

**QUALITATIVE IDENTIFICATION OF FERULIC ACID IN
ANGELICA SINENSIS (DONG QUAI)**

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WITH HONOUR**

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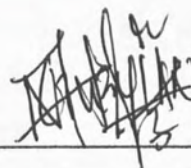
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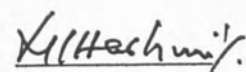
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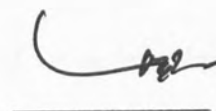


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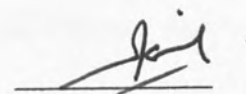
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ABSTRAK

IDENTIFIKASI ACID FERULIK DALAM *ANGELICA SINENSIS* (DONG QUI) SECARA KUALITATIF

Kajian ini dijalankan untuk menentukan kompaun aktif yang terdapat dalam *Angelica sinensis* telah dijalankan dengan menggunakan analisis TLC dan HPLC. *Angelica sinensis* telah diekstrak dengan menggunakan tiga jenis pelarut yang berlainan iaitu air, metanol and metanol – asid formik (95:5). Hasil daripada analisis didapat pelarut yang paling berkesan mengekstrakan komponen fenolik ialah metanol dan metanol-asid formik. Hasil menunjukkan kira- kira 11.5% asid ferulik diekstrak dengan air, 17.5% asid ferulik diekstrak dengan metanol dan 7.81% asid ferulik diekstrak dengan metanol – asid formik (95:5). Daripada komatografi lapisan nipis didapati ketiga-tiga ekstrak memberikan lapan bintik di mana antaranya terdapat hanya satu bintik yang mempunyai nilai R_f yang paling dekat dengan asid ferulik. Masa ketahanan juga dapat dicatatkan daripada analisis HLPC untuk mengenalpastikan kehadiran komponen ini dalam *Angelica sinensis*.



ABSTRACT

QUANTITATIVE IDENTIFICATION OF FERULIC ACID IN ANGELICA SINENSIS (DONG QUAI)

A research was conducted to determine the active ingredient in Angelica sinensis using TLC and HPLC analysis. Angelica was extracted by using three different solvents; which is water, methanol and methanol – formic acid (95:5). From the analysis it was found that methanol and methanol – formic acid (95:5) are best for extracting the phenolic compounds. Results showed that about 11.5% of ferulic acid was extracted by water, 17.5% of ferulic acid was extracted by methanol and 7.81% of ferulic acid was extracted by methanol – formic acid(95:5). The thin layer chromatography of the three extracts showed the presence of eight spots out of which one spot has a Rf value close to ferulic acid. The retention time found from the HPLC analysis of the sample also confirmed the presence of this compound in Angelica sinensis.



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4.1: *Angelica sinensis* extracted by water, methanol and methanol-formic acid (95:5)

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LIST OF SYMBOLS

WHO	World Health Organization
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
m	Meter
cm	Centimeter
g	Gram
eNOS	Endothelial nitric oxide synthase
ALP	Alkaline Phosphate
OPC-1	Osteoprecursor cells
H ₂ O ₂	Hydrogen peroxide
PBMCs	Peripheral blood mononuclear cells
GRAS	Generally regarded as safe
FT – IR	Fourier transform infrared spectroscopy
2D – IR	Two-dimensional correlation infrared spectroscopy
GC	Gas chromatography
GC – MS	Gas chromatography – mass spectrometry
PLE	Pressurized liquid extractin
ml	Milliliter
°C	Celsius degree
%	Percentage



CHAPTER 1

INTRODUCTION

This thesis describes the isolation of active ingredient of a Chinese herb, *Radix Angelica sinensis*, which is used commonly used for the treatment or prevention of women diseases such as tonifying the blood and treating female irregular menstruation and amenorrhea (Lin *et al.*, 1998). Because of its broad pharmacological effects it has been used for thousand of years (Sun *et al.*, 2005). The analysis is done by using difference solvent to extract the active ingredient and followed by analyzed qualitatively by using thin layer chromatography and then followed by using high performance liquid chromatography.

The World Health Organization (WHO) estimated that over 80% of populations in the world are using herbal medicine (Jennifer *et al.*, 2003). The WHO had defines the herbal medicines as "finished, labeled medicinal product that contains as active ingredient aerial (above ground) or under ground parts of plants material, or combination thereof, whether in crude state or as plant preparation (Bascom, 2002). Other definition of herbs are as "Plant or plant part that are used in fresh, dried or extracted form for promoting, maintaining or restoring health" or "Botanists describe an herb as a small, seed-bearing plant with fleshy, rather than woody parts" or "herbs are valued for their flavor, fragrance, medicinal and healthful qualities, economic and industrial uses, pesticidal properties and dyes" (Bascom, 2002).



Thousands years ago, people have been using herbs successfully to treat a wide variety of ailments. The ancestors passed their knowledge down through the ages and developed numerous remedies that proved effective for their health challenges. Herbs may be used in combination with conventional medical treatment to help a person feel healthy (Henry, 1994). Modern researchers have proven that herbs are effective because they contain the necessary nutrients to help the body heal itself (Louise and Lee, 2004).

Herbs contain many substances that will clean, balance and build health because it properties to enrich blood, activate blood circulation, regulate menstruation, relieve pain and relax bowel and also for treatment of anemia, hypertension, chronic bronchitis, asthma, rheumatism and cardiovascular diseases (Lu *et al.*, 2005 and Lin *et al.*, 1998). They contain minerals, vitamins, oils, enzymes and many other nutrients in their natural and most stable state (Kee, 1999). They provide materials for the body to correct imbalances and create harmony within the body. Herbs are part of a natural plan designed to keep all living creatures on our planet in optimum health. When used with care, they cause no harmful effects. Herbs provide nutrient in such a way that the body takes what it needs and eliminates what is not utilized (Louise and Lee, 2004).

Herbs are defined in several ways depending on the context in which the word is used. In botanical nomenclature, the word refers to non-woody seed-producing plants that die to the ground at the end of the growing season (Robbers and Tyler, 2002). In the other words it can refers as vegetable products which can used to add flavor or aroma to food. Whereas in the field of medicine, herbs is defined as crude drugs of vegetable origin utilized for the treatment of disease state, often of a chronic nature, or to attain or to maintain a



condition of improved health. Herbs plants have always provided an important source of medicines (Richard, 2004).

Conspicuously, an herbal product is start as plants. They make the journey from plant to medicine by either harvested from the wild (called wild-crafting) or grown for the purpose of creating and herbal medicine (Bascom, 2002). After harvesting an herb, it must be dry to reduce the moisture content but without destroying the plant's active chemical compounds. The herbs should be dried by spreading it loosely on a rack to let air circulate around it to prevent grow of mold. The procedure for harvesting and preparing each herb are depending on the time of year and the part of the plant that will be used for medicinal purpose. Herbs that been prepared should be stored in dark glass containers with tight-fitting lids and must be keeping away from sunlight and heat to prevent changes of the chemical compound (Bascom, 2002).

1.1 Concept of Chinese herbs

1.1.1 Classification of Chinese herbs

Chinese herbs are as old as Chinese history, and the ancient Chinese classified herbs are according to their basic action, which means what they could do to the human body. These are including the four energies, the five flavors, the four directions and their relationship to the internal organs. Herbs are also described as food-like, mildly toxic and very toxic plants (Henry, 1994).

The Four Energies categories are cold, cool, warm and hot (Lucas, 1987).
Sicknesses had been classified as a cold or hot in nature so that herbs that have the



ability to oppose or counterbalance a cold or hot disease. Herbs that have a cold energy are used to treat inflammatory and toxic conditions (Fang, 2000). In western herbalism these might be classified as alterative, blood purifying or detoxifying.

The Five Flavors are spicy, sweet, sour, bitter and salty (Fang, 2000). Herbs may have a complex of more than one flavor. This organoleptic classification of herbs is a way that Chinese herbalism relates to the biochemical action of herbs. The flavors of herbs do not always relate to the actual perceived flavors but are used to indicate the actions of specific herbs. In most cases, herbs with the same flavor have similar properties, while those of different flavors have different properties (Henry, 1994). However, some herbs may have similar flavors but different properties or different properties with similar flavors. Because of this both the nature and flavors are jointly taken into consideration (Henry, 1994).

1.1.2 Processing of herbs

Herbs should be processed before using or making into various forms. Many Chinese herbs now are standardly processed for use into capsules, pill, tablets and extracts form (Jennifer *et al.*, 2003). The purposes of preparation are to enhance the curative effects, to neutralize its toxicity effects and to change the properties of herbs and expand their uses.

1.1.3 Form of herbal preparations and dosage

Herbs are available in various forms, depending on their purpose and the body system involves; they may be bought individually or in mixtures formulated for



specific conditions (Jennifer *et al.*, 2003). Drying is always the first stage in preparation, because fresh herbs are not readily available. The dried plants, leaves, or root may be used as teas and infusion (steeps in hot water), extracts and decoctions (soaked in alcohol or vinegar) or powdered in capsules (Philp, 2004). Freeze-dried preparations have appeared more recently (Philp, 2004). Pharmaceutical preparations made by extracting herbs with various solvents to yield tinctures, fluidextracts, extract or the like are known as phytomedicinals (Robbers and Tyler, 2002).

The most common form of herbs preparation is decoction. Traditionally it is prepared in a clay pot. Now a day, it can also be prepared in glass, unchipped enamel or high quality stainless steel without interfering with their properties (Bascom, 2002). Herbs should not prepare in iron, copper, aluminum or any type of metal that can alter the chemical compound of the herbs (Fang, 2000).

The dosage of Chinese herbs is calculated mainly in weight. In the past, an obsolete system of weight was used in China with 1 Jin equal to 16 liang and 1 liang equal to 10 qian. At present, the metric system of weight has been introduced with gram as the unit.

Table 1: Standard weight and measures of Chinese herbs

1 fen	0.3 grams approx.
10 fen	1 qian = 3 grams approx.
10 qian	1 liang = 30 grams approx.
16 liang	1 jin = 480 grams approx.

(Source: Tierra, 2002)

When a Chinese client gets a prescription from an herbalist, he or she usually brings it to an herb shop for filling. The clerk at the herb shop will place the herbs in small paper bags, with instructions for the contents of each bag to be decocted two to three times for oral administration. Normally, each decoction is taken all at once as one dosage, usually in 1 or 2 cups, and two dosage are taken a day (Henry, 1994).

1.1.4 Toxicity

Toxicity is referred to as harmful effects or toxic effects of herbs on the human body (Melanie, 2000). In general, the toxic dose of a poisonous herb is close to its therapeutic dose with a relatively small safety coefficient in its clinical use. If used improperly, a poisonous remedy could cause severe damage to tissues and organs of the human body and even lead to death (Lucas, 1987).

There are few things that have to be take care when taking herbs. These are including the dose of a poisonous herb using should be strictly controlled, attention should be paid when use the toxic herbs correctly and preparation technique of herbs should be to reduce or eliminate the toxic effects of drugs (Louise, 2005).



Chinese herbal products are safer than synthetic or lab-produced drugs because they are naturally rich in both biologically active and inert substances. Many of these nutrients have strong antioxidant, detoxicant, scavenging, and dual-functioning properties (Joseph and Jin, 2005). Herbs do not act like chemical drugs but more closely resemble the orthomolecular substances of vitamins, minerals, and enzymes that naturally exist in human bodies (Louise, 2005). And also, medicinal herbs usually do not give immediate reactions but their effects are usually subtle and gradual. They tend to fill the needs of the body's system as a whole. In TCM, it is stated simply that these herbal properties balance the yin and yang, gradually bringing the entire body to a natural state. (Joseph and Jin, 2005)

1.5.5 Status of Angelica in Chinese Medicine

Angelica in Chinese medicine, the part of root is most often used in combination with other herbs. Within the Chinese medical framework, Angelica root is used as a component of formulas for liver *qi* stasis and spleen deficiency (Fang, 2000). It is believed to work best in patients with a *yin* profile, and is considered to be a mildly warming herb (Fang, 2000). Angelica root is thought to return the body to proper order by nourishing the blood and harmonizing vital energy. So, the name of Angelica root in Chinese "*Dong Quai*" is translates as "return to order" based on its alleged restorative properties (Henry, 1994).

Radix Angelica sinensis, also known as Chinese Angelica root or Dong Guai and it is come from the family of *Umbelliferae* (Philp, 2004). It has been used for thousand years in traditional Chinese including also in Japanese and Korean medicine.



Chinese angelica root is an aromatic herb that grows in China, Korea, and Japan and it was known as Toki in Japan while Tanggwi in Korean (Henry, 1994). There are several species of Angelica which are used as the herb Dong Quai such as *Angelica Acutiloba* where the Japanese regard this herb as equal or even surpassing the blood tonic properties of *Angelica Sinensis*. The Angelica root usually is finely sliced and soaked in wine to make it warmer and more bio-available (Robert and Tyler, 2002). The body of the root is more for blood while the heads are considered to be more for the 'qi of the blood' meaning blood circulation (Fang, 2000).

Angelica root is well known as "female ginseng" and is the most important female tonic in Chinese medicine. The reputation of Angelica root is second only to Ginseng and is considered the ultimate, all-purpose woman's tonic herb. Angelica root helps in promote uterine health, regulating the menstrual cycle and relieve menstrual cramping which is caused by the hormonal changes (Philp, 2004). Angelica root is frequently used by the Chinese as a strengthening treatment for the heart, spleen, liver and kidneys (Ebadi, 2002). Both men and women use the herb as a general blood tonic. According to Andrew Weil, reported in the Journal of Ethnopharmacology (1981) that Chinese tonic herbs are some of the world's most potent immune system enhancers (Joseph & Jin, 2005).

Angelica is a fairly easy to grow plant from seed (Philp, 2004). They are best planted as soon as they are gathered, but some will germinate if kept in freezer. It requires a deep moist fertile soil in dappled shade. Plants are perennial up to 3 years if prevented from setting seed (Philp, 2004). The part of the plant most often cultivated for medicinal use is the root, which is divided into three parts (head, body,



and tail) (Jennifer *et al.*, 2003). Each section is thought to have different actions within the body. For example, the head is proposed to be best for promoting blood circulation, while the tail is thought to be the worst. Angelica is used extensively and in alternative medicine. Angelica is edible and medicinal; the young shoots are edible in salad or boiled as a pot herb (Jennifer *et al.*, 2003). It has a sweet taste similar to celery. Angelica root, leafstalks and stems are often candied.

Angelica root contains vitamins A, E and a significant amount of B₁₂ (Pedersen, 2000). Researchers have isolated at least seven coumarin derivatives that exert antispasmodic and vasodilatory effects. Antispasmodics are a remedy for menstrual cramps. The essential oil in Angelica root contains Ligustilide, butylphthalide and numerous other minor components. Ferulic acid and various polysaccharides are also found in Angelica root (Pedersen, 2000). These elements can prevent spasms, reduce blood clotting and relax peripheral blood vessels. Research has shown that Angelica root produces a balancing effect on estrogen activity (Philp, 2004). The other constituents of volatile oils, valeric acid, angelic acid, angelicin, safrole, scopoletin, and linoleic acid also useful in the treatment of fevers, colds, coughs, flatulent colic and other stomach disorders (Lucas, 1987). A medicinal infusion made from stems, seeds, and root is carminative, diaphoretic, emmenagogue, sedative, stomachic and tonic.

Angelica is a very good tonic herb for women and children, the elderly or general debility, it is said to strengthen the heart (Lucas, 1987). Powdered root is said to cause disgust for liquor. It has an antibacterial action, preventing the growth of various bacteria. An infusion of Angelica root, used as a wash for the face, is said



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