in northwest Yunnan the average field size was 837m^2 (n=6, SD=242m^2) and in east Yunnan 1039m^2 (n=5, SD=1118m^2), respectively.

In Gansu farmers mentioned that cultivation starts in February or March by planting seedlings from the preceding year. Harvest usually takes place in October (n=5). In Yunnan, cultivation and harvest last longer. Typically, farmers start between February and May by planting seedlings sown in the preceding year and harvest is between October and December of the same year (n=7). Interestingly, in east Yunnan all interviewed farmers said that they sow the plants in January and harvest in November of the same year (n=7). Also some farmers in northwest Yunnan cultivate *dang gui* this way (n=2).

Generally, *Angelica sinensis* roots were called *dang gui* and only the interviewed bai people (n=5) in 马厂 (Mǎchǎng) mentioned *qīng guī* as their local name and lisu people (n=2) in 腊八底 (Làbādǐ) called *dang gui* after the province Yunnan *yún guī*.

In Gansu the interviewed farmers have been cultivating *dang gui* throughout their life as their parents already cultivated *dang gui* (n=3). Similarly, in northwest Yunnan most farmers have been cultivating *dang gui* for more than 20 years or throughout their life (n=4). But unlike in Gansu, there are some farmers who started cultivation 1-5 years ago (n=3). Interestingly, in east Yunnan the vast majority of farmers started cultivating *dang gui* less than 15 years ago (n=6). Consequently, in Gansu and northwest Yunnan the cultivation of *dang gui* was regarded by most farmers as a family tradition or even as a tradition of the whole village (n=13). In contrast, in east Yunnan farmers did not consider the cultivation of *dang gui* to be a tradition (n=8).

Medicinal plants and important cash crops cultivated in addition to *dang gui* are given in figure 14. In Gansu farmers most commonly cultivate *Codonopsis pilosula* (n=4) and *Astragalus membranaceus* (n=3). In northwest Yunnan *Aconitum wilsonii* (n=7), *Ligusticum wallichii* (n=5) and *Lepidium meyenii* (n=2) are mainly cultivated besides *dang gui*. If not *dang gui* is cultivated, the most common alternative in east Yunnan is *Nicotiana tabacum* (n=4), a typical cash crop.

Across the three cultivation areas most of the farmers said that they mainly rely on organic fertilizer and only little chemical fertilizer (n=17). Five of them exclusively use organic fertilizer (sheep or goat dung), arguing that chemical fertilizer is harmful to health or renders the soil hard. In Gansu and in northwest Yunnan most farmers emphasized that they explicitly avoided pesticides (n=14), whereas in east Yunnan all farmers mentioned to use pesticides (n=7).

In all areas cultivation was usually carried out without the use of machines (n=17) and most of the farmers do not process *dang gui* themselves (n=15). *Dang gui* is dug out and sold fresh (n=15) and only in a few cases farmers cut *dang gui* into pieces (n=2) or remove the small branches of the roots (n=2).

Throughout the cultivation areas farmers think essential factors for successful cultivation of *dang gui* are altitude of about 2000 to 3000m above sea level (n=10) and plenty of rain during the growth of the seedlings (n=5). As ideal soil type red soil (n=5), black soil (n=4) as well as white soil (n=3) were named, whereas red soil is only mentioned in east Yunnan. Due to the influence of the weather and the unpredictable prices, farmers consider the cultivation of *dang gui* risky (n=5). In Gansu one farmer told us that cultivating *dang gui* is like gambling, i.e. high investment and dependence on unpredictable weather.

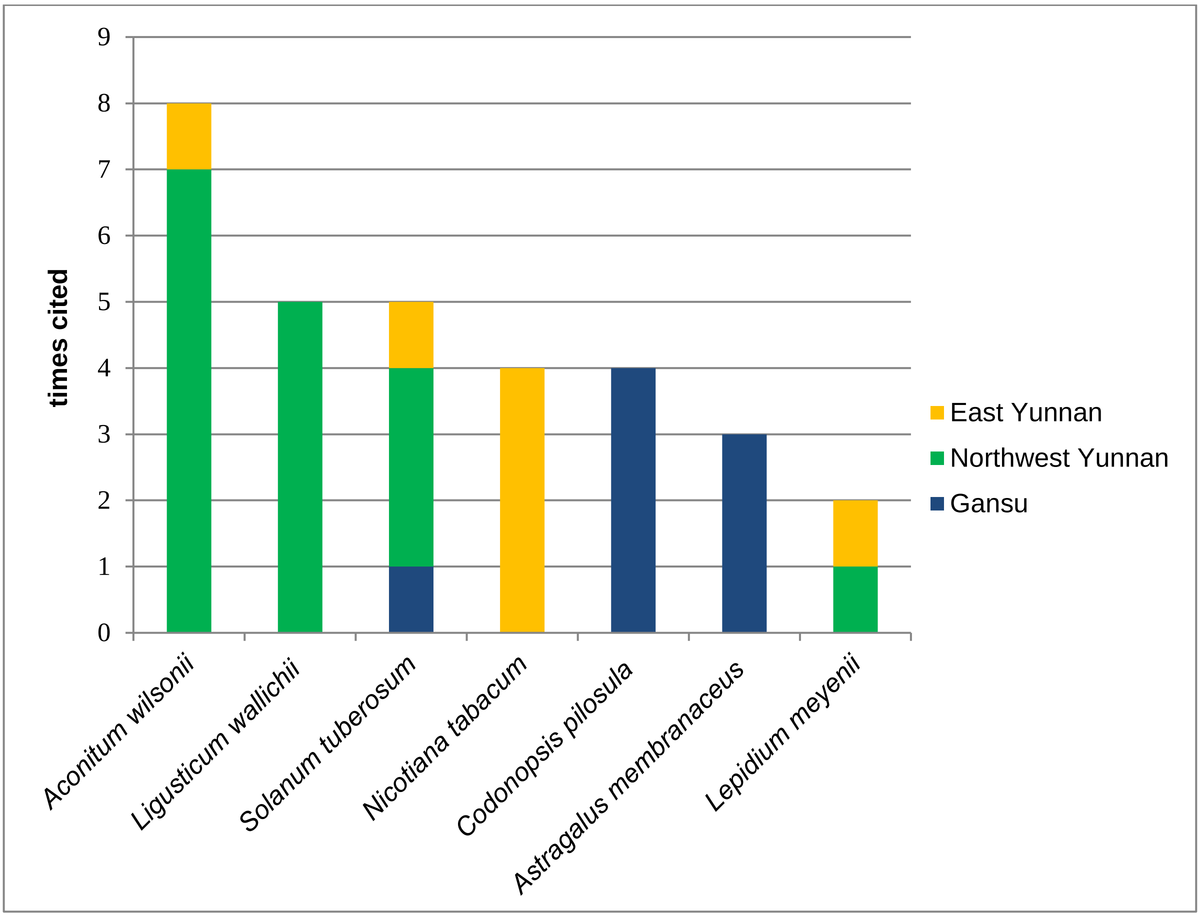

Fig.14 Most common cash crops or medicinal plants cultivated by farmers across the three cultivation areas in addition or alternatively to *Angelica sinensis* (n=22). Complete plant names in Latin and Chinese are in Appendix I.

Quality of dang gui

Along the value chain different criteria were mentioned to be indicative of good quality. For the farmers size of the root often serves as quality criterion in all cultivation areas, that is to say that the bigger the root the better the quality (n=7). Only one of the interviewed farmers said that adulteration of *dang gui* has happened in the past by mixing roots of *Angelica pubescens*, as these are difficult to tell apart from *dang gui*.

In addition to the size of the root, most of the interviewed dealers also mentioned that smell (n=6) as an important factor for good quality.

For the interviewed doctors place of cultivation matters most and they regard *dang gui* from Gansu to be the best one (n=6). Most of them even think that *dang gui* from Gansu is more effective in therapy than *dang gui* from Yunnan (n=7). They argue that the weather in Gansu benefits the quality (n=4) or that Gansu is the authentic place of production, hence *dang gui* from there is an authentic and superior medicinal material (n=2) (道地药材: Dàodì yàocái). However, there are doctors who argue that there is no difference in quality between *dang gui* cultivated in Gansu and *dang gui* from Yunnan and that only the smell or the taste is different (n=3).

Of all the interviewed pedestrians scarcely anybody knew where high quality *dang gui* comes from (n=17f, 19m).

Livelihood and value chain

As a cash crop the cultivation of *dang gui* plays an important role in assuring farmer's livelihood, but to what extent the cultivation contributes to it varies among the different areas. In Gansu the cultivation of *dang gui* or other medicinal plants is often the main income source of the farmers (n=4), whereas in northwest Yunnan it is jobbing (n=5) and in east Yunnan the cultivation of *Nicotiana tabacum* (n=3). Therefore the average contribution of the cultivation of *Angelica sinensis* to the total income is higher (p=0.054) in Gansu (n=4, 62.5%, SD=22.2) compared to Yunnan (n=5, 29.2%, SD=18.7).

Governmental support is found rarely and was only documented for east Yunnan by either providing free seeds (n=3) or seedlings (n=1). Furthermore, there are no regulations by the government regarding the cultivation or quality of *Angelica sinensis* known to the farmers. In Gansu and in northwest Yunnan the *dang gui* harvest is mainly sold to local dealers (n=11) and farmers usually do not act in cooperation with companies. In contrast, in east Yunnan the vast majority of the interviewed farmers sells *dang gui* to pharmaceutical companies (n=4) or to the government, that is to say to the local bureau of agriculture (n=3). However, one farmer in east Yunnan explained to us that most farmers sell *dang gui* themselves at the local market in order to achieve the highest prize. Furthermore, he was of the opinion that if farmers acted in cooperation with a company there would be a fixed prize which may be lower than the price at the local market. Therefore there are only few farmers who sell *dang gui* directly to local companies.

Trading and prices

Participants of the value chain look at the prize of *dang gui* and how it is defined from various angles. Farmers think the price is defined by the dealers themselves (n=3), by the big companies (n=2) or just very general by the market (n=4). Others have no idea who defines the price (n=4). As for the selling price, there are considerable fluctuations (n=6) and the price can even change from one day to another, as one farmer explained. Being asked what they know about the value chain, two farmers in northwest Yunnan stated that dealers sell *dang gui* in Dali or in Kunming (n=2). In Gansu one farmer said that dealers store *dang gui* to sell it for the best price and to do so they need to smoke *dang gui* with sulfur. Another farmer in northwest Yunnan thinks that middlemen process *dang gui* only to get higher profit.

Most of the interviewed dealers sell *dang gui* from Gansu (n=19) and only few ones sell *dang gui* both from Gansu and from Yunnan (n=4) or exclusively from Yunnan (n=5). They told us that the selling price has been decreasing over the past years (n=16), but the demand for *dang gui* remains unchanged (n=19).

There are striking differences in prize between whole roots and heads (see table 6 and 7). The whole roots are on average sold for a significant lower prize (p= 0.043) of 222 CNY/kg[6] (n=21, SD=209.0) and the heads for 372 CNY/kg (n=21, SD=255.2). Furthermore, the selling price of *dang gui* differs considerably between shops in Kunming, Lijiang and Shangrila, also depending on place of cultivation of the sold roots. The average prize of whole roots from Gansu is highest in Lijiang with 540 CNY/kg (n=2, SD= 28.3) and lowest in Kunming with 67 CNY/kg (n=9, SD=5.7). Whole roots from Yunnan are sold for 173 CNY/kg in Shangrila (n=9, SD=203.7) and for 75 CNY in Kunming (n=1). It is striking that in Kunming mainly whole roots from Gansu are sold and in Shangrila only roots from Yunnan. Heads from Gansu are sold in Lijiang for 300 CNY/kg (n=1) and in Kunming for 95 CNY/kg (n=3, SD=32.8). Interestingly, heads from Yunnan are much more common and are sold at much higher prizes with an average of 600 CNY/kg (n=5, SD= 387.6) in Shangrila and 190 CNY/kg (n=4, SD=40.3) in Kunming.

Table 6 Selling prize of whole roots in Kunming, Shangrila and Lijiang in CNY. The number of shops visited is indicated by n.

	Kunming (n=10)		**Shangrila (n=9)**		**Lijiang (n=2)**	
Shops	9	1	-	9	2	-
Place of cultivation	Gansu	Yunnan	Gansu	Yunnan	Gansu	Yunnan
Average selling price	67.2	75	-	173.3	540	-
SD	5.7	-	-	203.7	28.2	-

[6] 1 CNY = 0.1613 USD, July 1, 2015

Table 7 Selling prize of heads (the combined head and body) in Kunming, Shangrila and Lijiang in CNY. The number of shops visited is indicated by n.

	Kunming (n=7)		Shangrila (n=5)		Lijiang (n=9)	
Shops	3	4	-	5	1	8
Place of cultivation	Gansu	Yunnan	Gansu	Yunnan	Gansu	Yunnan
Average selling price	95	190	-	600	300	398.8
SD	32.8	40.3	-	387.6	-	106.4

Medicinal use of dang gui

As one of the most commonly used medicine in China, *dang gui* is known and used by all participants of the value chain. Farmers throughout all cultivation areas described *dang gui* as a medical plant (n=17) and sometimes they knew about the medical indications such as supply and quicken the blood (n=7). *Dang gui* is frequently used by farmers themselves as a medicine throughout Gansu and Yunnan (n=15), since farmers preferred Chinese medicine to Western medicine considering CM to have no side-effects and being able to eliminate the root of disease (n=10).

The dealers told us that customers who buy *dang gui* are mainly women (n=9) or elderly people (n=5) and use it in order to supply blood (n=20), to move blood (n=7) or to adjust menstruation (n=5).

The interviewed pedestrians said that they rather go to Western medicine doctors (n=11f, 10m) than to Chinese medicine doctors (n=2f, 11m) and only few ones mentioned that they see both Western and CM doctors (n=4f, 2m). Consequently, the number of interviewed pedestrians who have used *dang gui* is rather low (n= 14f, 12m) and about the same as the ones who have never used *dang gui* (n= 13f, 14m). Strikingly, the ones who see CM doctors are mainly men (n=11). However, the majority of the pedestrians considers CM to be better than Western medicine (n=15f, 15m). They argue that CM has no side effects (n=6f, 6m) and can eliminate the root of a disease (n=1f, 3m) and traces back to a long history (n=2m). The therapeutic effect of *dang gui* such as supplement and quickening of the blood is only known to some (n=10f, 5m). *Dang gui* is most often taken in as decoction in form of a soup (n=20), especially chicken soup (n=8), according to instructions given by the doctors (n=10). The interviewed pedestrians gather information on the use of *dang gui* mainly from television and books (n=5) or by asking their parents and grandparents. (n=5). In contrast, they think Western medicine is faster in action than CM (n=5f, 4m) and more convenient to use (n=4f, 2m), because CM medicines often need to be prepared at home and have a bitter taste. In addition, whether CM or Western medicine is chosen depends on the nature of the ailment. To treat a chronic disease pedestrians most often choose CM, whereas to treat acute diseases Western medicine is used (n=8f, 6m). Western medicine is used especially in case of cold (n=7f, 9m), but also to treat acute pain, fever and cough (n=4f, 4m). If Western medicine is not effective some pedestrians use CM alternatively (n=4f, 2m).

The Chinese medicine doctors pointed out that *dang gui* is a frequently used herb in their therapy (n=14) and most commonly used in combination with other herbs. Figure 15 lists all herbs mentioned to be frequently combined with *dang gui* in CM formulations. The CM doctors said that *dang gui* is mainly used in order to supplement and quicken the blood (n=12), to adjust menstruation (n=2) or to stop pain (n=1). According to the doctors each part of *dang gui* has a different effect and/or usage. The head is used to supplement the blood (n=4), the body to supplement (n=3) and quicken (n=2) the blood. The tail is used to quicken the blood (n=3), primarily to dispel blood stasis (n=3) or resolve hematomas (n=3). The doctors mentioned that *dang gui* is used in the treatment of anemia (n=7), *qi* insufficiency (n=5), blood stasis (n=3), hematoma (n=2) and skin ulcer (n=2). CM doctors most often recommend *dang gui* to be taken in as a decoction in form of a soup (n=12), but it can also be administered as a pill (n=7), or as an injection (n=2).

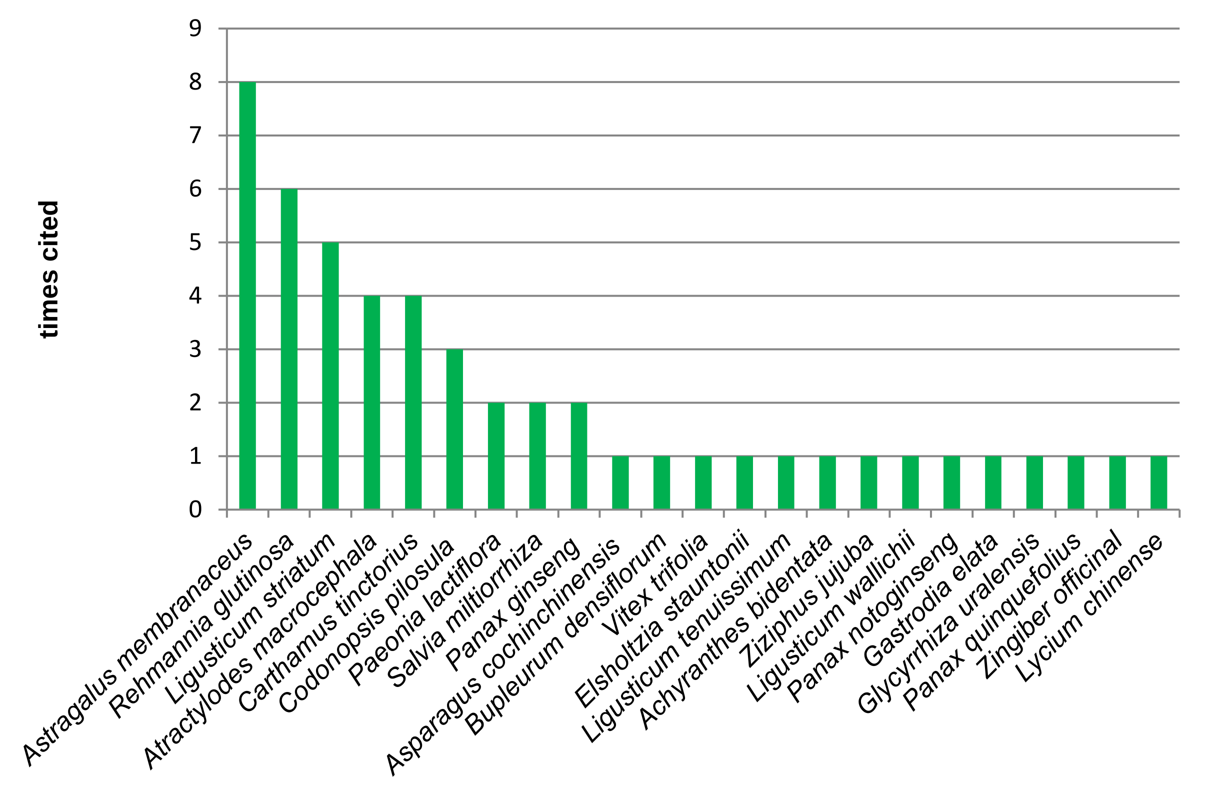

Fig.15 Herbs most commonly combined with *dang gui* in CM formulations based on interviews with CM practitioners in Kunming (n=14).

3.2 HPTLC analysis

Comparison of roots from Gansu and Yunnan

The comparison of the obtained HPTLC fingerprints from roots cultivated in Gansu and Yunnan reveals that with the naked eye no obvious differences are detectable in color, intensity, amount and order of zones (see Fig.16). Thus chromatograms alone cannot be used to unambiguously differentiate between samples of the two provinces. The quantification of ferulic acid showed no significant difference between samples of the two provinces (p=0.0866), but *dang gui* cultivated in Gansu tends to have higher amounts of ferulic acid (see Fig.17). In contrast, Z-ligustilide levels differ significantly and higher values are found in roots cultivated in Gansu (p=0.0025).

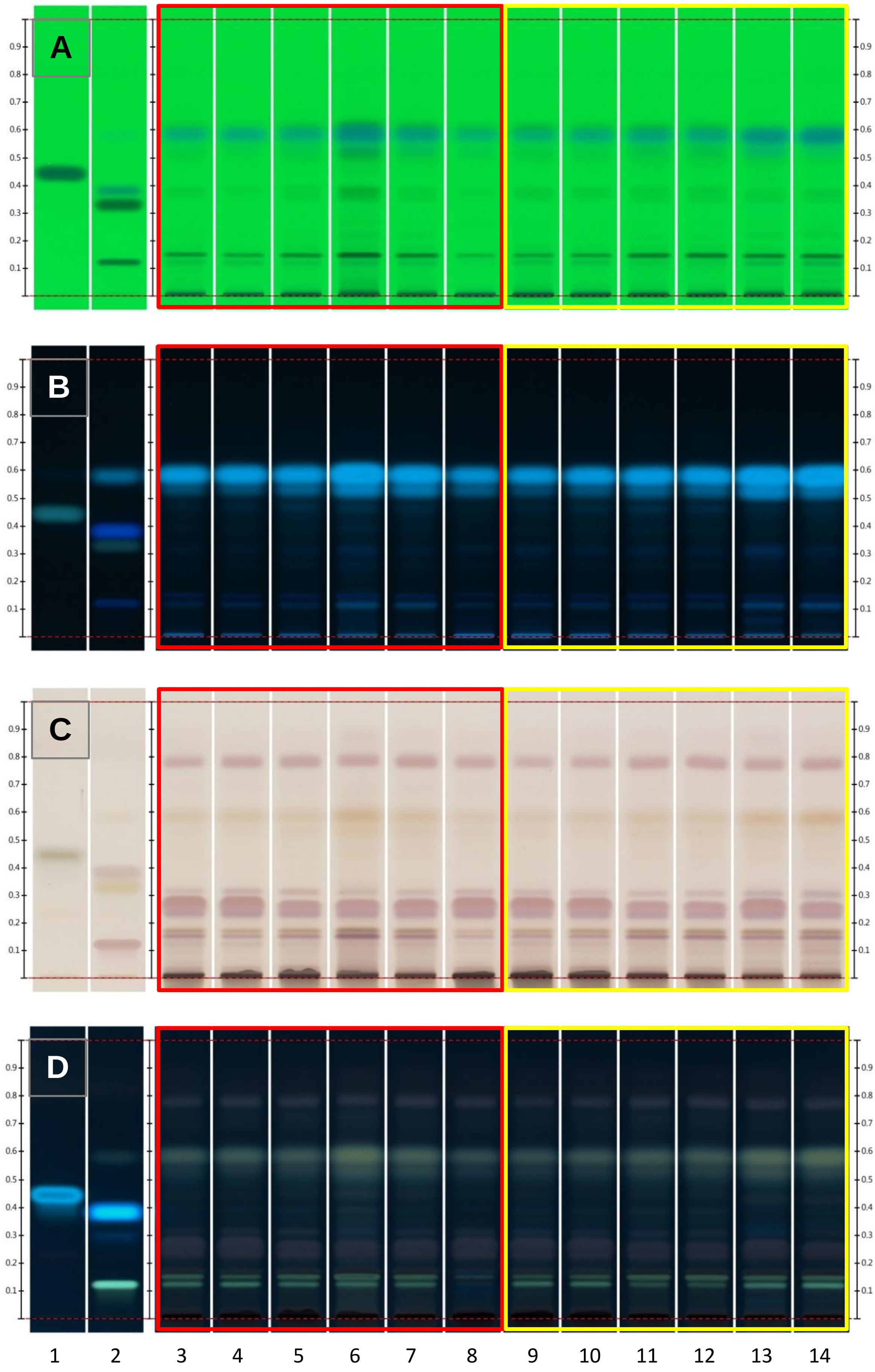

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Fig.16 HPTLC fingerprints *Angelica sinensis* cultivated in Gansu (3-8) and Yunnan (9-14). Only unsulfured whole roots were used. Isoimperatorin (R_f=0.46), Imperatorin (R_f=0.38), ferulic acid (R_f=0.12), osthole (R_f=0.33) and Z-ligustilide (R_f=0.59) were used as reference substances. **(A)** Image of the plate prior to derivatization under UV 254nm. **(B)** Image of the plate prior derivatization under UV 366nm. **(C)** Image of the plate after derivatization under WRT. **(D)** Image of the plate after derivatization under UV 366nm.

Table 8 track assignment of Fig.16

Track	Sample Nr.	Production area
1	Isoimperatorin	
2	Imperatorin, ferulic acid, osthole, Z-ligustilide	
3	Sample 4	Huize, Yunnan
4	Sample 16	Yunnan
5	Sample 38	Yunnan
6	Sample 14	Bole, Yunnan
7	Sample 40	Dali, Yunnan
8	Sample 42	Lijiang, Yunnan
9	Sample 43	Min County, Gansu
10	Sample 41	Min County, Gansu
11	Sample 2	Min County, Gansu
12	Sample 39	Gansu
13	Sample 3	Huichuan, Gansu
14	Sample 5	Min County, Gansu

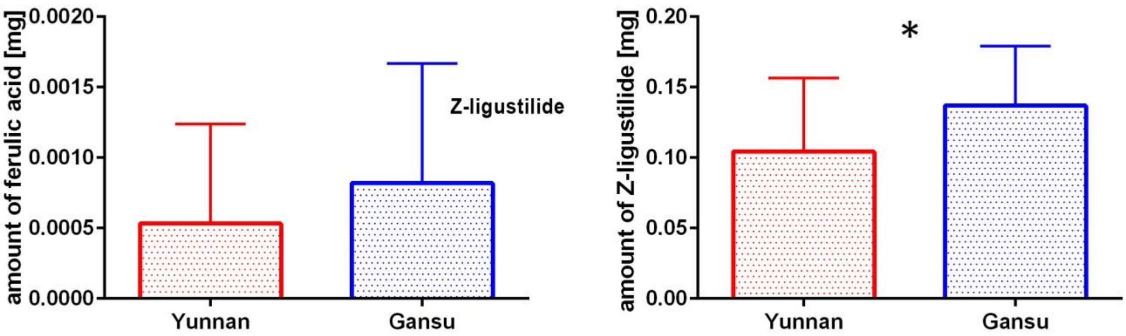
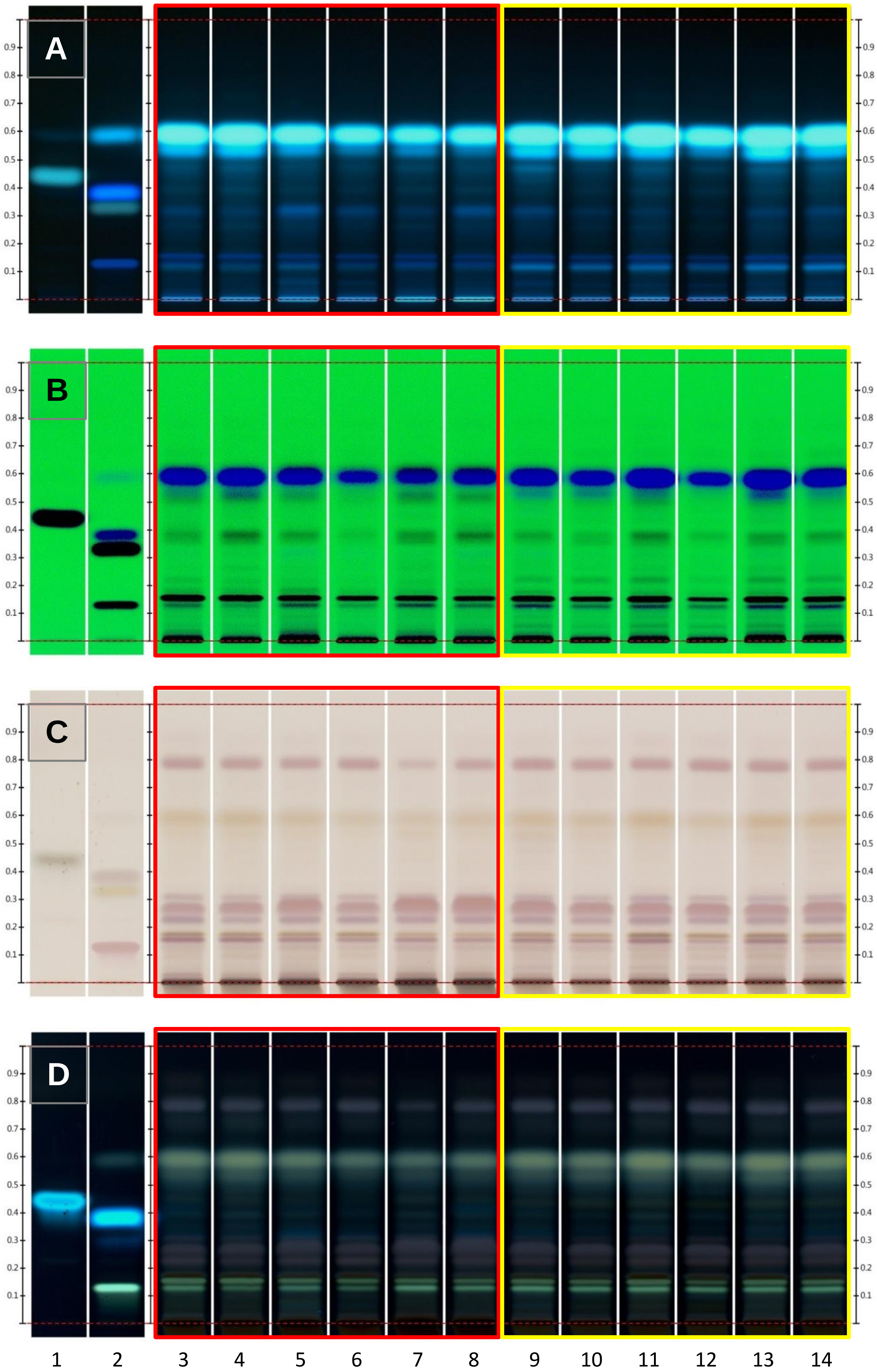


Fig.17 Comparison of ferulic acid and Z-ligustilide levels between roots cultivated in Yunnan and Gansu. An unpaired t-test was performed. Error bars represent the mean ± SD (Yunnan, n=53; Gansu, n=36).

Comparison of sulfured and unsulfured roots

The comparison of sulfured and unsulfured samples showed that no specific chromatograms are obtained and hence based only on the chromatographic fingerprint no differentiation can be made (see Fig.18). Interestingly, both ferulic acid (p=<0.0001) and Z-ligustilide (p=<0.0001) are significantly reduced in sulfured roots (see Fig.19). Sulfur-smoking of the roots hence has a negative impact on the amount of both constituents.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0.6
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0.4
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Fig.18 HPTLC fingerprints of sulfured (3-8) and unsulfured roots (9-14). Isoimperatorin (R_f=0.46), Imperatorin (R_f=0.38), ferulic acid (R_f=0.12), osthole (R_f=0.33) and Z-ligustilide (R_f=0.59) were used as reference substances. **(A)** Image of the plate prior derivatization under UV 254nm. **(B)** Image of the plate prior to derivatization under UV 366nm. **(C)** Image of the plate after derivatization under WRT. **(D)** Image of the plate after derivatization under UV 366nm.

Table 9 track assignment of Fig.18

Track	Sample Nr.	Production area
1	Isoimperatorin	
2	Imperatorin, ferulic acid, osthole, Z-ligustilide	
3	Sample 19-1	Min County, Gansu
4	Sample 9-1	Min County, Gansu
5	Sample 22-1	Min County, Gansu
6	Sample 19-2	Min County, Gansu
7	Sample 43-51	Min County, Gansu
8	Sample 43-50	Min County, Gansu
9	Sample 31-2	Huichuan, Gansu
10	Sample 31-1	Huichuan, Gansu
11	Sample 23-2	Min County, Gansu
12	Sample 32-1	Min County, Gansu
13	Sample 24-2	Min County, Gansu
14	Sample 24-1	Min County, Gansu

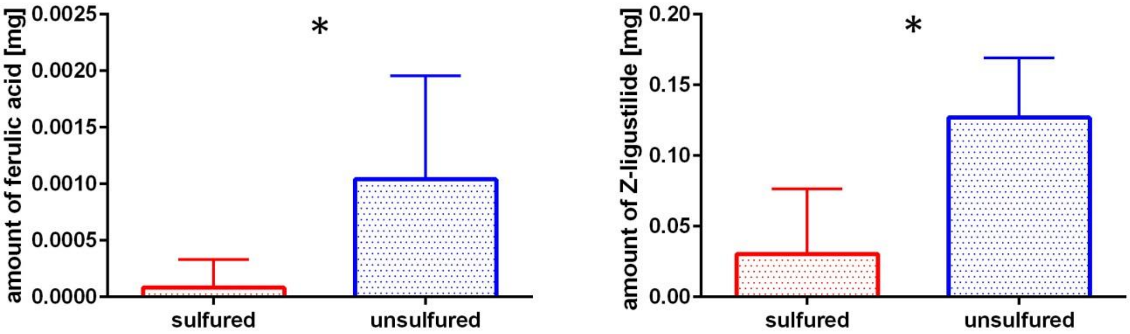
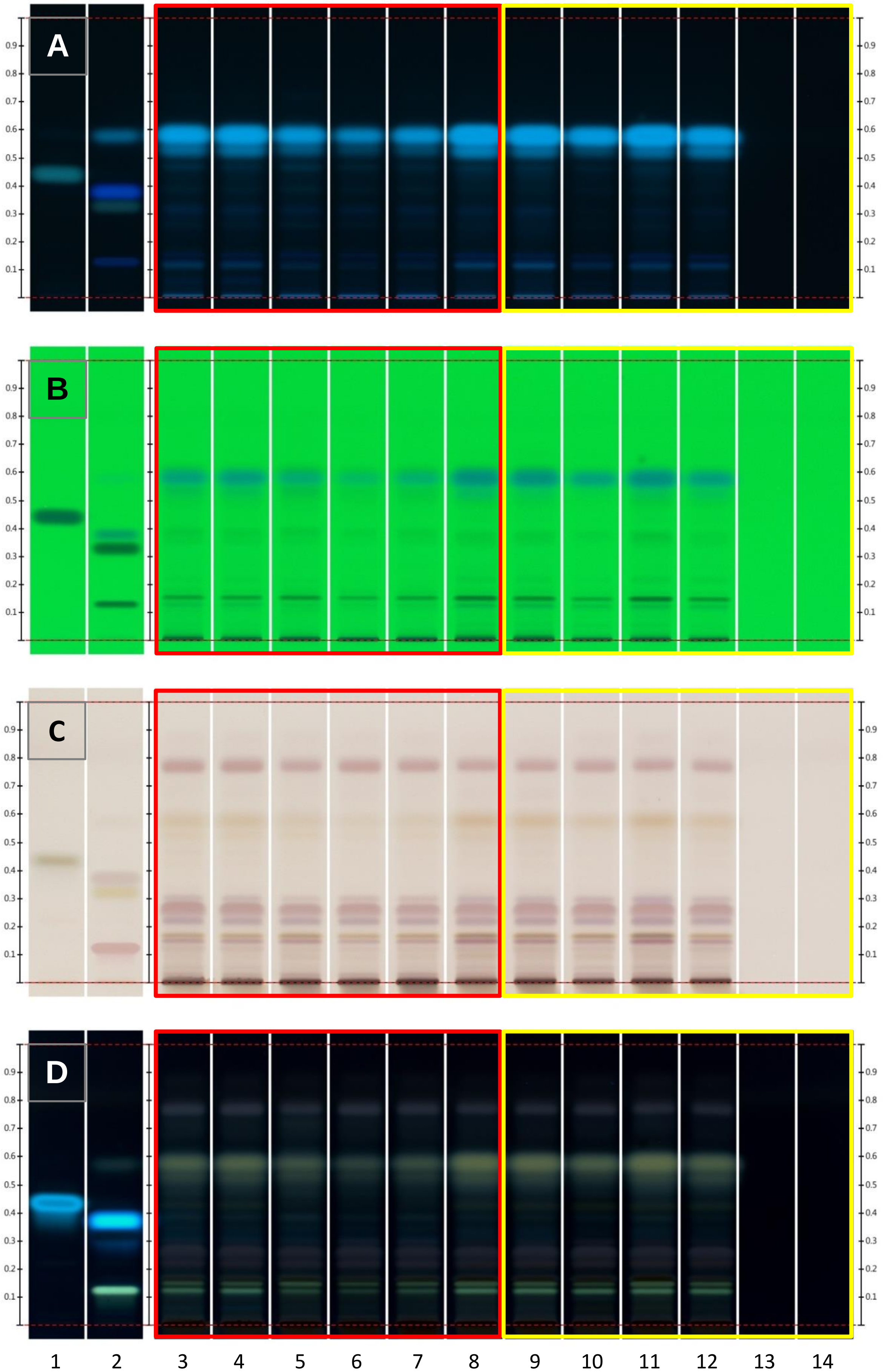


Fig.19 Comparison of ferulic acid and Z-ligustilide levels between sulfured and unsulfured roots. An unpaired t-test was performed. Error bars represent the mean ± SD (sulfured, n=64; unsulfured, n=24).

Comparison of sliced and non-sliced roots

Again, the HPTLC chromatograms do not show any evident differences in color, intensity, amount and order of zones and therefore slices and whole roots cannot be told apart only by means of their chromatograms (see Fig.20). However, the quantification shows that ferulic acid (p=0.0062) as well as Z-ligustilide (p=0.0083) are significantly less abundant in sliced samples and hence slicing interferes with the abundance of both main active compounds (see Fig.21).


A
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0.4
0.3
0.2
0.1
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig.20 HPTLC fingerprints of sliced (3-8) and whole roots (9-14). Isoimperatorin (R_f=0.46), Imperatorin (R_f=0.38), ferulic acid (R_f=0.12), osthole (R_f=0.33) and Z-ligustilide (R_f=0.59) were used as reference substances. **(A)** Image of the plate prior derivatization under UV 254nm. **(B)** Image of the plate prior to derivatization under UV 366nm. **(C)** Image of the plate after derivatization under WRT. **(D)** Image of the plate after derivatization under UV 366nm.

Table 10 track assignment of Fig.20

Track	Sample Nr.	Production area
1	Isoimperatorin	
2	Imperatorin, ferulic acid, osthole, Z-ligustilide	
3	Sample 12-1	Min County, Gansu
4	Sample 13-1	Min County, Gansu
5	Sample 18-1	Min County, Gansu
6	Sample 27-1	Min County, Gansu
7	Sample 29-1	Min County, Gansu
8	Sample 2-1	Min County, Gansu
9	Sample 5-1	Huichuan, Gansu
10	Sample 7-1	Huichuan, Gansu
11	Sample 23-1	Min County, Gansu
12	Sample 32-1	Min County, Gansu

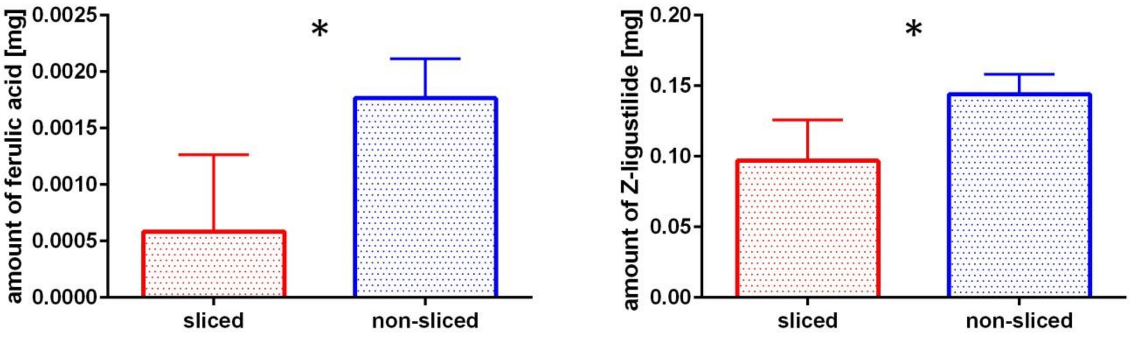

Fig.21 Comparison of ferulic acid and Z-ligustilide levels between sliced and non-sliced roots**.** An unpaired t-test was performed. Error bars represent the mean ± SD (sliced, n=4; non-sliced, n=6).

Comparison of the three parts head, body and tail

The quantification shows that there is significant variation of ferulic acid levels within the root with lower amounts found in the body (*p*=0.44; see Fig.22). In a pairwise comparison significant differences were found between head and body and tail and body, whereas head and tail compared to each other did not show significant different ferulic acid levels. As to Z-ligustilide, there is no significant variation found between different parts, but a similar pattern found as for ferulic acid (p=0.7441).

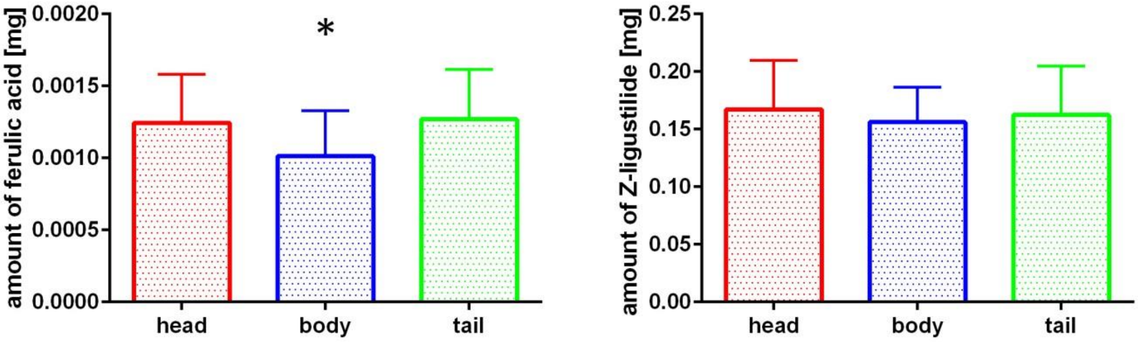

Fig.22 Comparison of ferulic acid and Z-ligustilide levels between parts within root
As there are more than two independent samples an univariate ANOVA was chosen. Error bars represent the mean ± SD (head, n=20; body, n=18; tail, n=20).

Table 11 Comparison of ferulic acid and Z-ligustilide levels among the three parts. Means and standard deviation of ferulic acid and Z-ligustilide levels in the three parts of the root are shown. For pairwise comparison of means, a Duncan *post hoc* test was used. Means sharing the same superscript letters are not significantly different from each other (p<0.05) and consequently can be grouped.

	part of root				
	head	**body**	**tail**	**F**	***p***
ferulic acid [mg]	0.0012±0.00034[a]	0.0010±0.00032[b]	0.0013±0.00035[a]	3.32	0.044
Z-ligustilide [mg]	0.1671±0.0425[a]	0.1561±0.0305[a]	0.1624±0.0425[a]	0.373	0.069

Comparison of outer and inner part of the root

For both ferulic acid and Z-ligustilide higher amounts are found in the outer part of the root. The here presented results are only of preliminary nature.

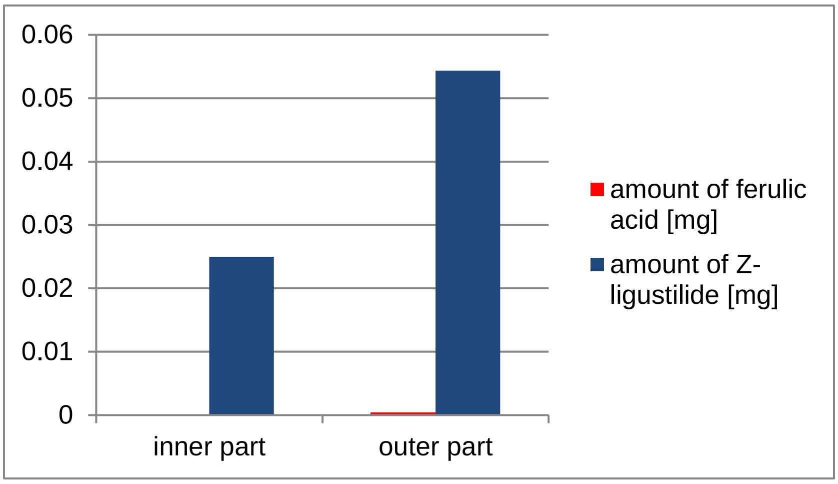

Fig.23 Comparison of ferulic acid and Z-ligustilide levels between inner and outer parts of the root**.** Due to the small sample size (n=2) no statistical tests were performed.

Correlation of head size and amount of main active compounds

Angelica sinensis roots cultivated in Yunnan as well as in Gansu show a significant negative correlation between the size of the head (measured as diameter) and the amount of ferulic acid (Yunnan, p=0.0006; Gansu, p=0.0018; see Fig.24). This means that the concentration of ferulic acid tends to decrease as head size increases. In contrast, there is no significant statistical correlation found for Z-ligustilide and head size (Yunnan, p=0.7926; Gansu, p=0.2922).

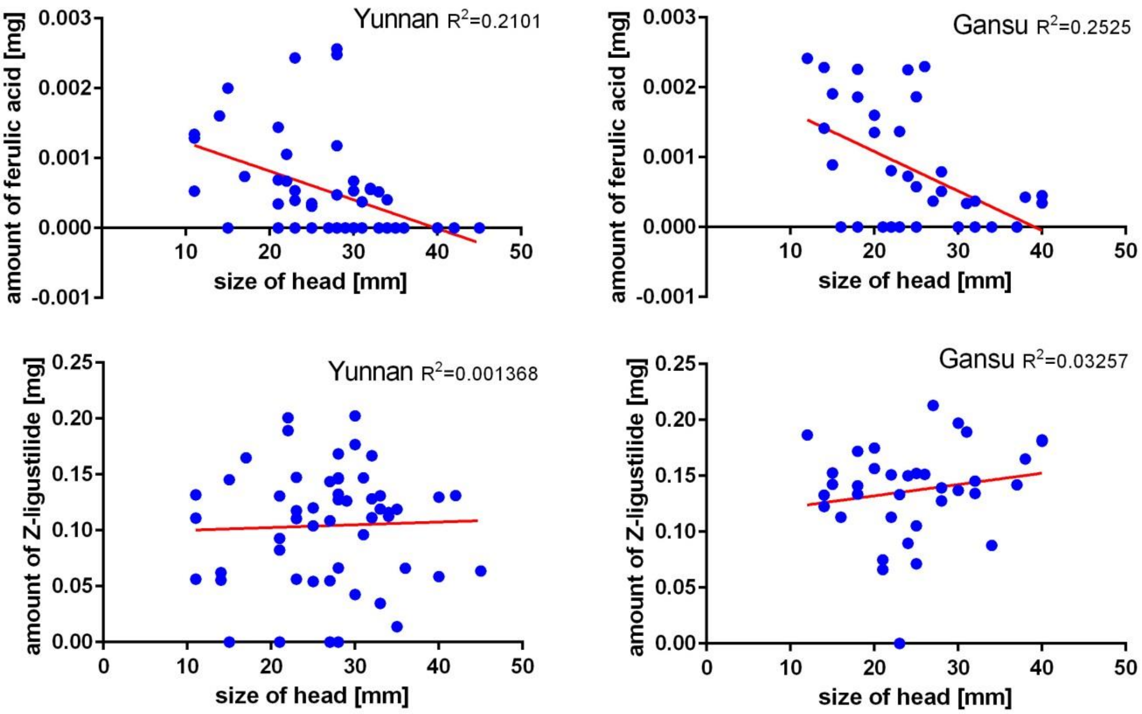

Fig.24 Linear regression analysis of ferulic acid and Z-ligustilide levels and root head size.

Correlation of root length and amount of main active compounds

As far as the root length is considered, similar correlations as for the head size are found (see Fig.25). There is a significant negative correlation found between the amounts of ferulic acid and the root length (Yunnan, p=<0.0001; Gansu, p=0.0483), whereby it should be noted that the correlation is stronger for roots cultivated in Gansu. But again, there is no significant correlation found for Z-ligustilide and root length (Yunnan, p=0.5757; Gansu, p=0.9316).

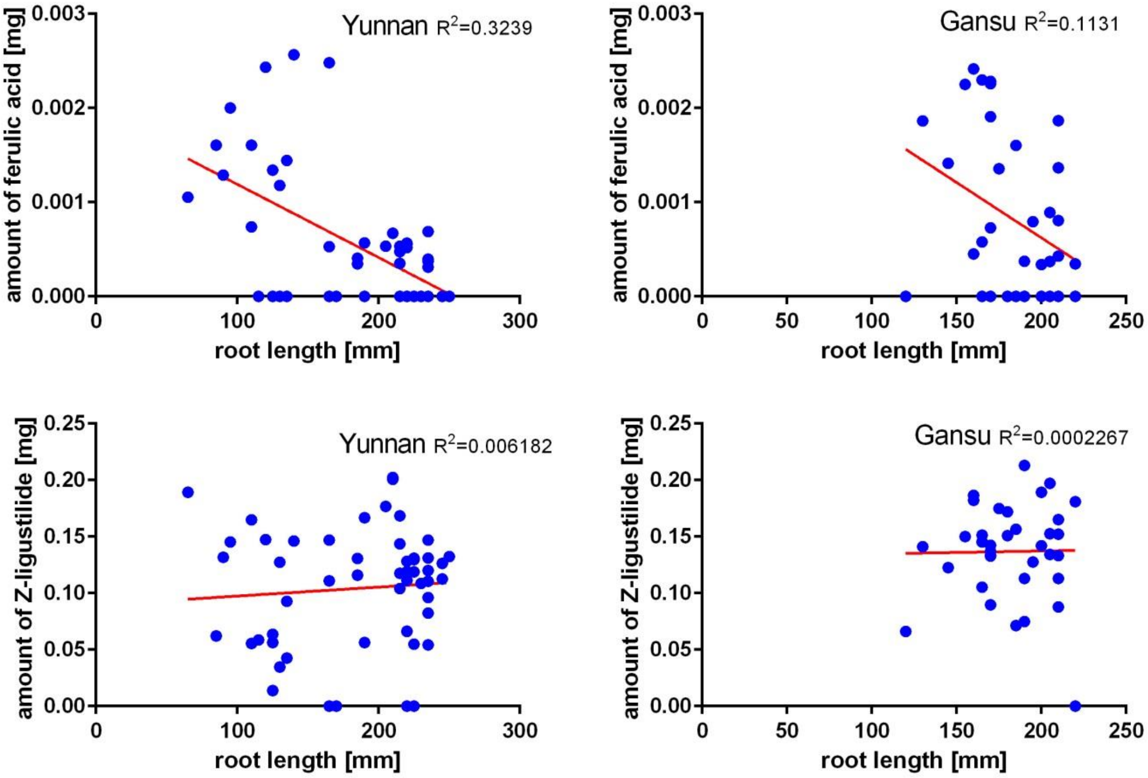

Fig.25 Linear regression analysis of ferulic acid and Z-ligustilide levels and root length.

4. Discussion

4.1 Qualitative fieldwork data on the value chain of *Angelica sinensis*

The interviews allow an overall insight into different steps of the value chain of *Angelica sinensis* and provide a comprehensive frame of reference for analyzing and contextualizing each step. In the following, several aspects of each step are chosen to be interpreted and discussed in greater detail.

Regional cultivation differences

The cultivation of *Angelica sinensis* documented and described in this study is in large part consistent with what has been reported in recently published Chinese literature (DGBS 2009; YYMZJC 2013; Zhang et al. 2012). The already known differences in cultivation between

Gansu and Yunnan such as harvest time and drying technique were also documented in this study. But in addition to provincial differences, significant differences were also found between cultivation areas within Yunnan. The eastern cultivation area contrasts strongly with the one in the northwest of Yunnan in several aspects. It is the only cultivation area where pesticides are used, where one-year old plants are harvested and farmers sell their harvest mainly to pharmaceutical companies or the government. Furthermore, the cultivation of *A. sinensis* is not regarded as a tradition there. Several reasons might explain these differences. First, farmers of the eastern cultivation area are exclusively Han Chinese, whereas in the northwest most farmers belong to ethnic minorities such as Bai or Lisu. The different traditional lifestyles and customs of these ethnic minorities could find expression in the different cultivation and trading of *A. sinensis*. But in turn, the eastern cultivation area in Yunnan sets itself also apart from the one in Gansu, where *A. sinensis* is cultivated by Han Chinese. Ethnic background might therefore not be the only reason to explain these differences (Huber et al. 2015). Much more likable factors are the time when *A. sinensis* was introduced and the political context of the respective area. *Angelica sinensis* was already introduced in the early 19th century to the northwestern cultivation area, but in east Yunnan cultivation in a larger scale started after turn of the millennium (YYMZJC 2013). Therefore the cultivation has not been carried out over generations like in Gansu and the northwest of Yunnan and hence is not perceived as a part of their tradition by farmers in east Yunnan. Furthermore, in the Zhanyi County in east Yunnan the cultivation has been pushed since 2004 by businessmen on the one hand and by the government on the other hand, which explains why famers get governmental support and sell their harvest to pharmaceutical companies or the local bureau of agriculture (YYMZJC 2013).

Role of the cultivation of Angelica sinensis *in farmer's livelihood*

Cultivation areas in Gansu and northwest Yunnan are remote mountainous regions with limited job opportunities inhabited by rather poor farmers (YYMZJC 2013). The cultivation of *Angelica sinensis* therefore serves as ideal means to diversify the income of these farmers for several reasons. First of all, the cultivation has a long tradition over several generations and hence most farmers are familiar with it. Second, the cultivation does not demand for many labors and can be carried out by just one family without special equipment (small-scale household cultivation). Third, *dang gui* can be sold easily at high prize, much higher than staple foods. As for most crops, the cultivation of *A. sinensis* is an annual household decision, meaning that farmers trade off their expenses against benefits before cultivation. This in turn means that if the cultivation of *A. sinensis* is not economic anymore other crops are cultivated instead, as many interviewed farmers explained during the interviews. Furthermore, during fieldwork we experienced that especially in northwest Yunnan many famers, even whole villages, gave up the cultivation of *A. sinensis* and started to cultivate other medicinal plants instead. As high as the profit of the cultivation of *A. sinensis* may be, as risky is its business. Many farmers explained that unpredictable factors like weather and market fluctuations greatly influence the cultivation and therefore there is no guarantee for economic profitability. If the weather is good, the yield is increased, but because of a market surplus for instance the selling price might decrease. This also means that the cultivation areas of Yunnan and Gansu may influence each other negatively. If there is a rich harvest in Gansu, the biggest cultivation area in China, the selling prizes of *dang gui* cultivated in Yunnan will be decreased, as dealers in Yunnan mentioned. In addition, a lot of *dang gui* is exported worldwide and is thus linked to foreign markets. In conclusion, the cultivation of *A. sinensis* is an ever dynamic and complex business restricted to rather poor farmers in remote areas, but interlinked not only with local but also with global economy.

Agricultural practice and notion of quality

According to literature, the overuse of chemical fertilizers and pesticides is a problematic aspect of the cultivation of *Angelica sinensis* (DGBS 2009). As farmers seek high profit by maximizing yield and because of a lack of adequate skills and technologies, overuse and use of wrong pesticides are prominent (ibid). This led to excessive pesticide residues and harmful heavy metal content in *dang gui*. In addition, because of long-term use of high doses of pesticides, resistance of plant diseases has increased fast (ibid). However, in this study we have documented that across the three cultivation areas most of the farmers mainly rely on organic fertilizer and only little chemical fertilizer, whereas some exclusively use organic fertilizer. Furthermore, only farmers in east Yunnan mentioned to use pesticides. In respect of such discrepancy between literature and statements by the farmers a "social desirability bias" that can occur in in-person surveys should be taken into account (Leggett et al. 2003). This is a phenomenon whereby the presence of an interviewer leads respondents to answer questions in a more socially desirable or respectable manner that is viewed favorably by others (Bernard 2011; 250; Krosnick 1999). Therefore, even though farmers stated in the interviews that mainly organic fertilizers are used, it is likely that this does not reflect their actual handling with fertilizers and pesticides.

The interviews with the farmers have shown that they know only a little about the value chain of *dang gui* and its participants and there seems to be little information exchange among participants. By better integrating not only the farmers and dealers, but also pharmaceutical companies and regulating authorities such as local agricultural bureaus, the safety of the medicinal product and economic sustainability could be improved (Singh 2005). For example, collaborations between farmers and pharmaceutical companies including secure trade contracts that guarantee fair prizes to the farmers and impose restrictions on the use of pesticides and chemical fertilizers would create incentives to propagate skilled and economic sustainable cultivation (Sher et al. 2014). By doing so, middlemen can be bypassed, which would effectively shorten the value chain and increase the farmer's profit margin. Also quality and safety of the medicinal product would be easier to monitor and improve.

This leads to the notion of quality, another aspect worth discussing. Famers and dealers use odor and size of the root as quality criteria. Especially dealers point out the superior quality of big roots and sell roots according to size, with big roots always sold at a higher prize irrespective of cultivation area. It is also reported in the American Herbal Pharmacopoeia and Therapeutic Compendium (Upton 2003) and the Materia Medica (Dan Bensky et al. 2004) that good quality consists of thick and long main roots. In addition, in the Chinese literature diameter of the main root and odor are mentioned as quality criteria (YYMZJC 2013; 31-32). Interestingly, to the Chinese medicine doctors cultivation area matters most and to them *dang gui* from Gansu is more effective than the one from Yunnan. This is linked to the concept of 道地药材 (Dàodì yàocái), according to which *dang gui* from Gansu is authentic medicinal material and thus surpasses the quality and therapeutic effects of *dang gui* produced elsewhere. This concept applied in Chinese medicine has been widely recognized in the Chinese pharmaceutical industry. The interviewed pedestrians on the other hand seem not to know about how quality is defined. Apparently, this concept is known only to CM practitioners or professionals in the pharmacy but not to other participants of the value chain resulting in different perceptions of quality.

Trading of Angelica sinensis *in different cities of Yunnan*

Selling prizes of *dang gui* vary drastically depending on city in Yunnan. The prizes for whole roots and heads were significantly higher in Lijiang and Shangrila, both of which are typical tourist destination visited not only by foreign but also by Chinese tourists. At the Chinese medicine market in Kunming I have not encountered any foreign or Chinese tourists, whereas in Shangrila and Lijiang tourists were everywhere. According to my impression the Chinese tourists encountered there mainly belonged to the fast increasing wealthy middle class. Therefore the high prizes might be a consequence of the tourist abundance and their shopping behavior in these cities. Furthermore, I myself as an obvious foreigner must have been perceived as a tourist and the documented prizes are definitely influenced by this fact. All shops visited in Kunming were located in the same supermarket side by side. For this reason the competition by neighboring shops is strong and salesmen consequently sell *dang gui* for a similar price as their competitors. This would explain the lower prizes and the slight standard deviation of the prizes in Kunming and the fact that there were almost no outliers. As mentioned before, according to Chinese medicine the three parts of *dang gui* are treated as therapeutically distinct parts, which is reflected in the striking differences in prize between whole roots and heads customers obviously are willing to pay.

Angelica sinensis *in Chinese medicine*

The medical use of *dang gui* is well-known by most participants of the value chain, which shows how commonly used *dang gui* is in Chinese medicine formulations and how popular CM is in China. Despite the fact that the interviewed pedestrians rather go to Western medicine doctors than to CM doctors, the vast majority of them considers CM to be better than Western medicine. This preference for CM by Chinese people has also been reported by Nestler et al. (2010) and Scheid (1999). This might be due to the fact that CM has been linked to "China's project of social transformation" and is also used by the Chinese state to promote "China's culture". Thus Chinese people regard CM as being consistent with their own culture and believe that it plays a crucial role in helping the Chinese nation to flourish (Da-Yuan et al. 1996). In addition, whether CM or Western medicine is chosen depends on the nature of the ailment, whereas to treat chronic diseases most often CM and to treat acute diseases Western medicine is chosen. This is also mentioned in Scheid (1999; 354) supposing that particularly for chronic diseases the side-effects of Western medicine may be judged to outweigh its benefits. However, even if in contemporary China CM is practiced side by side and complementary to Western medicine, the pressure to modernize, westernize and compete globally will potentially reduce the cultural significance of CM in the future (Burke et al 2003).

4.2 Analytical analysis

4.2.1 Chromatographic fingerprints

The obtained HPTLC fingerprints do not show obvious differences in color, intensity, amount and order of zones in all analyzed samples. Among the analyzed samples no additional zones were found. All fluorescent zones in the chromatograms of the collected samples correspond in position and color to the zones of the reference substances used. Minor variations in zone intensity occur though. HPTLC fingerprints might be a fast and powerful tool for the identification of *Angelica sinensis* (Zschocke et al. 1998), but without quantification, no conclusions about cultivation area, type and manner of processing can be drawn from these HPTLC fingerprints. However, for the distinction between closely related species of *A. sinensis* such as *Angelica archangelica*, *Angelica dahurica* and *Angelica pubescens,* the chromatographic fingerprints are sufficient (Zschocke et al. 1998). Therefore, HPTLC fingerprints are a valid means to detect adulteration of *dang gui* with closely related *Angelica* species. For the discrimination of *dang gui* from different cultivation areas a novel approach has been proposed recently. This approach includes odor detection by an electronic nose enabling a rapid and reliable identification of *dang gui* from different geographic origin (Zheng et al. 2015). However, this method has not been tested in the present study.

4.2.2 Semi-quantitative analyses

Comparison of dang gui *from Gansu and Yunnan*

The quantification of samples from Gansu and Yunnan shows significant differences for Z-ligustilide, with higher values found in roots cultivated in Gansu. Ferulic acid is also found to be present in higher amounts in roots from Gansu, but there is no statistically significant difference. These findings are consistent with previous studies using different analytical techniques. By means of HPLC Zhao et al. (2003) showed that *dang gui* cultivated in Gansu contains a 2-fold higher amount of both Z-ligustilide and ferulic acid compared to *dang gui* cultivated in Yunnan. The province Gansu (especially Min County) is traditionally thought to produce the best *dang gui* and above all considered to be the authentic region, whereas provinces like Yunnan are considered unauthentic regions (Liu et al. 2014; Zhang et al. 2012; Zhao et al. 2003). In additon, in the medicinal industry and according to Chinese medicine *dang gui* from Gansu is regarded as a *daodi* medicinal product (道地药材) and hence of superior quality (Zhao et al. 2012). There are several possible reasons for the comparatively higher amounts of these marker compounds in *dang gui* from Gansu. As is generally known, environmental factors greatly affect the quality of herbal medicines. Especially secondary metabolites such as ferulic acid and Z-ligustilide can serve as adaptations to fluctuating temperature and light conditions, stress, infections or herbivory (Canter et al 2005). It has already been shown that differences in quality of *dang gui* among different cultivation areas are not due to intraspecific genetic diversity, but rather to cultivation and environmental conditions (Song et al. 2014; Zhao et al. 2003). Gansu has a semi-arid to arid continental climate, which means high temperatures during summer and low temperatures in winter and most of the precipitation being delivered in the summer months (Zhang and McBean 2014). In contrast, much of Yunnan lies within the subtropical highland or humid subtropical zone, with mild winters and tempered summers (YYMZJC 2013). The higher levels of ferulic acid and Z-ligustilide found in roots from Gansu might therefore be natural adaptations to the different weather conditions. Furthermore, one needs to keep in mind that *Angelica sinensis* is a stenotopic species whose natural distribution was mainly confined to places in Gansu and Qinghai with cool and moist habitats (Lin et al. 2008; Zhang et al. 2012). Therefore, environmental properties in Gansu might be more suitable for the cultivation of *Angelica sinensis* than in Yunnan. Furthermore, unlike in Gansu, where roots are dug in their second year of growth, in Yunnan *dang gui* is sometimes sown in January and already harvested in December of the same year. These one-year old plants are considered to be of inferior quality (Upton 2003). Cultivation techniques of *Angelica sinensis* are not uniform between different cultivation areas (e.g. use of pesticides, harvest time). All these differences could interfere with chemical composition of *dang gui* and be a reason for different levels of ferulic acid and Z-ligustilide. The way of how *dang gui* is processed after harvesting is another factor affecting the ferulic acid and Z-ligustilide contents. This will be discussed in the following sections.

Comparison of processed dang gui

In this study we have found that both ferulic acid and Z-ligustilide are significantly reduced in sulfured roots and hence sulfur-smoking has a negative impact on the abundance of these two marker compounds. Using a quantitative analysis by HPLC in *dang gui* Zhao et al. (2003) showed that the content of ferulic acid was reduced after smoke-drying by 30 to 60% and even more after sulfur-smoking, but the content of Z-ligustilide is not affected as much as ferulic acid. Several reasons are taken into consideration why sulfur-smoking has an impact on ferulic acid and Z-ligustilide. First, during the sulfur-smoking process sulfur dioxide gas might chemically interact with the two marker components. Second and most importantly, the volatile oil Z-ligustilde is known to be very sensitive to heat and air and therefore smoking reduces its abundance in *dang gui* (Upton 2003). Similarly, volatilization could also be the reason for the reduced amounts of ferulic acid. But unlike Z-ligustilide, ferulic acid, whose melting point is 168-171°C, is not volatile and should be less affected by the low temperatures (exact temperatures not known) during sulfur-smoking. So it is not really clear why ferulic acid is found in reduced amounts in sulfur-smoked roots. Other studies point towards the changes in quality and quantity of the main active components in both crude and prepared drugs entailed by sulfur-smoking (Lou et al. 2014). With respect to this Zhao et al. (2003) propose to use vacuum-drying in order to avoid potential volatilization, even though it is more expensive than the conventional method of sulphur-smoking. Moreover, health aspects also need to be taken into account when choosing the right preservation technique, as it is known that sulfur-smoking leaves considerable residues of sulfur dioxide and harmful heavy metals in the processed materials. These residues are not only harmful to human health but also reduce the curative effects of the medicinal material (Lou et al. 2014)**.**

The comparison of sliced and non-sliced roots revealed that ferulic acid as well as Z-ligustilide are significantly less abundant in sliced samples and hence slicing interferes with the quality of the roots. Zschocke et al. (2001) has reported that the amount of Z-ligustilide is much higher in roots purchased as whole roots compared to already sliced roots. A possible reason for this is that slicing increases the surface to air area and therefore leads to an increased volatilization reducing the ferulic acid and Z-ligustilide contents.

Comparison of the different parts of dang gui

Quantification of ferulic acid and Z-ligustilide in each part of the root (head, body, tail) shows that there is significant variation of ferulic acid levels among parts within one root. In my pairwise comparisons significant differences were found between head and body and tail and body, whereas head and tail compared to each other did not show significant different ferulic acid levels. As to Z-ligustilide, no significant variation is found between the three parts. In a previous study examining the three distinct parts of *dang gui* by Wei et al. (2008) using gas chromatography coupled with mass spectrometry (GC-MS) similar findings were made. Wei et al. (2008) also found differences of *Z-ligustilide*, butylidene phthalide and ferulic acid content among the three parts. However, he found that differences between tail and other parts are larger than that between body and head and assumes that head and body have a similar chemical composition. Based on this data he concludes that the higher amounts of ferulic acid and butylidene phthalide found in the tail might account for the traditionally believed therapeutic effect of enhancing blood circulation of the tail of *dang gui*. However, the alleged distinct therapeutic activities of different parts of *dang gui* are grounded on empirical observations by Chinese medicine doctors throughout centuries without modern scientific foundation (Wei et al. 2008). The presented results in my study and by Wei et al. (2008) show that the chemical composition varies within the root, which in the first instance may support the idea of CM. Nevertheless, chemical knowledge of the three parts of *dang gui* at a molecular level is not sufficient to elucidate possible pharmacological effects (see general considerations), especially as it is not known whether the measured differences of a few compounds are responsible for the different therapeutic activity. Therefore, this is no convincing validation or explanation of the therapeutic effects according to CM. Further investigation should include pharmacological examinations of the three parts using clinical trials.

The comparison of inner and outer parts of the root showed that both ferulic acid and Z-ligustilide are found in higher amounts in the outer part of the root. Due to time constraints only two samples were analyzed and further investigation of this aspect is needed. In addition, the separation into inner and outer part is not premised on root physiological properties, but merely arbitrary as the roots were scarcely cuttable. A separation into plant physiologically relevant sections could provide insights into how ferulic acid and Z-ligustilide are distributed in the root, which might help to elucidate their role in the plant.

Correlation of root size and amount of main active compounds

In this study I have demonstrated that *Angelica sinensis* roots cultivated in Yunnan as well as in Gansu show a significant negative correlation between the size of the head (measured as diameter) and the amount of ferulic acid. This means that the concentration of ferulic acid tends to decrease as head size increases. In contrast, there was no significant statistical correlation found for Z-ligustilide and head size. As far as the root length is concerned, similar correlations were found. There was a significant negative correlation found between the amounts of ferulic acid and the root length, but no significant correlation for Z-ligustilide. One possible explanation for this finding is that from a certain stage of root growth ferulic acid is not accumulated anymore, meaning that with increasing size the absolute amount does not increase anymore, but as a consequence the concentration decreases. Z-ligustilide in turn might be accumulated in the root throughout root growth and hence its concentration is maintained at a certain level. However, this is only a rough guess and understanding of the physiological function of ferulic acid and Z-ligustilide within the root might shed new light on this finding.

4.3 General considerations

Throughout this work only the two chemical components ferulic acid and Z-ligustilide were analytically examined, as these are regarded to be the main active compounds and hence related to the therapeutic effects of *dang gui* (Zhao et al. 2003). For this reason, they are commonly used as primary analytes for the quality assessment of *dang gui*. Not surprisingly, these two chemical compounds have gained a lot of interest in current pharmacological research of *dang gui* done mainly in China (Cheng et al. 2011; Li et al. 2015; Ou and Kwok 2004; Wang et al. 2006). Furthermore, in the Pharmacopoeia of the people’s republic of China (volume 1, 2010) ferulic acid is listed for the quality assessment of *dang gui* (no less than 0.050% of ferulic acid with reference to the dried drug). Many papers dealing with elucidating the quality or pharmaceutical activity of *dang gui* focus on ferulic acid and Z-ligustilide (Wei et al. 2008; Zhao et al. 2003). However, even though a lot of current studies use ferulic acid and Z-ligustilide for quality assessment of *dang gui*, this attempt has drawn criticism. First, unlike conventional drugs, natural products like Chinese medicinal herbs contain hundreds of bioactive constituents, which as an integrative and complex result are responsible for the therapeutic effects (Chavez et al. 2006; Huang et al. 2004; Lao et al. 2004; Song et al. 2014).

Therefore, the analysis of few active constituents does not represent the complete activity of a herb and may not be a sufficient means to assess the quality of herbal drugs, not to mention pharmacological effects (Jiang 2005; Liang et al. 2009; Zhao et al. 2013). In addition, composite formulae in Chinese medicine comprise a multitude of natural products, each consisting of a different chemical composition, complicating matters further.

Moreover, taken into consideration how common among plants ferulic acid is and given the fact that also Z-ligustilide is not unique to *dang gui*, the measured amounts of them alone is not sufficient for the quality assessment of *dang gui* (Upton 2003). According to a review by Yi et al. (2009), measured concentrations of ferulic acid in previous studies quantified by HPLC and GC vary within the range of 0.211–1.75mg/g (Yi et al. 2009). Interestingly, the estimated sum of the daily ferulic acid intake through consumption of cereals, vegetables, fruits, coffee and juices may reach 150-250mg, which means that in order to reach this amount of ferulic acid one would need to consume 100-1000g *dang gui* on a daily basis (Zhao and Moghadasian 2008). Moreover, despite the detailed information on chemistry, synthesis and availability of ferulic acid in a number of foods, there is only limited knowledge on pharmacokinetic and pharmacodynamic properties up to now (Zhao and Moghadasian 2008). Another main issue in the clinical use of ferulic acid is its low bioavailability after oral administration (Mancuso and Santangelo 2014).

The reported concentrations of Z-ligustilide using a variety of analytical methods vary within 1.26–37.7mg/g (Yi et al. 2009). As a volatile oil, instability and rapid chemical degradation are the issues of highest concern of Z-ligustilide (Schinkovitz et al. 2008). For this reason the method of extraction used for quantification might influence the measured contents, apart from natural variation (Yi et al. 2009). The measured concentration does therefore not reflect the actual contents of Z-ligustilide in *dang gui*. Nevertheless, Z-ligustilide is considered to be the main active ingredient of many Apiaceae medicinal plants (Cheng et al. 2011; Li et al. 2015) and might be more relevant in the pharmacological context of *dang gui* compared to ferulic acid.

In addition to the above mentioned issues, drawbacks of the fieldwork influencing the analytical analysis also need to be brought forward. First of all, the samples analyzed in this study were bought by my translator and me at local markets. Therefore, we heavily relied on information given by dealers and thus there is no guarantee for the correctness of information such as place of production and manner of processing of the collected *dang gui*. Only by judging with the naked eye it was impossible for us to tell roots from different cultivation areas apart. Also, if roots are sulfur-smoked is not visible to the naked eye. Another major problem is that no information was available about when *Angelica sinensis* was harvested and processed. It is known that dealers can store sulfur-smoked roots for several years. Therefore the purchased plant material is likely to be of different year's harvest. Zschocke et al. (1998) have shown that the Z-ligustilide content is low in *dang gui* samples stored for a long time. Thus, there are many unknown variables in the analytical part of this study and to what extend these interfere with the results is not clear. In any case these uncertainties somehow impact on the analytical analyses done in this study and need to be kept in mind when interpreting the validity of the presented results.

4.4 Conclusion

This study has provided an overview of the complexity of the value chain and has helped to develop a more accurate picture of the cultivation and trade of *Angelica sinensis*. With respect to this, some problems and potential areas for improvement have been pointed out. The cultivation of *A. sinensis* plays an important role in poor farming families in remote rural areas and helps to increase and diversify their income. Better understanding of the value chain and integration of all participants could improve cultivation and trading conditions of *A. sinensis*.

HPTLC has turned out to be a valid and powerful tool not only for the identification of *Angelica sinensis*, but also for quantitative measurements. The HPTLC analysis revealed significant differences of ferulic acid and Z-ligustilide contents depending on where *A. sinensis* is cultivated, how it is processed and which part of the root is used. These findings are in large part consistent with previous results using different analytical techniques. Nevertheless, the quality of *dang gui* is not assessed adequately by exclusively using the present evaluation paradigm for single chemical compounds as done in this study. With respect to this, more attention should be paid to quality in terms of cultivation and processing, instead of linking quality of herbal medicines only to certain main active compounds. Cultivation practice and processing are fundamental for ensuring both the correct identity and quality of herbal medicines without the need of complex chemical analyses and set the cornerstone for an economic and sustainable value chain.

Finally, this study should be seen as an initial effort to elucidate both the potential and problems associated with the value chain of *A. sinensis* and serves as basis for future investigations.

4.5 Outlook

As the fieldwork of this project was confined to five months, only few pieces of the whole puzzle were gathered. Together with the knowledge gained from this study, a longer stay in the field could provide a deeper comprehension of the value chain and more plant material could be collected. A possible aim could be to assess the role of environmental factors in the cultivation of *Angelica sinensis* by collecting weather and soil data in Yunnan and Gansu throughout at least a year. This can show how weather conditions vary among the different cultivation areas in Gansu and Yunnan and how the growth of *A. sinensis* is affected. A stay in the field for more than a year would also enable one to make observations of the entire cultivation process from sowing of the seeds to harvesting of the roots. In addition, the actual use of fertilizers and pesticides could be documented and using HPTLC the impact on ferulic acid and Z-ligustilide content assessed. Furthermore, it would be interesting to know if pesticide residues and harmful heavy metal content are found in *dang gui*, as reported in Chinese literature (DGBS 2009). If roots could be collected directly from the field, uncertainties of the oral information given by informants (e.g. dealers) would be eliminated. Due to time constraints, also the analytical analysis left room for improvements. Instead of the semi-quantitative attempt made in this project using the profiles generated from the images, quantification could be carried out using the TLC SCANNER 4 (CAMAG AG), by which quantitative evaluation usually is done in HPTLC. Quantification by the TLC SCANNER 4 would allow for more accurate measurements.

5. References

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Appendix I Plant names

Table 12 List of plant names in Latin and Chinese.

Astragalus membranaceus (Fisch.) Bunge	黄芪 (huángqí)
Achyranthes bidentata Blume	牛膝 (niúxī)
Aconitum wilsonii Stapf ex Veitch	附子 (fùzǐ)
Asparagus cochinchinensis (Lour.) Merr.	天门冬 (tiānméndōng)
Atractylodes macrocephala Koidz.	白术 (báizhú)
Bupleurum densiflorum Rupr.	柴胡 (cháihú)
Carthamus tinctorius L.	红花 (hónghuā)
Codonopsis pilosula (Franch.) Nannf.	党参 (dǎngshēn)
Elsholtzia stauntonii Benth.	木香薷(mùxiāngrú)
Gastrodia elata Blume	天麻 (tiānmá
Glycyrrhiza uralensis Fisch.	甘草 (gāncǎo)
Lepidium meyenii Walp.	玛卡 (mǎkǎ)
Ligusticum tenuissimum (Nakai) Kitag.	细叶藁本 (xìyègǎoběn)
Ligusticum wallichii Franch.	川芎 (chuānxiōng)
Lycium chinense Mill.	枸杞 (gǒuqǐ)
Nicotiana tabacum L.	烟草 (yāncǎo)
Paeonia lactiflora Pall.	杭芍 (hángsháo)
Panax ginseng C.A.Mey.	人参 (rénshēn)
Panax notoginseng (Burkill) F.H.Chen	三七 (sānqī)
Panax quinquefolius L.	西洋参 (xīyángshēn)
Salvia miltiorrhiza Bunge	丹参 (dānshēn)
Solanum tuberosum L.	洋芋 (yángyù)
Vitex trifolia L.	蔓荆子 (mànjīngzi)
Zingiber officinale Roscoe	姜 (jiāng)
Ziziphus jujuba Mill.	大枣 (dàzǎo)

Appendix II Sample collection

Table 13 List of all collected samples in Gansu and Yunnan.

Sample -Nr.	Date [yyyy/mm/dd]	Place of cultivation	Price [RMB/kg]	Form	Quantity	Notes
1	2014.09.09	Ludian	80	whole	2	
2	2014.08.13	Min County	27-30	whole	3	
3	2014.08.09	Huichuan	28	whole	2	
4	2014.08.03	Huize	70	whole	2	wild
5	2014.08.14	Min County	60	whole	1	
6	2014.10.11	Liongjiao	-	whole	2	has worms
7	2014.08.14	Min County	50	whole	2	
8	2014.09.02	???	-	head	4	gift, received in shaxi
9	2014.08.13	Min County	40	whole	1	sulphur
10	2014.10.06	Bole	-	whole	1	fresh root from field
11	2014.09.26	Labadi	-	whole	3	fresh roots from field
12	2014.08.14	Min County	40	slices	2	no sulphur, 2 bags
13	2014.08.14	Min County	38	slices	2	2 bags
14	2014.10.05	Bole	40	whole	5	
15	2014.08.09	Huichuan	50	head	1	
16	2014.08.29	Yunnan	200	whole	2	purchased in shaxi
17	2014.08.21	Machang	200+	head	4	
18	2014.08.09	Huichuan	28	slices	2	2 bags
19	2014.08.14	Min County	38	whole	2	sulphur
20	2014.08.03	Gansu	65	whole	1	
21	2014.10.07	Yanfang	-	???	Some	gift
22	2014.08.13	Min County	30	whole	1	sulphur
23	2014.08.14	Min County	42	whole	2	
24	2014.08.13	Min County	20	whole	3	
25	2014.08.09	Huichuan	47	head	2	

26	2014.08.14	Min County	30	whole		lost
27	2014.08.14	Min County	38	slices	2	Sulphur, 2 bags
28	2014.09.15	Shangrila	160	whole	3	wild
29	2014.08.14	Min County	38	slices	2	Sulphur, 2 bags
30	2014.08.24	Machang	-	head	7	gift
31	2014.08.09	Huichuan	50	head	2	
32	2014.08.13	Min County	50	whole	1	No sulphur
33	2014.08.14	Min County	80	whole	1	International standard; only six roots
34	2014.08.13	Min County	40	whole	1	
35	2014.08.03	Lijiang	240	head	1	Best quality
36	2014.08.03	Lijiang	180	head	1	
37	2014.08.03	Lijiang	150	head	1	
38	2014.11.17	Yunnan	65	whole	9	
39	2014.11.17	Gansu	70	whole	10	
40	2014.11.17	Dali	75	whole	14	
41	2014.11.17	Min County	65	whole	10	
42	2014.11.17	Lijiang	80	whole	14	
43	2014.11.19	Min County	60	whole	60	sulphured
44	2014.11.19	Qujing	110	whole	20	

Appendix III Interview guides

Interview guide farmers

1. Name
姓名
Sex
性别
Age
年龄
Occupation/profession
职业
Ethnic group
民族
Location/village/town
住址

2. What Angelica species do you cultivate? Are there local names (local taxonomy)?
你们中什么当归?有没有本地的名字?

2.1 How are the species identified? Based on what characteristics?
你们怎么鉴别品种?根据什么特性?

2.2 Why do you cultivate this species?
你为什么种植这种品种?

2.3 Are there specific varieties of *Angelica sinensis*?
有没有特定的品种?

2.4 Do you collect wild *Angelica sinensis* plants?
你是否采当归?

2.5 What is the main use of the plant?
当归的主要用途是什么?

3. How big is your field area in total?
当归的主要用途是什么?

3.1 How big is your field area, where *Angelica sinensis* is cultivated? How long have you been cultivating *Angelica sinensis*?
种多大面积?在哪种?种当归有多长时间了?

3.2 Are there alternative crops or medicinal plants you cultivate?
有没有替代作物或者药用植物?

3.3 How do you know how to cultivate *Angelica sinensis*? Where does the knowledge come from?
你怎么知道(学习)种当归?这些知识从什么地方来?

3.4 When do you start cultivating? When is harvest time? How is *Angelica sinensis* harvested? When does harvest time end?
什么时间开始种?

3.5 Has harvest time an inpact on quality and/or price?
什么时间收?为什么?怎样收?

3.6 How old are the plants you harvest?
收获时间对价格或质量有影响吗?

3.7 What chemical/organic fertilizers are used? Why? Are there plant infections?
你收的当归是几年的?

3.8 What pesticides are used? Why?
用什么化肥或农家肥?为什么?对植物有影响吗?

3.9 What technology/machine is used for cultivation?
用什么农药?为什么?

3.10 What soil and weather conditions are necessary for growing *Angelica sinensis*? Does altitude play a role?
中的时候用什么农机或技术?

3.11 How is *Angelica sinensis* processed? Do you process the plant raw material yourself?
当归生长所必需的土壤和天气条件是什么?海拔对当归的生长有什么影响?

3.12 How do you check the quality?
谁还参加了种植?谁在地里管理?有没有雇工?

3.13 Is the production every year the same or fluctuating/decreasing/increasing?
当归怎么加工?自己加工生当归吗?

3.14 What has changed over the past years regarding the cultivation and processing?
每年的当归产量一样还是有波动?

3.15 Why don't you cultivate staple foods? Isn't cultivating *dang gui* risky?
这些年当归的产量有什么变化?

4. How many people live in your household?
你家有多少人?

4.1 What is your household income? What are your income sources? What is your main income source?
你家的收入是什么?家庭的经济来源是什么?主要的经济来源是什么?

4.2 How much (%) does the *Angelica sinensis* production contribute to your total income?
当归占总收入的多少?

4.3 Do you get subsidies? Why?
能不能得到补助?

4.4 Are there any regulations by the government about the cultivation/production/quality?
对当归的种植、产量、质量政府有没有规定?

4.5 Is the cultivation of *Angelica sinensis* a family or village tradition? Are there neighbors who also cultivate Angelica?
种当归是不是一个家庭或一个村子的传统?有没有邻居或邻近的村子也在种当归?

4.6 Do you use *Angelica sinensis* as a medicinal product yourself?
你是否把当归当药用?

4.7 What is your personal view on traditional Chinese medicine?
你个人对中药的看法?

5. Where do you sell the plant material? To whom do you sell the plant material (middlemen/wholesalers/pharmaceutical companies/healers)?
往哪儿出售药材?给谁 (中间人、大老板、制药厂、土医生)?

5.1 For what reasons do you sell it there/to him/to her?
为什么卖到那儿或卖给他?

5.2 What do you know about the participants of the value chain (middlemen/wholesalers)?
关于、利益链条的参与者你知道什么? (中间人、大老板)

5.3 Who is responsible for quality control?
谁负责产品质量?

5.4 Does adulteration occur? At which stage of the value chain is it most likely to occur? Why?
有没有掺假?在那个阶段最可能掺假?为什么?

5.5 Who defines the prizes? Are there fluctuations? Do you know about the market prizes of your plant material?
谁决定价钱?有没有波动?你知道药材的市场价吗?

5.6 Do fluctuations regarding demand occur (market fluctuations)?
波动是市场需求决定的吗?

5.7 Are there cooperation projects with companies?
和公司有无合作项目?

6. Can you tell us about your personal outlook?
你自己对未来的看法是什么?

Interview guide trading

1. How is the business this year?

2. Where does the *dang gui* come from?

3. Who comes here to buy *dang gui* (age, sex)?

4. For what reason do they buy *dang gui*?

5. Has there been a change in demand over the past years?

6. How has the price changed over the past years (Selling price, if not stated otherwise)?

7. How do you check the quality?

8. What's the future of *dang gui*?

Interview guide Chinese medicine doctors

1. Where does the best *dang gui* come from?

2. Is there a difference between Yunnan *dang gui* and Gansu *dang gui*?

3. How often is *dang gui* used?

4. What's the effect of *dang gui*? What's the effect of each part of *dang gui*?
5. *Dang gui* can cure what kind of diseases?
6. What does move and support blood mean?
7. What herbs are usually used in combination with *dang gui*? Why?
8. How is *dang gui* prepared?

Interview guide pedestrians

1. If you are sick do you use western medicine or Chinese medicine?
2. What do you consider to be better? Why?
3. Are there diseases you rather use western medicine and some you rather use Chinese medicine?
4. Do you use *dang gui* yourself?
5. Where does the best *dang gui* come from?
6. What kind of diseases can *dang gui* cure?
7. How dou you prapare *dang gui* to take in?
8. How do you know how to use *dang gui*?