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Eidesstattliche Erklärung

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Ich bin mir bewusst, dass eine falsche Erklärung rechtliche Folgen haben wird.

Wien, August 2011

Kristina Marchart

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1. Introduction and Aim of this Thesis

1.1. Introduction

Traditional Chinese Medicine (TCM) had its beginning in China and is more than 5.000 years old. Its medical treatment and philosophy was developed over centuries. TCM contains versatile theories, diagnoses as well as various therapeutic treatments and preventions of diseases. TCM treatments contain herbal prescriptions, acupuncture, moxibustion, cupping, diet therapy, tui na, qi going and so on. The aim of these treatments is the return of the patient to homeostasis and energetic balance and to become healthy within mind, body and spirit (Ehling and Swart, 2002).

In the last 20 years the interest and practice of complementary and alternative medical (CAM) treatments in Western countries has increased. In Asian countries a lot of research has been done in TCM, but only a few evidence-based studies on its effects on various diseases are available in Western countries. In the last years, TCM became very popular in Europe and it also attracts the interest of European scientists in researching this health system.

In China, children grow up by taking herbs and most of them are readily available in stores. Taking herbs and combining with modern medical treatment is well integrated in Chinese culture. In the West, people grow up by taking pharmaceutical drugs, but more and more western patients are looking for alternative treatments with fewer side effects, like herbs. Scientific research is very important to guarantee a safe and good quality and therapy of traditional Chinese herbs. It is important to understand the side effects, potential herbal interactions and to identify improper herbs. In some plants, only the roots are medically used, in others only the flowers, the seeds, the stems or leaves and some are used as a whole. To reach the greatest medicinal properties the herbs are specially pretreated like cooked in ginger, boiled in vinegar, honey-fried or cut in a special way (Ehling and Swart, 2002).

"*The World Health Organization (WHO) defines herbal medicines as containing active ingredients from plants, or plant materials, or complex herbal preparations (Wan et al., 2010).*"

In China the health system fights against the same lifestyle diseases like Europe, for example diabetes and cardiovascular diseases (CVD). Herbal treatment of myocardial infarction is well developed in China. It is important to make an exchange of knowledge, because treatment with herbs is much cheaper than pharmaceutical drugs and when accordingly taken, it is safe. Our interest is on herbs with protective properties using for treating age related diseases.

Aging affects to all of us, but it is a difference in getting healthy or diseased old. The aging process is unique among health conditions. Over the past century the age has been steadily increased and this is very expensive for the health system. Cardiovascular disease costs the European Union € 169 billion in 2003 (Leal et al., 2006). Common age related diseases are cardiovascular disease, Alzheimer disease, type 2 diabetes, atherosclerosis and osteoporosis. Cardiovascular disease is the number one cause of death worldwide, it kills 17.1 million people every year. 15 million people suffer a stroke every year and 5 million are left permanently disabled (World Heart Federation, 2011). The risk factors of those age related killing diseases are well known. It is important to prevent them. Herbal treatment has a long history in TCM and it is well developed and accepted in China. Now we have the great chance and possibility to combine the knowledge of the eastern and western medicine to discover gentle and effective methods for prevention and treatment of ailment.

WHO definition of traditional medicine:

"Traditional medicine has a long history. It is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses. The terms complementary/alternative/non-conventional medicine are used interchangeably with traditional medicine in some countries (Anderson et al., 2000)*."*

1.2. Aim of the Thesis

The aim of this thesis was to perform the quality control of Traditional Chinese Herbs for promoting the safe and effective use of Chinese herbal medicine in Europe. We developed methods that can be used for quality control of TCM formulas and single herbs in the future. Our research experiments comprise High Performance Liquid Chromatography (HPLC), Bicinchoninic Acid Protein Assay (BCA), Sodium Dodecylsulfatepolyacrylamide Gel Electrophoresis (SDS-PAGE), N-terminal Sequencing and analysis of the sequence data.

We analyzed proteins of the herbal decoctions and developed methods for extraction of bioactive compounds. The Chinese Herbs using for my thesis are well known in the TCM for preventing and treating age related diseases like cardiovascular, neurological diseases and diabetes.

2. Traditional Chinese Herbs

2.1. Prescriptions of Herbs

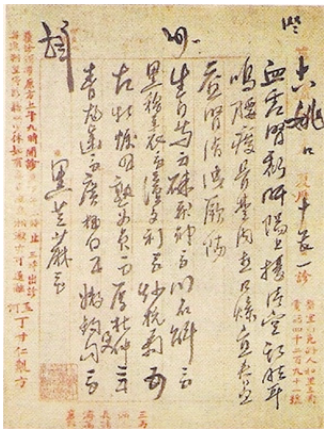


Figure 1: Prescription written by a TCM doctor in the early 20th century (Yugun, 2006).

It is said that Shen Nong (God of Agriculture 3737 – 3697 BC) became fascinated with the use of various plants and their medicinal properties. Legendary story tell that he tasted hundreds of herbs, minerals and animal substances and because of that he was poisoned many times. He is worshiped as the father of medicine and his researches are collected in the Shen Nong Ben Cao Jing – Classic Materia Medica. It contains 365 drugs on which 252 are herbs, 67 are from animals and 46 are from minerals (Ehling and Swart, 2002; Yugun, 2006).

In ancient China, the way to use drugs was compared to the way to command an army, because to treat diseases certain principles and strategies are needed like in the army.

The combined use of drugs is called prescription and they can be processed to decoction, granule, powder, paste, plaster, pill, tablet, bolus, injection and so on. In the composition of a formula, the herbs are categorized into four groups, monarch, minister, assistant and guide.

The "monarch drug" (Jun) is the key ingredient in the prescription and the "minister drug" (Chen) promotes it. The "assistant drug" (Zuo) is responsible for strengthen the curative effect or reduce toxin and the "guide drug" (Shi) leads compounds of the plants to work on the affected part.

After the Song Dynasty (960 – 1279) prescriptions were classified into their actions, namely sweating, vomiting, purgation, regulation, warming, clearing, nourishing and resolving (Yugun, 2006).

2.2. Processing of Herbs

Figure 2: Li Shizhen collecting herbs. (Yugun, 2006)

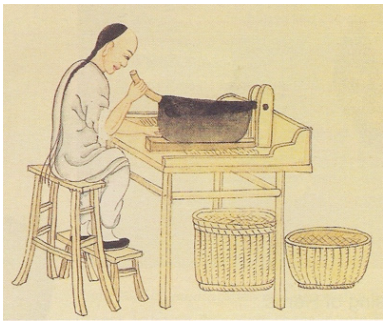


Figure 3: Processing herbs in Qing Dynasty (1644-1911). (Yugun, 2006).

Figure 4: Grounding herbs in Qing Dynasty (1644 - 1911). (Yugun, 2006)

Before the Eastern Han Dynasty (25 – 220), herbs were taken to the patients to cure their diseases. The form of decoction came into existence in the late Eastern Han Dynasty. The doctors began to decoct ingredients of a prescription to cure the patient.

Before clinical use, the herbs must be processed, like baking, roasting, washing, soaking, purifying, steaming and stewing. Another technique is adding wine, honey, vinegar and salt to the herbs. The method of processing is important for the effect of the herbs, because they are easier to store and use, toxic elements are removed, the nature of the plant is changed, the curative effect is increased and impurity is removed (Yugun, 2006).

Figure 5: Decoct preparation of Jiu Gancao

We prepared decoctions of the herbal formula Sinitang and as well of single herbs at the Medical University of Vienna.

The herbs were soaked for about 1.5 hours and afterwards they were gently cooked for 30 minutes in a ceramic pot. The extract was filtrated and fresh water added again and gently cooked for 30 minutes. At the end, both extracts were combined.

2.3. Absorption of Herbs

The main absorption occurs in the intestine, where herbs or drugs well pass through the intestinal wall to enter the blood stream. Several mechanisms influence the absorption of herbs through the intestine. For example if herbs are administered with other drugs, which may promote binding in the gastrointestinal tract, then the absorption is negatively influenced. Drugs such as cholestyramine (Questran), colestipol (Colestid) and sucralfate (Carafate) may bind to certain herbs and build an insoluble complex. Because of the large size, no molecules of either substance can pass through the intestine wall. Other drugs decrease the gastro intestinal motility and herbs stay in the intestine for a longer time of period, which means the herb absorption is increased. After absorption, the herbs will be delivered and released to different parts of the body. The majority of herbs and drugs have no clinically - significant interactions affecting distribution and thus herbs and drugs can safely be taken together. Drugs with a narrow range of safety index and a highly protein bound ratio like warfarin (Coumadin) and phenytoin (Dilantin) can lead to negative interactions and it must be taken with attention. After metabolizing the herbs and drugs by the liver the derivates are inactive and neutralized. Now the kidneys are responsible to eliminate the substances and their metabolites from the body (Chen et al., 2004).

2.4. Sinitang

Figure 6: Herbs of the Chinese formula Sinitang

Sinitang is a Chinese formula and was first described by Zhang Zhongjing (150 – 219 A.D.) in his book "Shang Han Lun". He described this formula as a remedy that acts on the heart, kidney and spleen meridians. It warms the inner body, dispels colds and is used as an herbal remedy of acute yang deficiencies.

In China, this herbal mixture has a longstanding tradition in the treatment of cardiovascular disease. It is very useful in the prevention of chronic heart failure post myocardial infarction. It has been used in Chinese clinics to improve blood circulation, relieve blood stasis and treat myocardial damage and cardiac exhaustion.

Sinitang is one of the simplest formula including four herbs:

Zhi Fuzi (*Aconitum carmichaeli* Debeaux)

Ganjiang (*Zingiberis officinale* Roscoe)

Rougui (*Cinnamomum cassia* Presl)

Jiu Gancao (*Glycyrrhiza uralensis* Fisch)

Studies published in the Chinese literature reported pharmacological properties of Sinitang like

- it increases the blood pressure (Tian, 1972)
- positive influence on cardiac function
- anti-apoptotic effect (Dietl et al., 2008)
- prevents cardiogenic shock (1983)
- anti-bacterial
- anti-inflammatory

- anti-oxidative (Liu et al., 2008b; Liu et al., 2006d)
- alleviates intimal hyperplasie and vascular stenosis (Liu et al., 2006c)
- shortens colon transmission time (Jin et al., 2006)

Free radicals and nitric oxide (NO) correlates with coronary heart disease (CHD). In a study of WU in 1999, the therapeutic mechanisms of Sini Decoction (SND) were investigated in patients with coronary arteriostenosis. A percutaneous transluminal coronary angioplasty (PTCA) can successfully delay a coronary atheromatous plaque stenosis and it can rebuild the blood supply. This effect was also discovered in post-PTCA. In this study, 40 patients received a successfully PTCA operation and 20 of them belonged to Deficiency Syndrome (DS) and 20 belonged to Excess Syndrome (ES). One group received three days before and after surgery daily oral 25 ml of SND and the control group had no treatment with SND.
Venous blood was taken to test oxygen free radicals (OFR) after operation and the concentration of superoxide dismutase (SOD), malonyldialdehyde (MDA) and nitric oxide (NO) were examined. Sini Decoct increases the superoxide dismutase activity and scavenge the toxic oxygen free radicals and decreases the malondialdehyde production. The effect of Sini Decoction was better in patients of DS because they are characterized by a high superoxide dismutase activity (Wu et al., 1999).
Another study of Su in 2000 determined the influence of Sini Decoction on quality of life (QOL). This research was performed with 40 patients who had an angina pectoris attack. Twenty patients received 3 days before and after percutaneous transluminal coronary angioplasty each day 25 ml of Sini Decoction. The other group was the control group and did not consume SND. The formula of Sini Decoct was the same like in the study described before of Wu. The quality of life of coronary heart disease patients after PTCA was general much better than before surgery. The blood perfusion was restored and the function of the heart improved. Sini Decoct administration improved the quality of life in somatic symptoms like palpitation and short breath, as well the well-being was improved and the depression degree lower than in the control group.
Sini Decoct scavenge toxic free radicals and this is important before surgery because it guards the myocardial tissues. This effect improves the restoration of myocardial blood supply and promotes the healing effect after PTCA. In this study, the herbal formula Sini Decoct was effective and safe and it improved the quality of life of coronary heart disease patients (Su et al., 2000).

"Sinitang Decoction recuperates the depleted yang and rescues the patient from collapse. For syndrome of yang exhaustion, it is usually Ganjiang combined with Fuzi, for maximal effect to decrease the toxicity of Fuzi as well as to strengthen the action of Fuzi (Yanfu, 2002a)."

Fuzi is extremely pungent and hot in nature and acts as monarch drug to recuperate depleted yang. Shengjiang acts as a minister drug and in compatibility with Fuzi, it strengthens the efficacy of recuperating depleted yang and dispelling cold (Yanfu, 2002b)*."*

Traditional use:

Medicinal properties: *"This recipe is used to treat syndrome of hyperactivity of yin due to yang exhausting, marked by cold limbs, aversion to cold, lying huddling up, vomiting, absence of thirst, abdominal pain, diarrhea, mental fatigue and sleepiness, whitish and slippery fur, feeble and thready pulse. It is applicable to myocardial infarction, acute cardiac failure, excessive vomiting from acute or chronic gastroenteritis (Yanfu, 2002b)."*

Actions: *"Recuperating depleted yang to rescue the patient from collapse (Yanfu, 2002b)."*

2.4.1. Fuzi and Zhi Fuzi

Figure 7: Fresh upper part of *A. carmichaelis*

Figure 8: Fuzi

Figure 9: Zhi Fuzi

Botanical Name: *Aconitum carmichaelis* Debeaux
Pharmaceutical Name: Radix Aconiti Lateralis Praeparata
English Name: aconite, prepared daughter root of common monks´ hood
Literal Name: "appendage"
Family: Ranunculaceae
Origin: Shandong Region, China
Part: root and tuber
Properties: acrid, hot
Channels entered: heart, kidney, spleen
(Chen et al., 2004)

The use of *Aconitum* species has a long history in the Traditional Chinese Medicine. They have been used for more than 2000 years. Usually the tubers and roots are used for treating the various diseases, like rheumatic fever, gastroenteritis, diarrhea, oedema, bronchial asthma, collapse and irregular menstruation (Singhuber et al., 2009).
Fuzi is the untreated root or tuber of *Aconitum carmichaelis* and it is a toxic herb for the heart muscle, but used as a cardiotonic herb in China. Prepared as an isolated heart preparation, it causes a temporary increase in myocardial contractility, followed by a decrease and then an irregular cardiac rhythm.
This herb is toxic because of its alkaloids like aconitine, hypaconitine, mesaconitine and talatisamine. Fuzi, the untreated aconite contains a high amount of those compounds,

whereas Zhi Fuzi has its alkaloids potency reduced. It is pretreated with special treatments like soaking, roasting and than decoction. The herbal pharmaceutical industry uses well-developed modern techniques, such as pressure steaming.

The first toxic symptoms caused by alkaloids, is loosing sensation in the mouth and tongue after consumption. The alkaloids are quickly intestinal absorbed and other toxic side-effects are nausea, vomiting, spasm of extremities and cardiac arrhythmias (Huang, 1999). Aconite is a cardiotonic herb, but it seems that it can positively influence heart related diseases and surgeries on the heart. Most important point is the dose of administration. The beneficial effects are probably from the alkaloids or the combination with other herbs.

The pharmacological effects of aconite are:

- anti-fungal and anti-bacterial
- anti-convulsive
- anti-shock
- anti-inflammatory
- rescue from heart and kidney failure
- removes water retention and oedema
- induces apoptosis in several tumor cells

It must always keep in mind that aconite contains toxic substances and it has to be handled with care (Singhuber et al., 2009).

Traditional use:

"Fuzi is used in the traditional Chinese medicine as a cardiotonic, to restore yang in the treatment of collapse and shock, to warm the kidneys, and to reinforce yang in the relief of pain (Huang, 1999)."

Medicinal properties: *"Pungent and sweet in flavor, hot in nature, toxic and attributive to the heart, kidney and spleen meridians* (Yanfu, 2002a)."

Actions: *"Recuperate the depleted yang for resuscitation, supplement fire and strengthen yang, expel cold to relieve pain (Yanfu, 2002a)."*

2.4.2. Shengjiang and Ganjiang

Figure 10: Ganjiang – dried ginger

(Bencao, 2007)

Figure 11: Fresh upper part of Shengjiang

(Bencao, 2007)

Botanical Name: *Zingiber officinale* Roscoe
Pharmaceutical Name: Rhizoma Zingiberis
English Name: fresh ginger and dried ginger
Literal Name: "fresh ginger" and "dried ginger"
Family: Zingiberaceae
Origin: Shandong Region
Part: root
Properties: acrid, hot
Channels entered: heart, lung, spleen, stomach
(Chen et al., 2004)

Ginger is widely used in Chinese, Ayurvedic, Tibb-Unani herbal medicine and all over the world. It is popular because of its flavor and ability to medicate sore throats, cramps, constipation, indigestion, vomiting, hypertension, fever, dementia, muscular aches, infectious diseases and much more. Ginger especially warms the body and is enjoyable to consume it as a tea to get rid of the cold. Shengjiang and Ganjiang contain a lot of compounds that are responsible for its pharmacological activities and taste. The rhizome consists as well volatile oil, which comprises monoterpenoids as camphene, cineole, geraniol, curcumene, citral and sesquiterpenoids like α-zingiberene, zingiberol, β-sesquiphellandrene, arcurcurmene and much more. Fresh ginger is much hotter than dry ginger and this is caused by gingerols, which belong to phenols. In ginger identified gingerols are [6]-, [4]-,[7]-,[8]-,[10]-gingerol.

During thermal processes gingerols become dehydrated and turn into shogaols. So in dry ginger (Ganjiang) the concentration of shogaols is increased whereas gingerols are reduced and the taste is not so intense like in fresh rhizomes (Shengjiang).

Biological activity:

- strong antioxidant
- immuno-modulatory
- anti-tumorigenic
- anti-inflammatory
- anti-apoptotic
- anti-hyperglycemic
- anti-lipidemic
- anti-emetic

An *in vitro* study demonstrated a dose dependent anti-microbial activity of ginger extract (10 mg/kg) against *Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli and Candida albicans*. The anti-helmintic activity was shown in sheep naturally infected with mixed species of grastrointestinal nematodes. It was used crude powder and crude aqueous extract of dried ginger (1-3 g/kg) and both extracts had a dose- and time-dependent anti-helmintic effect (Ali et al., 2008). Loads of *in vitro* and laboratory animal studies show an effective inhibitory effect of Shengjiang on the carcinogenic process. Nowadays ginger is mostly used as a prevention of travel sickness or as cancer chemoprevention because of its anti-inflammatory and its strong anti-oxidative effects. Masuda et al. isolated 50 antioxidants from the rhizomes of ginger in 2004. A study reports the inhibition of arachidonic acid induced platelet aggregation and formation of thromboxane B2 and prostaglandine D2 caused by [6]-gingerol (Shukla and Singh, 2007). Shogaols have a much stronger growth inhibitory effect on H-1299 human lung cancer cells and on HCT-116 human colon cancer cells than gingerols. The compound [6]-shogaol is much more effective than [6]-gingerol (Sang et al., 2009b). Another research of Ozgoli et al. in 2009, demonstrated a decrease of nausea intensity and vomiting incidence in pregnancy during an intake of ginger capsules (250 g for four times a day). The women who used the capsules where satisfied with this effect (Ozgoli et al., 2009). The anti-emetic effect can be used during chemotherapy as well. Some studies show a positive effect for the patients

some did not. This herb can be used complementary and the right dosage is important (Pillai et al., 2010). *Zingiber officinale* is a well-tolerated herb with less or none significant side effects.

Traditional use:

Medicinal properties: *"Pungent in flavor, hot in nature and attributive to the spleen, stomach, heart and lung meridians* (Yanfu, 2002a)."

Actions: *"Warm to the middle-energizer to expel cold, restore yang and dredge channels, warm the lung to resolve phlegm* (Yanfu, 2002a)."

2.4.3. Rougui

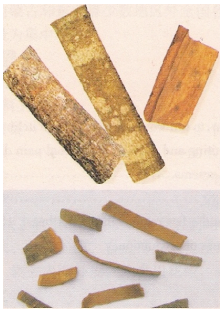


Figure 12: Rougui processed

(Bencao, 2007)

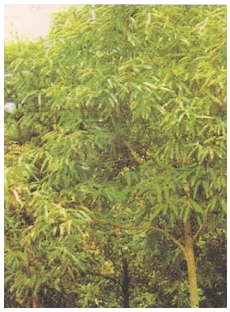


Figure 13: Rougui tree

(Bencao, 2007)

Botanical Name: *Cinnamomum cassia* Presl
Pharmaceutical Name: Cortex Cinnamomi
English Name: cinnamon bark, cassia bark
Family: Lauraceae
Origin: Shandong Region
Part: bark
Properties: acrid, sweet, hot
Channels entered: heart, kidney, liver, spleen
(Chen et al., 2004)

The aromatic evergreen Cinnamon tree belongs to the genus *Cinnamomum* and its twigs and bark are used worldwide as a favored spice. Cinnamon has a long history in use as spice and in naturopathic medicine. Especially in the cold season, cinnamon is used in tea and biscuits, because of its good taste and its warming effect. In Chinese books it was first mentioned about 4.000 years ago (Kirkham et al., 2009). In the traditional Chinese herbal medicine it is used against inflammation, chronic bronchitis, to improve blood circulation, to alleviate fever and induce perspiration. In the last years this spice got very popular because of the positive effects in the treatment of diabetes. *Cinnamomum cassia* contains a lot of bioactive compounds such as cinnamaldehyde, cinnamic acid, coumarins, diterpenoids and polyphenols. Most of the pharmacological effects are limited to its major compound cinnamaldehyde.

Biological activities:

- bactericidal
- sedative
- hypotensive
- peripheral vasodilation
- anti- platelet aggregation effect
- blood anticoagulation effect
- anti-fungal
- anti-pyretic
- anti-microbial
- anti-oxidant

(Kim et al., 2010)

In an *in vivo* study on Wistar rats and *in vitro* using INS-1 cells, the antidiabetic effect of *Cinnamomum cassia* was tested. The research group came to the result, that *C. cassia* has a direct insulin stimulatory effect and an anti-diabetic potency, which should be used in the future (Verspohl et al., 2005). A study on patients with type 2 diabetes demonstrated that an intake of 1, 3, or 6 g of *Cinnamomum cassia* per day reduced respectively the serum glucose, triglycerides, low-density lipoprotein cholesterol (LDL-C) and total cholesterol. After 40 days, there were significant changes in the cinnamon group, but no changes in the placebo group (Dugoua et al., 2007).

Even the anti-allergic effect of *C. cassia* extract was tested on mice with eczematous conditions and infiltration of various inflammatory cells into the skin lesions. Because of the anti-inflammatory effect of the cinnamon extract, the atopic dermatitis-like skin lesions in the mice were inhibited. This was caused by a suppressed T-helper 2 cell response (Sung et al., 2011).

Traditional use:

Medicinal properties: *"Pungent and sweet in flavor, hot in nature and attributive to the spleen, kidney and heart meridians* (Yanfu, 2002a)."

Actions: *"Supplement fire and strengthen yang, expel cold and alleviate pain, warm channels to promote the circulation of the blood* (Yanfu, 2002a)."

2.4.4. Gancao and Jiu Gancao

Figure 14: Dried Gancao

(Bencao, 2007)

Figure 15: Fresh upper part of Gancao

(Bencao, 2007)

Botanical Name: *Glycyrrhiza uralensis* Fisch
Pharmaceutical Name: Radix Glycyrrhizae
English Name: licorice root
Literal Name: "sweet herb," "sweet grass"
Family: Leguminosae
Origin: Shandong Region
Part: root
Properties: sweet, neutral
Channels entered: spleen, stomach, lung and heart
(Chen et al., 2004)

Gancao is the ordinary *Glycyrrhiza uralensis*, whereas Jiu Gancao is *Glycyrrhiza uralensis* processed with honey.

The dried roots and rhizomes of the species *Glycyrrhiza* have a long tradition in the herbal medicine and even in the food industry because it is used as a natural sweetener. It is added in many food or drug preparations as a flavoring adjuvant. Licorice contains the saponine glycyrrhizic acid, which is 50 times sweeter than sugar. Gancao is yellow because of the flavonoids and chalcones such as liquiritin, liquiritigenin, isoliquiritin, licoflavonol, isotrifiliol, glabrolide and much more. Also present are isoflavones, coumarins, stilbenoids, fatty acids, starch, polysaccharides and sterols.

Because of those and other compounds licorice has a lot of bioactivities such as:

- anti-inflammatory
- anti-microbial
- anti-viral
- anti-protozoal
- anti-oxidative
- hepatoprotective
- anti-tumor

Gancao is used for the treatment of bronchitis, tuberculosis and peptic ulcer. In Asia, glycyrrhizic acid is very popular for treating chronic hepatitis B and C. One example is Stronger Neo-Minophagen C (SNMC). The study of van Rossum in 1998, showed that SNMC decreases the aminotransferase levels in patient with chronic hepatitis (van Rossum et al., 1998). The intravenous administration of the formulation SNMC contains 40 mg glycyrrhizin and SNMC showed a clinical improve on patients with liver disease at various stages because of the anti-inflammatory effect (Fiore et al., 2008).
It has been shown that licorice has a direct hepatoprotective effect and glycyrrhizin prevents the development of hepatocellular carcinome (HCC) in patients with Hepatitis C Virus (HCV)- associated chronic hepatitis (Asl and Hosseinzadeh, 2008).
Gancao is a strong antioxidant. In the classic Chinese medical text it was written that *"Gancao can detoxify hundreds of toxic substances* (Huang, 1999)*."* Tests with glycyrrhinic acid showed that it can lower the toxicity of several toxic substances, like strychnine, snake venom, diphtheria toxin, tetanus toxin, histamin, arsenate, chloral hydrate and many more. In traditional herbal mixtures, Gancao is often combined with toxic compounds like *Aconitum carmichaelis* to bind its toxic compounds (Huang, 1999). A high consume of glycyrrhizin can cause hypertension and hyperkalemia (van Rossum et al., 1998).

Traditional use:

"Chinese medical texts describe Gancao as an agent to improve the tone of the middle Jiao (the digestive system) and replenish qi, to remove heat and toxic substance, to moisturize the lungs and arrest coughing, and to relieve spasms and pain (Huang, 1999)."

Medical properties: *"Sweet in flavor, mild in nature, and attributive to the heart, lung, spleen and stomach meridians* (Yanfu, 2002a)*."*

Actions: "Enrich qi and invigorate the stomach and spleen, moisten the lung and resolve phlegm, clear away heat and toxin, relieve spasm and alleviate pain (Yanfu, 2002a)*."*

2.5. Anti Aging Herbs

2.5.1. Hongqu

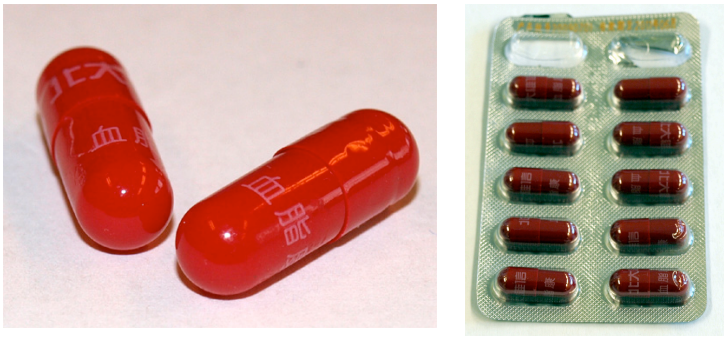


Figure 16: Pills of Hongqu

Botanical Name: *Monascus purpureus* Went.

Pharmaceutical Name: Monascus

English Name: Red Yeast Rice

Family: Poaceae

Origin: the pills for our studies are produced at the Beijing University for Pharmacy

Part: grain

Properties: sweet, acrid, warm

Channels entered: spleen, liver, large intestine

(Chen et al., 2004)

Package insert of the Red Yeast Rice pills, which were used for the experiments:

The normal dosage is twice a day after breakfast and after dinner, each time two pills of Red Yeast Rice. The lower dosage is one pill after dinner. One pill contains 0.3 g pulverized Red Yeast Rice. The tablet can be taken for 3 months and the blood have to be controlled regularly and if there are any side effects, the intake of Red Yeast Rice has to be stopped.

Persons who are allergic against Red Yeast Rice, who have an acute hepatitis, or pregnant and nursing women should not take the medicine.

It eliminates wetness and removes the body from phlegm. It boosts the blood circulation and releases blockades and nourishes the spleen and promotes the metabolism.

Red Yeast Rice treats weariness, dizziness, breathlessness, loss of appetite, high blood sugar, high cholesterol level, high blood pressure, foamed abdomen and the feeling of pressure on the heart.

Red Yeast Rice is also known as "Red Koji", "Angkak", "Hongqu" or "Xuezhikang" and its first appropriation was documented at 800 AD (Hong et al., 2008).
It has a traditional use in the Chinese cuisine because of its flavor, color and medicinal agent. Red Yeast Rice has a long history and is even mentioned in the ancient Chinese pharmacopoeia Ben Cao Gang Mu-Dan Shi Bu Yi during the Ming Dynasty (1368 – 1644) (Liu et al., 2006b). Hongqu has been used in the TCM as a remedy to improve the circulation, aids digestion and treats diarrhea. In China it is used for centuries as a therapy for cardiovascular disorders. Nowadays it is even popular in the Western because of its effect in decreasing the serum lipid (Bauer and Franz, 2010; Lin, 2010).
The production of Red Yeast Rice is a fermentation process. The washed white rice is fermented with the strain of the red yeast *Monascus purpureus* for about nine days at 25 °C and at a pH range of 5 to 6. Afterwards the rice gets dried, pulverized and encapsulated (Journoud and Jones, 2004). This process has to be well controlled because the nephrotoxin citrinin can occur.
The yeast *Monascus purpureus* produces a lot of substances like monounsaturated fatty acids, isoflavones, phytosterols and monacolins, which inhibit the enzyme 3-hydroxy-3-methylglutaryl (HMG) co-enzyme A reductase, an enzyme which is important in synthesizing cholesterol. Because of this effect it prevents cardiovascular events and can reduce mortality (Li et al., 2010b). All those substances in combination are able to lower the total cholesterol and the low density level (LDL) cholesterol level. Studies demonstrate that Red Yeast Rice therapy (1.2 g/day – 3.6 g/day) is an effective treatment for persons who are highly intolerant to daily statin use (Venero et al., 2010). A high dose of statins increases the risk of statin-associated myalgia (SAM). Hongqu contains as well statin-like metabolites like monacolin K (lovastatin) and 13 other monacolins. The treatment with Red Yeast Rice pills for 12 weeks significantly reduced the rates of myalgia and lowered the LDL cholesterol level, too (Halbert et al., 2010).
A study over 4.5 years on Chinese patients with hypertension and previous myocardial infarction demonstrated a cholesterol-lowering effect because of an intake of 0.6 g Red Yeast Rice twice a day. The mechanism is not fully understood, but anti-inflammatory,

neuroprotective, antithrombotic, direct vascular and plaque-stabilizing effects could be the important factors for this.

Extract of Hongqu inhibits prostate cancer cell line proliferation (LNCaP) and it induces the apoptosis (Li et al., 2010a). *In vitro* effects also demonstrated repression of proliferation and a stimulation of apoptosis in human colon cancer cells HCT-116 and HT-29 in a dose dependant manner. Even Red Yeast Rice without monacolin K still decreased the cell proliferation. Hence the inhibiting effect on the colon cancer cells is independent of monacolin in Red Yeast Rice (Hong et al., 2008).

It is important that patients are monitored during supplementation of Red Yeast Rice, because too long intake can lead to abnormal liver functions (Klimek et al., 2009).

Traditional use:

"*Hongqu is sweet in flavor and warm in property. It promotes digestion and blood circulation, can strengthen the spleen and dry the stomach. Another pharmacologist of the Ming Dynasty, Miao Xiyong, reported that Hongqu has an effect on spleen and stomach as described in the Ying system (a concept of traditional Chinese medical theory as it relates to the blood circulation), whereby blood depends on the principle of like attracting like* (Ma et al., 2000)."

2.5.2. Jiaogulan

Figure 17: Fresh leaves of Jiaogulan

Figure 18: Dried leaves of Jiaogulan

Botanical Name: *Gynostemma pentaphyllum* (Thunb.) Makino
Pharmaceutical Name: Rhizoma seu Herba Gynostemmatis
English Name: gynostemma, herb of immortality or five-finger leaf
Family: Cucurbitaceae
Origin: Anhui Region, China
Part: leaf
Properties: slightly bitter, cold
Channels entered: lung, heart

Gynostemma pentaphyllum Makino grows in wild fields in China, Japan, Korea, Thailand and Malaysia. In China it is named Jiaogulan and it is a perennial liana plant and traditionally used in food, tea and in the folk medicine. In the TCM it is used to lower the cholesterol level, regulate the blood pressure, strengthen the immune system, treats bronchitis and gastritis and reduce inflammation (Liu et al., 2004). The herb contains flavonoids like rutin and quercetin which possess many positive biochemical effects like antimicrobial, antioxidant, anticancer and many more (Ashok Kumar et al., 2009). The main compounds are saponins called gypenosides. Jiaogulan contains as well ginsenosides which where intended to be unique in *Panax ginseng* C. A. Mey or in Chinese it is Renshen (Xie et al., 2010). Compared to Ginseng, Jiaogulan is easier available and much cheaper. *Gynostemma pentaphyllum* is an annual plant so it can be harvest every year, but the production of Panax ginseng takes about 5 or even more years. The rhizomes are more worth, while the older they are. About 90 gypenosides have

been isolated and about six of them are the same with the ginsenosides from *P. ginseng (Huang et al., 2007b)*. It is reported that gypenosides have antioxidant activities for example studies demonstrated that gypenosides suppress lipid peroxidation, cell injury induced by hydrogen peroxide, reduce superoxide anion and hydrogen peroxide contents in human neutrophils. These antioxidant activities are important for preventing aging-associated health problems like chronic inflammation and cardiovascular diseases. Gypenosides **III** and **VIII** are responsible for the protective effect on the cardiovascular system (Circosta et al., 2005). One compound of Jiaogulan is phanoside and in a study tested on rats it induced the insulin release (Hoa et al., 2004).

Biological activities:

- may reduce the risk of cardiovascular diseases
- hypoglycemic activity
- anti-inflammatory
- anti-cancer
- hepatoprotective effect
- anti-gastric ulcer
- activates the immune response for cancer patients

(Huang et al., 2007a; Xie et al., 2010)

In a randomized, double-blind, placebo-controlled study the anti-diabetic effect of *Gynostemma pentaphyllum* was investigated. The patients who are diseased with type 2 diabetes mellitus got daily a tea of 6 g for twelve weeks of this herb and information about diet and exercise. After three month of treatment the fasting plasma glucose levels was totally decreased. Another study on ovalbumin-sensitized mice (OVA) tested oral administration of *G. pentaphyllum*, 5 days a week for 4 weeks. The consumption of this drug inhibited the inflammation of the airways and reduced asthma symptoms in mice (Liou et al., 2010).
In conclusion, these results suggest that an effective, safe and easy therapy for typ 2 diabetic patients can be developed in Europe in the future. Clinically, there were no side-effects on liver or kidney and the *Gynostemma pentaphyllum* tea was generally well tolerated (Huyen et al., 2010).

Traditional use:

Medicinal properties: *"Rhizoma seu Herba Gynostemmatis moistens the lung, promotes the generation of body fluids and dispels phlegm* (Chen et al., 2004)*."*

Actions: *"Jiaogulan has a general effect to nourish and strengthen the body. It is commonly used to treat chronic disorders, such as asthma, migraines, neuralgia, impaired function of the respiratory and gastrointestinal tracts, and syndromes characterized by deficiency. Clinical manifestations may include vomiting or nausea with drooling of saliva, shortness of breath and chest congestion* (Chen et al., 2004)*."*

2.5.3. Baiguo

Figure 19: Baiguo

(Bencao, 2007)

Botanical Name: *Ginkgo biloba* Linnaeus
Pharmaceutical Name: Semen Ginkgo
English Name: ginkgo nut, ginkgo seed
Literal Name: "white fruit seed"
Family: Ginkgoaceae
Origin: Jiangsu Region, China
Part: seed
Properties: sweet, bitter, astringent, neutral
Channels entered: lung
(Chen et al., 2004)

The *Ginkgo biloba* tree is a living fossil, because it is more than 150 million years old. *Ginkgo biloba* seeds and leaves have been used in the early Chinese herbology 2.800 years ago. Nowadays in the modern Chinese pharmacopeia the extracts of *Ginkgo biloba* leaves and the seeds are still recommended for treating heart and lung (asthma and bronchitis) problems. The seed also called Pak Ko treats phlegm, urinary incontinence, spermatorrhea and stop coughing and wheezing. The seeds are also very popular in Asia for using in the cuisine, but in Europe or America this usage is unknown. In the West, over the past 20 years, the use of leaves got more common and so they are taken as well, in tablets or in form of teas.
The therapeutic effect of Ginkgo biloba leaves and seeds results from their chemical constituents, which are essentially the same. Both contain flavonoids such as quercetin,

kaempferol, ginkgetin and bilobetin that are supposed to have antioxidant activity. The terpenoids like ginkgolide A, B, C, J and bilobalide are antagonists to platelet-activating factor (Singh et al., 2010).

Biological activities:

- anti-oxidant
- anti-inflammatory
- inhibition of platelet activating factor
- improves cerebral blood flow
- reduces corticosteroid production
- increases mitochondrial metabolism
- neuroprotective effect

(Chan et al., 2007)

The major use of *Ginkgo biloba* is the attendance of cerebral dysfunction. A well known standardized extract is EGB 761® in the treatment of dementia. It is produced from ground up leaves and contains 24 % flavone glycosides and 6 % terpene lactones (Gorby et al., 2010). An actual evidence based evaluation of the Institute for Quality and Efficiency in Health Care (IQWiG) confirms that a higher dosage of 240 mg of this extract per day has a positive effect on Alzheimer-Dementia. The Ginkgo-Extract EGb 761® was compared with Donepezil, a cholinesterase inhibitor and treatment for Alzheimer´s dementia. All of them improved the cognitive activity, the neuropsychiatric symptomatic, the daily living, but the patients with the EGb extract had less unwanted events compared to the other patients. In a study of Wettstein the results showed that EGB delayed the symptoms of Alzheimer´s dementia compared with placebos. Because of those results, this *Ginkgo* extract is an alternative to treat patients with dementia (Kasper et al., 2009; Weinmann et al., 2010).

Traditional use:

Medicinal properties: *"The seed is bitter, acerbity and sweet in taste and natural in nature."*

Actions: *"To arrest persistent cough and asthma, and to reduce leucorrhea and urination. Persistent cough and asthma with profuse expectoration; morbid leucorrhea with whitish discharge; enuresis, frequent urination"* (Bencao, 2007)."

2.5.4. Yinxinye

Figure 20: Yinxinye

Botanical Name: *Ginkgo biloba* Linnaeus
Pharmaceutical Name: Folium Ginkgo
English Name: ginkgo leaf
Literal Name: "silver fruit leaf"
Family: Ginkgoaceae
Origin: Jiangsu Region, China
Part: leaf
Properties: sweet, bitter, astringent, neutral
Channels entered: lung

Leaves are ideally harvested before or during the blooming of flowers. At this time the leaves contain the highest levels of active ingredients and aroma (Chen et al., 2004).
Le Bars tested in a placebo-controlled, double-blind, randomized trial with 309 patients over 52 weeks the effect of *Ginkgo biloba* leaves for treatment of dementia. Yinxinye was concluded to be safe and improved the cognitive performance and the social function of demented patients for 6 months to 1 year. Patients who take anticoagulant and antiplatelet medications, such like warfarin should use Yinxinye with caution, because it could influence the bleeding time (Chen et al., 2004).

Traditional use:

Medicinal properties: *"It astringes the lung, calms wheezing, stops pain and relieves chest oppression* (Chen et al., 2004)*."*

Actions: *"Yinguoye relieves wheezing, and alleviates the shortness of breath and dyspnea characteristic of Lung deficiency. Yinguoye improves circulation in the chest to relieve feelings of chest oppression and pain. Current applications of this herb include coronary artery disease, angina, hypertension and hyperlipidemia.* (Chen et al., 2004)."

2.5.5. Danshen

Figure 21: Dried roots of Danshen

Botanical Name: *Salvia miltiorrhizae* Bunge
Pharmaceutical Name: Radix Salviae Miltiorrhizae
English Name: salvia root
Literal Name: "red ginseng"
Family: Labiatae
Origin: Jiangsu Region, China
Part: root
Properties: bitter, cool
Channels entered: heart, pericardium and liver

Danshen used as a single herb or in combination with other herbs is a very popular drug in China. It has been used for more than thousand years because of the treatment of various diseases.
It was mentioned in Tao Hongjing (456 - 536) and also in the Ming Dynasty (1368 - 1644). The most important application in clinical use is the treatment of coronary artery diseases.
In China, it is the most common therapy of angina, because Danshen improves the microcirculation, inhibits platelet adhesion and aggregation, protects against myocardial ischemia and causes coronary vasodilation (Cheng, 2007). The major constituents of this drug are for example the lipid soluble diterpenoid quinones like tanshinone and cryptotanshinone and water soluble phenolics like salvianolic acid A, salvianolic acid B, rosmarinic acid, danshensu, protocatechuic acid and protocatechuic aldehyde (Yue et al., 2006). In a meta-

analysis a compound salvia pellet (CSP), which contains Danshen (*Salvia miltiorrhizae*), Sanqi (*Panax notoginseng*) and Borneol (*Cinnamomum camphora*) was determined in its efficacy and safety for the treatment of stable angina pectoris (SAP). The usage of CSP compared with nitrates had a significant improvement of the angina symptoms and even on the electrocardiogram results (Wang et al., 2006a).

In China, Korea and Japan, Danshen is a well established traditional medicine against hypertension, even for pregnancy induced hypertension. Responsible for this effect are the lithospermic acid B and salvianolic acid B because of the inhibition of an angiotensin converting enzyme, which is an essential regulatory enzyme of the renin-angiotensin system (Cheng, 2007). It is demonstrated *in vitro* and *in vivo* that salvianolic acid B (Sal B) has neuroprotective and anti-inflammatory activities. An *in vivo* study of ischemia-reperfusion induced injury in rat brain showed as well protective properties of salvianolic acid B. Lipopolysaccharide (LPS) increases the production of reactive oxygen species (ROS) which are mediators for inflammations. When the microglial cells were pretreated with salvianolic acid B and then exposed to LPS, the ROS production was significant lower than without Sal B. Because of this anti-inflammatory activity the microglial cells had a lower production of NO, TNF-α and IL-1β in a dose-dependant way (Wang et al., 2010).

Many compounds of TCM herbs have an anti hepatitis B virus (HBV) activity. This is very important for the future, because safe and potent drugs as a treatment of HBV are strongly needed. In China, Danshen is used as a medicine for heart diseases and it is as well a traditional drug for liver diseases. The lipophilic and the hydrophilic extracts have biological activities. For example protocatechuic aldehyde (PA) is a water soluble substance of *Salvia miltiorrhizae*. It is characterized as an anti-HBV ingredient. PA significantly inhibited the production of HBV DNA on HepG2.2.15 cells and the expression of HBsAG and HBeAg was respectively repressed. Even in clinical studies the anti-HBV activity is shown (Cui et al., 2010). Another study shows that Danshen elevates the activity of superoxide dismutase and reduces malondialdehyde in a chronic hepatic damaged animal model, which was induced by CCl_4. In diabetic rats the herb protects the eye and aorta of oxidative damage and because of those cognition Danshen supplementation may be useful in the treatment of diabetic complication (Yue et al., 2006).

Danshen has no major side effects but it may interact with other drugs, which can cause serious complications.

Warfarin is a medicine for patients with heart problems because of its anticoagulant action.

S. miltiorrhizae potentiates the effect of Warfarin. This is a very important fact for the patient safety, because warfarin is a very common medicine for cardiac patients in western medicine and Danshen is widely used in herbal medicine for patients with heart problems. Danshen can be administered orally or taken in a nebulizer or even some Chinese cigarette brands contain this drug.

Biological activities:

- anti-oxidant
- anti-coagulant and anti-thrombotic
- kidney function regulation
- liver protective effects
- cardiovascular effects
- anti-tumor
- anti-HIV
- anti-bacterial
- anti-inflammatory and anti-immunological effects
- anti-allergic

(Wang et al., 2007)

Salvia miltiorrhizae has really a wide range of positive effects on the human body. Danshen seems to be like Ginseng (*Panax notoginseng*), a remedy for almost all diseases. Sometimes those two herbs are combined and known as the "Cardiotonic Pill" or "Fu Fang Danshen Di Wan" (Compound Formula of Salvia and Rehmannia) in Chinese (Cheng, 2007).

Traditional use:

Medicinal properties: *"Bitter in flavor, slightly cold in nature and attributive to the heart, pericardium and liver meridians."*

Actions: *"Promote blood circulation to remove blood stasis, regulate menstruation to relieve pain, cool the blood to relieve carbuncle, and clear away heat from the heart and tranquilize the mind"* (Yanfu, 2002a)*."*

2.5.6. Salvia officinalis

Figure 22: Dried leaves of *Salvia officinalis*

Figure 23: Fresh leaves of *Salvia officinalis*

Family: Lamiaceae
English name: sage
Origin: Austria and Albania
Part: root and leaf

Sage is widely cultivated and a popular and culinary herb because of its flavour. The name *Salvia officinalis* has a nice meaning because it is attributed to its medicinal importance. In latin, *salvare* means "to cure" and *officinalis* comes from "medicinal" (Miura et al., 2002). The leaves of *Salvia officinalis* are well known as antioxidant and used in the food industry and in the human health area.
Sage is an important domestic herbal remedy for sore throat or digestive disorders. It is used in the treatment of menopausal problems for example sweating, or in tuberculosis it profuses the perspiration and it is as well used against anxiety, depression and female sterility (Oboh and Henle, 2009).

The plant has a wide range of biological activities such as:

- anti-oxidative
- anti-bacterial
- virustatic
- fungistatic
- eupeptic

Because of its anti-microbial properties and the astringent activities, sage is a benefit in the reduction in plaque growth and is an active compound of dental care preparations. Sage has anti-inflammation activities and inhibits gingivits and is an effective prophylaxis for caries.
Ursolic acid is the main compound of sage, which is responsible for the anti-inflammatory activity and it can be used as a quality control measure (Baricevic et al., 2001).
A study on rats showed, that an hydroalcohlic extract (HE) of *S. officinalis* protected the gastric mucosa which got induced gastric lesions with ethanol. The effect is caused because the extract inhibits the H^+, K^+-ATPase and has a high free radical scavenger potential (Mayer et al., 2009).
In another study the extract of *S. officinalis* was tested on the brain and liver tissues of Wister strain albino rats. The amount of Vitamin C in the leaf was higher than in other related spices like basil, cinnamon, oregano and rosemary and the total phenol content was high as well. Vitamin C has the ability to eliminate aqueous peroxyl radicals before they can damage the lipids. Especially the brain contains a lot of unsaturated fatty acids and is more exposed to lipid peroxidation than the liver. The herbal extract caused a dose dependent inhibition of malondialdehyde (MDA) production in brain and liver tissues because of the antioxidant effect. A high concentration of tissue iron is associated with liver and heart disease, cancer, neurodegenerative disorders, diabetes and hormonal abnormalities. $Fe(\mathbf{II})^{2+}$ can catalyze one-electron transfer reactions which generates reactive oxygene species (ROS). The extract of *S. officinalis* had as well a dose dependent inhibition of $Fe(\mathbf{II})^{2+}$-induced lipid peroxidation. Sodium nitroprusside (SNP) is an antihypertensive drug and it relaxes the vascular smooth muscle and it dilates peripheral arteries and veins. The problem is that SNP releases cyanide and/ or nitric oxid (NO) and NO can cause neuronal damage in attendance with other ROS. In the presence of SNP the production of MDA is higher, but in combination with the sage extract the MDA concentration was reduced. The extract of *S. officinalis* leaf has an antioxidant and protective effect and it could be used in prevention of degenerative diseases associated with oxidative stress (Oboh and Henle, 2009).

3. Materials and Methods

3.1 Materials

Chemicals	Suppliers
Coomassie Brilliant Blue R-250 (CBB) Dithiothreitol (DTT) Tris-(hydroxymethyl) aminomethane Glycin p.A. Aminoacetic acid	Biomol, Hamburg, Germany
Page Ruler™ Plus Prestained Protein Ladder	Fermentas, St. Leon-Rot, Germany
Ammonium peroxodisulphate (APS) ultra pure	Gibco BRL, Paisley, UK
Ammonium Persulfate	Life Technologies, Gaithersburg, USA
CH_3COOH (HAc) Acryl amide Bis-acryl amide Bromphenol blue Isopropanol	Merck, Darmstadt, Germany
Ginkgolide A Ginkgolide B Lovastatin Rutin Salvianolic acid B Tanshinone II A	NICPBP, Beijing, China
Cinnamaldehyde 8 - Gingerol	Phy Proof, Phytolab, Vestenbergsgreuth, Germany
Glycyrrizic acid	Present from the Institute of Pharmacy, University of Vienna
Complete EDTA-free Protease inhibitor cocktail tablets	Roche, Mannheim, Germany
Bovine serum albumin (BSA)	Roth, Karlsruhe, Germany

HCl	
N,N,N,N-tetramethylethylenediamine (TEMED)	
Sodium dodecylsulphate (SDS) ultra pure ≥ 99 %	
Acrylamide	
Bromphenol blue sodium-salt	
Glycerine	
TrisPufferan®, Buffer Grade	
Acetonitril (AcN) HPLC Grade, Rotosolv®	
TrisPufferan®≥ 99.3 %, Buffer Grade	
Acetonitril (AcN) HPLC Grade, Rotosolv®	
Methanol (MeOH) HPLC Grade, Chromosolv®	Sigma-Aldrich, St. Louis, USA
Hydrochloric acid min. 32 %	
Acetic acid 100 %	VWR, New Jersey, USA
Ethyl alcohol absolute	

Table 1: Chemical Materials

Herbs in Latin	**Chinese**	**Use**	**Source**
Aconitum carmichaelis	Fuzi	roots and tubers	06/2006
Aconitum carmichaelis praep.	Zhi Fuzi	roots and tubers	02/2009
Zingiber officinale	Ganjiang	roots	02/2009
Cinnamomum cassia	Rougui	bark	02/2009
Glycyrrhiza uralensis	Gancao	roots	06/2009
Glycyrrhiza uralensis praep.	Jiu Gancao	roots	04/2006
Monascus purpureus	Hongqu	grain	07/2009
Gynostemma pentaphyllum	Jiaogulan	leaf	04/2009
Ginkgo biloba	Baiguo	seeds	12/2009
Ginkgo biloba	Yinxinye	leaf	07/2010
Salvia miltiorrhizae	Danshen	roots	10/2009
Salvia officinalis	----------	leaf dry; Austria	09/2009
Salvia officinalis	----------	leaf fresh; Austria	12/2010
Salvia officinalis	----------	leaf dry; Albania	02/2010
Salvia officinalis	----------	roots; Austria	01/2010

Table 2: Herbal Materials

3.2 High Performance Liquid Chromatography (HPLC)

The High Performance Liquid Chromatography is based on the Liquid Chromatography (LC) and it is the most powerful chromatographic method to analyse and separate substances (Bio-Rad, Richmond, CA, USALindsay, 1996). HPLC has many advantages for analyzing herbal medicines, because it is high sensitive, reproducible, it can analyse multiple constituents and it is automated (Zhang and Ye, 2009).

The dissolved sample gets injected and pumped into a column packed with a solid separating material.

The principle of the HPLC is the separation of a liquid sample because of the adsorption and distribution between the mobile and the stationary phase with an eluent from low until high pressure.

There are two different ways of separation, the normal-phase chromatography and the reversed-phase chromatography (RP).

The adsorption in the reversed-phase system is the opposite of the normal-phase system.

In our experiment the measurements were performed by RP system. That means, the stationary phase is unpolar (hydrophob) and the mobile phase is polar (hydrophil). The retention of unpolar analytes is strong and the retention of polar analytes low. Most separations are performed on RP C_{18} columns. Each analyte elutes from the column at a characteristic time (retention time) and the peaks can be used to identify and quantify the substances (Hann and Stingeder, 2004). Just to mention a few possibilities for detection, here are some examples. The eluats can be detected by UV/Vis, photodiode array detector (DAD) or the HPLC is combined with a mass spectrometer (LC-MS) or infrared spectroscopy (LC-IR).

3.2.1 Decoct Preparation

Decoction has a long tradition in the Traditional Chinese Medicine (TCM) and it is a method to extract plant material containing stems, roots, bark and rhizomes in boiling water. In the TCM it is used as medicine.

Protocol:

The herbs were soaked in 300 ml bi-distilled cold water for 90 minutes in a ceramic pot. The mixture was cooked (Cooking plate, Severin, Sundern, Germany) until boiling. Afterwards it

was gently cooked on the lowest level of the cooking plate for 30 minutes. The extract was filtrated and 300 ml bi-distilled cold water was added again and re-extracted by cooking 30 minutes again. Both extracts were combined, filtrated and collected and spun at 8.000 rounds per minute (rpm) (Sorvall RC5C Plus, GMI, Minnesota, USA) for 40 minutes at 4 °C.
Before measuring by HPLC, the sample was filtered with a 0.2 µm non-pyrogenic, sterile syringe filter (Filtropur, Sarstedt, Nümbrecht, Germany). Some samples were lyophilized with a lyophilizator (Christ Alpha 1-4 Rieger, Vienna, Austria) and the other samples were frozen at – 20 °C (Liebherr Comfort, Ochsenhausen, Germany) for later using.
Before lyophilization, the batches were frozen at – 80 °C (Polar 550 H, Angelanton, Massa Martana, Italy) or put into liquid nitrogen.

Lyophilization:

It is also known as freeze-drying and it is a process of dehydration. This technology is often used in the pharmaceutical-, biotechnology- and food industry, because of the gentle way of conservation. Another positive effect is the easement of transport. The sample is frozen and put into a vacuum. It is heat needed, that sublimation starts. The frozen material turns directly from the solid phase into the gas phase. After lyophilizing, the lyophilizate has to be stored dry, because the powder is hydrophil.

Figure 24: Lyophilizator

Running process:

To save the pump, the instrument has to be switched on 30 minutes before use. The bell shaped top is put on the instrument and press the button *KM1*. Now, the pressure is switched on by pushing the button *MV Druck*. The vacuum pump is switched on by pushing *KM1* and afterwards switch off the *MV Druck*.

After 30 minutes, the frozen samples, which have small holes in the cap, can be stored in the instrument. The holes in the cap are very important for the sublimation process. The evacuation of the instrument can start by pushing the button *MV Druck*. The freeze-drying process starts and the amount of time depends on the amount of sample. If there is no ice left in the sample, the process is over and the instrument can be switched off. At first, the valve at the back must be opened slowly to remove the vacuum. Afterwards the valve must be closed again. Now you are able to take of the bell shaped top and take the lyophilized samples out of it. The instrument produces ice during freeze-drying and with the use of the button *thawing* the freeze-dryer can be used again faster. After thawing, the instrument has to be emptied of water and dried with paper towels (Working Instruction of the Department of Pathophysiology and Allergy Research at the Medical University of Vienna, Wagner S.).

3.2.2 HPLC Parameter

Figure 25: HPLC LC-10 AD, Shimadzu; Department of Pathophysiology and Allergy Research; Medical University of Vienna

HPLC: LC-10 AD, Shimadzu, Korneuburg, Austria
Medical University of Vienna
Pre-column: Guard-Pak, Inserts Delta-Pak C18 300A, Waters Corporation Milford, MA, USA
Column: LiChrosorb® RP-18,5 µm, Hibar®Pre Packed Column RT 4 mm x 250 mm, Merck
Autosampler: SIL-10A, Shimadzu
Pump: LC-10 ADVP, Shimadzu
Detector: SPD-M10A-DAD, Shimadzu

Software: Class VP

Temperature: This HPLC has no control for the temperature, the measurement was performed at room temperature.

Figure 26: HPLC Agilent 1100 Series; Department of Systematic and Evolutionary Botany; Faculty Centre of Biodiversity of the University of Vienna

HPLC: Agilent 1100 Series, Waldbronn, Germany

Pre-column: Hypersil® BDS C18, 5 µm 4 x 4 mm; Agilent 1100 Series

Column: Hypersil BDS C-18, 250 x 4,6 mm, 5 µm

Autosampler: G 1367A

Pump: G 1312A BinPump

Detector: UV-DAD G 1315C

Degasser: G 1322A

Software: Chem Station for LC 3D Systems Rev.B.04.02 SP1 Agilent Technology® (2001-2010)

Column Temperature: 23 °C

The measurements of *Salvia officinalis* and Danshen were performed with this HPLC.

Protocol:

Before HPLC measurement, all eluents were degassed for 5 minutes in a supersonic (Bandelin Sonorex super RK 106, Berlin, Germany).

Acetic acid was mixed with milli-Q water with a magnetic stirrer (Thermo scientific, Waltham, MA, USA) and pH was adjusted to 3 with the pH meter (Metrohm, 827 pH lab, Herisau, Switzerland).

Standards:

Name	Concentration	Shortcut
8-Gingerol	1 mg/1 ml Methanol	1
Cinnamaldehyde	1 mg/1 ml Methanol	2
Glycyrrizic acid	1 mg/1 ml Milli-Q water	3a
Glycyrrizic acid	1 mg/1 ml Ethanol	3b
Lovastatin	1 mg/1 ml Methanol	4
Rutin	0.6 mg/0.6 ml Methanol	5
Ginkgolide A lyophilized for 12 hours	1.5 mg/0.5 ml Methanol	6a
Ginkgolide B lyophilized for 12 hours	2 mg/0.5 ml Methanol	6b
Salvianolic acid B	1 mg/1 ml Milli-Q water	7a
Salvianolic acid B	1 mg/1 ml Milli-Q water	7b
Tanshinone II A	1 mg/1 ml Ethanol	8a
Tanshinone II A	1 mg/1 ml Ethanol	8b

Table 3: Standards for HPLC quality control

3.2.3 Developing methods for detection of bioactive compounds in Traditional Chinese Herbs via fingerprint

3.2.3.1 Sinitang Decoct

Protocol:

Aconitum carmichaeli praeperata, tuber

Zingiber officinale, root

Cinnamomum cassia, bark

Glycyrrhiza uralensis, root

The herbs for the Sinitang decoction were prepared and pretreated at the Shandong University of TCM, China in cooperation with Prof. Ruirong Xu.

The herbal decoction was performed at the Medical University, Department of Pathophysiology and Allergy in Vienna.

All herbs are stored at 7 °C (Liebherr ProFi-Line, Ochsenhausen, Germany) and all decoctions were stored at – 20 °C (Liebherr Comfort, Ochsenhausen, Germany). For every measurement 4 ml of the frozen decoction are taken and defrosted. Before using, the samples were spun at 13.000 rounds per minute (rpm) for 10 minutes at 4 °C, to remove the sediments (Sigma Laborzentrifugen 3K10, Berlin, Germany).

To shield the column, the decoction got filtrated through a non-pyrogenic sterile 0.2 µm syringe filter (Filtropur, Sarstedt, Nümbrecht, Germany) and filled in a vial (1.5 ml, VWR, Illinois, USA) for the HPLC measurement.

Sinitang normal amount of herbs

2 ml Sinitang was centrifuged and filtrated through a non-pyrogenic sterile 0.2 µm syringe filter before measurement.

Method A:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 1ml/min

Column Temperature: 23 °C

Wave length: 240 nm

Running process:

Minute	% Eluent B
18	0
23	12
40	20.5
63	43.9
73	60

80	100
85	100
85.1	0

Table 4: Method A

(developed by my colleague Mag.ª Karoline Peter)

Sinitang double amount of herbs

Protocol:

A decoct with the double amount of herbs was prepared as usually.

Aconitum carmichaelis praeperata, tuber

Zingiber officinale, root

Cinnamomum cassia, bark

Glycyrrhiza uralensis, root

3 ml Sinitang decoction was centrifuged as usually and filtered through a 0.2 µm non-pyrogen and sterile syringe filter.

Method A was performed and used for all herbs, which are compounds of Sinitang, and for the standards too.

3.2.3.2 Zhi Fuzi Decoct

Protocol:

With this single herb, a decoction of the tubers of *Aconitum carmichaelis praeperata* was prepared. Afterwards 11 ml of decoct got frozen with liquid nitrogen and lyophilized for two days.

For the measurement, 1 mg lyophilized aconite decoct was mixed with 1 ml of milli-Q water and vortexed. Afterwards spun up at 4 °C for 10 minutes at 13.000 rpm and filtrated through a 0.2 µm non-pyrogenic syringe filter.

Method A was used for the measurement.

3.2.3.3 Ganjiang Decoct

Protocol:

A decoct was cooked as described before, but just with the dried root of the single herb *Zingiber officinalis*. Afterwards 45 ml of decoct got frozen with liquid nitrogen and lyophilized for two days. For the measurement, 20 mg of lyophilized ginger was mixed with 1 ml of milli-Q water and vortexed. Afterwards spun up at 4 °C for 10 minutes at 13.000 rpm and filtrated through a 0.2 µm non-pyrogenic filter.

Method A was used for the measurement.

1: 8-Gingerol

It is a main compound in fresh ginger. The phenol with an alkyl side chain and 2 hydroxy groups and one oxo group, looses during cooking or drying one OH-group and turns into shogaol. Gingerols are anti-inflammatory and anti-tumor agents (Ali et al., 2008).

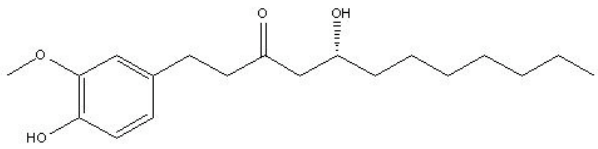


Figure 27: Chemical structure of 8-Gingerol

IUPAC: (R)-5-hydroxy-1-(4-hydroxy-3methoxyphenyl)dodecan-3-one

3.2.3.4 Rougui Decoct

Protocol:

A decoct of *Cinnamomum cassia* was prepared as described before, but just with the single herb Rougui. Afterwards 45 ml of decoct got frozen with liquid nitrogen and lyophilized for two days. For the measurement, 7.5 mg of lyophilized cinnamon was mixed with 1 ml of milli-Q water and vortexed. Afterwards spun up at 4 °C for 10 minutes at 13.000 rpm and filtrated through a 0.2 µm non-pyrogenic filter.

Method A was used for the measurement.

2: Cinnamaldehyde

The main compound of the species *Cinnamomum* is cinnamaldehyde. Its phenyl group is in conjugation with an α, β - unsaturated ketone.

Cinnamaldehyde is a spice and flavoring substance, which improves the quality of taste. It is often added to beverages, medicines or food. Studies have proven its bactericidal, sedative, hypotensive, peripheral vasodilation, and platelet anti-aggregation effects (Sung et al., 2011).

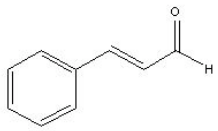


Figure 28: Chemical structure of Cinnamaldehyde

IUPAC: (2E)-3-phenylprop-2-enal

3.2.3.5 Jiu Gancao Decoct

Protocol:

A decoct was cooked as described before, but just with the single herb Jiu Gancao. Afterwards 47 ml of decoct got frozen with liquid nitrogen and lyophilized for two days.

For the measurement, 20 mg of lyophilized licorice was mixed with 2 ml of milli-Q water and vortexed. Afterwards spun up at 4 °C for 10 minutes at 13.000 rpm and filtrated through a 0.2 µm non-pyrogenic filter.

Method A was used for the measurement.

3: Glycyrrhizic acid

Glycyrrhizic acid is the main component of the licorice root and tastes sweet. The acid is metabolized to Glycyrrhetinic acid and inhibits enzymes, which are involved in the mechanism of corticosteroids. Glycyrrhizic acid is widely used as an antioxidant agent (PubChem a, 2011).

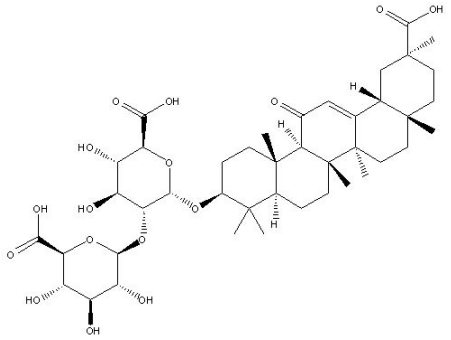


Figure 29: Chemical structure of Glycyrrhizic acid

IUPAC: 6-[6-carboxy-2-[(11-carboxy-4,4,6a,6b,8a,11,14b-heptamethyl-14-oxo-2,3,4a,5,6,7,8,9,10,12,12a,14a—dodecahydro-1H-picen-3-yl)oxy]-3,4,5trihydroxyoxane-2-carboxylic acid

3.2.3.6 Hongqu

Protocol

For the Red Yeast Rice extracts we used pills produced from Beijing University of Pharmacy in China.

3.2.3.6.1 Hongqu Milli-Q water Extract; 240 nm

Protocol:

1 tablet of Red Yeast Rice 0.31 g mixed with 1.5 ml of Mill-Q water was vortexed and extracted in a supersonic for 30 minutes.

Afterwards the extract was spun at 4 °C for 10 minutes at 13.000 rpm and filtrated through 0.2 µl filter.

Method B:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 0.8 ml/min

Column Temperature: 22 °C

Wave length: 240 nm

Running process:

Minute	**% Eluent B**
0.01	2
30	60
32	100
47	100
47.1	2

Table 5: Method B

3.2.3.6.2 Hongqu Alcohol Extract

Protocol:

Two tablets of Red Yeast Rice 0.65 g and 10 ml autoclaved milli-Q water got stirred to a homogenous compound for 15 minutes.

To separate the extract from the residues the mixture was centrifuged at 4 °C for 20 minutes at 14.000 rpm (Sigma Laborzentrifugen 3K10, Osterode am Harz, Germany) and filtrated through a 0.2 µl filter.

15 ml 100 % Alcohol + 5 ml Milli-Q water = 20 ml 75 % Alcohol

20 ml of 75 % alcohol got added to the pellet and extracted for 60 minutes in the supersonic (Bandelin, Sonorex super RK 106, Berlin Germany).

The sample was spun at 4 °C for 10 minutes at 14.000 rpm and filtrated through a 0.45 µl filter (Rotilabo®, PVDF, Carl Roth GmbH, Karlsruhe, Germany).

Method C:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 20 µl

Flow rate: 1ml/min

Column Temperature: 22 °C

Wave length: 240 nm

Running process:

Minute	**% Eluent B**
0.01	20
20	60
30	80
32	90
33	100
38	100
38.10	0

Table 6: Method C

This method was as well performed with the standard Lovastatin (4).

3.2.3.6.3 Hongqu Methanol Extract; 240 nm

Protocol:

1 tablet of Red Yeast Rice 0.31 g mixed with 1.5 ml methanol (99.9 %) was vortexed and extracted in a supersonic for 60 minutes.

Afterwards the extract was spun at 4 °C for 10 minutes at 13.000 rpm and filtrated through 0.2 µl syringe filter.

Method D:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 1ml/min

Column Temperature: 22 °C

Wave length: 240 nm

Running process:

Minute	**% Eluent B**
0.1	2
1	8
13	8
15	100
24	100
25	5

Table 7: Method D

This method was as well performed with the standard Lovastatin (4).

3.2.3.6.4 Lovastatin

It is also known as monacolin K and member of the drug class statins. Lovastatin is an isolated metabolite of the fungi *Monascus purpureus*. It is popular because of its anticholesteremic potential. The statin inhibits the Hydroxymethlyglutaryl Co A reductases, which is in the cholesterol biosynthesis rate-limiting. Monacolin K stimulates as well the receptors of low-density lipoprotein in the liver (PubChem b, 2011).

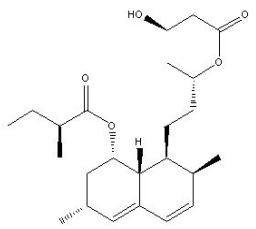


Figure 30: Chemical structure of Lovastatin

IUPAC: (S)-((1S,3R,7S,8S,8aR)-8-((S)-3-(3-hydroxypropanoyloxy)butyl)-3,7-dimethyl-1,2,3,7,8,8 hexahydronaphtalen-1-yl)2-methylbutanoate

3.2.3.7 Jiaogulan

3.2.3.7.1 Jiaogulan Decoct, 240 nm

Protocol:

25 g of the leaf of *Gynostemma pentaphyllum* was decocted and afterwards centrifuged and filtered as described before.

The proportion of the sample is one part Jiaogulan (0.214 ml) and 6 parts milli-Q water (1.286 ml).

Method E:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 1ml/min

Column Temperature: 22 °C

Wave length: 240 nm

Running process:

Minute	**% Eluent B**
0.01	33
40	33
45	100
50	100
50.10	33
51	0

Table 8: Method E

This method was as well performed with the standard Rutin (5).

3.2.3.7.2 Jiaogulan Methanol Extract

Protocol:

460 ml of Jiaogulan decoct was lyophilized. 1 mg of this sample got mixed with 1 ml methanol. Next step is to centrifuge it for 10 minutes at 4 °C at 13.000 rpm.

Method F:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 1ml/min

Column Temperature: 22 °C

Wave length: 240 nm

Running process:

Minute	**% Eluent B**
0.10	80
15	100
25	100
25.10	0

Table 9: Method F

This Method was as well performed with Rutin (5).

3.2.3.7.3 Rutin

The flavonol glycoside is a compound in many plants and it has been used therapeutically to decrease capillary fragility. Rutin has anticancer, antioxidative and antiproliferative effects (Lin et al., 2011; PubChem c, 2011; Xie et al., 2010)

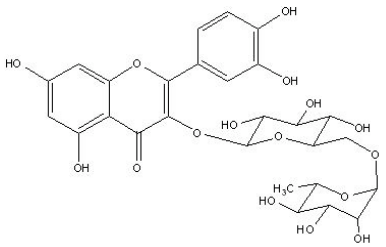


Figure 31: Chemical structure of Rutin

IUPAC: 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yloxy)methyl)tetrahydro-2H-pyran-2-yloxy)-4H-chromen-4-one

3.2.3.8 Baiguo

3.2.3.8.1 Baiguo Milli-Q water Extract, 280 nm

Protocol:

With 20 pieces of fresh *Ginkgo biloba* seeds (26.87 g including shell) an extraction was performed. The shell of the *Ginkgo* seeds were broken and cooked with a microwave (Panasonic 600 Watt, Germany) for 3 minutes.

When the seeds are cooled down, they got peeled and cut into small pieces with a knife and 50 ml of autoclaved Aqua bi-distilled water got added and grinded with a mortar.

To get a homogenous mixture the extract was added in a plastic beaker glass, 50 ml of Aqua bi-distilled water was added and pureed with a mixer (Ultra Turrax T25, VWR, USA). At the beginning a speed of 8.000 rpm was chosen, then increased to 9.500 rpm and at the end to 13.500 rpm. It is necessary to use plastic because ordinary glass would break.

The homogenous extract was cooled on ice and 2 µl protease inhibitor added.

Protease Inhibitor: 0.1 % x 200 = 20 ml

20ml/25 x Protease Inhibitor = 0.8 ml = 80 µl ~ 100 µl

1 pill was diluted in 20 ml Aqua bi-distilled water and 100 µl got taken and added to the *Ginkgo biloba* seed extract.

The alloy was stirred with the magnetic stirrer for 30 minutes and adjacent spun at 18.000 rpm for 30 minutes at 4 °C. The huge pellet of the *Ginkgo biloba* seed extract at the bottom got frozen at – 20 °C. Subsequently the extract got centrifuged a second time at 19.000 rpm

for 45 minutes at 4 °C and a third time at 18.000 rpm for 30 minutes at 4 °C. The soft pellet of the extract got frozen at – 20 °C.
The extract was filled in tubes and spun down at 14.000 rpm for 20 minutes at 4 °C and the supernatant centrifuged again at 15.000 rpm for 30 minutes at 4 °C. The supernatant got taken for the third time and spun down at 15.000 rpm for 20 minutes at 4 °C.

Stability Test of Baiguo Milli-Q water Extract; 280 nm

The *Ginkgo biloba* seed extract got defrosted, spun down at 13.000 rpm for 10 minutes at 4 °C and filtrated through a 0,2 µl non pyrogenic syringe filter.

Method G:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3
Eluent B: 99.9 % Methanol
Injection volume: 10 µl
Flow rate: 1ml/min
Column Temperature: 22 °C
Wave length: 280 nm
Time: 2; 4; 6; 8; 10 hours
Before each run, the column was cleaned with 99.9 % Methanol p.a., purged and equilibrated.
To test the stability of *Ginkgo biloba* seeds extract, the same sample was measured five times in an interval of two hours at the same conditions.

Running process:

Minute	**% Eluent B**
0.10	0
40	100
45	100
45.10	0

Table 10: Method G

3.2.3.8.2 Baiguo Methanol Extract; 223 nm

Protocol:

The frozen pellet (54.4 mg) of the *Ginkgo* seeds got defrosted and 2 ml of methanol got added and extracted in the supersonic for 60 minutes. Afterwards centrifuged for 10 minutes at 4 °C and 13.000 rounds per minute and filtrated through a 0.2 µl non-pyrogenic syringe filter.

Method H:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 1ml/min

Column Temperature: 22 °C

Wave length: 223 nm

Running process:

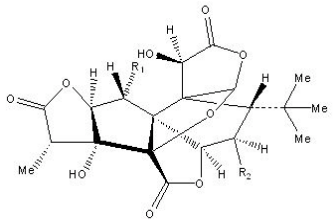
Minute	% Eluent B
0.10	80
15	100
25	100
25.10	0

Table 11: Method H

3.2.3.8.3 Ginkgolide

Ginkgolides are highly complex C_{20} terpenes containing 6 five-membered rings, which is, a spiro[4,4] nonane carbocylic ring, three lactones and a tetrahydrofuran. *Ginkgo biloba* L. contains many ginkgolides such as ginkgolide A, B, C, K, L and M. All have shown a specific and selective antagonism of platelet activating factor (PAF). Ginkgolide B is the most potent compound with many beneficial actions, such as anti-inflammatory, anti-allergic, antioxidant and neuroprotective effects (Esposito and Carotenuto, 2010; van Beek, 2005).

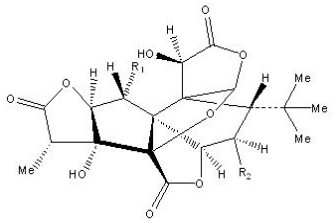
6a: Ginkgolide A



Ginkgolide A: R_1=R_2=H, R_3=OH

Figure 32: Chemical structure of Ginkgolide A

6b: Ginkgolide B



Ginkgolide B: R_1=R_3=OH, R_2=H

Figure 33: Chemical structure of Ginkgolide B

3.2.3.9 Yinxinye

3.2.3.9.1 Yinxinye Milli-Q Extract, 223 nm

Protocol:

A piece of *Ginkgo biloba* leaf (0.90 g) was extracted with 5 ml of milli-Q water including 200 µl of protease inhibitor and pestled.

Protease inhibitor: 1 tablet in 2 ml milli-Q water

2 ml……...50 ml

x……5 ml

x = 200 µl

Afterwards the extract was centrifuged at 13.000 rpm for 10 minutes at 4 °C and filtered through a 0.2 µl non pyrogenic syringe filter.

Method I:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 1ml/min

Column Temperature: 22 °C

Wave length: 223 nm

Running process:

Minute	% Eluent B
0.10	0
40	100
45	100
45.10	0

Table 12: Method I

This method was as well performed with the standards Ginkgolide A (6a) and B (6b).

3.2.3.9.2 Yinxinye Methanol Extract, 215 nm

Protocol:

A piece of *Ginkgo biloba* leaf (0.78 g) got ruptured and 5ml milli-Q water and 200 µl protease inhibitor added and pestled.

Protease inhibitor: 1 tablet in 2 ml milli-Q water

2 ml……50 ml

x…….5 ml

x= 200 µl

The extract was centrifuged at 13.000 rpm for 10 minutes at 4 °C and 13.000 and filtered through a 0.2 µl non-pyrogenic syringe filter.

Method J:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 1ml/min

Column Temperature: 22 °C

Wave length: 215 nm

Running process:

Minute	% Eluent B
0.10	10
1	100
17	100
17.10	10

Table 13: Method J

This method was as well performed with the standard 6a and 6b.

3.2.3.10 Danshen

3.2.3.10.1 Danshen Decoct

Protocol:

25 g *Salvia miltiorrhizae* are decocted and prepared as before. For measuring, the frozen decoct was defrosted, afterwards it was centrifuged and filtrated. 1.5 ml of Danshen extract was diluted into a proportion of 1 part herb extract (0.214 ml) to 6 part milli-Q water (1.286 ml).

Stability Test of Danshen Decoct; 300 nm

To test the stability of this decoction, Danshen was measured with the same method and the same conditions for five times in an interval of two hours.

Method K:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 1ml/min

Column Temperature: 22 °C

Wave length: 240 nm

Time: 2; 4; 6; 8; 10 hours

Before each run, the column is cleaned with 99.9 % methanol p.a., purged and equilibrated.

Running process:

Minute	% Eluent B
0.10	0
40	100
44.50	100
44.70	50
45	0

Table 14: Method K

3.2.3.10.2 Danshen Methanol Extract; 300 nm

Protocol:

1.5 mg Danshen was lyophilized and mixed with 1.5 ml 99.9 % methanol. Method K was performed.

3.2.3.10.3 Salvianolic acid B

Salvianolic acid B is a derivate of the caffeic acid. It is a polyphenol with two ester, two carboxylic acid groups and a dihydrobenzofuran core. This phenol acid is an active hydrophil major compound of the root of *Salvia miltiorrhizae* with biological functions such as anti-platelet activity (Liu et al., 2007a).

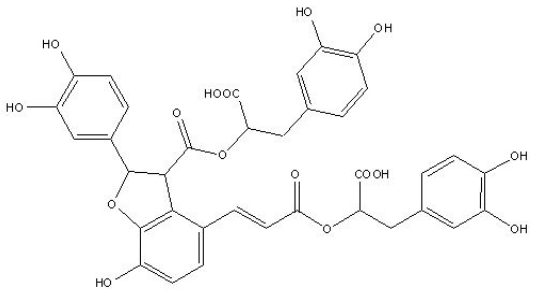


Figure 34: Chemical structure Salvianolic acid B

IUPAC: (E)-2-(4-(3-(1-carboxy-2-(3,4-dihydroxyphenyl)ethoxy)-3-oxoprop-1-enyl)-2(3,4dihydroxyphenyl)-7-hydroxy-2,3-dihydrobenzofuran-3-carbonyloxy)-3-(3,4-dihydroxyphenyl)propanoic acid

3.2.3.10.4 Tanshinone II A

This lipophilic diterpenoid is an active major compound and a selected marker for quality control of Danshen. Tanshinones can dilate coronary arteries, increase coronary flow, modulate mutagenic activity and protect the myocardium against ischaemia. The ability as a broad-spectrum bactericide is reported as well (Shi et al., 2005).

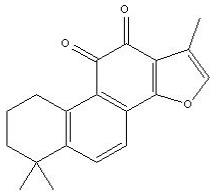


Figure 35: Chemical structure of Tanshinone II A

IUPAC: 1,6,6-trimethyl-8,9-dihydro-7H-naphtho[1,2-g][1]benzofuran-10,11-dione

3.2.3.11 Comparison of Salvia officinalis with Salvia miltiorrhizae (Danshen)

The following samples were detected with the HPLC Agilent 1100 Series at the Department of Systematic and Evolutionary Botany; Faculty Centre of Biodiversity of the University of Vienna.

Method K:

Eluent A: Milli-Q water and 100 % Acetic acid, pH = 3

Eluent B: 99.9 % Methanol

Injection volume: 10 µl

Flow rate: 0.8 ml/min

Column Temperature: 22 °C

Wave length: 300 nm

Before each run, the column is cleaned with 99.9 % methanol p.a., purged and equilibrated.

Running process:

Minute	**% Eluent B**
0.10	0
40	100
44.50	100
44.70	50
45	0

Table 15: Method K

3.2.3.11.1 Salvia officinalis

Origin: Austria, supermarket

Part: leaf and stipe, fresh

Protocol:

35 g fresh *Salvia officinalis* was cooked in 300 ml bi-distilled water and the decoction was prepared as described before. The decoction was lyophilized and 1 mg of the sample was mixed with 1 ml milli-Q water.

3.2.3.11.2 Salvia officinalis

Origin: lower Austria, Fam. Zach, organic

Part: leaf, dried

Protocol:
25 g of the dried herb was soaked in bi-distilled water and decocted as usually and afterwards it was lyophilized.
For measuring we used 1 mg of lyophilized sample eluated with 1 mg of milli-Q water.

3.2.3.11.3 Salvia officinalis

Origin: Albania, bought in a pharmacy in Vienna, Austria
Part: leaf, dried

Protocol:
25 g of the dried *S. officinalis* was decocted as the dried S. *officinalis* from Austria and afterwards lyophilized.
The concentration for the HPLC measurement was 1 mg lyophilized sample diluted in 1 ml of milli-Q water.

3.2.3.11.4 Salvia officinalis

Origin: 22nd district of Vienna, Austria, dried
Part: rhizome

Protocol:
1.14 g *Salvia officinalis* rhizome was soaked in 13.68 ml bi-distilled water in a 20 ml beaker glass. Because of this small amount, the beaker glass was put in a water bath and cooked like the other decoctions. After 30 minutes, the extract was filtered and again 13.68 ml bi-distilled water added and cooked for 30 minutes. The extract was filtered and centrifuged at 4.000 rpm for 40 minutes at 4 °C (Thermo Megafuge 1.0.R, Heraeus, Thermo Fischer Scientific, Waltham, MA, USA).

3.2.3.11.5 Salvia miltiorrhizae (Danshen)

Origin: China
Part: rhizome

Protocol:
1 mg of the lyophilized sample was solute in 1 ml milli-Q water and used for the HPLC detection.

This method was as well performed with the standards Salvianolic acid B (7b) and Tanshinone IIA (8b).

3.3 Protein analysis

3.3.1 Total protein concentration via Bicinchoninic Acid Protein Assay (BCA)

The BCA Protein Assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantization of total protein (Pierce, 2007).

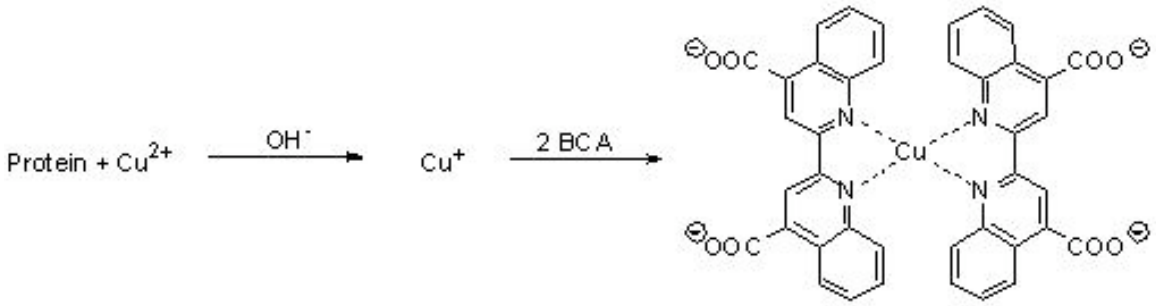


Figure 36: Biuret reaction

We used the Pierce® BCA Protein Assay Kit and the method is based on the Biuret reaction. Protein reduces Cu^{2+} to Cu^{+} in an alkaline medium and two molecules of bicinchoninic acid bind on one molecule cuprous ion and the effect is a purple-colored reaction. This complex is best absorbed at 562 nm and nearly linear with the protein concentration.
Responsible for the color formation with BCA are the macromolecular structure of protein, the number of peptide bonds and the presence of the amino acids cysteine, cystine,

tryptophan and tyrosine. Protein concentrations are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilutions of known concentrations are prepared from the protein and assayed alongside the unknowns before the concentration of each unknown is determined based on the standard curve (Pierce, 2007).

The following series of standards was prepared by using the same buffer as the sample:

Standard	Buffer	BSA [2mg/ml]	Final concentration
A	70 µl	10 µl stock	250 µl/ml
B	72 µl	8 µl standard A	200 µl/ml
C	148 µl	12 µl standard B	150 µl/ml
D	152 µl	8 µl standard C	100 µl/ml
E	156 µl	4 µl standard D	50 µl/ml
F	160 µl	- - - - - - - - - - - - - -	0 µl/ml = blank

Table 16: Preparation of BSA standard

Protocol:

The total volume of the BCA working reagent (WR) was determined using the following formula:

Total volume WR required =(# standards + # unknowns) x (# replicates = 2) x (volume of WR per sample = 200 µl)

The dilution of WR was mixed 50 parts reagent A and 1 part reagent B.

The 25 µl of the standards and of the unknown sample in duplicates were pipeted into a polystyren 96 microplate wells (Greiner bio-one, Frickenhausen, Germany).

200 µl WR was added per sample and the covered plate (Foil EASYseal™, Greiner, Frickenhausen, Germany) was incubated for 30 min at 37 °C. After cooling down to room temperature, the absorbances of the samples were measured at 562 nm on a plate reader (SPECTRAmax, Molecular Devices GmbH, Munich, Germany) and the evaluation was done with the program SoftMax Pro 4.8 (Molecular Devises, California, USA).

Equipment BCA

	Chinese	Formula	Volume
a	Sinitang	traditional formula	400 ml
b	Jiu Gancao	25 g	500 ml
c	Gancao	25 g	500 ml
d	Zhi Fuzi	18 g	400 ml
e	Fuzi	18 g	500 ml
f	Ganjiang	10 g	460 ml
g	Gancao & Ganjiang	25 g & 10 g	400 ml
h	Gancao & Zhi Fuzi	25 g & 18 g	450 ml
i	Jiu Gancao & Fuzi	25 g & 18 g	500 ml
j	Gancao & Zhi Fuzi & Ganjiang	25 g & 18 g & 10 g	420 ml
k	Red Yeast Rice	0.6 g	20 ml
l	*Ginkgo biloba* seeds	26.87 g	115.5 ml
m	Jiaogulan	25 g	460 ml

Table 17: Decoct samples

BCA™ Protein Assay Kit, Thermo Scientific, Rockford, USA

Polystyren 96 microplate wells, Greiner bio-one, Frickenhausen, Germany

Foil EASYseal™, Greiner, Frickenhausen, Germany

Microplate Spectrophotometer, Spectra max Plus 384 Molecular Devices, Orleans Drive Sunnyvale, USA

Software: SoftMax Pro 4.8, San Diego, California, USA

3.3.2 SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecylsulfatepolyacrylamide gel electrophoresis (SDS-PAGE) is a method to separate proteins according to their molecular mass. The SDS contains polyacrylamide gel and the polymerization of acrylamide and bisacrylamide is fastened because of N,N,N´,N´-tetramethylethylenediamine (TEMED) which is catalyzed from ammonium persulfate (APS). SDS denatures secondary and non-disulfide-linked tertiary structures and forms negatively

charged proteins. The amount of SDS (1.4 g SDS per 1 g protein) is in proportion to the mass of protein and is independent of its sequence. Bromphenol blue allows to recognize the protein bands in the gel during the electrophoresis. To evaluate the molecular weight of the proteins, it is necessary to use markers of known molecular weight. For the SDS-PAGE the tris-glycine electrode buffer system was used, according to Laemmli (Laemmli, 1970). To detect the proteins we used a protein marker (Page Ruler™ Plus Prestained Protein Ladder, Fermentas, Germany).

Solutions

Reagent C:

29.2 % Acrylamide

0.8 % Bis-Acrylamide

Dissolved in distilled water and filtered

Lower buffer:

1.5 M Tris-HCl, pH 8.8

0.4 % SDS

Dissolved in distilled water

Upper buffer:

0.5 M Tris-HCl, pH 6.8

0.4 % SDS

Dissolved in distilled water

Resolving gel 15 % (for 1 gel):

Reagent C	1.5 ml
Lower buffer	1.25 ml
Aqua dest.	1.25 ml
Temed	2.5 µl
10 % APS	25 µl

Stacking gel 4.5 % (for 1 gel):

Reagent C	300 µl
Lower buffer	500 µl
Aqua dest	1.2 ml
Temed	1 µl
10 % APS	20 µl

4 x Sample Buffer:

200 mM Tris-HCl, pH 6.8

300 mM DTT

4 % SDS

40 % Glycerine

0.04 % Bromphenol blue

Dissolved in distilled water

Electrophoresis buffer (Laemmli buffer):

25 mM Tris pure

192 mM Glycine

0.1 % SDS

Dissolved in distilled water

CCB-stain:

0.125 % CBB R-250

50 % Methanol

10 % of 100 % HAc

Dissolved in distilled water and filtered

Destainer:

20 % Methanol

15 % of 100 % HAc

Dissolved in distilled water

Guidelines for choosing the percent gel to be used for certain molecular weight proteins, based on 37:1 acrylamide : bis acrylamide ratio.

4 - 5 % gels:	> 250 kDa
7.5 % gels:	250 - 120 kDa
10 % gels:	120 - 10 kDa
13 % gels:	40 - 15 kDa
15 % gels:	< 20 kDa

Protocol:

The gels were prepared corresponding to the handling instructions (Bio-Rad, Richmond, CA, USA).

The resolving gel was prepared and poured into the gap between the glass plates. The gel was carefully overlaid with isopropanol, because it produces a smooth, completely level surface on top of the separating gel, so that bands are straight and uniform. After polymerization (about 45 minutes), the isopropanol was poured off and the stacking gel was poured onto the resolving gel. To get gel pockets, a clean spacer (Bio-Rad, 0.75 mm) was inserted into the stacking gel solution. The polymerization of the stacking gel also takes about 45 minutes.

The samples were mixed with 4 x sample buffer (SB), centrifuged at 13.000 rpm for 10 minutes at 4 °C, heated at 95 °C for 5 minutes, and centrifuged at 13.000 rpm for 10 minutes again.

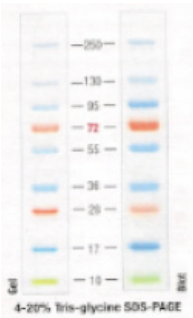


Figure 37: Marker PageRuler™ Plus Prestained Protein Ladder (Fermentas)

The spacer in the polymerized stacking gel was removed and the protein ladder and samples were loaded into the gel pockets and the electrophoresis was started (running conditions: 120 Volt, 75 minutes, 1 x Electrophoresis buffer).

To visualize the separated proteins, a Coomassie staining with Coomassie brilliant blue R-250 (CBB) was performed.
The gels were stained in a water bath for 30 minutes at 40 °C. After staining, the Coomassie was poured away and the gel rinsed with aqua distilled. The SDS-PAGE was destained in the destainer solution until the protein bands were detectable on a clear background.

3.3.3 Protein sequence analysis

3.3.3.1 Electroblotting

Blotting is the transfer of proteins from the SDS-PAGE gel to a nitrocellulose membrane. If an electric field is applied, the molecules can be transferred to the membrane. The direction of the protein transfer depends on the pH of the transfer buffer. In the Laemmli buffer system the proteins are negatively charged and they migrate from the cathode to the anode (Laemmli, 1970).

Solutions

Transfer buffer
10 mM CAPS, pH 11
10 % Methanol

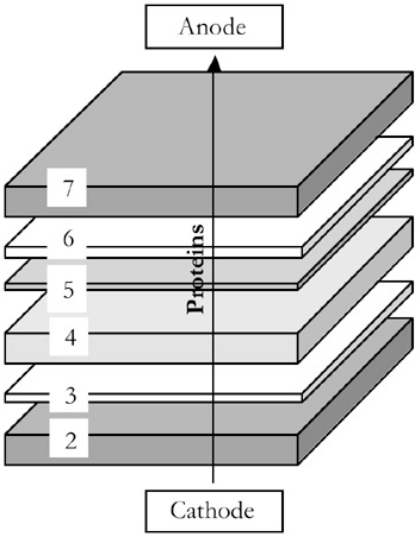
Staining solution
0.1 % CBB R-250
40 % Methanol
1 % Acetic acid
Dissolved in milli-Q water

Destaining solution
50 % Methanol
1 % Acetic acid
Dissolved in milli-Q water

Protocol:

The electrophoresed SDS-PAGE gel and Whatman paper (Chromatography paper, Whatman, Sanford, ME, US) were equilibrated in transfer buffer for 5 minutes. PVDF (poly vinylidenedifluoride) membrane (Westran®S, Whatman, Sanford, ME, US) was wet for a few seconds in methanol and as well equilibrated in transfer buffer for 5 minutes. This sandwich was put between 2 in transfer buffer wetted blotting sponges and build like in figure 38. It is important to remove all air bubbles between the gel and the membrane. The blotting cassette was placed into a Transpor Electrophoresis Unit (Hoefer Scientific Instruments, San Francisco, USA), which was filled with transfer buffer to cover the cassette. The PVDF membrane was facing the cathode (Towbin et al., 1979). The transfer of the proteins was carried out at 25 mA for 60 minutes at 4 °C.

The blotting cassette:



1.Cathode [-]
2.Blotting sponge
3. Whatman paper
4. SDS-PAGE gel
5. Pre wet PVDF membrane
6. Whatman paper
7. Blotting sponge
8. Anode [+]

Figure 38: Filling of the blotting cassette

(Bruckmueller, 2008)

3.3.3.2. N-terminal sequencing

Pehr Edman developed the Edman degradation in 1950. This is a technique for analyzing the primary structure of proteins and peptides by stepwise removal of the N-terminal amino acids. Originally it was a manual method, but in the late 1960s it was automated. The first commercialized sequencer was the Applied Biosystems model 470A.

The Edman degradation is based on three reaction steps:

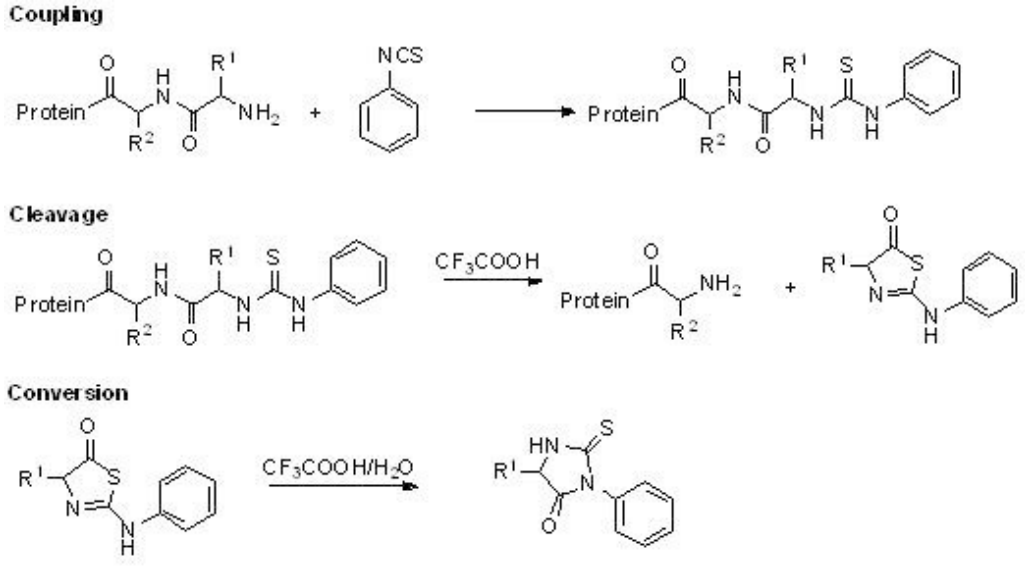


Figure 39: Edman Chemistry

Coupling: Phenyl isothiocyanate (PITC, Edman reagent) reacts with an α-amino group at the N-terminal end of the polypeptide chain. A phenylthiocarbamyl derivative is formed. If the amino group has become modified and does not react with PITC, the polypeptide is blocked. Acetyl, formyl, pyroglutamyl groups or even the handling of the sample in the laboratory can block many proteins.

Cleavage: In the presence of strong acid, cleavage is set in at the first peptide bond and the N-terminal amino acid is split off as anilinothiazolinone (ATZ) derivative and a shortened polypeptide with trifluoroacetic acid (TFA). The shortened polypeptide can be taken through another circle of coupling and cleavage to release the second residue. During cleavage a new N-terminus is generated and can be sequenced. In each cycle of the degradation sequentially only removes one amino acid from the amino terminal end.

Conversion: The unstable ATZ derivative is transferred into a more stable phenylthiohydantoin (PTH) derivative, which is separated by thin-layer chromatography or reversed phase HPLC. The PTH amino acid residue is identified and quantified by comparison with standards. The sequence is described from the N- to the C-terminus (Smith, 2011).

Solutions

Transfer buffer:

10 mM CAPS, pH 11

10 % Methanol

Staining solution:

0.1 % CBB R-250

40 % Methanol

1 % Acetic acid

Dissolved in milli-Q water

Destaining solution

50 % Methanol

1 % Acetic acid

Dissolved in milli-Q water

Protocol:

Sample preparation and electrophoresis was carried out as described in section 3.3.2. Before electroblotting, the PVDF membrane (Westran S, Whatman, Stanford, ME, US) was floated in 100 % methanol until it was completely saturated. The gel and membrane were equilibrated in the transfer buffer for 5 minutes. The blotting was carried out as described in 3.3.3.1.

After electroblotting the membrane was rinsed with milli-Q water 3 times for 5 minutes. For staining CBB solution is poured into unused plastic dishes and the membrane stained for only 30 seconds. For destaining, the destainer is changed as often as possible Afterwards the membrane is once rinsed briefly and 3 times for 5 minutes in milli-Q water. The blot dries between 2 sheets of Whatman paper. Afterwards the bands of interest are carefully cut with a scalpel on a clean glass plate and stored in a plastic micro centrifuge tube (Walsh et al., 1988).

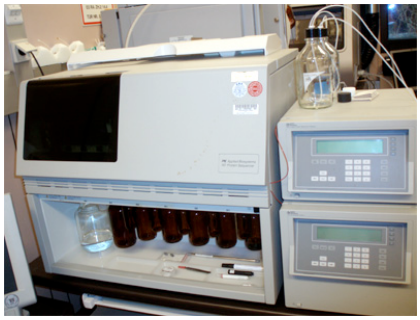


Figure 40: Gas-Phase Sequentor

The protein was sequenced with an automated gas-phase sequentor (Applied Biosystem 610A Procise 491 sequencer, Applied Biosystem, Perkin Elmer, Foster City, CA, USA) according to the manufacturer instructions. The N-terminal sequencing was performed at the Department of Pathopysiology and Allergy Research at the Medical University of Vienna.

4. Results and Discussion

4.1 High Performance Liquid Chromatography (HPLC)

4.1.1. Sinitang Decoct

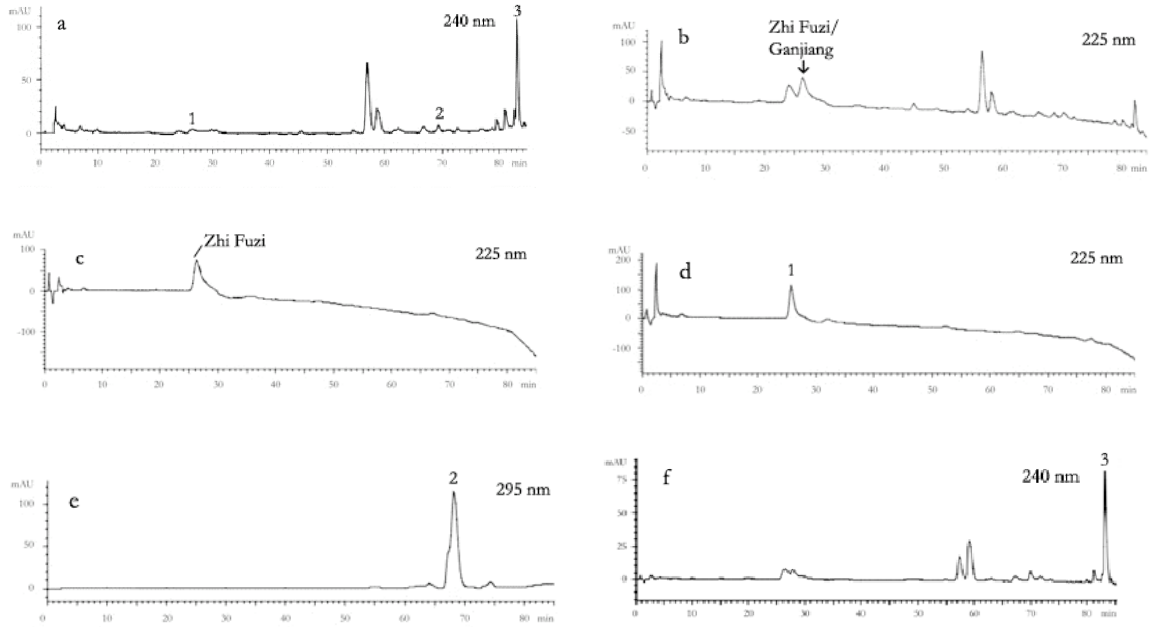


Figure 41: HPLC profiles from various batches of Sinitang and single herbs of Sinitang decoctions detected at 240 nm, 225 nm and 295 nm; (a) Sinitang at 240 nm (b) Sinitang at 225 nm (c) Zhi Fuzi (d) Ganjiang (e) Rougui (f) Jiu Gancao [8]-Gingerol (**1**) Cinnamaldehyde (**2**) Glycyrrhizic acid (**3**)

The chromatogram of Sinitang (a) detected at UV 240 nm was characterized by the main compound glycyrrhizic acid (3) and by the peaks of [8]-gingerol (1) and cinnamaldehyde (2). When Sinitang decoction detected at UV 225 nm (b), [8]-gingerol (1) was predominant. The Sinitang decoction of normal amount of herbs compared with Sinitang decoction double amount of herbs showed no differences. Decoctions of single herbs of Sinitang were prepared and detected too. Zhi Fuzi (c) was detected at a retention time (RT) of 27 minutes at a wavelength of 225 nm and is one of the important herb of the Sinitang decoction (b) as well. Ganjiang decoction (d) contained the bioactive compound [8]-gingerol (1) at a retention

time of 27 minutes. The Sinitang formula contained the same bioactive compound too. Rougui and Sinitang decoction contained cinnamaldehyde (2). The main ingredient of Sinitang belonged to Jiu Gancao. The peaks of Jiu Gancao (f) at a retention time of 57, 58, 81 and 83.5 minutes are the same like in chromatogram (a) of the Sinitang decoction at a wavelength of 240 nm. Glycyrrhizic acid, a common compound of licorice, was detected in both chromatograms at a retention time of 83.5 minutes.

4.1.2 Anti Aging Herbs

4.1.2.1 Hongqu

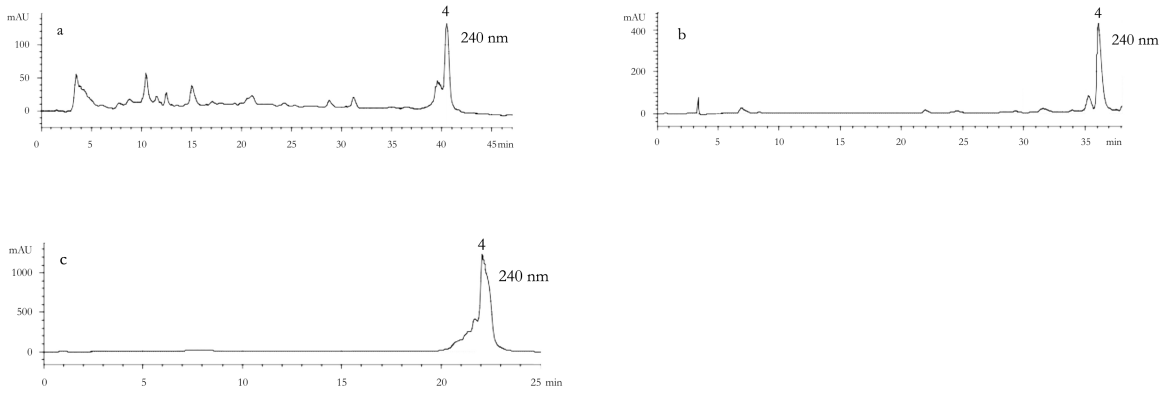


Figure 42: HPLC profiles of various Hongqu extracts detected at 240 nm; (a) Hongqu milli-Q water extract (b) Hongqu alcohol extract (c) Hongqu methanol extract; Lovastatin (**4**)

The standard Lovastatin (4) was detected in all three extracts. In the milli-Q water extract monacolin K (4) was detected at a retention time of 40.5 minutes, in the alcohol extract at 36 minutes and in the methanol extract at 22 minutes.

4.1.2.2 Jiaogulan

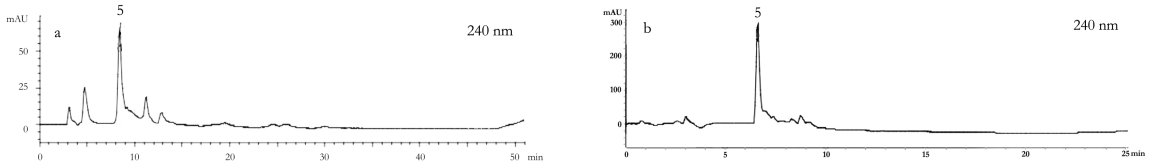


Figure 43: HPLC profiles from various Jiaogulan extracts detected at 240 nm; (a) Jiaogulan decoct (b) Jiaogulan lyo in methanol extract; Rutin (**5**)

The flavonoid rutin was detected in the Jiaogulan decoction (a) and in the methanol extract (b). Rutin was detected in chromatogram (a) at a retention time of 8 minutes and in the organic extract (b) at 6.8 minutes.

4.1.2.3 Baiguo and Yinxinye

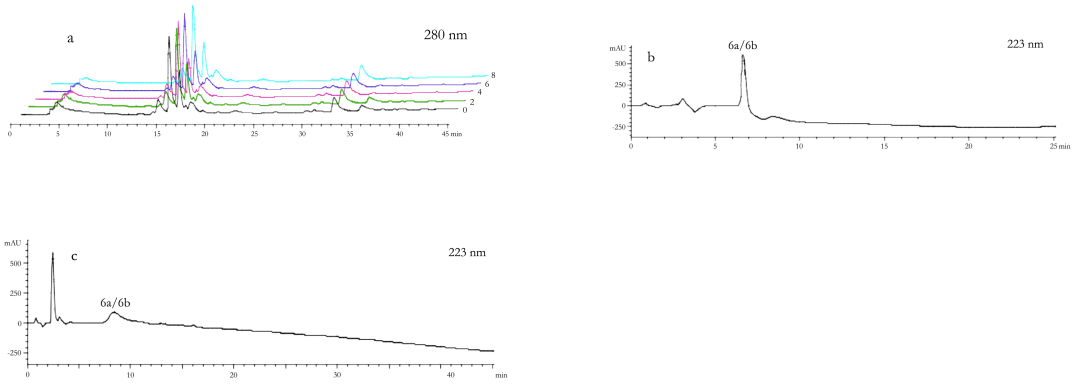


Figure 44: HPLC profiles from various Baiguo and Yinxinye extracts detected at 280 nm and 223 nm (a) reproducibility and stability of a Baiguo sample detected after 0, 2, 4, 6 and 8 hours (b) Baiguo methanol extract (c) Yinxinye milli-Q water extract; Ginkgolide A (**6a**) Ginkgolide B (**6b**)

To determine the reproducibility and stability, the Baiguo milli-Q water extract (a) was analyzed for five times consecutively. Analyzing the same sample solution injected at 0, 2, 4, 6 and 8 hours, tested the stability of the analytes. The Baiguo sample was reproducible and stable for five measurements in a row. The two bioactive compounds ginkgolide A (6a) and B

(6b) were detected in the Baiguo methanol extract (b) at a retention time of 6.8 minutes. Yinxinye milli-Q water extract and methanol extract contained ginkgolide A and B as well. In chromatogram (c) the standards were detected at a RT of 8.5 minutes.

4.1.2.4 Danshen

Figure 45: HPLC profiles of various extracts of Danshen detected at 300 nm; (a) reproducibility and stability of Danshen decoction (b) methanol extract of Danshen; Salvianolic acid B (**7a**)

To determine the reproducibility and stability, the Danshen decoction (Figure 45 (a)) was analyzed by successively injection into HPLC system for five times. Analyzing the same sample solution injected at 0, 2, 4, 6 and 8 hours, tested the stability of the analytes. Salvianolic acid B (7a), a hydrophilic bioactive substance, was detected at a RT of 25 minutes (Figure 45 (b)).

Conclusion:

The Sinitang formula contains the active component of Zhi Fuzi as well as the bioactive substances [8]-gingerol, cinnamaldehyde and glycyrrhizic acid. In a study of Guangguo Tan in 2010, [8]-gingerol was detected in a Sinitang formula as well. 59 bioactive compounds of the herbs *Aconitum carmichaelis*, *Zingiber officinalis* and *Glycyrrhiza uralensis* were detected with HPLC/DAD/TOFMS. The group of Guangguo Tan detected in *Aconitum carmichaelis* compounds like mesaconine, benzoylhypaconine, benzoylmesaconine and hypaconitine.

In the sample of *Zingiber officinalis*, four bioactive substances were detected like [6]-gingerol, [8]-gingerol, [6]-shogaol and [10]-gingerol. In *Glycyrrhiza uralensis*, 25 substances were identified, like liquiritin, isoliquiritin, and glycyrrhizin (Tan et al., 2010). A study of Liu et al. in 2005 detected flavonoids in Sinitang by HPLC/ESI-MS, like isoglycyrol, formononetin,

neoglycyrol, isoliquiritigenin, liquiritigenin, isoliquiritin, liquiritin, kumatatkenin B and more (Liu et al., 2005). A review of Qingying Zhang and Min Ye in 2009 described the identification and determination of glycyrrhizic acid in Gancao by HPLC/ESI-MS and HPLC/DAD. Pharmacological studies of licorice are mainly interested in glycyrrhizic acid and its aglycone glycyrrhetic acid, because those constitutes have extensive biological activities. It is believed that saponins and flavonoids are the main bioactive compounds of Gancao (Zhang and Ye, 2009). Wang et al. determined in 2006 alkaloids in four species of aconitum by HPLC. All of them contained the alkaloids benzoylmesaconine, mesaconitine, aconitine, hypaconitine and deoxyaconitine, but the quality of the drugs varied significantly (Wang et al., 2006b). My colleague Mag.[a] Karoline Peter could identify three alkaloids of the raw Fuzi, like aconitine, mesaconitine and hypaconitine (Peter, unpub. 2011).

The sample of Ganjiang (dry ginger) had a high amount of [8]-gingerol. This constitute is a main compound of Shengjiang (fresh ginger), whereas shogaols are predominant pungent constitutes from dry ginger. Sang et al. detected by HPLC/ECD (electrochemical detection) the profile of an extract of *Zingiber officinalis*, which contained [6]-gingerol, [8]-gingerol, [6]-shogaol, [6]-paradol, [1]-dehydrogingerdione, [10]-gingerol, [8]-shogaol and [10]-shogaol (Sang et al., 2009a).

As shown in chromatogram (e) in Figure 41, cinnamaldehyde is a major compound of *Cinnamomum cassia* (Kim et al., 2010).

Monacolin K is a main compound of Red Yeast Rice and was constitute in all our extracts (Figure 42). Monacolin K, also known as lovastatin or mevinolin is described in many studies as bioactive substance in Red Yeast Rice (Halbert et al., 2010; Huang et al., 2006; Tsukahara et al., 2009). Lovastatin, a pharmaceutical product, inhibits cholesterol synthesis in the liver by blocking the action of the HMG-Co A reductase. Figure 42 demonstrated that lovastatin is rich in Hongqu. Chromatogram (a) demonstrated many compounds of the Hongqu pills, which could be other monacolins like the hydroxy acid form of monacolin L, the hydroxy acid form of monacolin K, methyl ester of hydroxy acid form of monacolin L, monacolin L or monacolin K (Ma et al., 2000). Li et al. detected in 2005 by HPLC/DAD monacolin J acid, monacolin J, monacolin L acid, monacolin K acid, monacolin L, monacolin K and dehydromanacolin K (Li et al., 2005). In a study of Li et al. in 2004, 14 monacolin

compounds were identified in Red Yeast Rice by HPLC with photodiode array detector and tandem mass spectrometry (Li et al., 2004).
Lovastatin was stable in water, alcohol and methanol extraction.

Two extracts of Jiaogulan (Figure 43) contained the flavonoid rutin. Jiaogulan can contain many more compounds (Liu et al., 2008a). For example Kao et al. detected in 2008 by HPLC-ELSD, 34 compounds in *Gynostemma pentaphyllum* Makino. The extract contained 8 flavonoids like rutin, quercetin and kaempferol and the saponine extract contained gypenosides. For example gypenoside IV and gypenoside VIII, also known as ginsenoside Rb_3 and ginsenoside Rd were detected. Gypenosides are the saponins of *Gynostemma pentaphyllum* and ginsenosides are the saponins of *Panax ginseng* (Renshen) (Kao et al., 2008). Huang et al. published in 2008 the detection of 15 chlorophylls and their derivatives in Jiaogulan, determined by HPLC/MS.

The fingerprint of Baiguo and Yinxinye in Figure 44 of the chromatograms (a), (b) and (c) showed other components too. Those peaks could belong to ginkgolide A, B, C, J and bilobalide, which are detected in *Ginkgo biloba* extracts. A review of T. A. van Beek and P. Montoro of 2009 described a GC/MS profile of a *Ginkgo biloba* extract, which contained squalane, kaemfperol, isorhamnetin and quercetin. Also a number of biflavones occur in *Ginkgo* leaves like bilobetin, ginkgetin, isoginkgetin and sciadopitysin (van Beek and Montoro, 2009).

The Danshen sample in Figure 45 was reproducible and stable for five measurements within eight hours at room temperature. The methanol extract contained salvianolic acid B.

4.1.2.5 Comparison of Salvia officinalis and Danshen (Salvia miltiorrhizae)

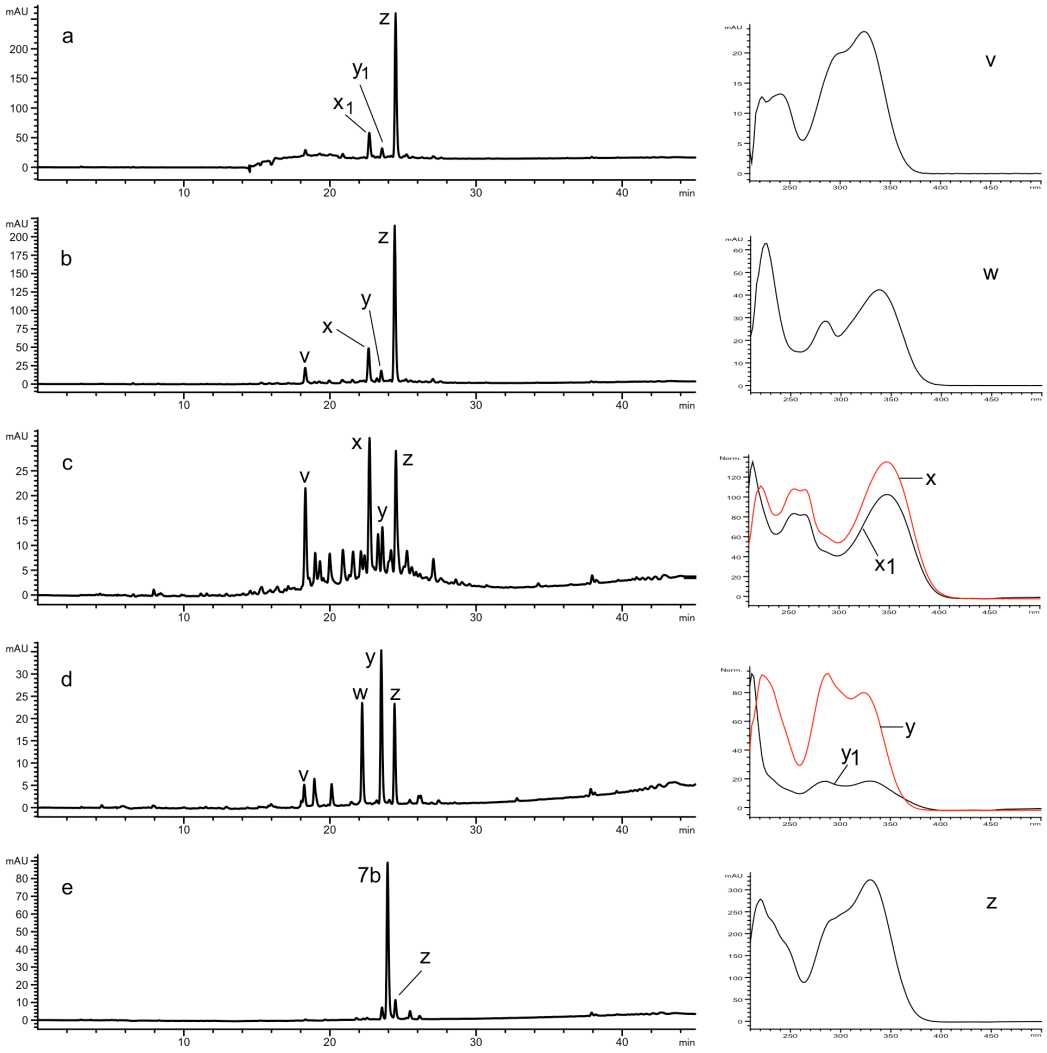


Figure 46: HPLC profiles detected at 300 nm and UV spectra of various Salvia species

(a) *Salvia officinalis* decoction of fresh leaves from Austria (b) *S. officinalis* decoction of dried leaves from Austria (c) *S. officinalis* decoction of dried leaves from Albania (d) *S. officinalis* decoct of dried rhizomes from Austria (e) Danshen decoction Salvianolic acid B (**7b**) the peaks with the letters **v, w, x, y** and **z** are unknown components of the decoctions

The HPLC profiles of decoctions prepared from dried leaves of *S. officinalis* originated from Austria and Albania were detected at 300 nm. Those decocts are clearly characterized by five

constitutes, marked as v, w, x, y and z. In the sample of Albania (c) the peak height ratio from z to x (z : x is about 1 : 1), is definitely deviating compared to the samples from Austria (z : x is about 4 : 1).

In the HPLC profile of the hydrophilic extract of Danshen (e), only compound z was detectable. On the other hand the compounds v, w, x and y could not be proven. Salvianolic acid B (7b) was detectable in this sample, in contrast to tanshinone II A, which is another major lipophilic component from Danshen. Tanshinone II A is a lipophilic main component in Danshen, because of this it was not detected in our milli-Q water extracts.

To sum up, the different peak height ratio form z to x in chromatogram (a), (b) and (c) in Figure 46, could be caused by environmental factors or due the treatment after harvesting. Another reason could be the different development stage of the plants during harvesting (Sheng et al., 2009).

Decocts	Tanshinone ll A	Salvianolic acid B	v	w	x	y	z
Austria, dry leaves	-	-	+	-	+	+	+
Albania, dry leaves	-	-	+	-	+	+	+
Danshen	-	+	-	-	-	-	+
Austria, fresh leaves	-	-	-	-	-	-	+
Austria, dry roots	-	-	-	+	-	+	+

Table 18: Various components in *Salvia* species

v, **w**, **x**, **y**, **z** refer to components in the HPLC chromatograms in Figure 46

In Figure 46, the decoction of *Salvia officinalis* fresh leaves from Austria (a) demonstrated three peaks marked with the letters x_1, y_1 and z. The retention time of the peak x_1 is at 22.69, of the peak y_1 at 23.56 minutes and of the peak z at 24.49 minutes.

The *Salvia officinalis* decoction of dried leaves from Austria (b) and the *Salvia officinalis* decoction of dried leaves from Albania (c) contained the similar substances of x_1 and y_1, demonstrated by peak x, y. The substance z is the same in all three herbs. Compound x_1 and y_1 are constitutes in the fresh leaves. The UV-spectra demonstrated a bathochromic shift to the dried leaves. The drying process could cause this effect.

The compound z was detected in all five samples. The chromatogram of *Salvia officinalis* decoction of dried rhizomes from Austria (d) and Danshen decoction (e) demonstrated compound z at a retention time of 24.49 minutes, too.

The hydrophilic standard salvianolic acid B (7a) was only proven in the decoction of Danshen (e), at a retention time of 23.5 minutes. The lipophilic standard tanshinone II A could not be detected in any extract because it is a lipid – soluble component.

UV – Maxima of peaks in nm

peak	v	w	x_1	x	y_1	y	z
	222	224	214	222	212	220	220
	240	284	254	254	----	224	300 sh
	300 sh	338	266	266	284	286	330
	324	----	348	348	330	322	----

Table 19: UV - maxima of the peaks **v**, **w**, **x_1**, **x**, **y_1**, **y** and **z** (in nm) sh = shoulder

The fingerprint of dried leaves of *Salvia officinalis* from Albania (c) demonstrated more peaks than the dried and fresh leaves of *S. officinalis* from Austria (a) and (b) in Figure 46. The reason could be caused by various effects on the herb due the environment or the time of harvesting. The herb sage belongs to the Labiatae family, which exhibits a high amount of antioxidants (Miura et al., 2002). *Salvia officinalis* contains many bioactive substances like rosmarinic acid, ursolic acid, carnosol, kaempferol, p-coumaric acid, rosmanol, epirosmanol, rosmadial, carnosic acid, apigenin, hispidulin, cirsimaritin and many more (Baricevic et al., 2001; Cuvelier et al., 1994; Imanshahidi and Hosseinzadeh, 2006). The substances v, w, x_1, x, y_1, y and z maybe belong to one of those mentioned constitutes.

More than 70 compounds have been isolated and structurally identified in the root of *Salvia miltiorrhizae* in various concentrations (Li et al., 2009). The major bioactive constitutes in Danshen are the hydrophilic caffeic acid derivatives such as salvianolic acids and the lipophilic diterpenoids such as tanshinones (Liu et al., 2007b). In 2004, Zhang Jin-lan et al. performed fingerprint analysis of Danshen and described danshensu, protocatechuic aldehyde and salvianolic acid B as main constitutes of Danshen injection, whereas salvianolic B was the main compound in the raw material of Danshen. Salvianolic B was decomposed and produced other phenolic acids. In this study, Danshen contained as well salvianolic acid G, D, E, A and C, rosmarinic acid and lithospermic acid (Zhang et al., 2005).
Other studies reported the presence of salvianolic acid B as well (Li et al., 2009; Liu et al., 2006a; Liu et al., 2007a).

Isotanshinone II A, dihydrotanshinone I, hydroxytanshinone II A, danshenxinkum A, tanshinone II B, tanshinone I, cryptotanshinone and tanshinone II A are bioactive substances of *Salvia miltiorrhizae* too and reported by Liu et al. in 2007 (Liu et al., 2007b).

4.2 Protein Analysis

4.2.1 Measurement of total protein concentration using BCA protein assay

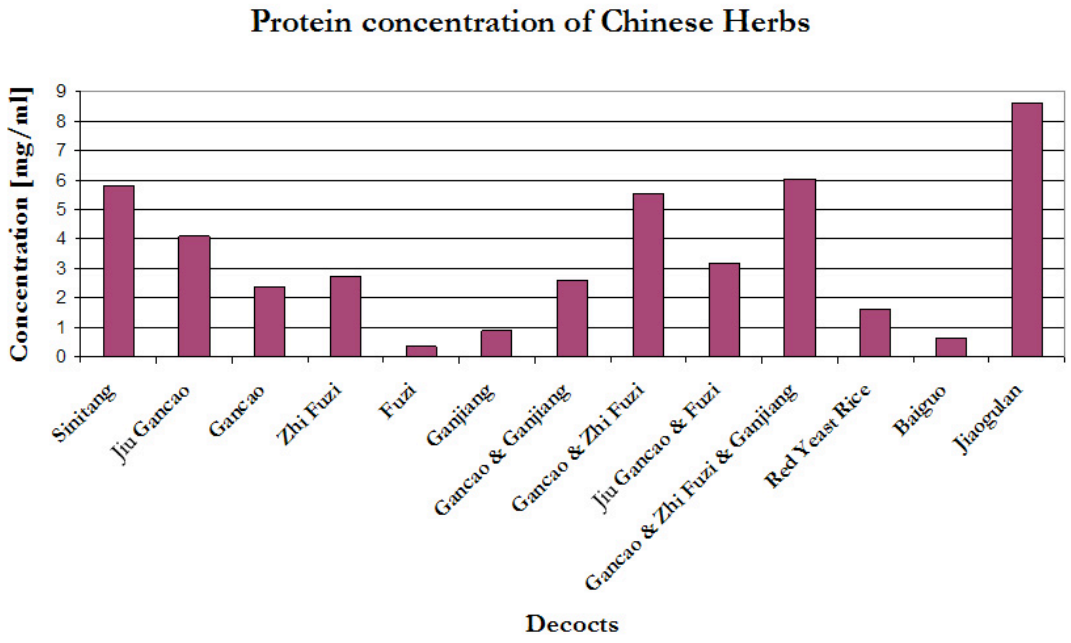


Figure 47: Total protein concentration of traditional Chinese herbs

The measurement of total protein concentration using BCA demonstrated that Jiaogulan had the highest amount of proteins, followed by Gancao & Zhi Fuzi & Ganjiang decoction and Sinitang formula. The pretreated herbs like Jiu Gancao and Zhi Fuzi have a higher amount than the untreated Gancao and Fuzi. The decoction of Gancao & Shengjiang has the same amount of proteins like the sum of the single decoct of Gancao and Shengjiang. The protein concentration of Gancao decoct plus Zhi Fuzi is nearly the same amount of the decoct combination Gancao and Zhi Fuzi.

The protein concentration of Chinese herbal decoctions and extracts were performed by BCA. It aids to choose the right concentration for SDS-PAGE.

4.2.2 SDS-PAGE

4.2.2.1 Sinitang

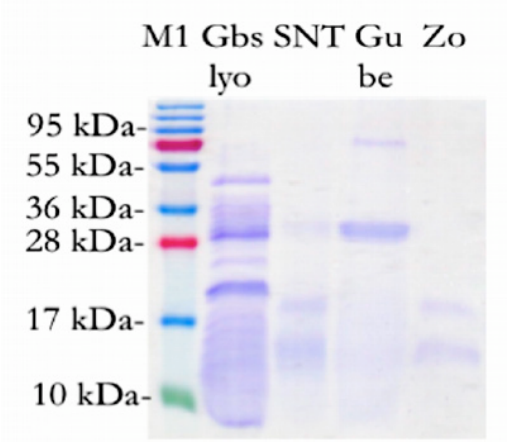


Figure 48: SDS-PAGE of Sinitang

SM1: marker **Gbs lyo:** Baiguo lyophilized **SNT:** Sinitang **Gu be:** Jiu Gancao **Zo:** Ganjiang

Figure 48 shows the Coomassie Brilliant Blue (CBB) stain of SDS-PAGE gel of Baiguo (Gbs), Sinitang formula (SNT) and the single herbs decoction of Jiu Gancao (Gu be) and Ganjiang (Zo). Baiguo contained many protein bands with a molecular weight from 10 kDa until 50 kDa. Sinitang contained three proteins with a molecular weight of 14 kDa, 19 kDa and 30 kDa. The protein of more than 30 kDa of Jiu Gancao is visible in the Sinitang sample with a molecular weight of 85 kDa. The CBB staining visualized two strong protein bands in Ganjiang sample. One protein had a molecular mass of about 14 kDa and the second one about 19 kDa. The protein of Jiu Gancao with 30 kDa and the two proteins of Ganjiang with 14 kDa and 19 kDa are shown in the lane of Sinitang, too.

Calculation of protein concentration:

Gb seed lyo: BCA: 0.62 mg/ml; (lyo = 10 mg)

0.62 * 1,5 = 0,93 mg protein/1.5 ml

0.93/10 = 0.093

(8 * 0,093/5) / 20 * 1000 = **7.44 µg/µl**

SNT: BCA: 5.83 mg/ml

5.83 * 90 = 524.7 mg/90 ml → lyo: 2370 mg

524.7/2370 = 0.2214

(8 mg lyo * 0.2214 = 1.77/mg protein/5)/20 * 1000 = **17.71 µg/µl**

Gancao: BCA: 4.09 mg/ml

4.09 * 180 ml = 739,2 mg/180 ml → lyo: 3060 mg

736.2/3060 = 0.2406

(8 mg lyo * 0.2406 = 1.9247 mg protein/5)/20 * 1000 = **19.25 µg µl**

Ganjiang: BCA: 0.89 mg/ml

0.89 * 196 ml = 174.44mg/196 ml → lyo: 2150 mg

174.44/2150 = 0.0811

(8 mg lyo * 0.0811/5)/20 * 1000 = **6.49 µg/µl**

4.2.2.2 Anti Aging Herbs

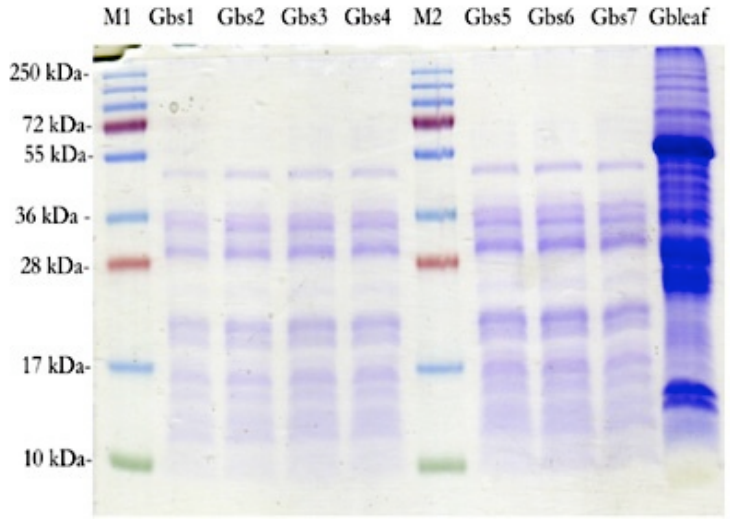


Figure 49: SDS-PAGE of Anti Aging Herbs

Gbs1 – Gbs7: Different concentrations of Baiguo lyophilized samples diluted in milli-Q water

M1 & M2: marker **Gbleaf:** Yinxinye milli-Q water extract

Figure 49 demonstrates the proteins of Baiguo in different sample concentrations. The best visible concentrations are shown in the last three lanes of *Ginkgo biloba* seeds (Gbs5, Gbs6, Gbs7).

Yinxinye extract showed many proteins. Baiguo contained at least 11 visible proteins, whereas Yinxinye contained 23 proteins.

The molecular weights of the estimated proteins of Baiguo were about 13 kDa, 13.8 kDa, 15.8 kDa, 17 kDa, 20.8 kDa, 22.1 kDa, 25.5 kDa, 30 kDa, 33.3 kDa, 36 kDa and 48.6 kDa according to the protein database of NCBI (National Center of Biotechnology Information).

In comparison the molecular weights of Yinxinye proteins were about 13 kDa, 15 kDa, 16 kDa, 16.5 kDa, 17 kDa, 20 kDa, 24 kDa, 25 kDa, 26.24 kDa, 28 kDa, 31 kDa, 34.5 kDa, 40.1 kDa, 42.8 kDa, 48.2 kDa, 53.3 kDa, 55 kDa, 79.6 kDa, 95 kDa, 112.5 kDa, 130 kDa, 190 kDa and 250 kDa.

Database protein search was performed using a program of Protein Translations of Life.

The protein about 13 kDa could be an allergen, non-specific lipid-transfer protein. The protein with a mass about 14.5 kDa could be the isoform antimicrobial protein Gnk2-1, antifungal protein ginkbilobin-2 or ginkbilobin-2-percursor.

Maturase K is about 15. 5 kDa.

Calculation of protein concentration:

dilution rate: 0.093

Gb 1: (0.4 * 0.093 2)/20 * 1000 = **0.93 µg/µl**

Gb 2: (0.6 * 0.093/2)/20 * 1000 = **1.4 µg/µl**

Gb 3: (0.8 * 0.093/2)/20 * 1000 = **1.86 µg/µl**

Gb 4: (1 * 0.093/2)/20 * 1000 = **2.33 µg/µl**

Gb 5: (1.2 * 0.093/2)/20 * 1000 = **2.79 µg/µl**

Gb 6: (1.4 * 0.093/2)/20 * 1000 = **3.3 µg/µl**

Gb 7: (1.6 * 0.093/2)/20 * 1000 = **3.72 µg/µl**

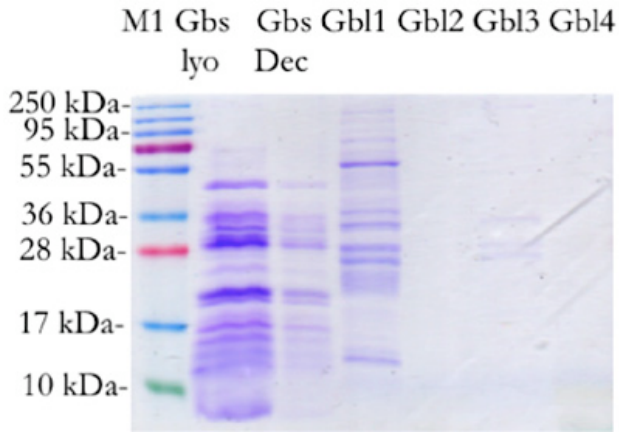


Figure 50: Comparison of Baiguo and Yinxinye extracts *via* SDS-PAGE

Gbs lyo: Baiguo lyophilized **Gbs Dec:** Baiguo milli-Q water extract & protease inhibitor

Gbl1: Yinxinye milli-Q water extract & protease inhibitor **Gbl2:** Yinxinye methanol extract & protease inhibitor

Gbl3: Yinxinye milli-Q water extract **Gbl4:** Yinxinye methanol extract

Figure 50 demonstrates proteins of different parts of *Ginkgo biloba* with different extract preparation. Lane 1 shows the sample of lyophilized Baiguo extract, lane 2 is the original Baiguo extract. Lane 3 to lane 6 shows different Yinxinye extracts. Gbl1 is the same sample like in Figure 49 the lane of Yinxinye and it looks the same as well. The proteins of Gbl2 and Gbl4 are missing. Gbl3 contains the same proteins like Gbl1, but the bands are brighter. The missing protease inhibitor in Gbl3 extract may cause this.

Calculation of protein concentration:

Gb lyo: 10 mg * 0.093 = 0.93 mg protein in 10 mg lyo

0.93/125 * 1000 = **7.44 µg/µl**

Gb Decoct: (0.62/50)/20 * 1000 = **0.62 µg/µl**

Table of proteins identified by SDS-PAGE:

kDa	**Name**	**Function**
G. uralensis		
30 kDa	Plasma membrane intrinsic protein	Watering stress protein
85 kDa	Beta-amyrin synthase	Catalyzes the cyclization of oxidosqualene into beta-amyrin Encodes beta-amyrin synthase involved in glycyrrhizin and soya saponin biosynthesis in licorice
Z. officinalis		
14 kDa	beta-1,3-glucanase or sesquiterpene synthase A1	Influence on metabolic process
19 kDa	violaxanthin de-epoxidase or cysteine protease gp3a	Dissipates excess energy in the photosystem II and protects photo-inhibitory damage MHC class II immune responses Important to bone development
G. biloba		
12.5 kDa	Non-specific lipid-transfer protein	Allergen

14.48 kDa	Antimicrobial protein Gnk2-1	Three proteins about 14.5 kDa are isoforms Inhibit the growth of the fungi *C. albicans* and *Fusarium oxysporum*
14.47 kDa	Antifungal protein ginkbilobin-2	
14.47 kDa	Ginkbilobin-2-percursor	
15.5 kDa	Maturase K	Usually encoded in the trunK tRNA gene intron. Probably assist in splicing its own and other chloroplast group II introns.
17.5 kDa	RNA polymerase C	Its subunits probably play an important role in DNA binding
19 kDa	ASR protein	Ripening protein: Specific immune activity to anti-His antibodies Inhibits the growing of the plants
26.3 kDa	Cytosolic absorbate peroxidase	Flooding stress protein: ↓cAPX→↑ROS→↓growth key AO enzyme in plants H_2O_2 detoxification in green leaves
29.5 kDa	RNA polymerase II larges subunit	Catalyzes the transcription of DNA to synthesize precursors of mRNA
48.46 kDa	Legumin or 11S-globulin	Storage protein Allergen of sesame

Table 20: Proteins of *Glycyrrhiza uralensis*, *Zingiber officinalis* and *Ginkgo biloba* identified *via* SDS-PAGE

(Bioinformatics Resource Portal, 2011; European Informatics Institute, 2011; National Center for Biotechnology Information, 2010)

4.2.3 N-terminal Sequencing

N-terminal Sequencing was performed with a protein of *Ginkgo biloba* seed with a molecule weight of about 20 kDa and a protein of *Glycyrrhiza uralensis* with a molecule weight of about 30 kDa. No results were obtained because the sequence was blocked in both times.

Sequencing was performed again with a protein of about 51 kDa of *Ginkgo biloba* seed. The obtained sequence is FVEEAGDA.

Result: There was no significant matching in the protein database with this sequence.

A longer sequence is needed to identify this protein.

5. Summary

Age related diseases have a dramatic impact on the health care budgets in developing countries. Early diagnosis, development of drugs and device therapies are worldwide efforts the fight those killing diseases. This thesis aims to contribute to the quality control of traditional Chinese medical herbs, which could be used for prevention and therapy of age related diseases.

Sinitang is a very famous Chinese herbal formula in TCM and has a long tradition to prevent and treat cardiovascular diseases. It consists of four herbs: Zhi Fuzi (*Aconitum carmichaelis praeperata*), Ganjiang (*Zingiber officinalis)*, Rougui (*Cinnamomum cassia)* and Jiu Gancao (*Glycyrrhiza uralensis praeperata*).
Hongqu (Red Yeast Rice), Jiaogulan (*Gynostemma pentaphyllum*), Baiguo (*Ginkgo biloba* seed), Yinxinye (*Ginkgo biloba* leaf) and Danshen (*Salvia miltiorrhizae*) are "anti aging" herbs used for lowering the cholesterol level, reducing the risk of cardiovascular diseases and improving cerebral and lung functions. All herbs comprise a high amount of antioxidants.
The Chinese herb *Salvia miltiorrhizae* was compared with European species of *Salvia officinalis*.

High Performance Liquid Chromatography (HPLC) fingerprinting, as well as protein analysis using Bicinchoninic Acid Protein Assay (BCA), Sodium Dodecylsulfatepolyacrylamide Gel Electrophoresis (SDS-PAGE) and N-terminal Sequencing performed the Quality control of the Chinese medical herbs.
The Chinese medical herbs contained bioactive substances and the developed HPLC methods were suitable and reproducible for the quality control.
The major active components [8]-gingerol, cinnamaldehyde and glycyrrhizic acid were identified in the Sinitang formula by HPLC. The main component belongs to glycyrrhizic acid of *Glycyrrhiza uralensis*.
Lovastatin, also known as monacolin K is a pharmaceutical product in Western medicine and was detected in the water, alcohol and methanol extracts of Hongqu.
The flavonoid rutin was identified in the Jiaogulan decoction and in the methanol extract. The ginkgolides A and B were detected in the methanol extract of Baiguo and in the water and methanol extract of Yinxinye.

The hydrophilic component salvianolic acid B was detected in the methanol extract of Danshen. Different parts of the herb *Salvia officinalis* from Austria and Albania were compared with the Chinese popular medical herb *Salvia miltiorrhizae*. Salvianolic acid B was only detected in Danshen. The active lipophilic component of Danshen, Tanshinone II A was not detected in any Salvia species, because of its solubility. The dried leaves of *S. officinalis* from Austria and Albania contained the same substances. The fingerprint of the fresh leaves was different to the dried leaves because of the drying process. One unknown substance appeared in all species of European and Chinese *Salvia*.

Herbal preparation procedures including the process of drying the herbal materials and environmental factors could influence the content ratio of the components in the herbs.

The total protein concentrations of each herb and the herb mixture were measured via BCA and analysed by SDS-PAGE. The proteins obtained from SDS-PAGE were further analysed by N-terminal sequencing.

We can conclude that HPLC is a simple and useful method for quality control of Chinese medical herbs as well as the European medical herbs.

The protein analysis including determination of protein concentration and protein identification by N-terminal sequencing could promote the development of quality control of traditional Chinese herbal medicine to a new level.

6. Zusammenfassung

Die anfallenden Kosten des Gesundheitssystems sind kaum noch zu tragen. Speziell altersbedingte Erkrankungen kosten dem Staat viel Geld. Um diese gefährlichen Erkrankungen zu bekämpfen sind Prävention, frühe Diagnosen, Entwicklung von Medikamenten und gezielte Therapien notwendig. Diese Diplomarbeit befasst sich mit der Qualitätskontrolle von chinesischen medizinischen Kräutern, welche zur Prävention und Therapie von altersbedingten Erkrankungen eingesetzt werden könnten.

Sinitang ist in der Traditionellen Chinesischen Medizin eine sehr bekannte Kräuterrezeptur und hat eine lange Tradition bei der Behandlung von kardiovaskulären Erkrankungen. Sinitang enthält vier Kräuter: Zhi Fuzi (*Aconitum carmichaelis praeperata*), Ganjiang (*Zingiber officinalis*), Rougui (*Cinnamomum cassia*) und Jiu Gancao (*Glycyrrhiza uralensis*).
Hongqu (Red Yeast Rice), Jiaogulan (*Gynostemma pentaphyllum*), Baiguo (*Ginkgo biloba* Frucht), Yinxinye (*Ginkgo biloba* Blatt) und Danshen (*Salvia miltiorrhizae*) sind „anti aging" Kräuter und wurden ebenfalls untersucht. Alle beinhalten viele Antioxidantien und haben daher einen protektiven Effekt auf den Körper. Sie werden verwendet zur Cholesterinsenkung, zum Schutz und zur Behandlung von kardiovaskulären Erkrankungen und sie können die Gehirn und Lungenfunktion verbessern.
Das sehr berühmte chinesische medizinische Kraut *Salvia miltiorrhizae* (Danshen) wurde mit der europäischen Spezies *Salvia officinalis* verglichen.

Die Qualitätskontrolle der chinesischen und europäischen Kräuter wurde mittels HPLC durchgeführt, sowie die Proteinanalyse mittels BCA, SDS-PAGE und N-terminaler Sequenzierung
Die chinesischen Kräuter enthielten bioaktive Substanzen und die entwickelten HPLC Methoden waren geeignet und reproduzierbar für deren Qualitätskontrolle.
In der Sinitang Rezeptur konnten die bioaktiven Inhaltsstoffe [8]-Gingerol, Cinnamaldehyd und Glyzyrrhizinsäure nachgewiesen werden.
Lovastatin, auch bekannt unter dem Namen Monakolin K wird als Medikament in der westlichen Medizin zur Cholesterinsenkung verwendet. Dieses Statin konnte im Wasser-, Alkohl- und Methanolextrakt von Hongqu (Red Yeast Rice) nachgewiesen werden.

Das Flavonoid Rutin wurde im Jiaogulan Dekokt und im Methanolextrakt identifiziert.
Die Ginkgolide A und B wurden im Methanolextrakt von Baiguo (*Ginkgo biloba* Frucht) und im Wasser- und Methanolextrakt von Yinxinye (*Ginkgo biloba* Blatt) detektiert.
Die hydrophile bioaktive Substanz Salvianolsäure B, wurde im Methanolextrakt von Danshen (*Salvia miltiorrhizae*) nachgewiesen. Es wurden verschiedene Pflanzenteile vom österreichischen und albanischen *Salvia officinalis* mit der chinesischen Heilpflanze *Salvia miltiorrhizae* verglichen. Salvianolsäure B konnte allerdings nur in Danshen gemessen werden. Aufgrund der Löslichkeit konnte der lipophile Inhaltsstoff Tanshinon II A in keiner *Salvia* Spezies nachgewiesen werden. Die getrockneten Salbeiblätter von Österreich und Albanien enthielten die gleichen Inhaltsstoffe. Es war ein Unterschied zwischen frischen und getrockneten Salbeiblättern zu erkennen. Dies dürfte auf den Einfluss des Trocknungsprozesses zurückzuführen sein. Eine uns unbekannte Substanz wurde in allen Spezies des europäischen und chinesischen Salbeis entdeckt.
Die Behandlung und Bearbeitung von Kräutern beinhaltet zum Beispiel Trocknen aber auch die Umgebung und der Standort der Pflanze haben einen Einfluss auf die Konzentration der Inhaltsstoffe der Kräuter.
Die Gesamtproteinkonzentration von den einzelnen Kräutern und Kräutermischungen wurde mit BCA und SDS-PAGE bestimmt. Die mittels SDS-PAGE erhaltenen Proteine wurden weiter analysiert durch N-terminale Sequenzierung.
HPLC ist eine einfache und geeignete Methode für Qualitätskontrollen von chinesischen und europäischen Kräutern. Die Untersuchung der Proteine und Proteinkonzentrationen von Kräutern, sowie die Proteinidentifizierung mittels Sequenzierung ist ebenfalls eine interessante Möglichkeit für Qualitätskontrollen.

7. List of Abbreviations

AcN	Acetonitril
AD	anno domini
APS	Ammonium Peroxodisulphate
ATZ	Anilinothiazolinone
BCA	Bicinchoninic Acid
BSA	Bovine Serum Albumin
CBB	Coomassie Brilliant Blue R-250
CHM	Chinese Herbal Medicine
CSP	Compound Salvia Pellet
CVD	Cardiovascular Diseases
DAD	Photodiode Array Detector
Dec	Decoct
DS	Deficiency Syndrome
DTT	Dithiothreitol
ES	Excess Syndrome
EtOH	Ethanol
g	Gram
Gbl	*Ginkgo biloba* leaf/ Yinxinye
Gbs	*Ginkgo biloba* seed/ Baiguo
Gu be	*Glycyrrhiza uralensis* pretreated/ Jiu Gancao
HAc	Acetic Acid
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinome
HCl	Hydrochloric Acid
HCV	Hepatitis C Virus
HE	Hydroalcohlic Extract
Herbs	
a	Sinitang
b	Jiu Gancao
c	Gancao

d	Zhi Fuzi
e	Fuzi
f	Ganjiang
g	Gancao & Ganjiang
h	Gancao & Zhi Fuzi
I	Jiu Gancao & Fuzi
j	Gancao & Zhi Fuzi & Ganjiang
k	Red Yeast Rice
l	Baiguo
m	Jiaogulan
HPLC	High Performance Liquid Chromatography
kDa	kilo Dalton
LC	Liquid Chromatography
LC-IR	HPLC combined with infrared spectroscopy
LC-MS	HPLC combined with a mass spectrometer
LDL-C	Low-Density Lipoprotein Cholesterol
LNCaP	Prostate cancer cell line proliferation
LPS	Lipopolysaccharide
lyo	lyophilized
M	Molar
mAU	Milli Absorbance Units
MDA	Malonyldialdehyde
MeOH	Methanol
mg	Milligram
min	Minute
ml	Milliliter
mM	Millimolar
mm	Millimeter
µm	Micrometer
µl	Microliter
NHIS	National Health Interview Durvey
nm	Wave Length
NO	Nitric Oxide

OFR	Oxygen Free Radicals
OVA	Ovalbumin
PA	Protocatechuic Aldehyde
PAF	Platelet Activating Factor
PITC	Phenyl Isothiocyanate
PTCA	Percutaneous Transluminal Coronary Angioplasty
PTH	Phenylthiohydantoin
PVDF	Polyvinyldifluorid
QOL	Quality Of Life
ROS	Reactive Oxygen Species
RP	Reversed-Phase
rpm	Rounds Per Minute
Sal B	Salvianolic Acid B
SAP	Stable Angina Pectoris
SDS	Sodium Dodecylsulphate
SDS-PAGE	Sodium Dodecylsulfatepolyacrylamide Gel Electrophoresis
SND	Sini Decoction
SNMC	Stronger Neo-Minophagen C
SNT	Sinitang
SOD	Superoxide Dismutase
Standards	
1	8-Gingerol diluted in Methanol
2	Cinnamaldehyde diluted in Methanol
3a	Glycyrrizic Acid diluted in Ethanol
3b	Glycyrrizic Acid diluted in Methanol
4	Lovastatin diluted in Methanol
5	Rutin diluted in Methanol
6a	Ginkgolide A diluted in Methanol
6b	Ginkgolide B diluted in Methanol
7a	Salvianolic Acid B diluted in Milli-Q water
7b	Salvianolic Acid B diluted in Milli-Q water
8a	Tanshinone II A diluted in Ethanol
8b	Tanshinone II A diluted in Ethanol

TEMED	N,N,N,N-Tetramethylethylenediamine
TCM	Traditional Chinese Medicine
TFA	Trifluoroacetic Acid
UV	Ultraviolet
WHO	World Health Organization
Zo	*Zingiber officinalis*/ Ganjiang
°C	degree Celsius
[c]	concentration
SAM	Statin-Associated Myalgia

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