Impact of minerals in Chinese medicinal herbs and decoctions on kidney function

A THESIS SUBMITTED FOR THE DEGREE OF

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BY

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ABSTRACT

Kidney function or renal function is essential and depends on the ability of the kidneys to filter the blood. Millions of people around the world suffer from kidney diseases, and these patients will eventually need a form of renal replacement therapy: dialysis or transplantation. Dialysis and kidney transplantation save lives but with great costs, that are becoming a major issue in western countries because they account for a significant proportion of healthcare expenditure. Due to cost constraints, the majority of the people with kidney diseases die in developing countries. Traditional medicine is gaining an increasing share of the public's awareness. Studies have documented that about half of the population and many industrialised countries use traditional medicine, and the proportion is as high as eighty percent in many developing countries.

Traditional Chinese Medicine is an ancient medical system that has been practiced for more than five thousand years to diagnose and cure illness. The elements that accumulate in medicinal plants have the healing power for numerous ailments and disorders. The absence or deficiency of an element brings abnormalities that can be connected to specific biochemical changes reversed by supplying the element. This study involved elemental analysis of fifty Chinese herbs and eight Chinese herbal formulations that are used to improve kidney function. The analyses were performed using atomic absorption spectroscopy for the study of elements calcium, iron, magnesium, manganese, potassium, sodium and zinc. Data analyses of elements in the Chinese herbs and herbal decoctions were done by Chemometrics.

Preliminary analysis of various parts of the Chinese herbal plants, such as leaves, flowers, fruits, seeds and roots, include analysis for Zinc and Lead by atomic absorption spectroscopy and near-infra red spectroscopy. Results by atomic absorption spectroscopy showed variations in the metal content for various parts of the Chinese herbs. Chinese herbs that are leaves showed higher contents of metals compared to other parts of the herbs. Herbs that are leaves: Lian Zi Xin and He Ye had the highest Zn concentration and also He Ye has highest Pb concentration when compared to the other herbs. The samples were scanned using near-infra red

spectroscopy. Data were analyzed using statistics and best prediction calibration model was obtained.

Fifty Chinese kidney tonifying herbs in dried form were analyzed by atomic absorption spectroscopy. Various parts of the plants such as seeds, whole plant/stem/twig/bark, roots, seeds, fruits, fungus, peel and animal fossils were analyzed for beneficial elements such as Ca, Fe, Mg, Mn, K, Na and Zn. Results showed that Ca and K were higher compared to other analyzed elements. Bie Jia and Long Gu that were fossils found to be high in Ca; Tong Cao that is whole plant was high in Mn and K; and Yin Chen that is stem was high in Fe. Data analysis was done by chemometrics such as ANOVA, CA, PCA and HCA.

Decoctions of eight TCM formulae that are used to improve kidney function, namely Liu wei di huang wan, Jin gui shen qi wan, Da bu yin wan, Gui lu er xian jiao, Er zhi wan, Qi bao mei ran dan, You gui wan and Zuo gui wan were analyzed and compared for Ca, Fe, Mg, Mn, K, Na and Zn at different time of infusion intervals: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min by atomic absorption spectroscopy. Data analysis was done by chemometrics such as ANOVA, CA, PCA and HCA. Results showed that there are variations in the leaching of elements at different decoction times. TCM‘s that proved to be in agreement with the TCM theory and that made highest contributions to ADDI were reported. Manganese had highest contribution to ADDI in Liu wei di huang wan (8.25%) and in Jin gui shen qi wan (12.5%). Iron and Calcium made highest contributions to ADDI in Da bu yin wan (9.84%, 5.93%) and in Gui lu er xian jiao (30.2%, 21.23%) respectively. Potassium made highest contribution to ADDI in Er zhi wan(25.7%) and magnesium made highest contribution to ADDI in Qi bao mei ran dan (10.2%). Iron and manganese made highest contribution to ADDI in You gui wan (15.1%, 12.7%) and in Zuo gui wan (25.32%, 19.4%) respectively.

DECLARATION

"I, Archana Kolasani, declare that the PhD thesis entitled 'Impact of minerals in Chinese medicinal herbs and decoctions on kidney function' is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

Signature Date

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LIST OF ABBREVIATIONS

General and Medical

ADDI	Adequate daily dietary intake
ARF	Acute renal failure
AUD	Australian dollar
BMD	Bone mineral density
CAM	Complementary and alternative medicine
CHM	Chinese herbal medicine
CKD	Chronic kidney disease
CRF	Chronic renal failure disease
CRRT	Continuous renal replacement therapy
DRI	Dietary reference intake
eGFR	Estimated glomerular filtration rate
EPO	Erythropoietin
ESA	Erythropoiesis stimulating agents
ESKD	End stage kidney disease
ESRD	End stage renal disease
GFR	Glomerular filtration rate
HD	Hemodialysis
HEF	Herba epimedii
HPO	Hypothalamus-pituitary-ovary
IHD	Intermittent hemodialysis
Hgb	Hemoglobin
NIM	Neuroendocrine imunomodulation
OVX	Ovariectomized
RRT	Renal replacement therapy
RDA	Recommended dietary allowance
SAM	Senescene accelerated mouse
SRM	Standard reference material
TCM	Traditional Chinese medicine
TM	Traditional medicine

UK	United Kingdom
US	United States
USA	United States of America
USD	United States dollar
WHO	World health organisation

Instrumental

AAS	Atomic absorption spectroscopy
ETAAS	Electrothermal atomic absorption spectroscopy
FAAS	Flame atomic absorption spectroscopy
GFAAS	Graphite furnace atomic absorption spectroscopy
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
INAA	Instrumental neutron activation analysis
NIR	Near infrared
NIRS	Near infrared spectroscopy

Chinese herbal formulae

BHT	Bai Hu Tang
EZW	Er zhi wan
DB	Da bu yin wan
DBT	Danggui Buxue Tang
GL	Gui lu er xian jiao
JG	Jin gui shen qi wan
LW	Liu wei di huang wan
QB	Qi bao mei ran dan
YGW	You gui wan
ZGW	Zuo gui wan

Measurements

g	Gram
dL	Decilitre
kg	Kilogram
L	Litre
M	Molarity
m2	Square metre
mg	Milligram
min	Minute
mL	Millilitre
mmol	Millimoles
µ	Micron
µg	Microgram
mΩ	Milliohm
nm	Nanometre
pmp	Per million population
ppm	Parts per million
R^2	Coefficient of determination
RPD	Relative percent difference
SD	Standard deviation
SEC	Standard error of calibration
SECV	Standard error of cross-validation
%	Percentage

Mathematical modelling

ANOVA	Analysis of variance
CA	Correlation analysis
HCA	Hierarchical cluster analysis
LDA	Linear discriminant analysis
MLR	Multiple linear regression
MPLS	Modified partial least squares
PASW	Predictive analytics software
PCA	Principal component analysis

PLS	Partial least squares
SPSS	Statistical package for the social sciences
XL-STAT	Excel Statistics

Chemistry

Ag	Silver
Al	Aluminium
As	Arsenic
Au	Gold
B	Boron
Ba	Barium
Br	Bromine
Ca	Calcium
Ce	Cerium
Cd	Cadmium
Cl	Chlorine
Cr	Chromium
Cs	Cesium
Co	Cobalt
Cu	Copper
EDTA	Ethylenediaminetetraacetic acid
Fe	Iron
Hg	Mercury
Hf	Hafnium
In	Indium
K	Potassium
La	Lanthanum
Li	Lithium
Mg	Magnesium
Mn	Manganese
Na	Sodium
Ni	Nickel
P	Phosphorous

Pb	Lead
Rb	Rubidium
S	Sulfur
Sb	Antimony
Sc	Scandium
Se	Selenium
Si	Silicon
Sm	Samarium
Sr	Strontium
Tb	Terbium
Th	Thorium
Ti	Titanium
U	Uranium
V	Vanadium
Zn	Zinc

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1 INTRODUCTION

The kidneys are a pair of vital organs that perform many functions to keep the blood clean and chemically balanced. Reduced kidney function may lead to kidney diseases that are characterized as hereditary, congenital or acquired. In Australia, approximately sixteen percent of the population have some form of kidney damage present and are therefore at a risk of renal disease. Due to cost constraints for treatment of renal diseases with western medicine, majority of people (80%) in developing countries rely on traditional medicine (TM). Traditional Chinese medicine (TCM) has been used for thousands of years to improve the kidney function. Over the years, hundreds of herbal formulae were created and refined for specific types of diseases. These herbal formulae involve various combinations of herbs that are used to treat a particular disease.

1.1 OVERVIEW AND FUNCTIONS OF KIDNEY

The urinary tract includes the kidneys, ureters, bladder and urethra. These organs together, serve to produce and excrete urine and regulate fluid and electrolyte content in the body. The kidneys are bean-shaped organs, and approximately the size of a child's fist. They are located just above the waist behind the abdominal cavity [1].

Filtering, secreting and reabsorbing of waste products occur in a complex system of tubules inside the kidneys called nephrons. Each kidney has about a million nephrons. In the nephron there is a glomerulus that resembles a twisted mass of tiny tubes that pass the blood. The glomerulus acts as a filtering unit, and keeps the normal proteins and cells in the bloodstream, allowing extra fluid and wastes to pass through. A complicated chemical exchange takes place, as waste materials and water leave the blood and enter the urinary system. First, the tubules receive a combination of waste materials and chemicals those the body can still use. The kidneys measure out chemicals like sodium, phosphorus, and potassium and release them back to the blood to return to the body. In this way, the kidneys regulate the body's level of these substances. The right balance is necessary for life. In addition to excretion of waste products, the kidneys release three important hormones [2]:

- Erythropoietin (EPO) that stimulates the bone marrow to make red blood cells
- Rennin that regulates blood pressure
- Calcitriol that helps maintain calcium for bones and for normal chemical balance in the body

1.1.1 Renal function

Kidney function or renal function depends on the efficiency of the kidneys to filter the blood and is estimated using glomerular filtration rate (GFR). The amount of filtrate that forms every minute (min) within the glomerulus is called the GFR. A person's estimated GFR (eGFR) is the best indicator of how well the kidneys are functioning. In adults, the GFR is approximately 120 mL/min [3]. Gradual loss of kidney function is called chronic kidney disease (CKD). A person with GFR less than 60 mL/min per 1.73m^2 of body surface area for three months or longer is classified as having CKD, irrespective of the presence or absence of kidney damage. Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies. All individuals with kidney damage are classified as having CKD, irrespective of the level of GFR. Risk factors for CKD include diabetes, high blood pressure, family history, older age, ethnic group and smoking. Evaluation methods for people at increased risk of CKD are to measure urine albumin and red blood cells in the urine to assess kidney damage and to estimate the GFR with an equation based on the level of serum creatinine. GFR of 90 or above is considered normal. The severity of CKD is described by five stages: [4-8]:

1) Stage 1 – Slightly diminished function; kidney damage with normal or relatively high GFR (above 90)
2) Stage 2 – Mild reduction in GFR of 60 to 89 with kidney damage
3) Stage 3 – Moderate reduction in GFR of 30 to 59
4) Stage 4 – Severe reduction in GFR of 15 to 29
5) Stage 5 – Established kidney failure with GFR of less than 15

Total and permanent kidney failure is called end stage renal disease (ESRD). People with ESRD must undergo dialysis or kidney transplantation for treating last stages of kidney failure.

Dialysis is the artificial process used to maintain the chemical composition of the blood. Hemodialysis (HD) and peritoneal dialysis are the two major forms of dialysis. HD uses a cellulose membrane tube or filter called a dialyzer that functions as an artificial kidney to clean a person's blood. Peritoneal dialysis uses the lining of the patient's abdominal cavity, known as the peritoneum as the dialysis membrane to clean a person's blood [9]. Kidney transplantation involves replacing a patient's failed kidney with a kidney from a healthy donor.

1.1.2 Chronic kidney disease: the global challenge

The worldwide rise in the number of patients with CKD's is reflected in the increasing number of people with ESRD treated by renal replacement therapy (RRT)-dialysis or transplantation [10]. The health burden of renal disease is high for patients and health services worldwide. In Australia, approximately sixteen percent of the population have some form of kidney damage present and are therefore at risk of renal disease [11]. Every year in Australia, almost one percent of adults developed CKD and another one percent of adults developed evidence of kidney damage [12]. The rate of people with a kidney transplant or receiving dialysis in Australia rose by twenty six percent between 2006 and 2007. CKD contributed to nearly ten percent of all deaths in 2006 and over a million Australian dollars (AUD) hospitalisations in the year 2006-2007 in Australia. Indigenous Australians were six times as likely as other Australians to be receiving dialysis or to have had a kidney transplant [13]. Australia's annual spending on end stage kidney disease (ESKD) is at least five hundred and seventy million dollars (AUD), and this is increasing annually by thirty million AUD. By 2012, the cost of maintaining people on hemodialysis will be thirty billion United States dollars (USD) for the United States (US) and eleven hundred billion USD worldwide [14].

More than fifteen percent of people in the US of age twenty or older have CKD [15]. In the United Kingdom (UK), analysis of prevalent transplants by CKD showed fifteen percent [16]. The overall incidence rate of RRT for ESRD across Europe in 2007 was hundred and sixteen per million population (pmp) [17]. CKD is more prevalent in Africa and seems to be of a more severe form than is found in Western countries. No reliable statistics describe CKD in all African countries, India and

Pakistan. The majority of those with CKD die because of lack of funds, as very few can afford regular maintenance dialysis in Africa [18, 19]. Due to the cost constraints, only about five percent of all patients with CKD in India and Pakistan end up having a transplant [20]. Nephrologic work in China started in early nineteen sixty's. Approximately five thousand patients receive renal transplantation every year [21]. The incidence of ESRD in China is estimated to be hundred and two pmp [22].

1.2 TRADITIONAL MEDICINE

World health organisation (WHO) defines TM as including diverse health practices, approaches, knowledge and beliefs incorporating plant, animal, and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness. TM refers to TM systems such as TCM, Indian ayurveda and Arabic unani medicine. A variety of indigenous systems have also been developed throughout history by Asian, African, Arabic, Native American, Oceanic, Central and South American and other cultures. The term complementary and alternative medicine (CAM) is used instead of TM in countries like Europe and/or North America and Australia to refer to a broad set of health care practices that are not part of a country's own tradition, or not integrated into its dominant health care system [23].

1.2.1 Widespread and growing use of traditional medicine

TM is widely used and is a rapidly growing health system of economic importance. In Africa up to eighty percent of the population uses TM to help meet their health care needs [24]. In China, TM accounts for around forty percent of all health care delivered, and is used to treat roughly two hundred million patients annually [25]. For Latin America, the WHO Regional Office for the Americas reported that seventy one percent of the population in Chile and forty percent of the population in Colombia have used TM [26]. The percentage of the population that have used CAM at least once is forty two percent in United States of America (USA), forty nine percent in France, seventy percent in Canada and forty eight percent in Australia [27-30]. In UK, almost forty percent of all general allopathic practitioners offer some form of CAM referral or access [31].

1.2.2 Expenditure of traditional medicine

In many parts of the world expenditure on TM/CAM is not only significant, but growing rapidly. Annual revenues in Western Europe reached five billion USD in 2003-2004. In China sales of products totalled fourteen billion USD in 2005. Herbal medicine revenue in Brazil was one hundred and sixty million USD in 2007 [32]. In Malaysia, an estimated five hundred million USD is spent annually on TM/CAM, compared to about three hundred million USD on allopathic medicine [33]. In Australia, an estimated annual national expenditure on CAM and CAM practitioners is almost one thousand million AUD. Of this, six hundred and twenty one million AUD is spent on alternative medicines [27]. A study of the practice of TCM in Australia, commissioned by the Victorian Department of Human services, New South Wales Department of Health, and the Queensland Department of Health, showed that the popularity of TCM was growing strongly. It is estimated that there are at least three million AUD TCM consultations each year in Australia. This represents an annual turnover of eighty four million AUD. The increasing popularity of TCM is also reflected in the four-fold increase in imports of Chinese herbal medicine (CHM) since 1992 [34]. In US, Canada and the UK, an annual CAM expenditure is estimated at twenty seven hundred million USD, twenty four hundred million USD and twenty three hundred million USD respectively [28, 29, 35]. The world market for herbal medicines based on traditional knowledge is now estimated at sixty thousand million USD [36].

1.2.3 Principles of traditional Chinese medicine

TCM is one of the oldest healing systems. Clinical diagnosis and Chinese herbology are very important components of TCM. CHM not only includes plants, but also includes medicinal uses of animals and minerals. Most of the principles of TCM were derived from the philosophical basis that contributed to the development of Taoism, and Confucianism. Taoism and Confucianism are traditional Chinese philosophical and religious principles of ethics, morals and politics that emphasize living based on the writings of authors Lao-tzu and Confucius respectively [37, 38]. Ancient Chinese scholars noted that all natural phenomena could be categorised into Yin and Yang. Yin and Yang describe the interdependent relationship of opposing but complementary forces believed to be necessary for a healthy life style and the goal is to maintain a balance of yin and yang in all things. According to TCM, everything in the universe consisted of five basic elements: wood, fire, earth, metal, and water, and the universe is constantly changing to achieve dynamic balance or harmony in all things, which is called five elements theory. Disease occurs due to falling out of balance and such knowledge was applied to understand, prevent, and cure disease [38].

The organs in TCM do not literally correspond to the anatomical organs such as heart, liver or spleen instead; they are better understood in terms of the functions they perform, which incorporate energetic, emotional and spiritual aspects as well as physical. There are two categories of organs in TCM. They are Zang and Fu organs, and these are not simply anatomical substances, but more importantly represent the generalization of the physiology and pathology of certain systems of the human body. The Zang or Yin organs are the solid organs and the Fu or Yang organs are the hollow organs. Each Zang organ is coupled with a Fu organ according to what is known as the "Husband-Wife Law." All of the organs are interrelated in a complex network as described by the five element theory. Zang organs are Lungs, Heart, Spleen, Liver and Kidneys and Fu organs are small intestine, large intestine, gall bladder, stomach and San Jiao: called as triple warmer or triple heater. The extra Fu organs are Brain, Uterus, Marrow, Bone and blood vessels. Yin refers largely to the material aspects of the organism and Yang to functions. There is a circulation of Qi and blood. Qi is an invisible energy force that flows freely in a healthy person, but is weakened or blocked when a person is ill. The organs work together by regulating and preserving Qi and blood through the so-called channels and collaterals (blood vessels). Disease occurs after a disturbance in Yin–Yang or flow of Qi or blood, or disharmony in the organs caused by pathogenic factors such as anger, joy, sadness, fear etc. and climatic factors such as wind, cold, damp, heat etc. Treatment aims to expel or suppress the cause and restore balance [39].

Diagnosis and treatment in TCM is entirely different to Western medicine. Western medicine is based on human anatomy and treatment is given only to the problematic area and patients with similar complaints or diseases usually will receive virtually the same treatment. TCM emphasizes on the role of entire body in healing and cures the "root cause of disease" and not merely treats the symptoms of disease. Patient's imbalance of harmony or diagnosis is assessed by four traditional examination methods: looking, hearing and smelling, questioning, and palpating. The diagnosis is derived with theories such as the eight diagnostic principles to differentiate between Yin–Yang, interior–exterior, deficiency–excess, and cold–hot, the five elements theory to assess the relations between organs and functions, and the visceral manifestation theory to establish the disease location [40].

The diagnosis that guides treatment in TCM is called Zheng, a temporary state at one time and which is like a syndrome defined by symptoms and signs. The same disease in western medicine can manifest in different Zheng's and vice versa. Thus, treatment in the same patient varies over time and the same disease can be treated differently. The treatment in TCM falls into four main categories: herbal medicine, acupuncture, dietary therapy, massage and exercise. A typical TCM prescription consists of a complex variety of many different herbal and mineral ingredients. Herbal formulas are usually given as teas, which differ according to the patient. For example, Kidney Yin deficiency as a Zheng has three components: kidney, Yin, and deficiency. For each or a combination of the components, there are specific herbs or treatments. For example, bitter herbs are cool in nature and can be used to treat heat-ridden diseases. TCM can make diagnoses and treat patients without needing a scientific understanding of cause and pathogenesis [40].

1.2.3.1 The Kidney in Chinese medicine

The Kidney is a Zang organ also known as a Yin organ. The Kidneys are situated in the lumbar region, each on either side of the spinal column and the space between them is known as the San Jiao or triple burner. The triple burner, however, is said to be primarily energetic and does not have a physical component, unlike all the other organs in TCM. The main functions of Kidney include [40]:

- The Kidneys store Jing and dominate reproduction, growth and development

Jing is the essence of life and is stored in the Kidneys. It is in part inherited from our parents and in part refined essence extracted from food. When kidney Jing is impaired in any way-often for constitutional reasons- then this can lead to retarded growth, learning difficulties, infertility, sexual disorders or premature senility.

- The Kidneys produce marrow, fill up the brain, dominate the bones, and manufacture blood

Kidney Jing is responsible for the production of Marrow. Marrow has a role in manufacturing blood. In TCM, Marrow is the essential element of bone, Bone Marrow, the spinal cord, and brain structure. Thus, healthy Kidney Jing results in strong bones, teeth and efficient brain function. If Marrow production is impaired in any way then this may result in blurred vision, impaired thinking, tinnitus, and blood deficiency.

- The Kidneys maintain the gate of vitality (Mingmen fire)

In TCM, the Mingmen fire is the source of all heat: Yang aspect in the body. If the Kidney Yang energy is deficient, this will result in general coldness, lethargy, and/or impaired sexual function.

- The Kidneys govern water

A central function of the Kidneys is that of regulating the fluid balance in the body. When the Kidneys are functioning well they are able to send the clear fluids back to the Lungs and excrete the impure fluids through the Bladder. If the Kidney function is at all impaired then this can lead to a whole range of urinary problems.

- The Kidneys control the reception of Qi

This function represents the harmonious relationship between the Kidneys and the Lungs. If the Kidney function is impaired, then this can lead to the Qi rebelling upward, causing breathing difficulties and leading, in extreme cases, to chronic asthma.

- The Kidneys open into the ear

  The ears rely on Kidney Jing for nourishment, and if this is in any way lacking, then it can lead to tinnitus and deafness.

- The Kidneys are made manifest in the hair

  The hair also relies on Kidney Jing for nourishment. If there is a Kidney Jing deficiency, then it is liable to lead to dull, lifeless and brittle hair. It also can lead to premature greying and thinning.

- The Kidneys house the will and control fear

  The connection between will power and the emotion of fear is seen in the Kidneys. The kidneys are seen as the root of life, and thus our sense of personal power and will to succeed in life are rooted in healthy Kidney functioning. Poor Kidney functioning will lead to feelings of weakness and timidity.

1.3 Conclusions

Healthy kidneys perform many essential services to our bodies mainly filtering the blood and producing waste products and urine. Kidney function or renal function is essential and depends on the ability of the kidneys to filter the blood that is estimated using GFR. Progressive loss of kidney function leads to CKD. When the CKD has worsened to the point at which kidney function is less than ten percent of normal, ESRD occurs. People with ESRD must undergo dialysis or kidney transplantation to stay alive. Millions of people around the world suffer from kidney diseases, and these patients will eventually need a form of RRT. Dialysis and kidney transplantation save lives but with great costs, that are becoming a major issue in western countries because they account for a significant proportion of healthcare expenditure. The condition in most developing countries is different in that the patient provides the bulk of fund for the dialysis or RRT. Due to cost constraints, the majority of the people with CKD die in developing countries. TM and CAM have claimed an increasing share of the public's awareness. Studies have documented that about half of the population and many industrialised countries use TM/CAM, and the proportion is as high as eighty percent in many developing countries.

TCM is an ancient medical system that has been practiced for more than five thousand years to diagnose and cure illness. Western medicine looks closely at a symptom and tries to find an underlying cause, whereas TCM looks at the body as a whole and its principle is treating the root cause of illness and not the symptoms. The clinical diagnosis and treatment in TCM are mainly based on the theories of yin-yang, five elements, internal organs, Qi, and blood. Treatment therapies of TCM include Chinese herbs, acupuncture, nutrition, massage and exercises (known as Tai Chi or Qi Gong). Herbal medicine is a major pillar of Chinese medicine. Herbal preparations have been used in China, for over three thousand years that involved plant, mineral, and animal substances to treat ailments.

2 LITERATURE REVIEW

2.1 INTRODUCTION

Chinese medicine has always been a medicine of empiricism. Chinese herbology is very important component of Chinese medicine. CHM not only includes plants, but also includes medicinal uses of animals and minerals. The chemical constituents in plants such as trace elements are partially responsible for their medicinal and nutritional properties. For human beings, trace elements are essential nutrients with a range of functions. Trace elements as essential components in medicinal herbs play important role in biological processes. The ultimate objective of the use of medicinal herbs is a positive interaction with the biochemistry of the body that aids in treating ailments. The use of Chinese herbology in the form of decoction to improve kidney function has accumulated over many centuries for their use worldwide.

2.2 UNDERSTANDING CHINESE HERBS

Traditional Chinese medical substances provide a rudimentary knowledge base – one of the “building blocks” used to develop the necessary clinical skills needed in the correct prescribing in Chinese herbs. Chinese herbs can be considered in terms of the following classifications [41]:

2.2.1 The four energies

The four essential energetic qualities of herbs, and their corresponding actions, relate to their perceived temperatures:

Cool or cold herbs relieve conditions where there is heat in the body, while warm or hot herbs relieve cold symptoms. Some herbs are neither hot nor cold, and in essence they describe a fifth energy, that of neutral herbs. For example: Sheng Di Huang: fresh rehmannia root is a cool/cold that relieves heat. Rou Gui: cinnamon bark is a warm/hot that relieves cold symptoms. Fu Ling: poria is a neutral herb. According to the yin-yang theory, the cold and cool natures are yin while the hot and warm natures are yang [41].

2.2.2 The five tastes

The five taste qualities of herbs relate to their action on the Qi of the body. These tastes describe the therapeutic effect of herbs.

- Pungent/acrid herbs disperse and promote the movement of Qi in the body and invigorate the blood
- Sweet herbs tonify and strengthen the Qi and nourish the blood
- Sour/astringent herbs absorb body substances and control the functions of the Zang-fu
- Bitter herbs reduce excess Qi and dry excess moisture
- Salty herbs soften lumps
- Some herbs described as "bland", are relatively neutral in terms of taste.

For example: Hong Hua: safflower that is pungent/acrid invigorates the blood. Ren shen: ginseng root that is sweet in nature tonifies the Qi. Wu Wei Zi: schisandra fruit that is sour in nature relieves spontaneous sweating. Huo Po: magnolia bark that is bitter in nature dries and transforms dampness. Mang Xiao: Glauber's salt that is salty in nature clears constipation [41].

2.2.3 The movement of herbs

Since herbs are said to move through the energetic system of body, specific herbs can be used to target specific parts of the body, or to facilitate the movement of other active herbal ingredients. The four basic "movements" are as follows:

Herbs that ascend and float tend to move upward and outward, influencing the top part of the body and the extremities. Herbs that descend and sink, on the other hand, move downward and inward, influencing the lower part of the body and the interior. For example Jie Geng, the platycodi root or "balloon flower" ascends, opens and dispels lung Qi. Da Huang: rhubarb descends and relieves constipation [41].

2.2.4 Entering the channels

In CHM, individual herbs are thought to "enter" specific channels or meridians and are therefore targeted toward the Zangfu system associated with the channel. For example, when it is said that the herb Da Zao: Chinese date enters the spleen and stomach channels, it is suggested that the function of the herb relates to the function of the theory of the Zangfu. Da Zao is therefore used by herbalists to tonify the spleen and augment the Qi.

The Chinese herbal medicine practitioner considers how the energetic qualities of an herb in terms of the four energies and the five tastes interact to describe its function and action. At the same time, the practitioner also considers of which Zang-fu system is being influenced. A Chinese herbal formula is a complex cocktail of energetic qualities, functions, directions, and foci, and it takes skill to pitch ingredients and dosage at the correct level in order to address the symptoms of a patient's disharmony [41].

2.3 COMBINING CHINESE HERBS

In TCM, herbal formulas are constructed according to set principles. Essentially, it has been an empirical process in which the properties of herbs and the effects of combining them have been observed and recorded over many centuries. Classically, this entails ranking herbs as "Emperor, Minister, Assistant and Emissary". The Emperor is the main herb, necessary for the treatment of the disease; the Minister is the herb which assists the Emperor; the Assistant herbs restrain and neutralise the unwanted side effects of the main herb; the Emissary is the herb which answers to the Minister.

When herbs are mixed together they undergo complex changes. Some herbs become stronger, some become weaker, and some even become harmful, which is called "The Seven Features of Interaction". Herbs that are combined are selected and classified based on these affects to modify their therapeutic action [41].

- Single action – use of single herb
- Reinforcement – use of two herbs with similar functions to strengthen each other's therapeutic effect

- Assistance – use of two herbs with different functions
- Restraint – use of one herb to restrain the action or toxicity of another
- Counteraction – use of one herb to control the nature of another
- Neutralisation – use of one herb to eliminate the toxicity of another
- Incompatibility – use of two herbs to create violent side effects

2.3.1 Understanding Chinese herbal formula

The art of prescribing Chinese herbs lies not so much in identifying which basic formula to use, but in knowing how and when to adapt that basic formula to meet the changing therapeutic needs of the patient. An example of how the formula works is explained, Du Huo Ji Sheng Tang is a formula that is prescribed for patients suffering from painful arthritic joints, stiffness, tiredness, and lethargy. These conditions in Chinese medicine are seen as the external invasion of the channels by wind, damp, and cold. The invading factors tend to lodge in the channels at the joints, producing the pain and swelling, which are characteristics of arthritis. The name of the formula is built up from two main herbs Du Huo and Sang Ji Sheng. The end of the name very often describes how the formula is prepared. Thus Tang means "soup" or "decoction". As described in Table 2.1, several herbs used in this formula warm the channels, expel wind, cold, and damp and relieve pain. This is the main focus of the formula. In addition, other herbs move and nourish the blood, encouraging the flow and lubricating and nourishing the joints, bones and sinews. The formula also contains Qi tonic herbs to help strengthen the spleen, promote the production of Qi, and build up the body's immunity [40].

In a complex formula containing a large number of herbs with their own energies and tastes, it is important that they all work well together. A practitioner may well start treatment with Duo Huo Ji Sheng Tang formula as given, but as the treatment process continues, the ingredients and doses may change in response to how the patient reacts.

Table 2.1: Herbs in Duo Huo Ji Sheng Tang and their actions

Herb	Action
Du Huo	Expels wind, damp, and cold from the lower part of the body, and from bones and sinews
Sang Ji Sheng	Expels wind and damp, tonifies the liver and kidneys
Xi Xin	Clears wind and damp from bones, scatters cold in the channels, stops pain
Fang Feng	Expels wind and clears damp
Qin Jiao	Expels wind and damp, relaxes the sinews
Du Zhong	Expels wind and damp, tonifies the liver and kidneys
Nui Xi	Expels wind and damp, tonifies the liver and kidneys
Rou Gui	Expels wind and damp, warms the channels, strengthens the Yang
Chuan Xiong	Nourishes and invigorates the blood
Sheng Di Huang	Nourishes and invigorates the blood
Bai Shao	Nourishes and invigorates the blood
Dang Shen	Tonifies the Qi, strengthens the spleen
Fu Ling	Drains dampness, tonifies the spleen
Zhi Gan Cao	Tonifies the Qi, harmonizes the action of the herbs

Source: [42]

2.3.2 Heating Chinese herbal formula – decoction

The herbs involved in the formula are placed in the water, and then boiled for a specific period of time. The liquid is strained from the mixture and ingested through the mouth. One of the primary advantages of decoction is that it is rapidly absorbed by the body; its effects are strong and immediately perceived by the patient. It is very important to correctly follow the procedure for decocting the formula as the result depends not only on the ingredients but also on the correct procedure [43].

Equipment

The preferred pots used for decoction should be ceramic, earthenware or stainless steel. The pots must have a tight fitting lid and be clean. Iron or aluminium pots should not be used as they can cause unknown chemical reactions when herbs are decocted in them [43] .

Water

Tap water is usually good enough for decoctions. In areas where the tap water is polluted or has high mineral content, distilled or clean bottled water may be substituted. Where special types of water are required, they are specifically noted under the method of preparation for the particular formula. The amount of water should generally cover the herbs by about one-half inch. For 30g of herbs, 200 – 300 mL of water should be used. Minerals and shells absorb very little water, while roots, leaves, and flowers will absorb more [43].

Type of Heat

The Chinese traditionally distinguish two types of heat for preparing herbs: the high flame or 'military fire', and the low flame or 'civilian fire'. Usually the decoction is brought to boil using a high flame, and then heated on a lower flame [43].

Method of decoction

When the herbs have been put together in the pot and covered with appropriate amount of water, they are allowed to soak for a while. This will facilitate the extraction of the active ingredients during the process of decoction. Most formulas are heated for 20 – 30 min. However, to be most effective for the formulas that release the exterior, clear heat, or contain herbs with volatile oils should be heated over a relatively high flame for a shorter period of time (10 – 15 min). Tonics and other formulas that containing rich and cloying substances should be heated over a relatively low flame for a longer period of time (45 – 60 min) to extract as much from them as possible [43].

2.4 Chemical Constituents In Plants - Elements

The chemical constituents present in plants are responsible for their medicinal as well as toxic properties which include vegetable bases comprising of alkaloids and amines, glycosides, essential oils responsible for their characteristic odour, toxic substances known as toxalbumin, resins and antibiotics. Whereby, trace elements play a very important role in the formation of these compounds. One important factor for the formation of active constituents in medicinal plants are the trace elements because they are known to play an important role in plant metabolism and active constituents of medicinal plants are metabolic products of plant cells [44].

Trace elements accumulated in medicinal plants have the healing power in numerous ailments and disorders. The absence or deficiency of an element brings abnormalities that can be connected to specific biochemical changes reversed by supplying the element. The process of absorption from the gastrointestinal tract and excretion with body fluids, therefore, are major ways in which the concentration and amount of an element can be controlled in the body. Even though a direct link between elemental content and its curative capability is yet to be established, the data on major, minor and trace elemental contents in plants is of vital importance to understand the pharmacological action of the herbs [44].

Minerals are of critical importance in the diet, even though they comprise only 4-6% of the human body. The exact classification of trace versus macro minerals is not clear cut, but traces are often considered as minerals required by the body in amounts less than 100 mg daily and make up less than 0.01% of bodyweight. Essential trace elements are zinc, iron, silicon, manganese, copper, fluoride, iodine and chromium. Major minerals are those required in amounts greater than 100 mg per day and they represent 1% or less of body weight. These include calcium, phosphorus, magnesium, sulphur, potassium, chloride and sodium that exist in body fluids or tissues. While some of them are vitally important for health, the roles of others are unclear. Recommended intakes have been set for some trace elements and their deficiency can lead to disease, but lack of others does not cause any recognised problems. The recommended dietary allowance (RDA) and adequate intakes, and tolerable upper intake level of some elements for adults are given in **Table 2.2** [42].

Table 2.2: Dietary Reference Intakes (DRIs) of elements

Element	Recommended Dietary Allowances [a] and Adequate Intakes-range [b] (mg/day)	Tolerable Upper Intake Level (mg/day)
Ca	1000 - 1300 [a]	2500
Fe	8 - 18 [a]	45
Mg	310 - 420 [a]	350
Mn	5 - 5.5 [b]	NE
K	2800 - 3800 [b]	NE
Na	460 - 920 [b]	2300
Zn	8 - 14 [a]	40

NE – not established
Recommended Dietary Allowances are set to meet the needs of all (Adults – women and men age from 19 to 70 yr and >70 yr).
Tolerable Upper Intake Level, the maximum level of daily nutrient intake that is likely to pose no risk of adverse effect.
Source: [42]

2.4.1 Role of nutrition in renal diseases

For decades, the treatment of cases of renal failure has progressively emphasized the relevance of nutrition as an integral part of medical care. Thus, long-term maintenance of tissue function, including growth of the child under treatment for renal insufficiency, becomes a challenge to our knowledge of nutrient needs. Dietary intake plays a crucial role in the management of abnormalities associated with chronic renal disease. These include retention of nitrogenous metabolites, decreased ability to regulate plasma or tissue levels of sodium, magnesium, phosphorous, calcium, potassium, water and other compounds, and certain vitamin deficiencies. In addition, wasting is commonly seen in uremic patients and may have adverse clinical effects. The wasting is engendered by poor dietary intake, losses of nutrients during dialysis, altered amino acid and protein metabolism associated with renal failure and impaired metabolic functions of kidney [45, 46].

2.4.2 Role of trace elements in renal diseases

For human beings trace elements are essential nutrients with a gamut of functions. They are for instance indispensable components of many enzymes, so they have some regulatory functions, and they may affect immune reactions and free radical generation. Abnormalities of trace elements are primarily the results of uremia, and they may be further modified and sometimes greatly exacerbated by the dialysis procedure. Hsieh *et al* found that hemodialysis patients had significantly lower serum concentrations of Zn, Se, and Mn [47]. Zinc deficiency has been suggested as a possible cause of some typical uremic symptoms, including immunological impairment, abnormalities of taste and olfaction, growth retardation in children, and testicular atrophy in men [48]. Kiziltas *et al* examined the status of trace elements (Cu, Zn, and Fe) and minerals (Mg, K, Na, and Cl) and the level of biochemical parameters (urea, creatinine, total protein, albumin, and glucose) in HD patients. The results showed that there is lower Zn, Fe and albumin levels and higher Mg, K levels in the after dialysis group of patients. The results also suggest that the relationship between creatinine and K, Mg and Zn could be ascribed to the loss of renal function [49]. Serum metal contents could be tested in dialytic patients before treating with appropriate decoctions.

Rapid decline in renal function leads to acute renal failure (ARF). As a result, excretion of nitrogenous waste is comprised and fluid and electrolyte balance cannot be maintained [50]. Malnutrition is common in patients with ARF and is likely contributor to increases morbidity and mortality [51, 52]. Alterations in blood and tissue trace element levels have been described in chronic renal failure (CRF) patients undergoing intermittent hemodialysis (IHD) [53-56]. Increased or decreased plasma zinc levels and increased plasma copper levels have been reported. A possible zinc deficiency – induced gonadal dysfunction in IHD patients has been proposed, which was reversed by adequate zinc supplementation [57-59]. Critically ill ARF patients are often treated with continuous renal replacement therapy (CRRT). Although CRRT removes waste products, it also removes beneficial medications and trace elements. Churchwell *et al* reported loss of measurable quantities of chromium, copper, manganese, selenium and zinc in the CRRT patients [60]. The elements considered for this study are discussed below:

2.4.2.1 Iron

Iron is critical to the functioning of all cells in the body and is the most essential nutrient for the creation of healthy erythrocytes with adequate haemoglobin (Hgb) content. One of the primary roles of iron is to carry oxygen as part of Hgb [61]. Iron deficiency: Anaemia is a common complication of CKD and is associated with poor outcomes including higher death risk in both maintenance dialysis patients [62], and in individuals with non-dialysis-dependent CKD stages 3 to 5 [63]. It is estimated that the number of adults with CKD associated anaemia is 480,000, 840,000, and 1,590,000, when anaemia was defined as Hgb <10, <11, or <12 g/dL, respectively [64]. Anaemia leading to low Hgb levels, is a pervasive problem in patients with ESRD on HD [65]. Erthyropoiesis stimulating agents (ESA) and iron supplementation therapy form the cornerstone of anaemia management in CKD [66].

2.4.2.2 Magnesium

Magnesium plays a major role in overall functions, including DNA and protein synthesis, glucose and fat metabolism, oxidative phosphorylation, neuromuscular excitability, and enzyme activity [67]. Up to 70% of total plasma magnesium is ionized and is freely filterable by glomerular function, while 30% is protein bound [68]. An adequate serum magnesium concentration may be necessary to maintain renal function and protect the kidneys from damage. The kidney is a major regulator of total body magnesium homeostasis. Hypomagnesemia is defined as a serum magnesium concentration of less than 1.8 mg/dL. Most patients with hypomagnesemia are asymptomatic and these patients should be treated with oral magnesium supplements [69]. Hypomagnesemia is often found in patients with renal failure [70, 71]. Hypomagnesemia often coexist with other metabolic disorders, such as hypocalcemia or hypokalemia [72]. Hypomagnesemia may results from inadequate magnesium intake, increased gastrointestinal or renal losses, or redistribution from extracellular to intracellular space. Increased renal magnesium wasting can result from genetic or acquired kidney disorders [73, 74]. Loss of magnesium inhibits the restoration of renal tubules, resulting in renal failure [75]. Magnesium wasting enhances drug-induced nephrotoxicity [76] whereas magnesium supplementation protects against post-ischemic ARF [77].

2.4.2.3 Manganese

Manganese is a transition element associated with a large number of enzymes (as a constituent of metalloenzymes and as an enzyme activator), e.g. hydroxylases, kinases, decarboxylases and transferases. Hosokawa *et al.* found serum concentrations of manganese to be low in chronic HD patients and noted deficiency was correlated with decrease in creatinine clearance and total serum protein levels [78]. Hsieh *et al.* also found decreased serum manganese concentrations in HD patients [47].

2.4.2.4 Potassium

Potassium is an essential dietary mineral. It constitutes the main intracellular electro- and osmolyte necessary for fundamental processes such as membrane excitability, ion and solute transport or cell volume regulation [79]. The kidneys execute the major share of removing the dietary potassium. About 90% of the daily ingested potassium is excreted in an average amount of 1.5 L of urine [80]. Augmented intestinal potassium excretion becomes a relevant quantitative phenomenon in ESRD, where the colon is able to partially substitute for the reduced renal potassium excretory capacity [81-83]. The concentrations of potassium in plasma should be within 3-5 mmol/L. Krachler *et al.* determined plasma concentrations of potassium in chronic HD patients. They found that plasma potassium concentration in HD patients decreased steadily during HD compared to levels of healthy adults [84].

2.4.2.5 Sodium

Sodium is the main representative of the extracellular electrolytes that are substantial for maintaining the osmotic balance between extra- and intracellular space. The plasma levels of sodium should be in the range of 135-145 mmol/L [84]. Uremic patients retain nitrogenous wastes and have an elevated plasma osmolality. While urea exhibits osmotic activity in serum, no sustained gradient can be established across cell boundaries because it readily diffuses through cell membranes. Thus, sodium remains the major indicator of body tonicity and determines the distribution of water across the intracellular-extracellular boundary, subsequent cell volume, thirst, and among patients with renal insufficiency, systemic blood pressure [85]. Dietary advice for the patients undergoing HD should be customized to ensure that they do not become malnourished [86]. Krachler *et al.* found that plasma sodium levels in HD patients did not vary much compared to the healthy adults [84].

2.4.2.6 Zinc

Zinc is one of the most important trace elements in the body. Being a catalytic component of more than 300 enzymes, zinc has a structural and biological role in many proteins, peptides, hormones, transcriptional and growth factors and cytokines, involved in the various steps of immune development and reactivity [87, 88]. Zinc deficiency has a prevalence of 40-78% in HD patients [89, 90]. Bozalioglu *et al.* showed that patients on maintenance hemodialysis for a short time exhibit zinc deficiency and disturbed immune response [91]. Roozbeh *et al.* studied the effects of zinc supplementation on the concentration of serum zinc levels in HD patients. They found that maintenance HD patients with both low serum zinc and low serum total cholesterol levels may benefit from zinc supplementation [92]. Tang *et al.* found that zinc supplementation can partially prevent the kidney from diabetes-induced pathological changes, likely through renal metallotheonien induction [93]. Esfahani *et al.* showed that Zn deficiency may contribute to various conditions and symptoms in children undergoing chronic HD [94]. Zinc deficiency could contribute to the development of atherosclerotic cardiovascular disease, mainly in obese CKD patients [95].

2.4.3 Mineral and bone disorders in renal diseases

Bone tissue is a complex, metabolically active organ of which bone mineral is composed essentially of calcium and phosphate salts. These salts are essential for normal skeletal growth, the maintenance of skeletal mechanical integrity and as a pool for the extra cellular calcium compartment [96]. Calcium also regulates parathyroid hormone secretion. Calcium, a major component of bone matrix, has received considerable attention in the prevention and treatment of bone loss. Calcium plays a messenger in universal cellular signal transmission. Calcium intake is positively related to calcium balance, and calcium supplementation benefits appendicular cortical bone mass [97]. In renal failure, hypocalcemia may result from reduction in calcitriol synthesis, elevated serum phosphorus and impaired intestinal calcium absorption [98, 99]. In adults, calcium supplementation reduces the rate of age-related bone loss [100]. CKD is highly prevalent in older people living in residential homes. Secondary hyperparathyroidism, poor calcium intake and deficiency of 1, 25-dihydroxyvitamin D may lead to decreased bone mass in people with CKD. Carter *et al* assessed bone mineral metabolism and its relationship to kidney function in older people who were predominantly not taking vitamin D and calcium supplements. They reported that vitamin D replacement with 25 hydroxyvitamin D plus calcium supplementation appears to be at least partially effective in ameliorating secondary hyperparathyroidism [101].

2.4.3.1 Effect of traditional Chinese medicine on bone metabolism: multi-component formulations

TCM use traditional formulae containing combinations of herbs in the treatment of osteoporosis. Zhang *et al.* tested the synergistic effect of trace elements and falvonoids from *'Epimedium koreanum naki'*, a TCM. They showed that the combination of icariin or total falvonoids with mineral elements (Zn, Ca, and Mn) revealed either synergistic, or antagonistic effect in increasing alkaline phosphatise activity and improving primary osteoblast viability [102]. *'Shu Di Shan Zha'* is a traditional Chinese herbal formula recommended for bone health. Xu and Lawson investigated its effects on 14 menopausal women who administered for 4 months. The formula had a positive effect on bone structure and function [103]. *'Shen Gu'* is another herbal preparation used for similar purposes, and its efficacy has been evaluated in a clinical trial by Mingyue *et al* that involved 96 osteoporotic patients. After six months bone mineral density (BMD) was enhanced and bone resorption was inhibited [104]. Chen *et al* also investigated the effects of TCM's on bone loss: *Hachimi-jio-gan* and *Juzen-taiho-to* significantly prevented bone loss in a murine model for senile osteoporosis. Two of the main components of Hachimi-jio-gan are Rehmanniae radix and Hoelen [105]. Liao *et al* examined the effects of *'Yanghuogubao'* on osteoporotic male rats and showed that it could arrest the decline of BMD [106]. *'Kami-kihi-to',* another herbal preparation traditionally used for bone health, was tested on ovariectomized (OVX) rats for 3 months showed an increased BMD [107]. *'Bushen Ningxin'* decoction also showed an increased BMD when given to OVX mice for 12 weeks [108]. *'Jiangu'* granules increased BMD in OVX rats [109]. *'Dang-gui-ji-hwang-yeum'* is a well-established formulation used throughout China, Japan and Korea for the management of osteoporosis. Chae *et al.* administered OVX rats with this formula and showed that trabeculae area and thickness increased significantly from OVX control rats [110]. Another traditional Chinese preparation consisting of ten herbs, *'Hochu-ekki-to'* was tested for bone health enhancing properties on rats treated with gonadotropin-releasing hormone agonist burserelin and found that BMD of the formula treated rats increased to 106.2% after 56 weeks [111]. 'Osteocare', another herbo-mineral preparation, significantly restored femoral weight and density after 16 weeks in OVX and calcium deficient rats. Reddy *et al.* reported that it also decreased the number of osteoclasts and reversed the effect of OVX on trabeculae [112].

2.4.3.2 Single Components - herbs

The majority of studies done to date on herbs are pharmacological, whereas clinical studies on herbs are minimal. The following herbs used in this study have all shown some beneficial effect on OVX rat models of osteoporosis *in vivo: Drynariae rhizome* (Gu Sui Bu) is a major component in 56 of 73 fracture prescriptions in TCM. Its effects on protease activity involved in the initiation of bone loss in rats and mice were tested by Jeong *et al.* They showed that concentrations of the herb (≤120 µg/mL) enhanced proliferation, whereas >250 µg/mL was inhibitory [113]. Later *in vivo* work has confirmed these results [114]. The herb *Epimedium* (Yin Yang Huo) has been used in TCM for many years in the treatment of bone disorders. Meng *et al.* investigated the activity of *Epimedium brevicornum Maxim* on osteoblast-like UMR-106 cell line, which showed proliferative activity [115]. Liu *et al.* found that Herba Epimedii (HEF) increased the alkaline phosphatase activity, osteocalcin secretion and calcium deposition in rat osteoblasts [116]. Qian *et al.* showed that high dose of HEF (160 mg/kg) for 12 weeks could stimulate osteocalcin expression [117]. *Eucommiae ulmoides* (Du Zhong) has been investigated by Ha *et al.* and is thought to enhance osteogenesis by activating osteoblasts and suppressing osteoclasts [118]. Fructus Ligustri Lucidi (Nu Zhen Zi) is a 'kidney-tonifying' Chinese herb. Its effects on markers of bone turnover, calcium metabolism and balance were evaluated in an OVX rat model. Hence, it is proved that it is a possible prospect for a natural supplement to help treat osteoporosis [119].

2.4.4 Chinese herbs known to be effective in CKD

For treatment of CKD, the therapeutic principle of TCM is to "drain damp": regulating immune system and promoting urination, "activate blood and resolve stasis": anticoagulation, "replenish vital energy and nourish blood": supportive treatment and antianemia, and "coordinate Yin and Yang in the body": stabilization status [120]. There are hundreds of Chinese herbs used to treat CKD. **Table 2.3** shows the herbs most commonly prescribed. Some prescriptions and single herbs are made into patent medicines in China. In Chinese literature, there are several clinical reports regarding the beneficial effects of different single herbs, decoctions, and patent medicines for dieresis, reducing proteinuria, improvement of hypoalbuminemia, hyperlipidemia, and renal insufficiency in both diabetic and non-diabetic CKD [121-124]. Their mechanisms of action are mainly related to antioxidation, antifibrosis, and improvement of metabolic disturbance in CKD.

Table 2.3: Names of common Chinese herbal medicine for treatment of CKD

Chinese Name	English Name	Species Name
Huang Qi	Mongolian milkvetch root	*Astragalus mongholicus*
Mo Jia Huang Qi	Mongolian milkvetch root	*Astragalus membranaces*
Da Huang	Rhubarb, sorrel rhubarb	*Rheum palmatum L*
Lei Gong Teng	Common threewingnut root	*Tripterygium wilfordii*
Dong Chong Xia Cao	Chinese caterpillar fungus	*Cordyceps sinensis*
Dang Gui	Chinese angelica	*Angelica sinensis*
Chuan Xiong	Szechwan lovage rhizome	*Ligusticum chuanxiong*
Dang Shen	Pilose asiabell root	*Codonopsis pilosula*
Di Huang	Adhesive rehmannia root tuber	*Salvia Miltiorrhiae*
Fu Ling	Poria	*Poria cocos*
Cang Zhu	Swordlike atractylodes rhizome	*Atractylodes lancea*
Fang Ji	Fourstamen stephania root	*Stephania tetrandra*
Ze Xie	Oriental waterplantain tuber	*Alisma orientale*
Yi Yi Ren	Ma-yuen jobstears seed	*Coix lacryma-jobi L*
Shan Yao	Common yam rhizome	*Dioscoreae opposita*
Gou Qi Zi	Barbury wolfberry fruit	*Lycium barbarum L*
Che Qian Zi	Asiatic plantain seed	*Plantago asiatica L*
Shan Zhu Yu	Common macrocarpium fruit	*Macrocarpium officinale*
Gan Cao	Liquorice root	*Glycyrrhiza uralensis*

Source: [125]

Cordyceps sinesis (Chinese caterpillar fungus) is believed to promote tissue repair. Animal studies have shown that *Cordyceps sinesis* delays progression of worsening kidney function in 5 or 6 nephrectomized rats through the inhibition of glomerular hypertrophy, reduction of proteinuria, and reversal of metabolic abnormalities of protein and lipid profile [126].

Species of *Astragalus* family have been observed to have diuretic actions [127], providing some support for their use in numerous Chinese formulas for renal disorders. In addition, *in vitro* [128] and *in vivo* [129] studies support their use in the protection against gentamicin toxicity, probably due to antioxidant effects and their ability to alter renal kinase activity. As Astragalus contains numerous active components, including falvonoids, polysaccharides, triterpene glycosides, amino acids and trace minerals, it is traditionally prescribed depending on the desired therapeutic effect and the exact diagnosis [130]. *Astragalus mongholicus* in combination with *Angelica sinensis* studied in an animal model reduced deterioration of kidney function and histologic damage [124].

2.5 TCM FORMULAE PRESCRIPTION

The TCM formulae prescription is a subject dealing with treatment of theories of compatibility of prescriptions as well as the clinical application. A prescription is composed of selected drugs and suitable doses on syndrome differentiation for etiology and the composition of therapies in accordance with the principle of formulating a prescription. It serves as a chief means of treating diseases clinically and links the basic theories with clinical practices. TCM formulae involved in this study are discussed below.

2.5.1 Liu Wei Di Huang Wan

Liu wei di huang wan (LW) decoction is a classical TCM prescription used clinically in the treatment of the diseases manifesting “kidney-yin” deficiency syndrome such as cancer, diabetes, autoimmune diseases, and the diseases related to disorder of endocrine function including climacteric syndrome, sexual inadequacy, sterility and hyperplasia of prostate. In TCM theory, “kidney” is thought to be the most important organ in modulating the functions of the other “organs”, and plays a very important role in modulating the growth, development, reproduction and ageing process of the organic body [131]. In modern clinical practice, LW has been used in the treatment of many kinds of diseases related to dysfunction of the hypothalamus-pituitary-ovary (HPO) axis such as menopausal syndrome and sterility, etc. In a study of the function of “kidney”, Zhang postulated that it is quite similar to the function of the neuroendocrine immunomodulation (NIM) network [131]. Researchers demonstrate that the balance of NIM network plays a key role in keeping the normal physiological function of the body. Therefore, kidney deficiency syndrome may possess some similar pathophysiological changes to those of the imbalance of the NIM network, and accordingly, the effect of kidney-nourishing effect of TCM may relate to the modulating effect of NIM network. It was studied that LW processes wide pharmacological effects such as ameliorating learning and memory effect, and modulating immune function [131]. Zhang *et al* studied the age-related functional change of HPO axis and the effect of LW in senescence-accelerated mouse (SAM) and suggested that LW modulates or improves the imbalance of HPO axis in SAM during aging process [132]. One of the important ways of the cognitive enhancing effect of LW is to correct the abnormal expressions of cognition-related genes in the hippocampus [133, 134] and the level of sex hormone [135]. A pharmacological study also revealed that the ameliorating effect of LW on memory dysfunction might be due to increased cholinergic nervous activity and decreased serotonergic nervous activity [136].

2.5.2 Er Zhi Wan

Er zhi wan (EZW), a famous TCM formulation, has been developed for hundreds of years. EZW is widely used to prevent and treat various kidney diseases for its actions of nourishing the kidney yin and strengthening tendon and bone. It possesses the actions of tonifying the liver and kidney yin, nourishing a body's essential fluid and arresting hemorrhage [137]. It is widely used in China to treat kidney and Alzheimer's diseases, invigorate the body, and modulate the immune system in clinical applications [138]. Modern research shows that anti-osteoporotic activity of EZW is carried out mainly by restraint of osteoclastic bone resorption, which is in accordance with the TCM theory on nourishing kidney yin. Therefore EZW has potency to develop a new anti-osteoporotic agent for clinical usage [139]. Cheng *et al.* showed that EZW had a definite anti-osteoporotic effect without hyper-plastic effect on uterus, and it might be a potential alternative medicine for treatment of postmenopausal osteoporosis [140].

2.5.3 Zuo Gui Wan

Zuo gui wan (ZGW), a TCM has effect to invigorate the kidney and is widely used for deficiency of body fluid, night sweat, sore waist, nocturnal emission, mental fatigue and dry mouth [137]. Modern pharmacological studies showed that it also had contribution to promote bone marrow cells forming into liver cells, delay aging, and protect from leucopenia induced by cyclophosphamide [141]. The functions of kidney in TCM include not only the function of the kidney as known in western medicine, but also the functions of the reproductive and endocrine systems. According to the theory of TCM, the pathogenesis of disorders of menstruation and reproduction are related to "kidney deficiency". Chao *et al* also found that ZGW restored ovarian function effectively and offers another option for treating infertility in patients with premature ovarian failure [142].

2.5.4 You Gui Wan

You gui wan (YGW), a classical yang-tonic prescription of TCM, is thought to boost the body function against diseases. YGW exerts an important function in modulating physiological based upon the 400-year clinical practice. The protruded pharmacological activity of YGW has been known to boost functions of the patients in stress condition [143, 144]. Yao *et al.* showed that YGW improve the immune function even in the immunosuppressive condition [145]. YGW mainly functions to warm and strengthen kidney-yang and to replenish essence and blood. It is primarily used to treat kidney-yang insufficiency, declining fire from the gate of life, cold limbs with aversion to cold, lassitude in the loin and knees, fatigue, lack of appetite, diarrhoea, impotence and seminal emission, and cold pain in the lower abdomen. Administered to rabbits of modelled kidney-yang deficiency, YGW can restore the lowered levels of copper, iron, manganese, chromium, nickel, and cobalt in the whole blood to normal [146, 147].

2.6 Elemental Analysis of Medicinal Plants

Medicinal plants are of special importance taking into account their role in health protection as preventative or supportive therapy for numerous diseases and disorders. It is well known that medicinal plants are rich sources of elements for humans. Environmental conditions such as type of soil, rainfall, vicinity of industry and extensive agricultural activity influence the level of available elements in plants [148, 149]. Medicinal plants help in enhancing or antagonizing the response of the individual components. It has been suggested that the herbal preparation of medicinal plants have an antioxidant activity arising from their content of antioxidants that act in a synergistic way [150]. The knowledge of elemental content of these plants is important because it may influence the production of their active constituent and their pharmacological action. Active constituent of medicinal plants are metabolic products of plant cells and a number of trace elements play an important role in their metabolism [151]. The role of these elements in Chinese medicine is of significant scientific interest since a number of drugs have been reported to contain abundant trace elements. The elements are present at varying concentrations in different parts of the plants - roots, seeds, leaves, fruits, bark, twig, and stem. Several attempts have been made to determine the element contents of herbs, medicinal plants, and tea leaves from many parts of the world by using various techniques such as flame atomic absorption spectrometry (FAAS) [149, 152-155], electrothermal atomic absorption spectroscopy (ETAAS) [156, 157], inductively coupled plasma optical emission spectrometry (ICP-OES) [149, 158-160], and inductively coupled plasma mass spectrometry (ICP-MS) [157].

2.6.1 Mineral analysis of Chinese medicinal herbs

Mineral content of twelve Chinese medicinal herbs used as diuretic treatments for Taiwanese children was studied using instrumental neutron activation analysis (INAA) by Chien-Yi Chen. Different parts of the plants such as rhizome, herb, stem and seed were used in the study. The herbs studied were Fang Chi, Ze Xie, Yin Chen Hao, Yu Xing Cao, Deng Xin Cao, Mu Tong, Zhu Ling, Che Qian Zi, Dong Gua Zi, Lian Zi, Shan Chih Zi and Yi Yi Ren. The concentration of the elements (Al, As, br, Cl, Co, Cr, Fe, K, La, Mn, Na, Rb, Sb, Sc, Se, Sm, V and Zn) present was in the range of 10^4 to 10^{-3}µg/g. The mineral contents and the maximum daily intake values of the tested herbs were compared with published values and with the recommended daily intakes for Taiwanese children as specified by the world health organization. It was reported that consuming these herbs is safe with respect to the intake of essential and toxic elements [151]. Another study of medicinal plants used in the treatment of diseases resulting from urinary tract disorders was done by Rajurkar and Damame. Ayurvedic medicinal plants in the form of leaves, bark, root, fruit, and seed were used in this study. The importance of some elements (Ca, Fe, K, Mg, Na and Zn) in renal disorders was briefly discussed. They reported that K, Ca and Cl were present at major level and other elements (Mn, Pb, Zn, Ni, Na, Fe and Hg) were present in minor level in the herbs studied [44].

Hong Xu and Hou-En Xu studied the bioactive elements (La, Sr, Zn and Se) in twenty two Chinese therapeutic foods and herbs by ICP-MS and atomic absorption spectroscopy (AAS). They suggested that the therapeutic foods may be used as nutritional supplements for preventing and treating elemental deficiency. These herbs include high level La (Shan Zha, Ge Gen), high level of Se (Jin Yin Hua, Ma Chi Xian), high level Zn (Hong Hua, Shan Zha, Ma Chi Xian, Jue ming Zi and Jin Yin Hua) and high level Sr (Ge Gen, Yin Xing Ye, Hai Zao, Jin Ying Hua and Ma Chi

Xian). The knowledge of the effects and concentrations of bioactive elements in herbs could guide the selection of Chinese herbs in clinical practice in conjunction with TCM theories [161].

Ling Zhi (*Ganoderma lucidum)* is a type of fungi that is widely used as Chinese medicine in anti-cancer and immunomodulatory therapy. Some mineral and toxic elements (Ca, K, Mg, P, Na, Al, Fe, Ba, V, As, Se, Cd, Hg and Pb) in Ling Zhi were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) and ICP-MS. The results showed that levels of mineral and toxic elements in all samples tested were safe for ingestion [162].

Elemental analysis of ten Chinese traditional herbs by instrumental neuron activation technique was done by Hamaz and colleagues. Different parts of the plants such as root, stem, flower, root and stem were used. The following herbs were used in the experiment: Dang Gui, Ju Hua, Chuan Xiong, Dang Shen, Ren Shen, Gan Cao, Bai Hua She She Cao, Jin Yin Hua, Chuan Lian and Ban Zhi Lian. K and Na were present at highest concentration in all of the herbs. Other elements present were Zn, Sc, Br, La, Co, Fe, Ce, Cr, Sb, Sm, Th, U, Au, Hf and Tb. All elements were found to be in the safe levels prescribed by health regulations [163].

Concentrations of certain essential elements in Chinese herbs were determined by AAS and INAA. The following herbs were used: Ren Shen, Fu Ling, Rou Gui, Bai Zhu, Bai Shao Yao, Dang Gui, Chuan Xiong, Di Huang, Gan Cao and Huang Qi. Different parts of the plants such as root, sclerotium, bark and rhizome were used. All herbs exhibit extraordinary enrichment capabilities from the environment for elements such as Mn, Zn, Ca, Mg, Cd, Cu, Pb and As. Higher contents of Cd, Pb and As in herbs may be attributed to the uptake of these elements from polluted soil due to industrial and anthropogenic activities [164].

Mineral analysis of fifty Chinese herbs for used to improve kidney function was determined by AAS. Chemometrics such as principal component analysis (PCA), hierarchical cluster analysis (HCA) and linear discriminant analysis (LDA) were applied to classify the data and to understand the relation between the elements. Two significant groups of samples with Group 1 containing samples Tong Cao that is high in Mn and K and Yin Chen, high in Fe and Group 2 containing samples Bie Jia and Long Gu, high in Ca were obtained from principal component and HCA [165].

Paris polyphylla, an herbaceous perennial plant is a well known folk medicinal herb from southern China. It has been used not only in folk medicine but also as an anti-cancer, antibiotic, and anti-inflammatory drug [166]. Trace elements in thirty samples of *Paris polyphylla* were determined by FAAS. Data evaluation was done by common chemometrics. Five significant groups were classified by PCA and HCA. The range of the elements given in µg/g were Fe (2.49 – 68.6), Mn (1.06 – 24.91), Ca (7.43 – 1710), K (9418 – 95, 800), Mg (10.74 – 64.27), Na (13.23 – 1073) and Zn (13.09 – 60.21) [167].

Mineral elements were determined in three types of popular Chinese herbal tea products such as *Gynostemma pentaphyllum*, *Camellia sinesis,* and *Morus alba* by ICP-MS. Ca and Mg were found to be the most abundant elements in all herbal samples (1384 – 34070 and 783 – 7739 mg/kg, respectively). The tea infusions were prepared with 5 min infusion time. *G. Pentaphyllum* infusion contained essential elements (Mg, Ca, V, and Fe) at higher levels than *C. sinesis* and *M. alba infusions.* The daily intakes of all elements were calculated from these herbal tea infusions (three cups/day) and found to be within average daily intake without causing any risk for human consumption [168].

Roots of *Astragalus membranaceus* (Huang Qi), *Angelica sinensis* (Dang Gui), *Glycyrrhiza uralensis* (Gan Cao), and *Codonopsis pilosula* (Dang Shen) which were often used as herbs in TCM were analyzed for trace elements by INAA. Ca, Fe and Na were found to be the most abundant in the samples in mg/g levels. The range of concentrations of Ca, Fe and Na were 1.96 – 19, 0.48 -0.93, and 0.27 – 2.12 mg/g respectively [169].

The root, stem and leaf of *Zanthoxylum nitidum* (Liang Mian Zhen), a TCM was analyzed for trace elements by ICP-AES. Lian Mian Zhen has the efficacy of removing rheumatoid arthralgia, resolutiving turgescence and controlling pain, etc., according to the theory of TCM [170]. It was found that the root, stem and leaves were rich in Mg, Na, K and Ca [171]. The trace elements in CHM *holoparasitism Cistanche tubulosa* were analyzed by Huang *et al. Cistanche tubulosa* is a parasitic plant that grows in Taklamakan desert has been used as kidney tonifying herb to improve male impotence, constipation and infertility [172]. The concentration of Fe, Mn, Cu, Zn and Mg were found to be 174.21, 4.48, 8.11, 4.31 and 1126.93 µg/g respectively, in 200-500 grams of *Cistanche tubulosa* [173]. The metal contents ofTCM, *Sophora tonkinensis* and *Flemingiae philippinensis*, grown in Guangxi province of China were determined by ICP-AES. *Flemingiae philippinensis* have been used as folk medicines for the treatment of various diseases, such as rheumatism, arthropathy, chronic nephritis and menopausal syndrome [174]. *Sophora tonkinensis* is commonly used in TCM for the treatment of eczema, colpitis, acute pharyngolaryngeal infection, sore throat, acute dysentery and gastrointestinal hemorrhage [175-177]. The results showed that *Sophora tonkinensis* contains microelements Cu, Zn, Fe, Mn and Cr and macroelements Ca, Mg, Na and K; *Flemingiae philippinensis* contains microelements Cu, Zn, Fe, Ni, Mn and Cr and macroelements Ca, Mg, Na and K. The content of zinc was higher than copper in both two herbs, which agrees with the phenomenon that the zinc content higher than that of copper exists in other antitumor TCM [178]. Seeds of *Capparis spinosa* L, a TCM were analyzed by Wang *et al.* Seeds of caper have medicinal value, due to the presence of ferulic acid and sinapic acid. The seeds, boiled in vinegar can be used to relieve toothache [179]. The seeds of *Capparis spinosa* L found to be rich in Fe, Zn, Cu and Cr [180]. Tan *et al.* analyzed the leaves, stems and roots of *Plumbago zeylanica Linn*, a TCM for trace elements by ICP-AES. For all the determined elements, the contents in the leaves and roots are relatively higher as compared to those in the stems and it is in concurrence with the active parts of *Plumbago zeylanica* for the cure of antioxidant and anticancer drugs, as the elements Na, K, Ca, Zn, Fe, Mn, Sr, Cu and Co are the highest in leaves, followed by those is in roots. Many anticancer herbs usually show comparatively rich Zn, Mn, Fe, as well as Cr, Sr and Cu. *Plumbago zeylanica* exists with abundant Zn, Mn and Fe and a certain amount of Cr, Sr and Cu [181].

2.6.2 Mineral analysis of herbal medicinal formula/infusions

Bai Hu Tang (BHT), a classic TCM formula that contains four herbs: *Gypsum fibrosum*, rhizomes of *Anemarrhena asphodeloides* Bge. (Liliaceae), roots and rhizomes of *Glycyrrhiza uralensis* Fisch. (Leguminosae), and the fruits of *Oryza sativa* L. (Gramineae) has been clinically used to treat febricity, diabetes, acute inflammation, etc [182, 183]. BHT was analyzed by Wang *et al.* for selected mineral elements by ICP-MS and FAAS. It was found that TCM compatibility could significantly affect the dissolution of selected elements (Mg, Fe, Mn, Cu, Zn, Al, and Co) and fruit of *Oryza sativa* L. (Gramineae) acted as solubilising agent in this formula for most elements except for Co [184].

Si Wu Tang, a classical TCM formula that contains four herbs: Dang Gui, Chuan Xiong, Bai Shao, Shu Di has been used for women suffering from gynaecological conditions such as irregular menstruation, abdominal pain and menopausal symptoms [185]. Millikan *et al.* analyzed Si Wu Tang decoction for Ca, Fe, Mg, Mn and Zn at 20 min by FAAS. It was reported that the decoction is rich in magnesium (25 mg/L), which is within the daily intake recommendations [186].

Sheng Mai San, a TCM formula that contains three herbs: Ren Shen (*Ginseng)*, Mai Men Dong (*Ophiopogon*) and Wu Wei Zi (Schizandra) has been used for chronic cough, shortness of breath, coronary diseases, etc. Li *et al.* analyzed Sheng Mai San for trace elements by ICP-AES. The contents and solubility of the trace elements in Sheng Mai San with Chinese and western ginseng were compared. It was reported that when western ginseng was used in the Sheng Mai San, the solubility of trace elements was the highest [187].

TCM formulae LW known as six-ingredient pill with Rehmannia and Jin gui shen qi wan (JG) known as kidney Qi pill from the golden cabinet were analyzed for Ca, Fe, Mg, Mn, Na, K and Zn at different decoction intervals byAAS. JG was higher in all elements than LW. K (1691.29 – 2372.71 mg/L) was highest in both formulae LW and JG followed by Ca (245.31 – 562.91 mg/L). Ca, Fe, Mg, Mn and K were highest at 40 min for LW and Fe, Mn, and K were highest at 40 min for JG. Mn made the highest contribution to average daily dietary intakes from the formulae [188].

Danggui Buxue Tang (DBT), a Chinese medicial decoction that is commonly used as a dietary supplement in treating woman with menopausal irregularity, contains two herbs: *Radix Astragali* (Huang Qi) and *Radix Angelica Sinesis* (Dang Gui). Wan *et al.* analyzed DBT decoction for trace elements by ICP-AES. The contents and solubility of the trace elements in *Angelica Sinensis* and *Radix Astragali* of different proportion were compared. It was reported that when *Angelica sinensis: Radix astragali* was 1 : 5, the solubility of trace elements was the highest [189].

Chinese herbal tea samples of 30 commercially available teas were analyzed by Xie *et al* at different infusion periods (1, 3, 5, and 10 mins). They reported that contents of K, Rb, Mn, Ni and Zn increased higher when compared to Ca and Fe with longer extraction time (10 min) [190]. Chinese teas (Lapsang Souchong and Gunpowder Green*)* were analyzed by Mehra *et al.* for Al, Cu, and Mn at 2, 5 and 10 min infusion times. They reported that the first infusion period: 2 min showed highest elemental content followed by the second: 5 min and third infusions: 10 min in decreasing order [191]. Herbal infusions of commercially available teas (peppermint, Echinacea, red clover, Siberain ginseng, dandelion, red raspberry leaf, blue berry leaf and green tea) were examined for the mineral contents by Galllaher *et al.* They reported that none of the infusions was a good source of Ca, Mg, P, K, Na, Cu, Fe, Mn, or Zn in a single serving. By comparison, standard reference levels for both brewed black tea and coffee indicate these beverages contain several times as much K as dandelion and Echinacea infusions. They reported that these infusions will not interfere with low Na diets [192]. Herbal tea infusions of *Salvia aucheri* var. *canescens* were analyzed by ICP-AES for mineral elements at different infusion periods (3 min to 11 min). Twenty five elements were detected out of which K, Ca, Na, Mg, and S in all infusions was comparatively higher. It was reported by Ozcan that Al, Ba, Ca, Cd, Co, Cu, Fe, Li, K, Mg, Na, Ni, P, Pb, Sr, Ti, and Zn contents were high in the first period of infusion: 3 min and may be proposed as the optimum infusion time for this herbal tea [159] . Metal contents of black and green teas were studied at 3 and 5 min infusion times by Fernandez *et al.* The results were analyzed by pattern recognition techniques such as principal components analysis and linear discriminant analysis (LDA). They reported that for green teas Zn, Mn and Ca were significantly higher in 5min infusions and for black teas the concentrations of Mg, Al, Ca and K were significantly higher in 5min infusions while Zn and Cu were lower. After making the comparison between black and green teas at 3min: Mn, Mg, Cu, Al, Ca, Ba and K were significantly higher in black teas whereas Zn concentration was higher in green teas. Similarly for black teas versus green teas at 5min: Mg, Al, Sr, Ca and K were significantly higher in black teas and Fe and Cu in green teas [193]. Another study analyzed 30 tea herbal infusions for Cu, Fe, Mn, and Zn at 5 min, 60 min and 24 h intervals by ICP-AES. It was reported that for Cu there was no significant difference between the infusion times, for Fe the content decreased with time and, for Mn and Zn the contents significantly increased with the time [194]. Mineral contents of some herbal decoctions at 10, 15 and 20 mins were studied by Ozcan *et al.* They reported that the minerals Ag, B, Cu, Co, Fe, In and Zn were diffused at higher concentrations at 10 mins and suggested this time as the optimum time for getting minerals into the teas [195]. Queralt and colleagues analyzed medicinal plant infusions for some major and minor elements at 2 hrs infusion period. They reported that the degree of solubility at 2hr infusion period was higher for the elements Na, P, K and Mg and lower for Fe, Ti, As and Si. For some elements such as Ca, Fe, Pb the range of solubility exhibited a high degree of variation [196]. Selected Polish herbal infusions at 20 mins were analyzed for micro elements by graphite furnace atomic absorption spectrometry (GFAAS). It was reported by Kalny *et al.* that contents of Ni and Zn were the highest and the lowest for Cd and Pb. The calculated daily intake of majority of the analyzed elements was very low: under 1% of daily requirements [197]. Malik *et al.* analyzed 31 plant stimulant infusions at 15 mins. The results showed that chamomile and mate are high in B, Ca and mate was also rich in Mn, as their contents approximate to the respective RDAs [198].

2.6.3 Mineral analysis of plants by Near Infrared Spectroscopy

Near Infrared Spectroscopy (NIRS) in reflectance mode was used to determine the concentration of Pb, Cu and Zn in plants of Indian mustard (*Brassica juncea)* grown on contaminated soils in Spain. Modified partial least squares (MPLS) was the regression method employed to correlate spectral information. Calibration equations for Pb, Cu and Zn were developed and then validated by cross-validation. Reference analysis was done by using AAS for Zn and Cu, and GFAAS for Pb. The results showed that the use of NIRS can be used as a rapid screening method for determining these metals in *B.juncea* [199].

Total arsenic content in prostrate amaranth (*Amaranthus blitoides* S. Watson) was analyzed by near infrared (NIR) reflectance spectroscopy. As reference values were obtained by AAS and they were regressed against different spectral transformations using MPLS regression model. The results showed that the use of NIRS for the determination of the As content in *A.blitoides* plants offers an important saving of time and cost of analysis [200].

Rapid and accurate prediction of leaf and mineral concentration of alfalfa (*Medicago sativa* L.) forage was done by NIR reflectance spectroscopy. Reference analysis was done by ICP. NIR reflectance spectroscopy calibration equations were developed by regressing total ash and specific mineral concentration values against the spectra. The method accurately predicted total ash and Ca, K, and P of leaves and stems of alfalfa hay but was less consistent in prediction of Mg and S and micro minerals Al, B, Fe, Mn, and Si in leaves and stems [201].

The concentration of Pb and Zn in Chinese herbs was determined by NIR reflectance spectroscopy. Reference analysis of Pb and Zn was done by AAS. Partial least squares (PLS) and multiple linear regression (MLR) models were applied to analyze the data. This study showed that NIRS was able to distinguish the metal content in various plant parts such as roots, stems and leaves and is a rapid method for determination of Pb and Zn in the Chinese herbs [202].

Visible-NIR spectrophotometric analysis on orange leaves [*Citrus sinensis* (L) Osbeck cv Tarocco] was done for P, K, Ca, Mg, Mn, Fe and Zn. Reference analysis was done with ICP-OES. For spectral acquisition, a 'pen probe' was used to measure the spectral reflectance response on each leaf. Mean reflectance values of all leaves for each treatment were compared by chemometric multivariate methods (PLS) to both: a single reference chemical value and to all chemical parameters used together. The best model for single reference chemicals coefficient of correlation r = 0.995 was obtained for K. For all chemical parameters used together, the analyzed elements gave correlations in a range from r = 0.883 for Mg to r = 0.481 for P with standard error of prevision ranging from 0.01 for P to 12.418 for Fe [203].

2.7 AIMS

The modern scientific reasoning for TCM theories behind the prescription and dosage of the herbs in TCM formulae that are used to improve kidney function has been investigated in terms of elemental analysis. There has been limited information available on elemental analysis of kidney tonifying herbs. The main aim of this research is to analyze mineral elements in Chinese medicinal herbs and traditional Chinese medicinal formulae that are used to improve kidney function. The methods chosen for the analysis of this study include AAS and NIRS. Fifty Chinese herbs and eight TCM formulae have been selected for the analysis of mineral elements in this study. The aims include:

- To conduct a preliminary study of selected metals in Chinese herbal plant parts by NIR.
- To evaluate mineral contents of dry Chinese medicinal herbs used to improve kidney function with chemometrics.
- To determine and compare mineral elements in traditional Chinese herbal formulae Liu wei di huang and Jin gui shen qi wan at different decoction times.
- To evaluate selected elements in traditional Chinese herbal formulae Da bu yin wan and Gui lu er xian jiao with empirical and chemometric approaches.

- To evaluate and compare mineral elements in Chinese herbal kidney and liver tonifying formulae Er zhi wan and Qi bao mei ran dan at different decoction times.
- To determine and compare mineral elements in chinese herbal kidney tonifying formulae You gui wan and Zuo gui wan at different decoction times.

The scientific evidence and the knowledge gained from this research on kidney tonifying herbs of mineral elements will benefit the general public and also assist Chinese medicine practitioners to determine the effectiveness of percolation of these elements with time that aids in the prescription of these herbs and TCM formulae to improve kidney function.

3 MATERIALS AND METHODS

3.1 CHEMICAL REAGENTS

All laboratory chemicals were of analytical reagent grade unless otherwise indicated and all solutions were made up using millipore Milli-Q water purified to a resistivity of 18 mΩ-cm. Plant digests were prepared using concentrated nitric acid (*Trace* SELECT , 69%) from Sigma-Aldrich, Australia. Stock standard solutions 1000 mg/L of metals: Ca, Fe, Mg, Mn, K, Na, Pb and Zn were from Merck, Australia. Further serial dilutions were made to give required standards in 100 mL. All standard solutions derived from the stock solution were prepared fresh on the day of analysis. Strontium Chloride: 5000 µg/mL, Caesium Nitrate: 1000 µg/mL, Potassium Nitrate: 2000 µg/mL and Ethylenediaminetetraacetic acid (EDTA): 0.1M solution, were added, where necessary to the solutions to suppress ionisation. Air, Acetylene and Nitrous oxide gases were of high purity grade from Brin's Oxygen Company, Australia. Standard Reference Material (SRM): 1515 – Apple leaves was obtained from National Institute of standards and technology of Gaithersburg, USA was used to verify the accuracy of results.

3.2 SAMPLES

The Chinese herbs were purchased from a local registered herbal supplier in Melbourne. The list of the Chinese dried herb samples used is listed in **Table 3.1.**

Table 3.1: List of Chinese herbs

Chinese Herbs	English Name
Leaves	
Bo He	Mentha, field mint
He Ye	Lotus leaf
Lian Zi Xin	Lotus plumule
Sang Ye	White mulberry leaf
She Wei	Pyrrosia leaves
Yin Yang Huo	Epimedium, Horny goat weed
Zi Su Ye	Perilla leaf
Whole plant/Stem/Twig/Bark	
Bian Xu Cao	Knotweed, Polygonum
Che Qian Cao	Herba plantaginis
Deng Xin Cao	Lamp wick herb
Du Zhong	Gutta-percha tree, Chinese rubber tree
Gu Sui Bu	Drynaria rhizome
Hai Jin Sha	Japanese climbing fern spore
Han Lian Cao	Herba ecliptae
Huang Jing	Dropberry, Solomon seal
Jin Qian	Lysimachia
Mu Tong	Akebia caulis
Suo Yang	Herba cynomorii
Tong Cao	Ricepaper plant pith
Xian Mao	Golden eye-grass rhizome
Yin Chen	Red stem wormwood
Ze Xie	Water plantain rhizome
Flowers	
Jin Yin Hua	Honeysuckle flower, lonicera
Ju Hua	Chrysanthemum flower
Mei Gui Hua	Rosebud, bud of Chinese rose
Mo Li Hua	Jasmine flower
Seeds	
Bai Guo	Ginkgo nut
Che Qian Zi	Plantain seed
Chi Xiao Dou	Rice bean
Dong Gua Ren	Wintermelon seed
Hei Zhi Ma	Black sesame
Jue Ming Zi	Cassia seeds, foetid cassis seeds
Mu Hu Die	Oroxylum seeds
Pang Da Hai	Sterculia seed
Sha Yuan Zi	Flatstem milkvtech seed
Tu Si Zi	Chinese dodder seeds
Yi Yi Ren	Job’s Tears
Yi Zhi Ren	Cardamon

Chinese Herbs	English Name
Roots	
Ba Ji Tian	Morinda root
Bi Xie	Dioscorea root
Chuan Niu Xi	Cyathula root
Gan Cao	Licorice root
He Shou Wu	Chinese knotweed, Flowery knotweed
Hong Da Ji	Knoxia root
Ren Shen	Ginseng root
Mu Dan Pi	Peony root
Shan Yao	Cinnamon vine, Chinese yam
Shu Di Huang	Rehmannia Root
Xi Yang Shen	American Ginseng
Xu Duan	Teasel root
Fossil	
Bie Jia	Turtle Shell
Gu Jia Jiao	Colla Plastrum Testudinis
Long Gu	Dragon’s bone
Lu jiao jiao	Deer horn gelatine
Zi He Che	Dried human placenta
Fruits	
Bu Gu Zhi	Psoralea fruit
Di Fu Zi	Kochia fruit
Guo Qi Zi	Lycium fruit, Chinese wolfberry
Long Yan Rou	Flesh of the longan fruit, longan
Nu Zhen Zi	Ligustrum, Pivet fruit
Sang Shen Zi	Mulberry, morus fruit
Shan Zhu Yu	Japanese Cornelian Cherry
Fungus	
Dong Chong Xia Cao	Caterpillar fungus
Fulingpi	Indian bread
Ling Zhi	Reishi mushroom
Zhu Ling	Polyporus fungus
Peel	
Dong Gua Pi	Chinese waxgourd peel

3.2.1 Sample Preparation

3.2.1.1 Herbs

The dried herbal samples were grinded to powder using a laboratory hardened stainless steel grinder (Sumeet, Melbourne). Such a grinder was used to ensure contamination of the products did not occur. The herbal powder was stored in airtight 150 mL white rectangular plastic bottles from Nylex, Australia.

3.2.1.2 Acid digestion of herbal samples

Powdered herb sample of 1g was accurately weighed using analytical balance (Mettler Instrument AG – Model AE 200) into a 50 mL conical flask. Next, 6 mL of concentrated nitric acid was added to the sample, flask covered with a watch glass and left overnight in the fume hood. The mixture was then gently heated on a hot plate with a watch-glass for 3 hours until the digestion process was complete. The digested sample was allowed to cool to room temperature and then transferred quantitatively into clean 25 mL volumetric flasks. The samples were then diluted to the mark with Milli-Q water [204]. All samples were prepared in triplicate. SRM 1515 was also prepared in the same procedure for analysis.

3.2.1.3 Herbal formulae preparation

The dried herbs without grinding were directly used for herbal formulae preparation. The method of preparation of decoction was done according to the traditional Chinese standard procedure [43]. The herbs that were involved in the formulae were accurately weighed as per the formulae and put together in a 500 mL conical flask. 200 mL of Milli-Q water was added to the flask and the herbs were allowed to soak for 20 min. Twelve samples were prepared for each formula in the same manner and heated over different time intervals: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min. The mixture was then allowed to cool to room temperature and then filtered with an ashless filter paper into 100 mL volumetric flasks. All samples were performed in triplicate.

3.3 INSTRUMENTATION

Elemental analyses were performed using a Varian SpectroAA-400 AAS (Varian Inc., Mulgrave, Australia) and NIRS (Foss-NIRSystems Model 6500, Inc., Silver Spring, MD, USA).

3.3.1 Atomic Absorption Spectrophotometer

Varian SpectroAA-400 AAS was used for elemental analyses. The appropriate instrument settings for the particular mineral being measured is given in **Table 3.2**. Iron, lead, manganese and zinc were measured using the single element hollow cathode lamps. For calcium, magnesium, potassium and sodium the dual-element hollow cathode lamps: K/Na and Mg/Ca were employed. Air-acetylene flame with a Varian Mark VI model burner was used for iron, lead, magnesium, manganese, potassium, sodium and zinc analyses. Nitrous oxide-acetylene flame with a Varian Mark V model burner was used for analysing calcium. Suppressant agents such as strontium chloride to analyze calcium and magnesium, caesium nitrate to analyze potassium, potassium nitrate to analyze sodium, EDTA solution to analyze lead were added to all the samples and blanks. The instrument was interfaced to a computer for operation and data analysis using the Varian proprietary software. All the standards for the respective elements were run to obtain a calibration curve. The sample solutions were run to obtain the mineral concentrations in the samples from the respective calibration curves.

Table 3.2: Operating parameters for working elements

Elements	Wavelength(nm)	Lamp Current(mA)	Slit(nm)
Ca	422.7	10	0.5
Fe	248.3	5	0.2
Mg	285.2	4	0.5
Mn	279.5	5	0.2
K	404.4	5	0.5
Na	330.3	5	0.5
Pb	217.0	5	1.0
Zn	213.9	5	1

3.3.2 Near Infra Spectrophotometer

3.3.2.1 NIR Equipment and Software

NIRS (Foss-NIRSystems, model 6500) was used to analyze the powdered herb samples equipped with a transport module. The monochromator 6500 consists of a tungsten bulb and a rapid scanning holographic grating with detectors positioned for transmission or reflectance measurements. To produce a reflectance spectrum, a ceramic standard is placed in the radiant beam, and the diffusely reflected energy is measured at each wavelength. The actual absorbance of the ceramic is very consistent across wavelengths. Each spectrum was recorded once from each sample, and obtained as an average of 32 scans of the sample, plus 16 scans of the standard ceramic before scanning the sample. The ceramic and the sample spectra are used to generate the final Log (1/R) spectrum. The total time of analysis was about 2 min. Mathematical transformations of spectra and regressions performed on the spectral and laboratory data obtained by using the VISION SOFTWARE 2.1.

3.3.2.2 NIRS Procedure: Recording Spectra and Processing Data

The powdered samples were packed tightly in to the Foss NIRS standard powder sample cup that was placed in to the sample transportation unit. The NIR spectra were obtained at 2 nm intervals in the reflectance mode, over the visible and the NIR regions acquiring spectra over a wavelength range from 400 to 2500 nm. Samples of herbs were recorded as an NIR file, and were checked for spectral outliers, using PCA. This procedure will detect and, if necessary, will remove possible samples whose spectra differed from the other spectra in the set [205]. In the second step, laboratory reference values (obtained from AAS) were added to the NIR spectra file.

Calibration equations were computed in the new file by using the raw optical data: log 1/R, where R is reflectance, and second derivatives of the log 1/R data, with several combinations of segment: smoothing and derivative: gap sizes. The use of derivative spectra instead of the raw optical data to perform calibration is a way of solving problems associated with overlapping peaks and baseline correction [206]. The second-order derivative calculation results in a spectral pattern display of absorption peaks pointing down rather than up, with an apparent band resolution taking place [207]. In addition, the gap size and amount of smoothing used to enable the transformation will affect the number of apparent absorption peaks.

To correlate the spectral information: raw optical data and derived spectra of the samples and the elemental analyses determined by the reference method, using both partial least squares and multi linear regression as regression methods, using wavelengths between 400 and 2500 nm every 8 nm. Standard normal variates and De-trending: SNV-DT transformations [208] were used to correct the baseline offset due to scattering effects, differences in particle size and path length variation among samples.

3.3.2.3 Cross-Validation

Cross-validation is an internal validation method that, like the external validation approach, seeks to validate the calibration model based on independent test data, but it does not waste data on testing only, as is the case with external validation. This procedure is useful because all available chemical analyses for all individual species can be used to determine the calibration model without the need to maintain separate validation and calibration sets [209].

The prediction ability of equations obtained was determined on the basis of their coefficient of determination (R^2) in the cross-validation. When a cross-validation is performed on the calibration set, NIR prediction error is defined as the standard error of cross-validation (SECV). Statistically, the SECV is the standard deviation(SD) for the residuals due to difference between reference and NIR predicted values for samples used in the calibration, using the calibration equation [210]. For a comparison of the potential of the prediction among the equations obtained, a standardisation of the different SECV's is needed. In this way, the relative percent difference (RPD), defined as the SD to SECV ratio was estimated for each equation. In this study, cross-validation was computed based on the calibration set for determining the optimum number of terms to be used in building the calibration equations.

3.4 DATA ANALYSIS

The statistical data was carried out using predictive analytics software (PASW) Statistical package for the social sciences (SPSS) 18: Release 18.0.0, Jul 30, 2009 and Microsoft Excel 2007 with add-in Excel Statistics (XL-STAT) 2010. Elemental analyses of the Chinese herbs were done by chemometrics such as Analysis of Variance (ANOVA), Correlation Analysis (CA) and HCA using SPSS and PCA using XL-STAT.

4 PRELIMINARY INVESTIGATION OF SELECTED METALS IN CHINESE HERBAL PLANT PARTS BY NEAR INFRARED SPECTROSCOPY

4.1 ABSTRACT

The vast majority of the medicinal herbal products are unlicensed and are not required to demonstrate efficacy, safety or quality. Little is known about the relative safety of medicinal herbs compared to synthetic drugs, although, for some medicinal herbs the risk may be less than conventional drugs. The toxic effects that occur in Chinese herbs are largely due to the presence of heavy metals. Measurement of metal content in the herbs by standard conventional wet chemical methods usually requires numerous reagents, skilled labour and expensive analytical equipment. The objective of this study was to analyze the ability of NIRS to determine the concentrations of lead and zinc in Chinese herbs, common industrial pollutants. The reference values for the Chinese herbs were obtained by AAS. The samples were scanned using a FOSS-NIRSystem (Model 6500) Spectrophotometer from 400-2500 nm. PLS and MLR statistics were applied to analyze the data.

4.2 INTRODUCTION

Phytotherapy, which is healing with plants, is old and has become as increasingly popular today with the increase in the use of botanical medications. Medicinal herbs not only are very commonly used in Asia but are also widely consumed all over the world [158]. The use of Chinese herbs for the treatment or prevention of various diseases is increasing worldwide. The traditional usage of these herbs involves soaking of one or more herbs and boiling the herbs before drinking the resulting decoction [211].

TM plays an important role in the general state of health of a population. It is important to have good quality control for medicinal herbs in order to protect consumers from contamination. Some medicinal herbs and their mixtures can present a health risk to humans due to the presence of toxic elements such as lead, cadmium, aluminium, mercury and chromium. The toxicity depends on the oxidation state of the elements and their concentration levels [156, 212, 213]. It would be advisable for medicinal herbs to be checked for heavy metals contamination prior to use in clinical applications. Medicinal herbs may be easily contaminated during growing and processing. An important link in the transfer of trace elements from soil to man is plants. The level of essential elements in plants is determined by the geochemical characteristics of a soil and the ability of plants to selectively accumulate some of these elements. Plants readily assimilate elements through the roots, which dissolve in water and occur in ionic forms. Additional sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents and fertilizers, which could be adsorbed through the leaf blades [214].

Several studies have been carried out to determine the metals by using AAS, ICP-MS, ICP-AES and electrochemical methods [54, 215, 216]. AAS gives higher sensitivity in detecting individual elemental concentrations which is more reliable compared to ICP-MS. The standard conventional wet chemical methods that are mentioned above which are used for trace metal determination are accurate but they need a large quantity of reagents, skilled labor and time for analysis including pre-treatment. For these reasons, there is a need of fast analytical quality control methodology, based on spectrometric data of untreated samples for trace metal determination.

NIRS, a rapid non-destructive, cost effective technique, has been used for qualitative and quantitative analysis in the fields of food, agriculture, textile and pharmaceuticals [217-219].

NIRS has been applied to the analysis of metal contents in the environmental field. Various authors have reported the determination of heavy metals, minerals and arsenic in plants as well as studies concerning the chemical characterization of soils and analysis of heavy metals in lake sediments by NIRS [199-201, 220, 221]. Sauvage *et al.* showed for the first time that NIRS could distinguish trace metals in white wine due to hydrogen bonding in the water sheath around the metals, where the reference method was AAS [222]. The literature shows that NIRS in reflectance mode with wavelength range from 400 to 2500 nm has been successfully used to determine the concentration of heavy metals, and macro and micro minerals of plants. Contamination of the general environment with toxic metals has increased. Abou-Arab and Abou Dania suggested that heavy metal uptake could be attributed to the use of contaminated irrigation water as well as the addition of some fertilizers and herbicides. Sewage sludge, industrial activities, fuel, and automobile tires can also be significant metal sources [152]. Bosque *et al.* also reported that the contamination of plants with lead depends on several factors, for example, traffic densities and distance from the road. They also suggested that heavy metals accumulated in plants through both foliage and root systems [223]. Zinc is a common pollutant in rivers and estuaries where heavy metal industry is located that can cause contaminated water to affect the plants grown in the region. Fatoki analyzed surface soil and grass samples for Zn and Cu that were collected from ten locations perpendicular to a major road in Alice, South Africa. He reported that there is an association between Zn concentration and distance from road traffic where as results for copper did not show any correlation between sample concentration and distance from road traffic. He also reported that there is an association between soil Zn and grass Zn, which suggests the same source for the metal in samples [224]. Moss samples of *Barbula lambarenensis* were taken along major and minor roads of ile-ife, Nigeria and were analyzed for Pb, Zn and Cu. It was reported that Pb and Zn were generally higher in areas with relatively high traffic density than low traffic density areas, and Cu was low in these areas and did not show any correlation with traffic density [225]. Dudka *et al.* conducted a four year field experiment to study the transfer of Cd, Pb, and Zn from soil contaminated by smelter flue-dust to crop plants grown in a rotation. They reported that the highest metal concentrations were found in potato tubers (intact), meadow bluegrass, and barley straw, and also observed that the suppression of crop growth may be a result of relative nutrient deficiencies and high Zn (and possibly Cu) in plant roots [226] .

To date, there has not been any study reported, to differentiate metal content in the plant parts of Chinese herbs by NIRS. This preliminary study, therefore, has been done to determine the contents of zinc and lead in plant parts of Chinese herbs that are commonly used as teas by the general public in Australia and other countries. Zinc and lead were chosen first in this study due to the references above indicating that these metals are major pollutants. A larger study to analyze more toxic metals was not conducted due to change in the emphasis of work.

4.3 MATERIALS AND METHODS

4.3.1 Samples

Samples of dried Chinese herbs were purchased from local importers of Chinese herbs. Detailed description of these herbs is presented in **Table 4.1.**

Table 4.1: Herbs analyzed and their medicinal use

Chinese herbs	English Name	Key Characteristics
Leaves		
Bo He	Mentha, field mint	Cools and clears the eyes and head, soothes the throat
He Ye	Lotus leaf	Clears heat
Lian Zi Xin	Lotus plumule	Clears heat from the Heart and Pericardium
Sang Ye	White mulberry leaf	Cools and drains the Lungs and Liver
Zi Su Ye	Perilla leaf	Revives the spleen
Flowers		
Jin Yin Hua	Honeysuckle flower, lonicera	Disperses heat, resolves toxicity, cools the blood
Ju Hua	Chrysanthemum flower	Cooling and tonifying the Liver
Mei Gui Hua	Rosebud, bud of Chinese rose	Harmonizes the Liver, Spleen, and Stomach
Mo Li Hua	Jasmine flower	Treat canker sores, dysentery, and blood accumulated in bruises
Seeds		
Bai Guo	Ginkgo nut	Treats Phlegm-heat cough and wheezing
Jue Ming Zi	Cassia seeds, foetid cassis seeds	Nourishes the Liver and Kidney yin
Mu Hu Die	Oroxylum seeds	Frees the flow of Liver qi
Pang Da Hai	Sterculia seed	Cools and moistens the throat and Intestines
Roots		
Gan Cao	Licorice root	Moistens the Lungs, moderates toxicity
Hong Da Ji	Knoxia root	Powerfully drives pathogenic fluids downward
Ren Shen	Ginseng root	Tonifies the primal qi of the five organs, revives from collapse, stops heavy bleeding
Fruits		
Guo Qi Zi	Lycium fruit, Chinese wolfberry	Nourishes the Liver blood, mildly tonifies the kidney yang
Long Yan Rou	Flesh of the longan fruit, longan	Nourishes the blood
Nu Zhen Zi	Ligustrum, Pivet fruit	Tonifies the Liver and Kidney yin
Sang Shen Zi	Mulberry, morus fruit	Nourishes and cools the blood and yin

Source [227]

4.3.2 Reference Analysis

All the dried Chinese herbs were ground to powder in a blender. To prepare the samples for elemental analysis, 1 g of powdered herb was accurately weighed. Next, 6 mL of concentrated nitric acid (*Trace* SELECT, sixty nine percent) was added and left overnight. The mixture was then heated on a hot plate for three hours. After cooling, the sample solutions were quantitatively transferred into twenty five mL volumetric flasks and made up to the mark with deionised water (with a specific sensitivity of 18 mΩ from Millipore Milli-Q purifier system) [204].

All the samples were analyzed in triplicate and mean concentration was reported. The total concentration of Zinc (Zn) and Lead (Pb) were determined by Varian SpectroAA-400 AAS (Varian Inc., Mulgrave, Australia) under optimized conditions using suitable hollow cathode lamps. Metal standards, 1000 ppm for Zn and Pb were purchased from Merck Pty Ltd, Australia. The reproducibility of the method was checked for Zn by serially diluting the 1000 ppm of Zn solution to give standards in the range from 0 to 1.0 mg/L. The standards were analyzed 10 times that produced a series of absorbance values as shown in **Table 4.2.** and then corresponding linear regression graph for these values was plotted and shown in **Figure 4.1.** A regression coefficient of 0.9975 was obtained from **Figure 4.1** and given in **Table 4.3.** The regression coefficient very close to 1 shows a highly linear relationship between zinc concentrations in standard solution and absorbance values of the instrument.

Table 4.2: Linearity of standard curve - relation between zinc standard concentrations and absorbance

	Standard Concentration (mg/L)					
	0.00	0.10	0.25	0.50	0.75	1.00
Run	Absorbance (abs units)					
1	0.0011	0.0311	0.0775	0.1698	0.2357	0.3270
2	0.0018	0.0353	0.0828	0.1770	0.2460	0.3403
3	0.0071	0.0358	0.0839	0.1790	0.2493	0.3475
4	0.0100	0.0370	0.0851	0.1830	0.2520	0.3502
5	0.0108	0.0374	0.0862	0.1834	0.2547	0.3541
6	0.0104	0.0367	0.0853	0.1831	0.2532	0.3516
7	0.0109	0.0364	0.0850	0.1828	0.2521	0.3513
8	0.0114	0.0371	0.0852	0.1828	0.2529	0.3518
9	0.0127	0.0379	0.0862	0.1846	0.2540	0.3526
10	0.0127	0.0373	0.0855	0.1828	0.2532	0.3515
Mean	0.0089	0.0362	0.0843	0.1808	0.2503	0.3478
SD	0.0042	0.0020	0.0026	0.0045	0.0057	0.0083
CL (95%)	0.0026	0.0012	0.0016	0.0028	0.0036	0.0051

SD: Standard deviation; CL (95%): 95% Confidence Level

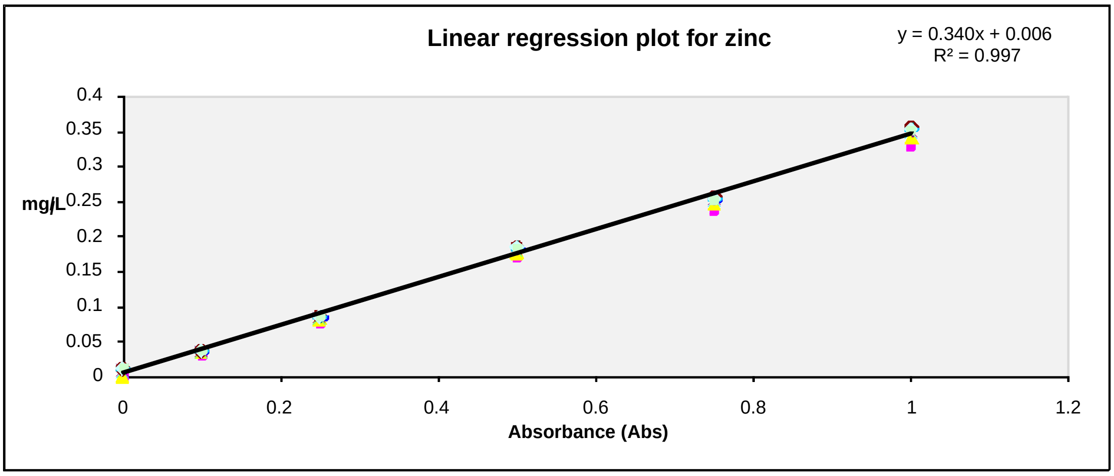

Figure 4.1: Linear regression plot for zinc

Table 4.3: Regression parameters from plot of absorbance against zinc concentration

Slope	Intercept	Regression Coefficient
0.3401	0.0064	0.9975

4.3.3 NIRS Analysis

All the powdered herb samples were scanned in the standard powder sample cup using the NIRS (Foss-NIRSystem Model 6500, Inc., Silver Spring, MD, USA) in the reflectance mode, acquiring spectra over a wavelength range from 400 to 2500 nm: visible and NIR regions. A reference scan was taken before each sample scan. Chemometric analysis was performed with the Vision 2.1 software.

The absorbance values: log 1/R, where R is reflectance were registered at 2 nm intervals. The calibration equations were computed using the raw optical data (log 1/R) and second derivative of log 1/R data, with several combinations of segment (smoothing) and derivative (gap) sizes. The use of derivative spectra instead of raw optical data to perform calibrations is to solve those problems associated with overlapping of peaks and baseline correction [228].

PLS and MLR statistics were applied to analyze the data. The R^2 relates to the NIRS predicted value regressed with the reference value obtained by analysis using the conventional method; the standard error of calibration (SEC) refers to the standard error of this regression.

4.4 RESULTS AND DISCUSSION

Reference data for the herbs, obtained by atomic absorption analysis are shown in **Table 4.4.** The mean concentration of zinc is slightly higher than that of lead. It can be observed from **Figure 4.2**, the following herbs that are leaves: Bo He, He Ye, Sang Ye and Zi Su Ye have higher concentrations of Pb when compared to other herbs that were flowers, seeds, roots and fruits. It can also be seen that the leaves Lian Zi Xin and He Ye has the highest Zn concentration and also He Ye has highest Pb concentration when compared to the other herbs.

Tan *et al.* analyzed the leaves, stems and roots of *Plumbago zeylanica Linn*, a TCM, for trace elements by ICP-AES. For all the determined elements, they reported that the contents in the leaves and roots are relatively higher when compared to those in the stems and it is in concurrence with the active parts of *Plumbago zeylanica* for the presence of antioxidant and anticancer drugs, as the elements Na, K, Ca, Zn, Fe, Mn, Sr, Cu and Co are the highest in leaves, followed by those is in roots [181]. Elemental analysis of ten Chinese traditional herbs determined by INAA where various parts of the plants: root, stem, and flower were used, as reported by Hamaz and colleagues. All elements were found to be in the safe levels prescribed by health regulations. It was reported that the concentration of elements varied depending on the origin of the herb [163]. Another study involved analysing the seeds of Chinese herb *Capparis spinosa* L, found to be rich in Fe, Zn, Cu and Cr [180].

Table 4.4: Concentration of metals (mg L^{-1}) in the herbs by AAS analysis

Herbs	Zn	Pb
Bo He	0.2815	0.4517
He Ye	0.6296	0.4942
Lian Zi Xin	0.6976	0.0772
Sang Ye	0.2583	0.4788
Zi Su Ye	0.3121	0.4903
Jin Yin Hua	0.2574	0.278
Ju Hua	0.2907	0.1699
Mei gui Hua	0.3392	0.1969
Mo Li Hua	0.4166	0.1699
Bai Guo	0.0455	0.0888
Jue Ming Zi	0.4168	0.0386
Mu Hu Die	0.1304	0.1042
Pang Da Hai	0.345	0.027
Gan Cao	0.0767	0.1004
Hong Da Ji	0.0809	0.0386
Ren Shen	0.0827	0.0927
Gou Qi Zi	0.0698	0.0386
Long Yan Rou	0.1465	0.2934
Nu Zhen Zi	0.3448	0.1506
Sang Shen	0.0822	0.2317
Range	**0.04-0.69**	**0.02-0.49**
Mean	**0.26**	**0.20**
SD	**0.18**	**0.16**

No of samples were twenty; SD: Standard deviation

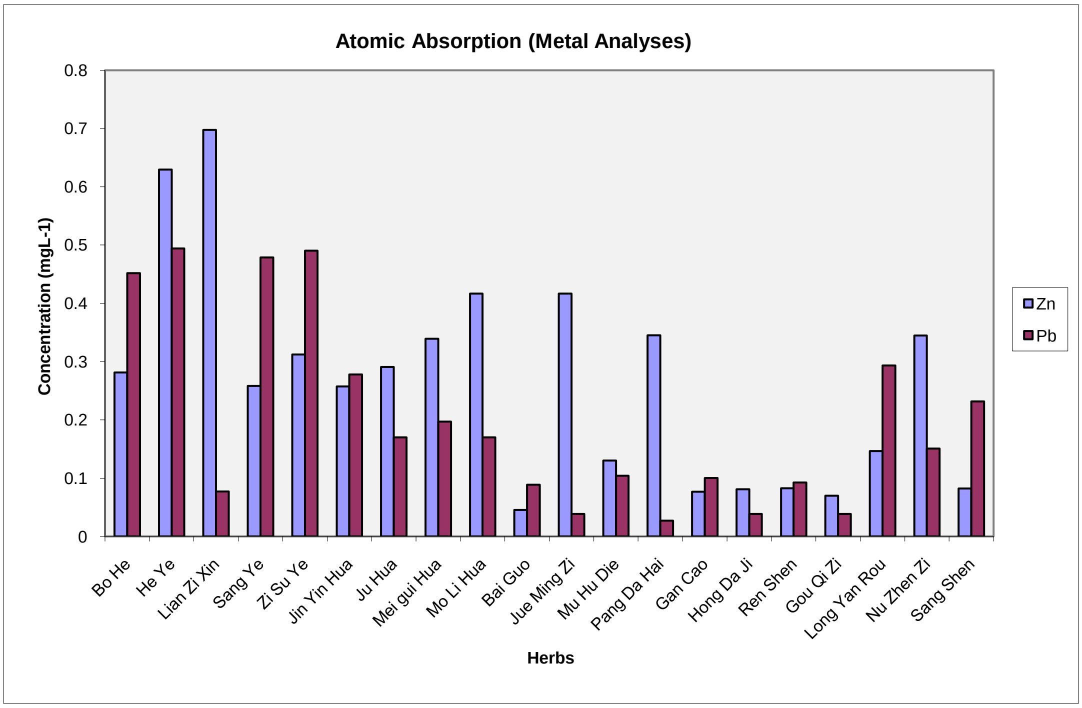

Figure 4.2: Concentration of Zn and Pb in mg L^{-1} of powdered herb samples

Figure 4.3 presents the raw spectra of powdered herb samples from NIR in the range of 400-2500 nm. This shows that the diversity of the samples agrees with the atomic absorption data in **Figure 4.2.**

The pre-treatment of the spectral data that operate on purified spectra, either fully or partially eliminates the systematic error that can be caused by various factors. The application of the second derivative to the raw spectra: log 1/R (**Figure 4.3**) resulted in substantial correction (**Figure 4.4**) of the baseline shift caused by differences in particle size and path length variation. Peaks and troughs in **Figure 4.3** correspond to the points of maximum curvature in the raw spectrum, and it has a trough corresponding to each peak in the original. The increase in complexity of the derivative spectra resulted in a clear separation of peaks, which overlap in the raw spectra.

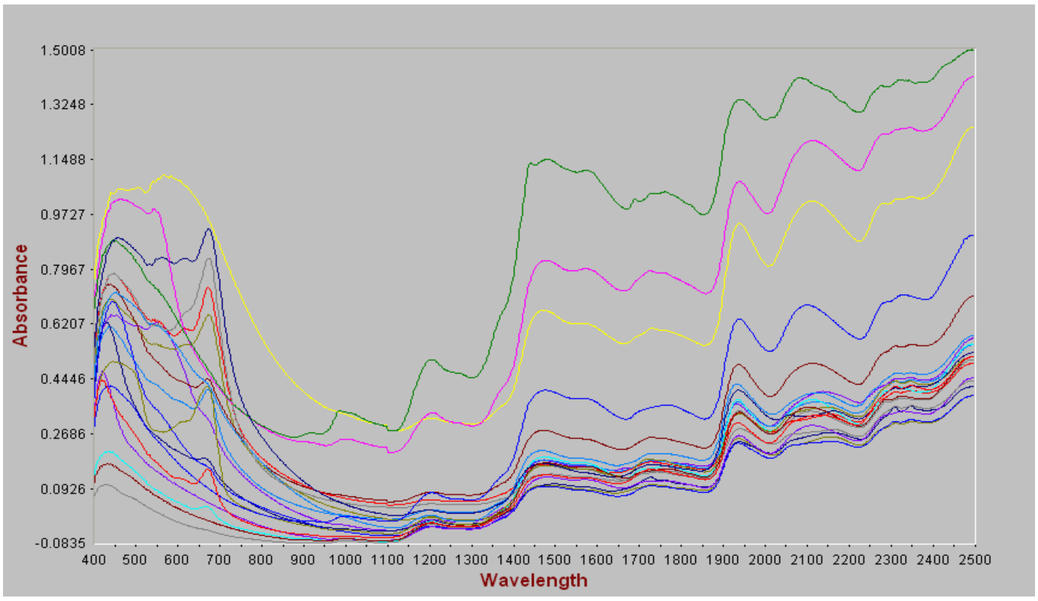

Figure 4.3: Raw spectra of powdered herb samples, in the range of 400 to 2500 nm

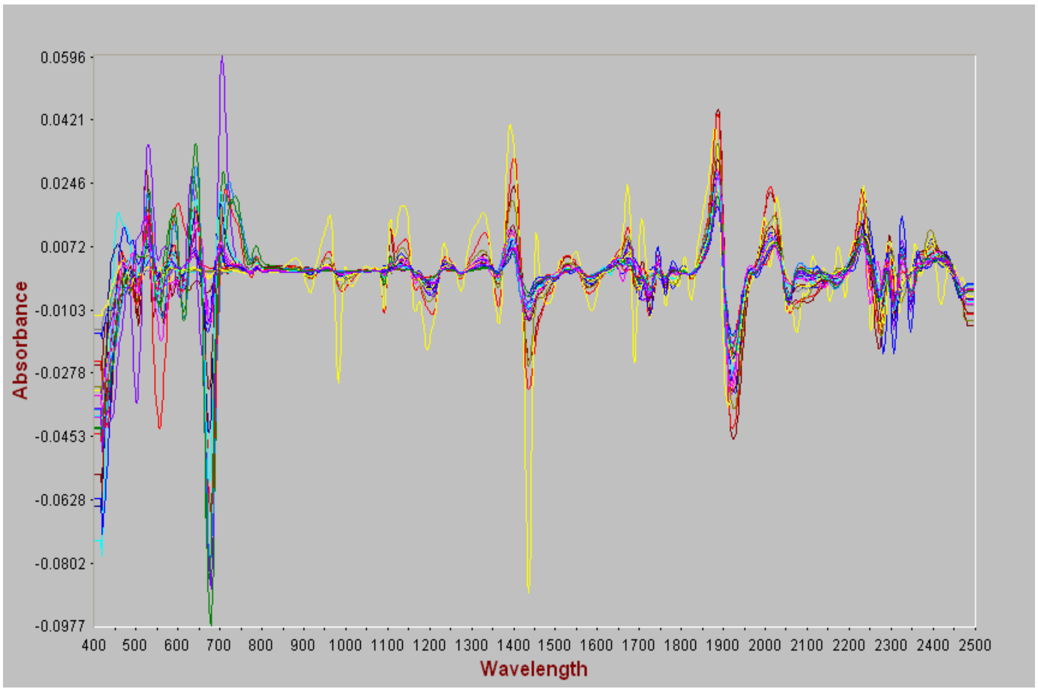

Figure 4.4: Second derivative spectra of the raw optical data of powdered herb samples in the range of 400 to 2500nm

It can be seen in **Table 4.5** and **Table 4.6**, the MLR and PLS methods were applied for both the metals using NIR spectral data and the atomic absorption data. The main statistical parameters of the developed calibration equations were the SEC and the R^2. **Table 4.5** shows the MLR calibration data for Zn and Pb, and **Table 4.6** shows the PLS calibration data for Zn and Pb. Lower SEC and higher R^2 are considered better and more accurate.

Table 4.5: MLR calibration data for Zn and Pb

MLR	Wavelength	R^2	SEC
Zn	2280	0.67	0.09
	622	0.74	0.08
Pb	730	0.86	0.06
	1736	0.92	0.04

R^2: Coefficient of determination in the calibration
SEC: Standard error of calibration

Table 4.6: PLS calibration data for Zn and Pb

PLS	Factor	R^2	SEC	SECV	RPD
Zn	1	0.41	0.13	0.15	1.20
	2	0.63	0.10	0.14	1.28
Pb	1	0.83	0.07	0.08	2.00
	2	0.91	0.05	0.08	2.00

R^2: Coefficient of determination in the calibration
SEC: Standard error of calibration
SECV: Standard error of cross-validation
RPD: ratio SD to SECV
Factors: only 2 factors were chosen due to small sample set

When a cross-validation is performed on the calibration set, NIR prediction error is defined as the SECV. The relation of the NIR-predicted and the reference values for determining the metal content is shown in **Figure 4.5**, **Figure 4.6**, **Figure 4.7** and **Figure 4.8**. For evaluation of the NIRS calibration models, standardization of different SECVs is required. So, the RPD, which is the ratio of SD to SECV, was estimated for each equation. As shown in **Table 4.6**, RPD for first and second factors for Lead (2.0) exhibited higher value than Zinc which agrees with the higher RPDs reported for Pb (1.72) in plants from the literature [199]. This low value is not acceptable for prediction or screening samples, because RPD is not above 2.5. RPD value for the second factor for Zn was 1.28, which is close to 1.0 and shows that the SD of differences between NIRS and reference data was close to SD of the overall variances in the reference data and hence there is insufficient diversity of samples for a reliable calibration model for prediction. Similarly RPD value for Pb (2.0), which is less than 2.5 and hence the same argument follows.

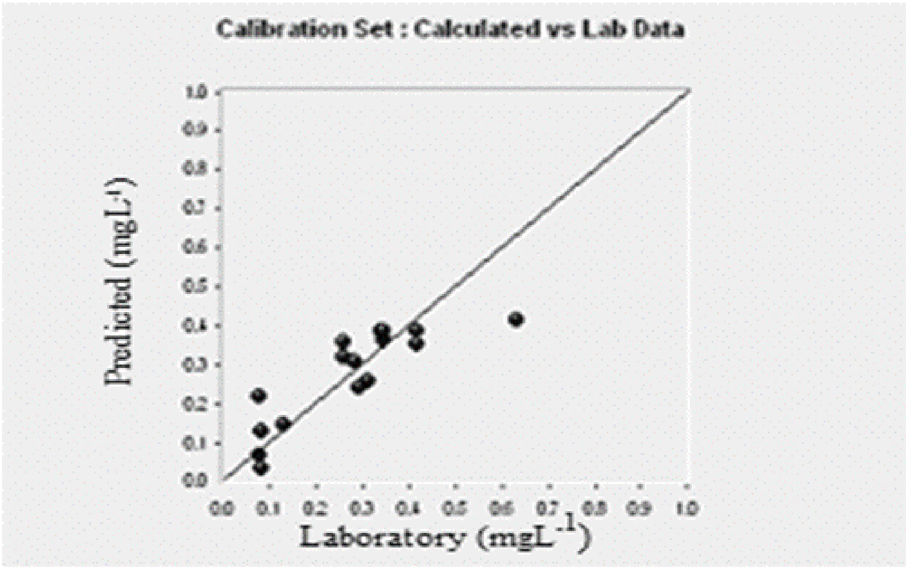

Figure 4.5: MLR Calibration plot for Zn

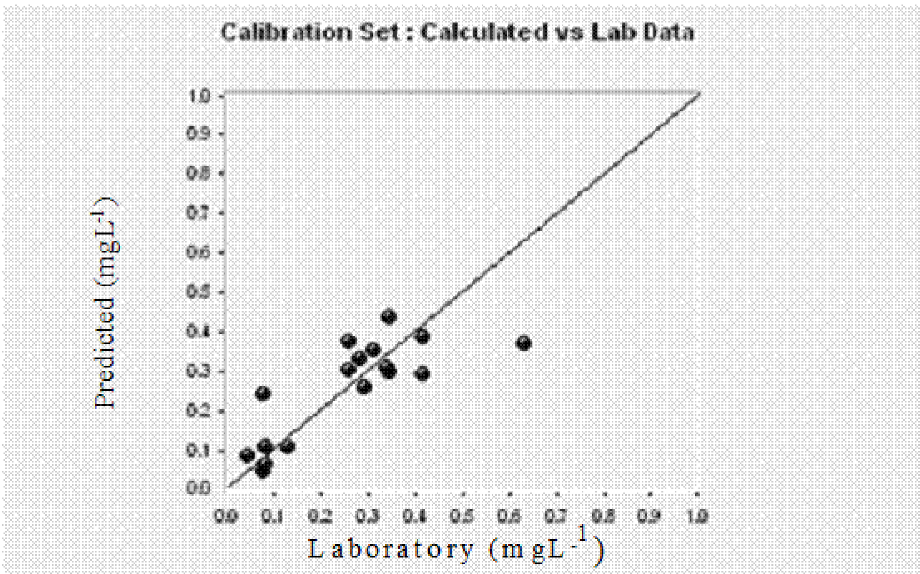

Figure 4.6: PLS Calibration plot for Zn

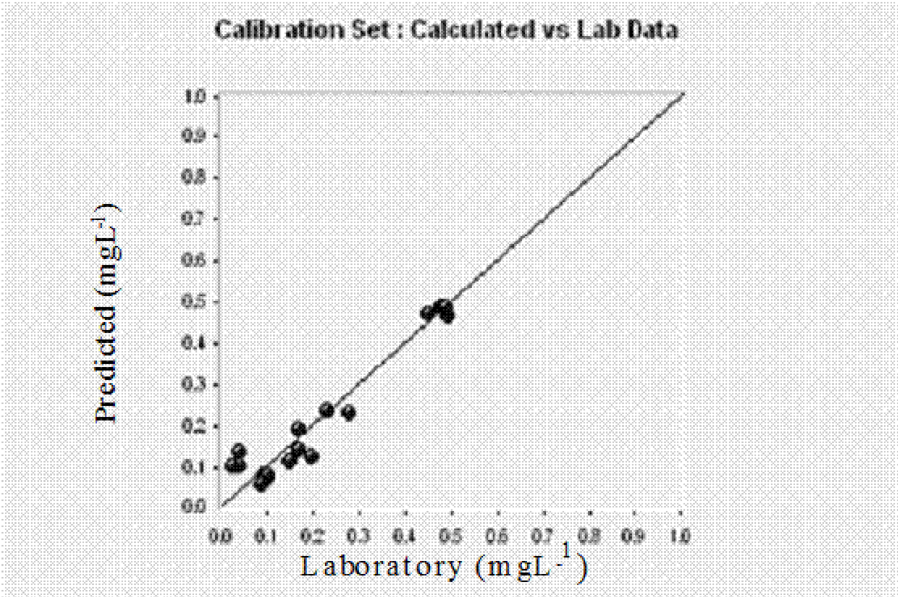

Figure 4.7: MLR Calibration plot for Pb

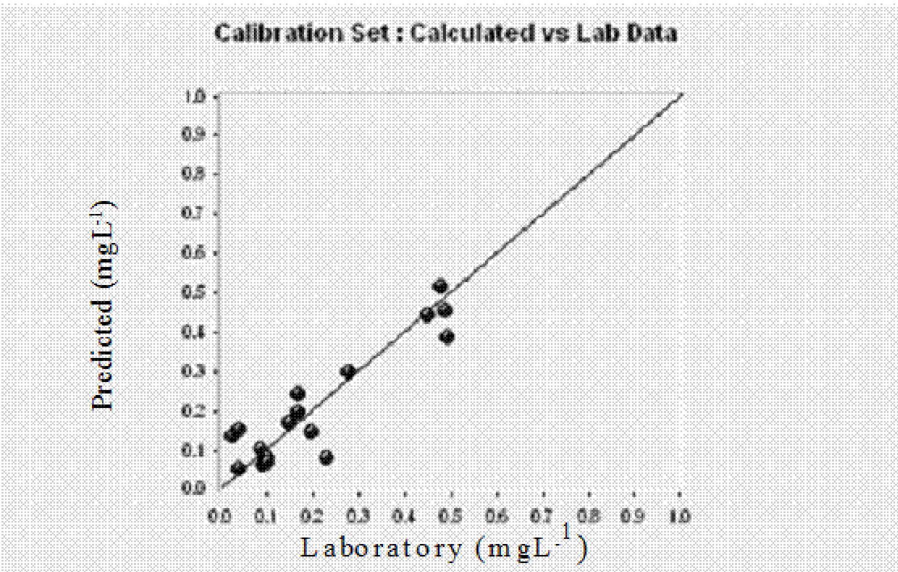

Figure 4.8: PLS Calibration plot for Pb

RPDs reported from the literature for Pb of different kinds of plants varied from 1 to 1.72 and for Zn from 0.87 to 1.48 [229, 230], but they do not represent high enough values for calibration model prediction.

4.5 CONCLUSION

This preliminary study shows that NIR spectroscopy is able to distinguish the metal content in various plant parts such as roots, stems and leaves of the studied Chinese herbs and looks very promising as a rapid method for metal content of herbs particularly for Zn and Pb which are reported here. In order to use NIR it is first necessary to analyse a large number of samples by wet chemistry which is time consuming. Once robust calibrations have been developed over three seasons on a very large number of diverse samples to allow for variation in plants due to climatic conditions, then it would be likely to replace the time consuming Atomic Absorption technique. From different parts of the analyzed herbs, the leaves were found to contain higher concentrations of Pb and Zn than other herbs that were flowers, seeds and roots.

5 EVALUATION OF MINERAL CONTENT OF CHINESE MEDICINAL HERBS USED TO IMPROVE KIDNEY FUNCTION WITH CHEMOMETRICS

5.1 ABSTRACT

Mineral elemental concentrations of fifty Chinese medicinal herbs in acid digests were determined by FAAS. The data were subjected to chemometric assessment to understand the association between the elements and to classify the herbal samples. Chemometric techniques such as PCA and HCA were used as classification techniques. PCA generated two principal components that explained 62% of the total variance in the data. HCA disclosed two significant groups of samples based on their elemental concentrations.

5.2 INTRODUCTION

Kidneys are a pair of vital organs that perform many functions including keeping the blood clean and chemically balanced. Kidney function depends on the ability of the kidneys to filter blood that is estimated using GFR. Progressive loss of kidney function leads to CKD [3]. The health burden of CKD is high for patients and health services worldwide. Millions of people around the world suffer from kidney diseases. The presence of kidney damage is indicated by proteinuria, hematuria, or reduced GFR. A study conducted by Australian Diabetes Kidney reported that age, diabetes mellitus, and hypertension were independently associated with proteinuria; age, gender and hypertension with hematuria; and age, gender and hypertension with reduced GFR. The study reported that approximately sixteen percent of the Australian population has either proteinuria, hematuria, and/or reduced GFR, indicating the presence of kidney damage [11]. In the view of Chinese medicine, kidney is considered as a system that include the functions of the urinary system and reproductive system [231]. Chinese herbalism has claimed an increasing share of the public's awareness. Herbal preparations have been used in China for over three thousand years that involved plant, mineral and animal substances to treat ailments [40]. Seventy to eighty percent of the population in many developed countries used some form of TM [32]. According to World Health Organization the percentage of the population that has used TM at least once is forty eight percent in Australia, seventy percent in Canada, forty two percent in USA, thirty eight percent in Belgium and seventy five percent in France [23].

Medicinal plants can synthesize a large variety of chemical substances that are of physiological importance. Therefore medicinal plants are a group of plants whose plant parts contain pharmacologically active compounds that can be used for the synthesis of useful drugs [232]. In the human body, the chemical constituents of medicinal plants interact directly or indirectly with the body chemistry. Once the active constituents are absorbed into the blood stream, these constituents circulate and influence the blood system to derive the required benefits. For example the chemical constituents present in the plant that include vegetable bases comprising alkaloids and amines, glycosides, essential oils are responsible for medicinal properties like characteristic odour of the plants. The toxic properties of plants chemical constituents are also responsible for toxic substances such as toxalbumin, resins and antibiotics. The trace elements play a very important role in the formation of these active compounds. One important aspect for the formation of active constituents in medicinal plants are the trace elements because the trace elements are known to play an important role in plant metabolism and the active constituents of medicinal plants are metabolic products of plant cells [233].

For human beings trace elements are essential nutrients with a gamut of functions. Trace elements are incorporated in the structures of proteins, enzymes, and the complex carbohydrates to participate in biochemical reaction. Trace elements with enzymes, for example, are necessary for the functioning and maintenance of the immune system [234]. Zinc is a critical nutritional component required for normal development and maintenance of immune functions in humans and animals. Zinc is also essential for the activity of numerous enzymes. Iron is an essential element for living cells, and a lack of iron is associated with anaemia. Magnesium plays a major role in overall cell functions. An adequate serum magnesium concentration may be necessary to maintain renal function and protect the kidneys from damage. Two of the most abundant ions that are controlled in dialytic patients are sodium and potassium. Sodium is the major indicator of body tonicity. Potassium is the most important intracellular cation that maintains the cell's osmotic pressure and acid-base balance in the human body. Calcium helps to form and maintain healthy bones [235]. Studies have shown a high prevalence of zinc deficiency in CKD patients, particularly those under either dialytic or conservative treatment [91, 236]. Other studies reported dialytic patients with low serum Iron, potassium and manganese levels [49, 84, 237]. Kiziltas *et al* reported high serum magnesium, potassium and normal sodium levels in dialytic patients. Studies indicate that calcium supplementation appears to be partially effective in people with CKD [101].

Chinese herbs are commonly used together in the form of decoctions according to the herbal formulae in which the properties of herbs and the effects of combining them have been observed and recorded over many centuries [40]. In this study, fifty Chinese herbs have been analyzed that are commonly used in Chinese formulae related to the regulation or improvement of kidney function. These herbs were analyzed for calcium, iron, magnesium, manganese, potassium, sodium and zinc by AAS. SRM was analyzed for data validation. Data analysis was done by standard chemometrics such as ANOVA, CA, PCA and HCA.

5.3 MATERIALS AND METHODS

5.3.1 Solutions and reagents

Doubly de-ionized water with a specific sensitivity of 18 mΩ obtained from Millipore- Milli-Q water purifier system (Milford, USA). Plant digests were prepared using HNO_3 (Trace SELECT, 69%) from Sigma-Aldrich, Australia. Stock standard solutions of individual metal elements (1000 or 10,000 mgL^{-1}) were supplied by Merck, Australia. SRM (1515), obtained from the National Institute of standards and technology of Gaithersburg, USA was used to verify the accuracy of results.

5.3.2 Instrumentation

Varian spectroAA-400 AAS (Varian Inc., Mulgrave, Australia) was used for the determination of metal elements. Measurements of elements were carried out in air/acetylene flame. The operating conditions for working concentrations of elements were set as recommended by the manufacturer, given in **Table 5.1.**

Table 5.1: The operating parameters for working elements

Elements	Wavelength (nm)	Lamp current (mA)	Slit (nm)
Ca	422.7	10	0.5
Fe	248.3	5	0.2
Mg	285.2	4	0.5
Mn	279.5	5	0.2
K	404.4	5	0.5
Na	330.3	5	0.5
Zn	213.9	5	1

5.3.3 Sample preparation procedure

Chinese herbal samples were purchased from local importers of Chinese herbs in dried form but not processed, which CHM practitioners prescribe to patients. Names of the herbs were listed in **Table 5.2.** Chinese herbs used in the experiment involved different parts of the plant. Sample numbers 1 to 2 were leaves, 3 to 17 were either whole plant/stem/twig/bark, 18 to 26 were roots, 27 to 34 were seeds, 35 to 40 were fruits, 41 to 45 were fossils, 46 to 49 were fungi and 50 was peel respectively. Sample numbers 42, 44 and 46 were in processed form and all other samples were dried Chinese herbs. The samples were ground using a pestle and mortar. The powdered samples were transferred into plastic bags. All samples were treated the same way. To prepare the samples for elemental analysis, 1g of the sample was accurately weighed into a pre-cleaned beaker. Concentrated nitric acid (6 mL) was added to the sample and the beaker covered with a watch-glass was heated gently for 3 h on a hot-plate until the digestion process was complete. The digested sample was allowed to cool to room temperature and then transferred quantitatively into clean 25 mL volumetric flasks. The samples were then diluted to the mark with Milli-Q water [204]. The same procedure was followed for SRM 1515. All the samples were analyzed in triplicate and quoted on a dry weight basis.

Table 5.2: English names of the analyzed Chinese herbs

Chinese herbs	English Name	Chinese herbs	English Name
Leaves		**Seeds**	
She Wei (no.1)	Pyrrosia leaves	Che Qian Zi (no.27)	Plantain seed
Yin Yang Huo (no.2)	Epimedium, Horny goat weed	Chi Xiao Dou (no.28)	Rice bean
Whole plant/Stem/Twig/Bark		Dong Gua Ren (no.29)	Wintermelon seed
Bian Xu Cao (no.3)	Knotweed, Polygonum	Hei Zhi Ma (no.30)	Black sesame
Che Qian Cao (no.4)	Herba plantaginis	Sha Yuan Zi (no.31)	Flatstem milkvtech seed
Deng Xin Cao (no.5)	Lamp wick herb	Tu Si Zi (no.32)	Chinese dodder seeds
Du Zhong (no.6)	Gutta-percha tree	Yi Yi Ren (no.33)	Job's Tears
Gu Sui Bu (no.7)	Drynaria rhizome	Yi Zhi Ren (no.34)	Cardamon
Hai Jin Sha (no.8)	Japanese climbing fem spore	**Fruits**	
Han Lian Cao (no.9)	Herba ecliptae	Bu Gu Zhi (no.35)	Psoralea fruit
Huang Jing (no.10)	Dropberry, Solomon seal	Di Fu Zi (no.36)	Kochia fruit
Jin Qian Cao (no.11)	Lysimachia	Gou Qi Zi (no.37)	Chinese wolfberry
Mu Tong (no.12)	Akebia caulis	Nu Zhen Zi (no.38)	Ligustrum, privet fruit
Suo Yang (no.13)	Herba cynomorii	Sang Shen (no.39)	Mulberry fruit
Tong Cao (no.14)	Ricepaper plant pith	Shan Zhu Yu (no.40)	Japanese Cornelian Cherry
Xian Mao (no.15)	Golden eye-grass rhizome	**Fossil**	
Yin Chen (no.16)	Red stem wormwood	Bie Jia (no.41)	Turtle Shell
Ze Xie (no.17)	Water plantain rhizome	Gu jia jiao (no.42)	Colla Plastrum Testudinis
Root		Long Gu (no.43)	Dragon's bone
Ba Ji Tian (no.18)	Morinda root	Lu jiao jiao (no.44)	Deer horn gelatine
Bi Xie (no.19)	Dioscorea root	Zi He Che (no.45)	Dried human placenta
Chuan Niu Xi (no.20)	Cyathula root	**Fungus**	
He Shou Wu (no.21)	Chinese knotweed	Dong Chong Xia Cao (no.46)	Caterpillar fungus
Mu Dan Pi (no.22)	Peony root	Fulingpi (no.47)	Indian bread
Shan Yao (no.23)	Cinnamon vine,Chinese yam	Ling Zhi (no. 48)	Reishi mushroom
Shu Di Huang (no.24)	Rehmannia Root	Zhu Ling (no.49)	Polyporus fungus
Xi Yang Shen (no.25)	American Ginseng	**Peel**	
Xu Duan (no.26)	Teasel root	Dong Gua Pi (no.50)	Chinese waxgourd peel

5.3.4 Data analysis

The statistical data analysis was done using PASW SPSS 18 (Release 18.0.0, Jul 30, 2009) and Microsoft Excel 2007 with add-in XL-STAT 2010. Analyses of elements in the Chinese herbs were done by Chemometrics such as ANOVA, CA and HCA using SPSS and PCA using XL-STAT.

5.4 Results and Discussion

The range, mean and SD obtained from triplicate analyses of the Chinese herbal samples were listed in Table 5.3. The relative SD was less than 10% for the analyzed elements. ANOVA was done to determine the significant differences between the mean values of elements in the samples. SRM analysis results were also presented in

Table 5.3. The association or correlation between the elements in the samples was categorized using CA and PCA. The herbs were categorized using PCA and HCA.

5.4.1 Total metal concentrations

Element concentrations of the samples are listed in Table 5.3. Ca content was highest in fossils (sample no's 41 and 43 respectively), followed by plants (sample no.14). Fe, K, Mn and Zn contents were highest in plants (sample no's 16, 14, 14 and 5 respectively). Mg content was highest in roots (sample no.20) and Na was highest in fruits (sample no.37). All the samples analyzed were found to be high in Ca and K compared to other elements. The results of one way ANOVA showed that significant differences ($p<0.05$) exist between various samples analyzed for Ca (130 – 560940 µg/g). The amount of Fe (20 – 8020 µg/g), K (270 – 90260 µg/g), Mg (90 – 5520 µg/g), Mn (20 – 1940 µg/g), Na (30 – 4500 µg/g) and Zn (10 – 1010 µg/g) were found to be similar ($p>0.1$) between the samples. The mineral contents of the herbs tested are within the range of recommended daily dietary intakes (Table 2.2).

The concentration of the elements for the herbs Che Qian Zi (No. 27), Dong Gua Ren (No. 29), Deng Xing Cao (No. 5), Mu Tong (No. 12), Yi Yi Ren (No. 33), Zhu ling (No. 49) and Ze Xie (No. 17) that are compared to the literature will now be discussed. The Fe content for these herbs studied was in the range of 150 – 2630 µg/g. Literature reported of Fe for the same herbs mentioned above in the range of the 65 – 580 µg/g [151]. For most of the analyzed herbs the Fe content is quite comparable, but for the herb Zhu ling, there exist a wide variation in the Fe content reported. The Mn content for the above herbs studied was in the range of 60 – 1250 µg/g. The range reported in the literature of Mn for the same herbs was in the range 35 – 138 µg/g [151]. Wide variation exists in the Mn content for the herb Deng Xing Cao. The Na content reported for the above herbs studied in this work was in the range 30 – 190 µg/g. Literature reported range of Na for the same herbs was in the range 90 – 1680 µg/g [151]. Wide variation exists in the Na content for the herb Dong Gua Ren in this study. The K content for the above herbs studied was in the range 560 – 5600 µg/g. Literature reported range of K for the same herbs was in the range 90 – 1680 µg/g [151]. The largest variation exists in K content for Che Qian Zi in this study. The Zn content for the above herbs studied was in the range 10 – 1010 µg/g. Literature reported range of Zn for the same herbs was in the range 6 – 83 µg/g [151]. Wide variation in Zn content exists for Deng Xing Cao in this work. Ca, Fe, Mg, Na and K contents studied for Ling Zhi were 2190 µg/g, 150 µg/g, 940 µg/g, 520 µg/g, and 3660 µg/g respectively. The Ca, Fe, Mg, Na and K contents for five herbal samples of Ling Zhi from various locations reported in the literature were in the range of 748 – 1637 µg/g, 64 – 124 µg/g, 418 – 775 µg/g, 205 – 293 µg/g and 704 – 5466 µg/g respectively [162]. The contents of Ca, Fe, Mg and Na were higher compared to the reported literature values. The contents of Ca, Fe, Mg, Mn, Na, and K for the herb Fu Ling in this study were recorded as 3030 µg/g, 20 µg/g, 720 µg/g, 30 µg/g, 40 µg/g and 3490 µg/g respectively. The contents of Ca, Fe, Mg, Mn, Na, and K for the herb Fu Ling reported in the literature were 529 µg/g, 333 µg/g, 358 µg/g, 20 µg/g, 211 µg/g, and 16220 µg/g respectively [164]. It can be seen that there is variation in the elemental contents for the studied herbs and literature. This may be due to the herbs grown in various regions where eenvironmental conditions such as type of soil, rainfall, vicinity of industry and extensive agricultural activity, influence the level of available elements in plants.

Table 5.3: Concentration of elements in Chinese herbal samples in µg/g

Sample ID	Ca	Fe	Mg	Mn	Na	K	Zn
SRM 1515 (Certified value)	15260 ± 150	83 ± 5	2710 ± 80	54 ± 3	24.4 ± 1.2	16100 ± 200	12.5 ± 0.3
SRM 1515 (Found value)	15450 ± 100	86 ± 5.5	2753 ± 50.2	56 ± 7.2	22.8 ± 2.4	16267 ± 152.7	12.6 ± 0.75
She Wei (no.1)	2500 ± 3.21	420 ± 2.51	1630 ± 7.81	80 ± 2.57	70 ± 3.22	5780 ± 4.61	10 ± 1.5
Yin Yang Huo (no.2)	3740 + 5.29	970 ± 6.38	2290 ± 4.52	580 ± 4.26	30 ± 1.27	3190 ± 5.24	10 ± 1.31
Bian Xu cao (no.3)	4530 ±4.72	1280 ± 4.23	4850 ± 6.93	120 ± 2.14	40 ± 1.84	8440 ± 5.38	30 ± 2.15
Che Qian Cao (no.4)	5450 ± 5.5	2360 ± 5.71	2290 ± 3.75	150 ± 3.38	40 ± 1.96	6910 ± 4.29	140 ± 2.51
Deng Xin Cao (no.5)	1750 ± 6.08	760 ± 8.12	1000 ± 5.94	1250 ± 2.76	160 ± 1.73	13200 ± 6.37	1010 ± 5.23
Du Zhong (no.6)	1720 ± 6.11	870 ± 4.11	290 ± 2.87	140 ± 1.69	40 ± 1.39	1580 ± 3.49	130 ± 2.14
Gu Sui Bu (no.7)	2950 ± 3.05	310 ± 1.74	1810 ± 3.69	280 ± 2.35	40 ± 2.75	5030 ± 4.82	20 ± 1.38
Hai Jin Sha (no.8)	2630 ± 2.51	230 ± 5.27	1000 ± 4.95	240 ± 2.46	40 ± 1.94	2970 ± 4.39	40 ± 2.37
Han Lian Cao (no.9)	11340 ± 7.37	1770 ± 7.83	3770 ± 7.36	330 ± 3.87	100 ± 2.76	31050 ± 6.81	20 ± 2.42
Huang Jing (no.10)	20610 ± 4.58	1440 ± 6.31	480 ± 2.43	190 ± 2.37	40 ± 1.34	3150 ± 2.94	40 ± 3.28
Jin Qian Cao (no.11)	36800 ± 9.53	3320 ± 9.78	850 ± 4.86	1090 ± 3.45	40 ± 1.65	2850 ± 3.27	20 ± 1.21
Mu Tong (no.12)	3680 ± 2.51	150 ± 3.24	490 ± 2.94	50 ± 1.38	40 ± 1.73	1890 ± 1.65	460 ± 4.23
Suo Yang (no.13)	2500 ± 9.07	300 ± 6.82	130 ± 1.49	30 ± 1.79	130 ± 3.84	3700 ± 6.37	20 ± 1.87
Tong Cao (no.14)	151050 ± 4.16	6650 ± 7.69	4970 ± 6.98	1940 ± 2.78	4050 ± 4.57	90260 ± 5.19	240 ± 4.26
Xian Mao (no.15)	64340 ± 7.07	1870 ± 3.41	2870 ± 3.72	350 ± 5.24	40 ± 1.37	5260 ± 4.25	40 ± 3.68
Yin Chen (no.16)	136540 ± 6.12	8020 ± 5.26	5010 ± 2.87	690 ± 3.76	200 ± 2.84	46490 ± 6.94	60 ± 2.36
Ze Xie (no.17)	1150 ± 3.15	180 ± 2.75	770 ± 2.16	370 ± 2.21	50 ± 1.62	5600 ± 3.18	100 ± 2.57
Ba Ji Tian (no.18)	2640 ± 6.97	1090 ± 6.92	440 ± 3.54	660 ± 3.58	70 ± 1.43	1320 ± 2.91	10 ± 1.17
Bi Xie (no.19)	910 ± 4.12	440 ± 4.26	200 ± 5.73	70 ± 1.29	40 ± 1.38	270 ± 2.46	10 ± 1.35
Chuan Niu Xi (no.20)	5010 ± 7.21	450 ± 3.57	5520 ± 4.76	210 ± 3.65	130 ± 1.94	11290 ± 5.82	10 ± 1.98
He Shou Wu (no.21)	1730 ± 3.98	420 ± 4.35	700 ± 2.83	50 ± 1.72	140 ± 1.67	6910 ± 3.67	10 ± 1.46
Mu Dan Pi (no.22)	5230 ± 5.26	210 ± 3.64	370 ± 5.84	30 ± 1.36	190 ± 2.49	1160 ± 2.82	210 ± 2.84
Shan Yao (no.23)	160 ± 3.28	90 ± 4.82	90 ± 1.21	20 ± 1.48	110 ± 2.73	810 ± 2.47	N.D
Shu Di Huang (no.24)	1020 ± 5.23	1080 ± 5.12	360 ± 4.73	60 ± 2.79	980 ± 1.64	5380 ± 3.64	10 ± 1.57
Xi Yang Shen (no.25)	1250 ± 6.36	130 ± 3.47	590 ± 2.34	130 ± 2.47	70 ± 1.95	4790 ± 4.58	10 ± 1.23
Xu Duan (no.26)	6730 ± 8.27	1320 ± 6.84	4090 ± 4.86	230 ± 1.26	110 ± 1.57	8130 ± 5.21	240 ± 2.16
Che Qian Zi (no.27)	1720 ± 4.25	310 ± 5.21	1380 ± 6.71	60 ± 1.47	70 ± 1.32	3390 ± 2.83	50 ± 2.48
Chi Xiao Dou (no.28)	660 ± 2.31	170 ± 4.26	820 ± 2.94	50 ± 1.65	40 ± 1.76	6880 ± 2.56	20 ± 2.31
Dong Gua Ren (no.29)	520 ± 6.31	290 ± 6.85	3360 ± 4.38	90 ± 1.74	100 ± 3.46	760 ± 2.35	50 ± 3.24
Hei Zhi Ma (no.30)	4190 ± 7.51	270 ± 3.69	2880 ± 7.42	110 ± 1.96	50 ± 2.35	800 ± 1.24	50 ± 2.73
Sha Yuan Zi (no.31)	940 ± 8.13	340 ± 2.87	940 ± 3.18	60 ± 1.27	30 ± 1.97	4380 ± 5.29	40 ± 2.14
Tu Si Zi (no.32)	770 ± 2.38	370 ± 5.64	810 ± 2.45	80 ± 1.49	50 ± 2.16	960 ± 1.95	20 ± 2.57
Yi Yi Ren (no.33)	130 ± 6.31	480 ± 6.18	850 ± 2.98	60 ± 2.49	30 ± 1.67	2250 ± 3.21	20 ± 1.59
Yi Zhi Ren (no.34)	1000 ± 1.39	150 ± 3.96	830 ± 6.32	710 ± 2.15	30 ± 1.48	15210 ± 5.73	20 ± 2.46
Bu Gu Zhi (no.35)	1880 ± 5.12	180 ± 2.64	800 ± 4.81	70 ± 2.18	60 ± 1.29	8320 ± 4.28	20 ± 2.33
Di Fu Zi (no.36)	4320 ± 2.34	180 ± 4.26	1940 ± 1.45	90 ± 1.39	190 ± 3.43	3780 ± 4.19	120 ± 2.79
Gou Qi Zi (no.37)	1010 ± 5.17	390 ± 6.57	550 ± 3.42	30 ± 2.28	4500 ± 2.94	8730 ± 5.82	20 ± 2.15
Nu Zhen Zi (no.38)	2100 ± 6.79	340 ± 4.19	590 ± 4.58	30 ± 3.22	60 ± 1.37	6250 ± 6.73	20 ± 1.64
Sang Shen (no.39)	3680 ± 4.28	1740 ± 2.47	1140 ± 2.31	80 ± 2.74	170 ± 2.19	6400 ± 6.34	10 ± 1.38
Shan Zhu Yu (no.40)	2100 ± 6.87	190 ± 5.28	620 ± 5.24	20 ± 1.52	50 ± 1.64	9640 ± 5.24	20 ± 2.46
Bie Jia (no.41)	560940 ± 8.74	140 ± 2.49	2080 ± 3.15	60 ± 1.02	170 ± 1.24	2150 ± 3.12	40 ± 2.19
Gu jia jiao (no.42)	5860 ± 6.82	410 ± 6.52	380 ± 1.82	30 ± 2.95	90 ± 3.68	1370 ± 2.79	10 ± 1.35
Long Gu (no.43)	382890 ± 8.35	3460 ± 8.24	2280 ± 6.73	370 ± 3.25	170 ± 2.72	2250 ± 4.57	50 ± 2.76
Lu jiao jiao (no.44)	5520 ± 4.52	300 ± 3.19	210 ± 4.58	20 ± 1.27	80 ± 1.95	1190 ± 5.89	10 ± 1.69
Zi He Che (no.45)	4310 ± 1.97	730 ± 2.56	340 ± 2.61	20 ± 1.21	110 ± 1.49	2020 ± 4.21	40 ± 2.43
Dong Chong Xia Cao (no.46)	15880 ± 6.79	510 ± 5.87	1360 ± 4.84	70 ± 3.22	180 ± 2.63	6240 ± 6.84	40 ± 2.67
Fulingpi (no.47)	3030 ± 5.76	20 ± 2.08	720 ± 3.72	30 ± 1.58	40 ± 1.92	3490 ± 4.16	N.D
Ling Zhi (no.48)	2190 ± 3.77	150 ± 3.57	940 ± 5.34	250 ± 2.74	520 ± 2.61	3660 ± 3.76	20 ± 2.48
Zhu Ling (no.49)	11150 ± 6.73	2630 ± 6.83	730 ± 4.53	110 ± 1.35	190 ± 1.75	560 ± 2.75	10 ± 1.26
Dong Gua Pi (no.50)	5300 ± 4.38	1940 ± 7.12	5380 ± 6.49	140 ± 1.18	120 ± 1.34	6440 ± 4.59	20 ± 1.48
Range	130-560940	20-8020	90-5520	20-1940	30-4500	270-90260	10-1010
Mean	30001	1036	1575	239	281	7690	72
Std. Deviation	97369 ± 2.03	1546 ± 1.88	1531 ± 1.73	362 ± 0.94	837 ± 0.77	14179 ± 1.56	157 ± 0.95

N.D - Not Detected

5.4.2 Correlation analysis

CA quantifies the relationship between the two variables and is measured by correlation coefficient r. r ranges from -1 to +1. The correlation analyses of the total elements content is presented in **Table 5.4**. The positive and negative correlation coefficients indicate positive and negative correlations respectively, between the two elements. When r is close to 1.0, the two variables are more similar and when r is close to 0, there exist very weak or no relation between the variables. All elements (**Table 5.4**) are positively correlated except that calcium is negatively correlated with zinc. Considering only the values of r greater than 0.5 (**Table 5.4**), it can be seen that there are strong correlations between Mg and Fe; Mn and Fe as well as K, Fe, Mg, Mn and Na.

Table 5.4: Correlation matrix for the element concentrations in Chinese herbal samples

	Ca	Fe	Mg	Mn	Na	K	Zn
Ca	1						
Fe	0.319	1					
Mg	0.220	0.529	1				
Mn	0.170	0.627	0.325	1			
Na	0.101	0.318	0.133	0.381	1		
K	0.177	0.731	0.521	0.716	0.572	1	
Zn	-0.011	0.065	0.023	0.440	0.066	0.178	1

5.4.3 Principal component analysis

PCA is a technique for reducing the number of variables by finding a linear combination of variables that explains the variance in the original variables. Principal component analyses give prominence to the relationship between the elements [167, 238]. The principal components with eigenvalues greater than 1 were extracted that lead to the formation of two principal components. The first and second components reported 45.2% and 16.8% variance respectively. The first two components described 62% variances for all the data. Varimax rotation method was used for the components rotation and the rotated loadings are listed in **Table 5.5** and plotted in **Figure 5.1.** Considering the values greater than 0.5, the loadings were larger for Fe, Mn, Na, K and Zn on the first component PC1 and for Ca, Fe and Mg on the second component PC2. **Table 5.6** gives the score values of the two principal components for each Chinese herbal sample and the scores were plotted in **Figure 5.2.** The score values of the herbs are considered in ascending order and these scores are interpreted with the larger loadings obtained for elements for both PC1 and PC2 respectively from **Table 5.5.** From the scores of the first principal component PC1, it can be interpreted that the concentrations of Fe, Mn, Na, K and Zn on the PC1 loadings are higher for sample no's 14, 5, 16, 11, 37 and 12 than sample no's 26, 9, 34, 4, 18, 17 and 2 and were lower for all the other samples. When the second principal component PC2 is interpreted, Ca, Fe and Mg concentrations on the PC2 loadings are higher for sample no's 16, 14, 41, 43, 50, 20, 9 and 3 than sample no's 15, 29, 49, 11, 39, 4, 2, 30, 26 and 1 and were lower for all the other samples.

Varimax rotated principal component loadings of elements and scores for Chinese herbal samples of the first two principal components are plotted in **Figure 5.1** and **Figure 5.2** respectively. The loadings indicate the association between the elements. The elements with larger loadings affect the separation of the samples. The loadings of PC1 and PC2 in **Figure 5.1** indicate individualized behaviour between the elements that appeared to be in order when interpreted with the loadings of the scores in **Figure 5.2** that are samples. The scores of PC1 and PC2 in **Figure 5.2** indicate the correlation between the samples. Sample no's 5, 14, 16, 41 and 43 were outliers. The sample no.5 had the highest content of Zn, sample no 14 had the highest content of Mn and K, sample no 16 had the highest content of Fe and Sample no's 41 and 43 had the highest content of Ca. Sample numbers 41 and 43 were fossils and supposed to have highest content of Ca than other samples and hence appear more dispersed in the score loadings. All other samples other than the outliers appeared to be more associated with each other.

Table 5.5: Varimax rotated factor loadings of the first two principal components for elements

Element	PC1	PC2
Ca	0.037	0.604
Fe	0.598	0.638
Mg	0.325	0.656
Mn	0.881	0.155
Na	0.553	0.201
K	0.786	0.467
Zn	0.664	-0.509

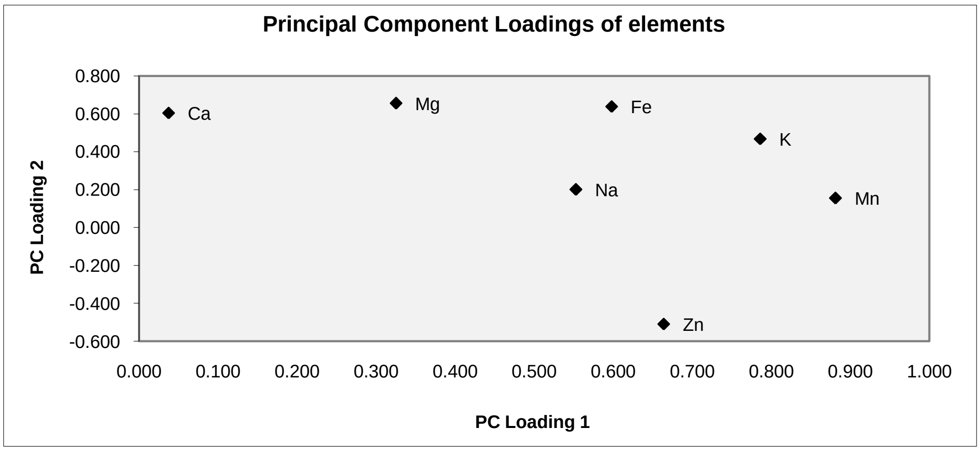

Figure 5.1: Varimax rotated principal component (PC1 versus PC2) loadings for elements

Table 5.6: Varimax rotated scores of the first two principal components for Chinese herbal samples

Chinese herbal samples	PC1	PC2	Chinese herbal samples	PC1	PC2
She Wei (no.1)	-0.441	0.005	Xu Duan (no.26)	0.462	0.040
Yin Yang Huo (no.2)	0.064	0.114	Che Qian Zi (no.27)	-0.402	-0.232
Bian Xu Cao (no.3)	-0.247	0.936	Chi Xiao Dou (no.28)	-0.448	-0.269
Che Qian Cao (no.4)	0.157	0.128	Dong Gua Ren (no.29)	-0.418	0.220
Deng Xin Cao (no.5)	3.722	-3.631	Hei Zhi Ma (no.30)	-0.414	0.106
Du Zhong (no.6)	-0.089	-0.697	Sha Yuan Zi (no.31)	-0.417	-0.297
Gu Sui Bu (no.7)	-0.237	-0.068	Tu Si Zi (no.32)	-0.505	-0.298
Hai Jin Sha (no.8)	-0.264	-0.362	Yi Yi Ren (no.33)	-0.498	-0.251
Han Lian Cao (no.9)	0.405	0.986	Yi Zhi Ren (no.34)	0.386	-0.376
Huang Jing (no.10)	-0.235	-0.161	Bu Gu Zhi (no.35)	-0.397	-0.258
Jin Qian Cao (no.11)	0.774	0.176	Di Fu Zi (no.36)	-0.157	-0.340
Mu Tong (no.12)	0.670	-1.834	Gou Qi Zi (no.37)	0.710	-0.226
Suo Yang (no.13)	-0.493	-0.432	Nu Zhen Zi (no.38)	-0.462	-0.287
Tong Cao (no.14)	4.973	2.277	Sang Shen (no.39)	-0.296	0.154
Xian Mao (no.15)	-0.060	0.682	Shan Zhu Yu (no.40)	-0.426	-0.273
Yin Chen (no.16)	1.541	2.978	Bie Jia (no.41)	-1.179	2.317
Ze Xie (no.17)	0.086	-0.643	Gu Jia Jiao (no.42)	-0.569	-0.324
Ba Ji Tian (no.18)	0.141	-0.370	Long Gu (no.43)	-0.320	2.140
Bi Xie (no.19)	-0.550	-0.407	Lu jiao jiao (no.44)	-0.593	-0.389
Chuan Niu Xi (no.20)	-0.205	1.010	Zi He Che (no.45)	-0.451	-0.367
He Shou Wu (no.21)	-0.430	-0.211	Dong Chong Xia Cao (no.46)	-0.342	-0.078
Mu Dan Pi (no.22)	-0.012	-1.026	Fulingpi (no.47)	-0.600	-0.275
Shan Yao (no.23)	-0.630	-0.453	Ling Zhi (no.48)	-0.181	-0.320
Shu Di Huang (no.24)	-0.179	-0.179	Zhu Ling (no.49)	-0.300	0.193
Xi Yang Shen (no.25)	-0.426	-0.342	Dong Gua Pi (no.50)	-0.218	1.209

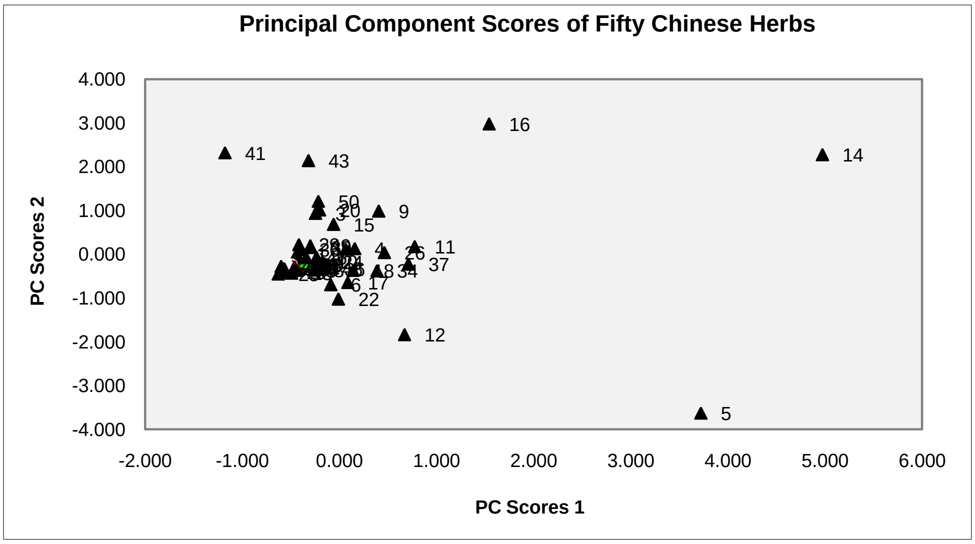

Figure 5.2: Varimax rotated principal component scores (PC1 versus PC2) of Chinese Herbal Samples

5.4.4 Hierarchical cluster analysis

HCA is the most widely used unsupervised pattern recognition technique in chemometrics. The technique involves hierarchically grouping samples on the basis of similarity without using prior information about these groupings. The cluster analyses were done using SPSS. In this study, Ward's method of clustering was applied with the squared Euclidean distance measurement. Close results to PCA were obtained from HCA. Three groups were obtained (Figure 5.3). Group 1 include sample no's 1 to 50 except 14, 16, 41 and 43. Group 2 include sample no's 14 and 16 (highest in Mn, K and Fe respectively from PCA). Group 3 include sample no's 41 and 43 (highest in Ca from PCA).

The results gave three significant groups of samples. Sample no 5 (Deng Xin Cao), which is a diuretic, is high in Zn. Sample no 14 (Tong Cao) which promotes urination according to Chinese medicine, is high in Mn and K. Sample no 16 (Yin Chen) removes damp heat (water metabolism disorder) and is high in Fe. Sample no's 41 and 43 (Bie Jia and Long Gu) strengthen the bones and are high in Ca. From total metal concentrations, Tong Cao is also high in Ca, K and Mn. Chuan Niu Xi (strengthen bone and invigorate blood circulation) is high in Mg. Gou Qi Zi (tonifying kidney function) is high in Na. In CHM practice, various kidney-tonifying herbs that were analyzed in this study are commonly used together as a formula to achieve better treatment outcomes in balancing or improving kidney function. The elemental concentration of the herbs obtained in this study can assist in the development of herbal formulae to improve kidney function.

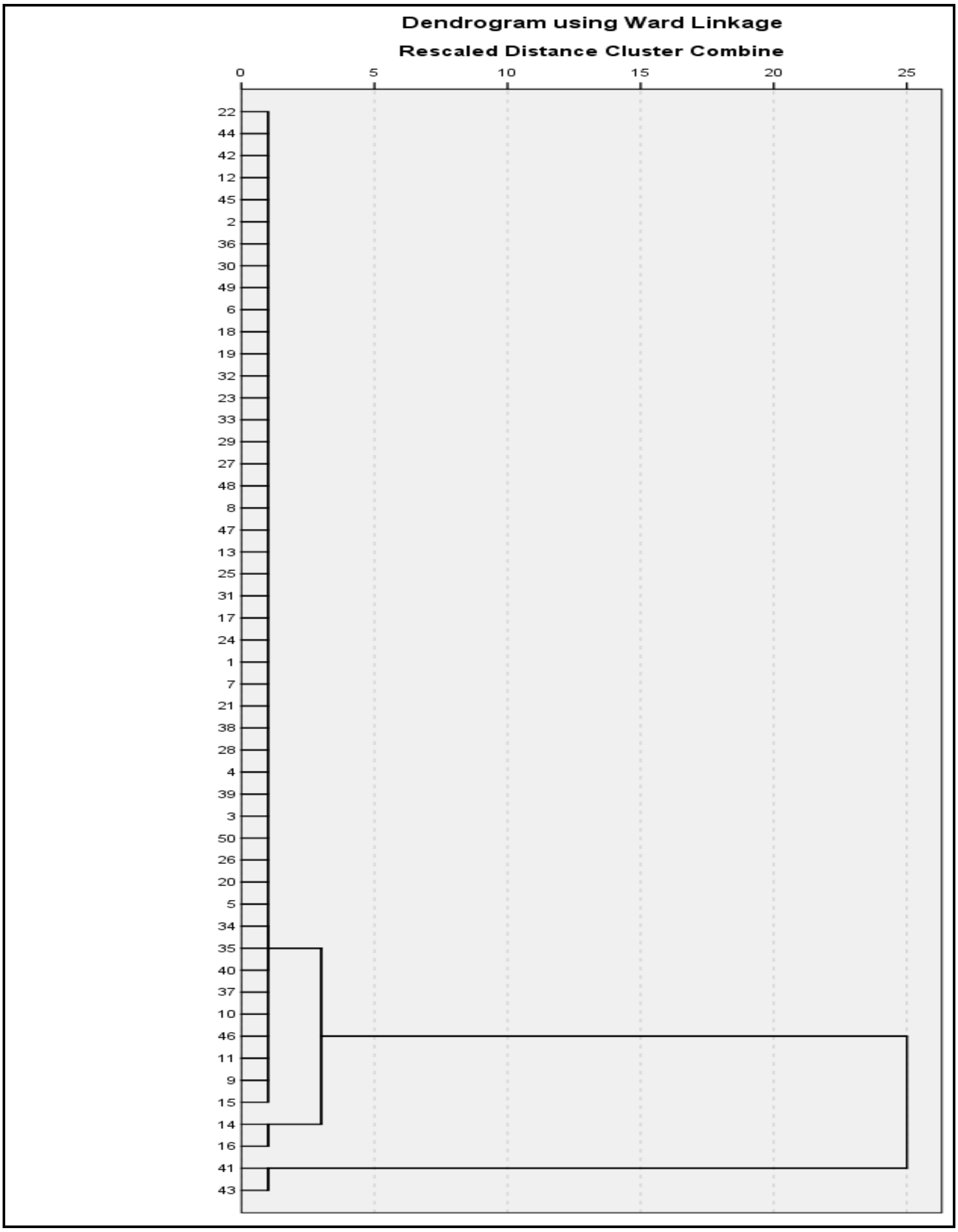

Figure 5.3: Dendrogram of hierarchical cluster analysis for the Chinese medicinal herbs

5.5 CONCLUSIONS

This study contributes to the analyses of mineral concentration of elements in fifty Chinese herbal samples. All the samples analyzed were found to be high in Ca and K compared to other elements. Data analysis was done using chemometrics such as ANOVA, CA, PCA and HCA. ANOVA showed that significant differences exist between various samples analyzed for Ca. CA showed that strong correlations exist between Fe, Mg, Mn, Na and K. Two significant groups of samples with group 1 containing samples Tong Cao that is high in Mn and K and Yin Chen, high in Fe and group 2 containing samples Bie Jia and Long Gu, high in Ca were obtained from PCA and HCA.

6 DETERMINATION AND COMPARISON OF MINERAL ELEMENTS IN TRADITIONAL CHINESE HERBAL FORMULAE: LIU WEI DI HUANG WAN AND JIN GUI SHEN QI WAN AT DIFFERENT DECOCTION TIMES USED TO IMPROVE KIDNEY FUNCTION – CHEMOMETRIC APPROACH

6.1 ABSTRACT

Traditional Chinese herbal formulae LW: six-ingredient pill with Rehmannia and JG: kidney Qi pill from the golden cabinet were analyzed for Ca, Fe, Mg, Mn, Na, K and Zn at different decoction intervals byAAS. LW and JG were used to improve the kidney function. JG was higher in all elements than LW. K (1691.29 – 2372.71 mg L^{-1}) was highest in both formulae LW and JG followed by Ca (245.31 – 562.91 mg L^{-1}). Ca, Fe, Mg, Mn and K were highest at 40 min for LW and Fe, Mn, and K were highest at 40 min for JG. Chemometrics such as PCA, HCA and LDA were applied to classify the data and to understand the relation between the elements. Metal intake related to the consumption of decoction has also been studied. Mn made the highest contribution to average daily dietary intakes from the formulae.

6.2 INTRODUCTION

TCM is one of the oldest healing systems. Clinical diagnosis and Chinese herbology are very important components of TCM. CHM not only includes plants, but also includes medicinal uses of animals. Most of the principles of TCM were derived from the philosophical basis that contributed to the development of Taoism, and Confucianism [37, 38]. Ancient Chinese scholars noted that all natural phenomena could be categorized into Yin and Yang, which are two opposite, complementary, interdependent, and exchangeable aspects of nature, everything in the universe consisted of five basic elements: wood, fire, earth, metal, and water, and the universe was constantly changing towards dynamic balance or harmony. Such knowledge was applied to understand, prevent, and cure disease. In TCM, the five Yin, solid organs are Lungs, Heart, Spleen, Liver and Kidneys and six Yang, hollow organs are small intestine, large intestine, gall bladder, stomach and San Jiao: called as triple warmer or triple heater. Yin refers largely to the material aspects of the organism and Yang to functions. Disease occurs after a disturbance in Yin–Yang or disharmony in the organs caused by pathogenic: anger, joy, sadness, fear and climatic factors: wind, cold, damp, heat. Treatment aims to expel or suppress the cause and restore balance [39].

The development of herbal formulae has been an empirical process in which the properties of herbs and the effects of combining them have been observed and recorded over many centuries. The resulting classic formulae comprise the basis of treatment in CHM. The herbs are classified as Yin or Yang and are selected to balance the Yin or Yang of the patient's illness. The qualities of the four energies in the herbs: hot, warm, cold, and cool are also used to balance the qualities shown in the illness [239]. Additionally, the herbs are chosen for their appropriate flavors', with the five flavors: hot, sweet, sour, bitter, and salty matching the five elements: metal, earth, wood, fire, and water, and treating the related organ. The special affinity of an herb and its drying or moistening nature also affect the selection. The simplest way of using the herbs is to make a decoction. This is done by boiling the herbs in a given amount of water to extract their substance [40].

TCM views kidneys as centre of body's Yin or Yang or 'the origin of life' where nutrients are stored and physiological functions takes place. Kidney Yin is the foundation of the Yin fluid (blood, Qi). Kidney Yin moistens and nourishes the organs and tissues of the whole body. Kidney Yang is the foundation of the Yang Qi and is crucial for normal bladder function. Kidney Yang warms and promotes the functions of the organs and tissues. One of the major functions of kidney's Yin Qi, which is the vital energy of the body, is to promote the transformation of blood. Ancient TCM classics mention that "Kidney Qi excerpts into the bones and transforms into marrow". The liver also plays an important role in the process of transforming Qi into blood because it stores the excess blood that is produced from the marrow. When either the kidneys or liver are not functioning properly, the production of blood will be affected and individuals can acquire a blood-deficiency syndrome [3]. A traditional Chinese herbal formula such as LW is used for kidney and liver Yin deficiency and JG is used for kidney Yang deficiency.

The medicinal plants traditionally occupied an important position in the sociocultural, spiritual, and ethnopharmacology of rural and tribal lives in different parts of the world. Modern pharmacopeia contains at least 25% drugs derived from plants. Approximately 70% of "synthetic" medicines are derived from plants. The consumption of specific medicinal plants for fighting inflectional diseases and other pathologies falls within a specialist herbivore behaviour. Quality of herbs depends on the presence of active principles. Among those, organic components have deserved most attention. Trace elements in medicinal plants can have a substantial influence on the therapeutic value of herbal remedies: a positive contribution as a source of essential nutrients or even as active principles, or a negative effect because of the accumulation of high concentrations of potentially toxic elements [240]. Medicinal plants can be rich in trace elements [158, 168, 241]. Despite the relatively small weight proportion of these plant products in the diet, their regular consumption can make a significant contribution to the daily recommended intake [242]. It has to be taken into account that only part of the trace elements present in the herbal product is leached into the infusions. Enhanced intake of minerals like Ca, K, Na and P can be useful in patients with chronic renal failure. Mineral concentrations in herbs used for renal and urinary tract disorders in India have been reported to be higher than in non-medicinal plants [44]. The knowledge of the effects and concentrations of bioactive elements in foods and herbs could guide the selection of Chinese herbs in clinical practice in association with TCM theories [243].

In this study, two TCM formulae used to improve the kidney function have been analyzed. LW, formula that nourish and tonify the Yin and JG, formula that warm and tonify the Yang. LW is known as six-ingredient pill with Rehmannia, formula that nourish and tonify the kidney Yin and used in treating disorders such as dizziness, tinnitus, diabetes, autoimmune diseases, urinary tract infection and sexual inadequacy, etc [134, 244]. JG is known as kidney Qi pill from the golden cabinet, formula that warm and tonify the kidney Yang, and that it supports its function of transforming water in particular and used in treating disorders such as interstitial nephritis, arthritis, chronic bronchitis, etc [43]. The formulae were analyzed for calcium, iron, magnesium, manganese, potassium, sodium and zinc at different decoction intervals by AAS.

6.3 MATERIALS AND METHODS

6.3.1 Solutions and reagents

Standard stock solutions of Ca, Fe, Mg, Mn, Na, K and Zn at a concentration of 1 gL^{-1} were obtained from Merck, Australia, and working solutions were prepared by dilution of stock solutions in 5% (v/v) HNO_3 (Trace SELECT, 69%) from Sigma-Aldrich, Australia. Ionization in AAS was controlled by adding a 5mL (1 g/L CsCl + 10 g/L La, Merck) buffer solution to all samples and standards and made up to final volume of 100 mL. Deionized water with a specific sensitivity of 18 mΩ obtained from Millipore- Milli-Q water purifier system (Milford, USA). All chemicals and reagents were of analytical grade.

6.3.2 Sample preparation procedure

The metal content was analyzed in two TCM formulae: LW and JG. The herbs involved in the formulae, the amount of each herb used that were generally prescribed by herbalist were listed in **Table 6.1**

Table 6.1: List of the traditional Chinese herbal formulae

Formula name	Chinese herbs	Botanical name	Amount used (g)
Li wei di huang wan	shu di huang	*Radix Rehmanniae Glutinosae Conquitae*	24
	shan zhu yu	*Fructus Corni Officinalis*	12
	shan yao	*Radix Dioscoreae Oppositae*	12
	fu ling	*Sclerotium Poriae Cocos*	9
	mu dan pi	*Cortex Moutan Radicis*	9
	ze xie	*Rhizoma Alismatis Orientalis*	9
Jin gui shen qi wan	sheng di huang	*Radix Rehmanniae Glutinosae*	24
	shan zhu yu	*Fructus Corni Officinalis*	12
	shan yao	*Radix Dioscoreae Oppositae*	12
	fu zi	*Radix Lateralis Aconiti Carmichaeli Praeparata*	3
	gui zhi	*Ramulus Cinnamomi Cassiae*	3
	ze xie	*Rhizoma Alismatis Orientalis*	9
	fu ling	*Sclerotium Poriae Cocos*	9
	mu dan pi	*Cortex Moutan Radicis*	9

The method of preparation of decoction was done according to the Chinese standard procedure [43]. The process of decoction combines the elements of various herbs involved as a means of treating a particular ailment. All the herbs involved in each formula were broken down into small pieces, weighed (**Table 6.1**) and kept in a glass beaker with a lid and 200 mL of water was added and allowed to soak for 20 min. Twelve samples were prepared in the same way and performed in triplicate for each formula over the different time intervals: 5 to 60 min. The mixture was first heated to boiling (100º C) and then kept at 60º C for different time intervals: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min. The decoction was allowed to cool to room temperature and then filtered with Whatman ashless filter paper of grade 41 and transferred into a 100 mL volumetric flask.

6.3.3 Instrumentation

The determination of Ca, Fe, Mg, Mn, Na, K and Zn was done on a Varian spectroAA-400 AAS (Varian Inc., Mulgrave, Australia) under optimized measurement conditions using hollow cathode lamps. The operating conditions for working concentrations of elements were set as recommended by the manufacturer, given in **Table 6.2.**

Table 6.2: The operating parameters for working elements

Elements	Wavelength (nm)	Lamp current (mA)	Slit (nm)
Ca	422.7	10	0.5
Fe	248.3	5	0.2
Mg	285.2	4	0.5
Mn	279.5	5	0.2
K	404.4	5	0.5
Na	330.3	5	0.5
Zn	213.9	5	1

6.3.4 Data analysis

The statistical data analysis was made using the SPSS for windows PASW SPSS 18 (Release 18.0.0, Jul 30, 2009) and Microsoft Excel 2007 with add-in XL-STAT 2010. To obtain more reliable information about the element content relationship, Pearson correlation analysis technique was used. To identify the elemental patterns in the data set, PCA was used. In order to classify the data, HCA – unsupervised pattern recognition technique and LDA – supervised pattern recognition technique were used.

6.4 RESULTS AND DISCUSSION

6.4.1 Metal content in formulae

According to the Chinese standard procedure of making decoctions, most formulas are heated for 20-30 min. The formulas that contain rich, cloying substances should be heated for 45-60 min. The metal contents of the two formulae LW and JG at different decoction times with 5 min interval from 5-60 min were given in **Table 6.3** and plotted in **Figure 6.1** and **Figure 6.2** respectively. The content of essential elements in plants is conditional, the content being influenced by the geochemical characteristics of the soil and by the ability of plants to selectively accumulate some of these elements [245, 246]. Ca, Fe, Mg, Mn, Na, K and Zn were higher in formula JG than LW. Independent sample t-test was done to determine the significant differences between the mean values of the elements in the formulae. The results showed that, except for Zn (0.91 – 1.04 mg L^{-1}), significant differences ($p<0.05$) exist between LW and JG for Ca (245.31 – 562.91 mg L^{-1}), Fe (10.85 – 16.92 mg L^{-1}), Mg (95.34 – 147.03 mg L^{-1}), Mn (3.33 – 5.16 mg L^{-1}), Na (40.98 – 85.54 mg L^{-1}) and K (1691.29 –

2372.71 mg L^{-1}). K was highest followed by Ca, Mg, Na, Fe, Mn and Zn in both formulae LW and JG.

Table 6.3: Metal content (mg L^{-1}) of the analyzed formulae (mean ± S.D)

Formula name	Time (min)	Ca	Fe	Mg	Mn	Na	K	Zn
Li wei di huang wan	5	215.26 ± 3.5	10.02 ± 1.56	81.85 ± 2.89	3.05 ± .75	20.81 ± 2.47	1833.57 ± 5.45	0.91 ± .23
	10	218.18 ± 4.2	10.97 ± .64	86.31 ± 2.7	2.90 ± .52	22.81 ± 2.21	1662.6 ± 6.89	1.41 ± .81
	15	225.14 ± 3.84	13.30 ± .89	98.57 ± 4.86	3.45 ± .98	46.09 ± 2.91	1708.07 ± 2.22	0.87 ± .27
	20	280.39 ± 3.71	13.55 ± 1.48	92.74 ± 6.0	3.52 ± .59	46.93 ± 2.56	1835.24 ± 6.35	0.85 ± .28
	25	250.16 ± 2.93	11.95 ± .22	91.06 ± 3.71	3.65 ± .43	53.09 ± 2.84	1676.64 ± 2.53	0.84 ± .29
	30	225.55 ± 3.47	10.98 ± 1.10	88.66 ± 1.84	2.36 ± .90	70.52 ± 2.62	1557.91 ± 2.33	0.83 ± .28
	35	220.13 ± 1.11	10.17 ± 1.74	86.93 ± 2.34	3.29 ± .68	41.51 ± 3.4	1505.48 ± 4.05	0.89 ± .34
	40	311.28 ± 5.43	17.46 ± 1.33	122.1 ± 4.38	4.11 ± 1.55	82.51 ± 5.04	1896.19 ± 2.84	0.89 ± .75
	45	269.42 ± 4.87	7.77 ± .83	98.43 ± 2.07	3.51 ± .83	35.19 ± 3.48	1644.68 ± 4.22	0.77 ± .38
	50	262.58 ± 5.47	5.19 ± 1.24	100.71 ± 5.4	3.24 ± .79	22.35 ± 2.55	1621.64 ± 6.29	0.96 ± .28
	55	242.06 ± 4.64	9.46 ± .86	102.55 ± 4.29	3.19 ± 1.06	20.96 ± 2.59	1672.03 ± 5.79	0.84 ± .56
	60	223.62 ± 3.35	9.30 ± 1.43	94.15 ± 3.88	3.65 ± .62	29.04 ± 2.48	1681.38 ± 5.82	0.77 ± .34
Mean		245.31	10.85	95.34	3.33	40.98	1691.29	0.91
Jin gui shen qi wan	5	432.72 ± 4.19	10.18 ± 1.02	97.85 ± 1.93	3.55 ± .57	70.49 ± 2.1	1859.56 ± 4.07	1.00 ± .31
	10	424.19 ± 3.57	11.15 ± 1.27	88.06 ± 1.48	3.49 ± .58	56.91 ± 1.92	1783.31 ± 4.24	0.84 ± .56
	15	608.64 ± 3.54	14.16 ± .58	123.55 ± 1.98	4.75 ± .33	86.91 ± 1.56	2160.1 ± 2.47	1.00 ± .67
	20	975.03 ± 3.03	16.30 ± .74	129.15 ± 1.70	5.43 ± .62	94.41 ± 1.35	2163.06 ± 4.29	1.26 ± .77
	25	1061.6 ± 4.04	18.95 ± .29	202.96 ± 1.9	5.84 ± .79	127.14 ± 1.63	2940.72 ± 3.25	1.53 ± .57
	30	482.85 ± 2.45	18.68 ± 1.03	156.77 ± 2.84	5.80 ± .4	72.53 ± 1.4	2447.06 ± 3.6	1.10 ± .96
	35	489.12 ± 2.29	21.65 ± 1.35	152.86 ± 1.83	4.84 ± .66	74.84 ± 1.84	2410.06 ± 4.22	1.50 ± .42
	40	470.51 ± 2.68	22.08 ± 1.71	191.31 ± 2.86	6.3 ± 1.37	92.03 ± 2.18	3179.18 ± 2.21	0.72 ± .65
	45	463.24 ± 2.8	14.58 ± 1.3	195.28 ± 2.85	5.08 ± .87	121.26 ± 2.89	2502.58 ± 4.0	0.74 ± .44
	50	456.37 ± 2.79	13.20 ± 1.24	130.32 ± 1.44	4.45 ± .55	71.07 ± 2.23	1998.01 ± 2.91	0.76 ± .63
	55	448.18 ± 2.78	21.20 ± 2.23	145.05 ± 2.32	5.94 ± .19	78.69 ± 1.85	2450.53 ± 3.38	0.93 ± .85
	60	442.41 ± 4.2	20.83 ± 2.6	151.22 ± 2.18	6.28 ± 1.38	80.14 ± 3.09	2578.37 ± 2.0	1.02 ± .9
Mean		562.91	16.92	147.03	5.16	85.54	2372.71	1.04

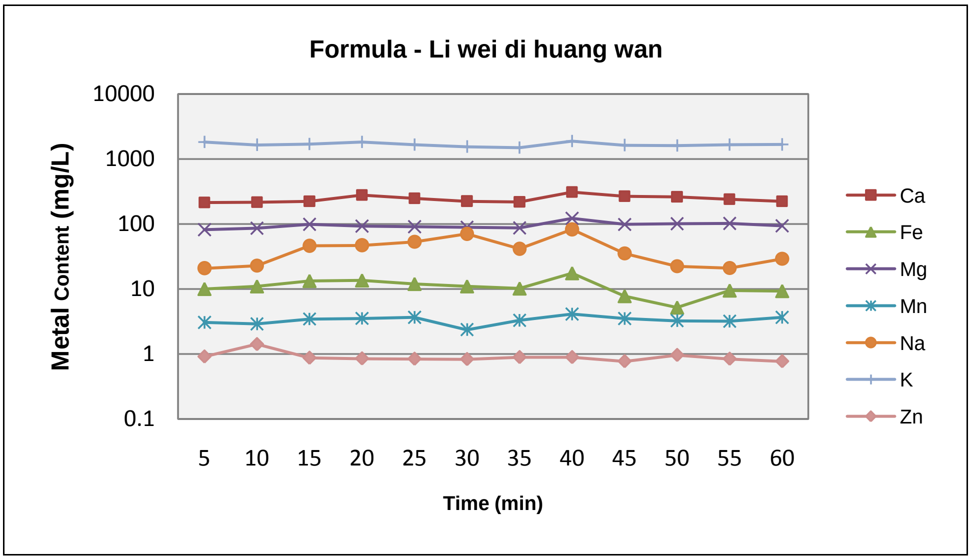

Figure 6.1: Metal content (mg L^{-1}) of formula LW

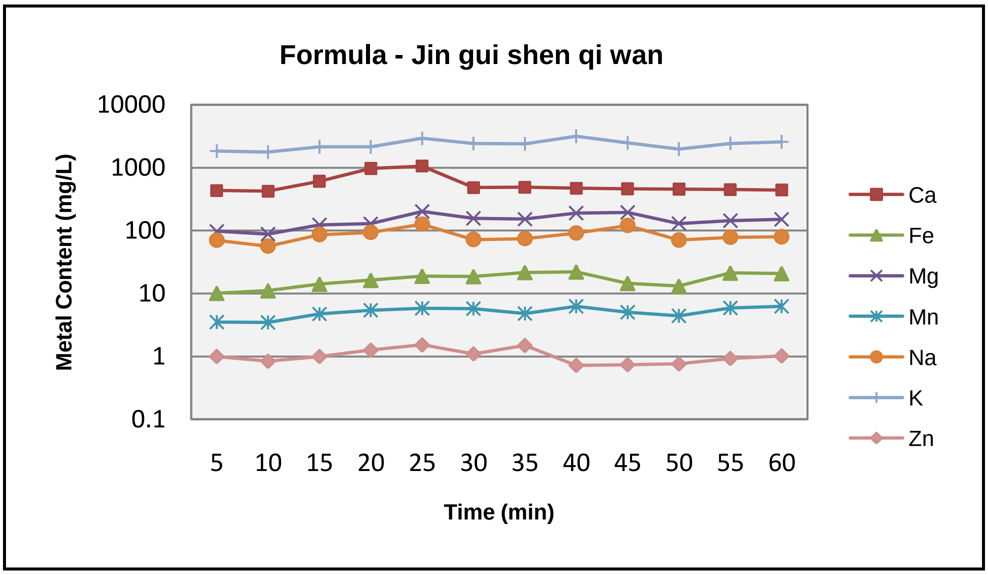

Figure 6.2: Metal content (mg L^{-1}) of formula JG

Correlation is a measure of association between two variables. The correlation matrix of the elements was presented in **Table 6.4**. Considering only the values of correlation coefficient greater than 0.5, it can be seen that good correlation exists between all the elements.

Table 6.4: Correlation matrix of elements (Pearson correlation)

Variables	Ca	Fe	Mg	Mn	Na	K	Zn
Ca	1						
Fe	0.531	1					
Mg	0.664	0.763	1				
Mn	0.683	0.857	0.872	1			
Na	0.768	0.707	0.828	0.738	1		
K	0.667	0.849	0.937	0.922	0.770	1	
Zn	0.558	0.355	0.268	0.253	0.241	0.295	1

In order to assimilate the elemental patterns that are meaningful, PCA was performed. The principal components (PC) with eigenvalues greater than one were extracted and subjected to Varimax rotation. The results after Varimax rotation: first and second factor are presented in **Table 6.5** and plotted in **Figure 6.3**. The percentages of variances after the Varimax rotation for the first two components were 64.4% and 21.6% respectively. The scores of the two PC's for formula LW and JG at different decoction times were given in **Table 6.6** and the scores were plotted in **Figure 6.4**. It can be seen from **Table 6.5** the larger loadings > 0.5 for PC1 were Ca, Fe, Mg, Mn, Na and K, and also Ca and Zn for PC2. From the loadings and scores of the PC1 (**Table 6.5** and **Table 6.6** respectively), it could be interpreted that for LW: Ca, Fe, Mg, Mn, Na and K were highest at 40 min as LW40 had highest score 0.259 (**Table 6.6**) and for JG: Fe, Mn and K were highest at 40 min that had highest score 2.302 (**Table 6.6**). From the loadings and scores of the PC2 (**Table 6.5** and **Table 6.6** respectively), it could be interpreted that for LW: Zn was highest at 10 min as LW10 had highest score of 1.642 (**Table 6.6**) and, for JG: Ca and Zn were highest at 25 min as it has highest score of 2.628 (**Table 6.6**).

Table 6.5: Varimax rotated factor loadings of the first two principal components for elements

Element	PC1	PC2
Ca	0.627	0.633
Fe	0.856	0.213
Mg	0.935	0.167
Mn	0.937	0.160
Na	0.853	0.230
K	0.946	0.184
Zn	0.093	0.965

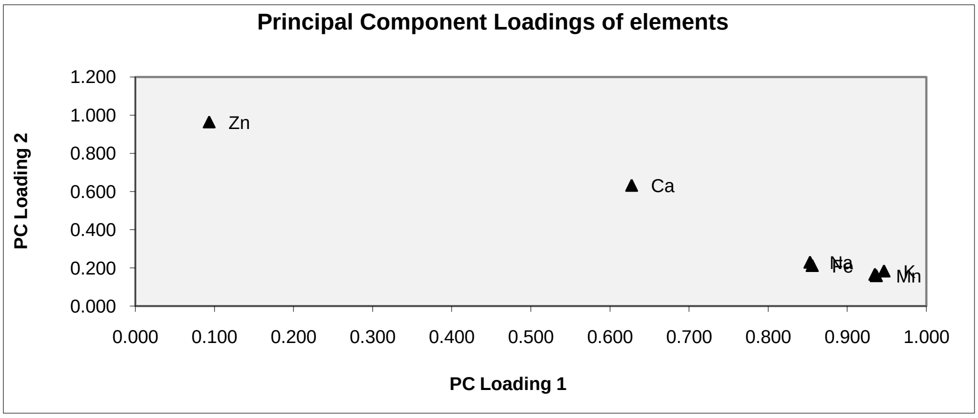

Figure 6.3: Varimax rotated principal component (PC1 versus PC2) loadings for elements

Table 6.6: Varimax rotated scores of the first two principal components for LW and JG

Formulae	PC1	PC2
LW5	-1.012	-0.235
LW10	-1.555	1.642
LW15	-0.533	-0.483
LW20	-0.452	-0.459
LW25	-0.539	-0.526
LW30	-0.802	-0.464
LW35	-0.938	-0.287
LW40	0.259	-0.463
LW45	-0.768	-0.686
LW50	-1.206	0.043
LW55	-0.879	-0.497
LW60	-0.719	-0.809
JG5	-0.525	0.352
JG10	-0.530	-0.195
JG15	0.342	0.398
JG20	0.433	1.920
JG25	1.476	2.628
JG30	0.912	0.271
JG35	0.412	1.825
JG40	2.302	-1.503
JG45	1.554	-1.158
JG50	0.323	-0.714
JG55	1.183	-0.424
JG60	1.263	-0.176

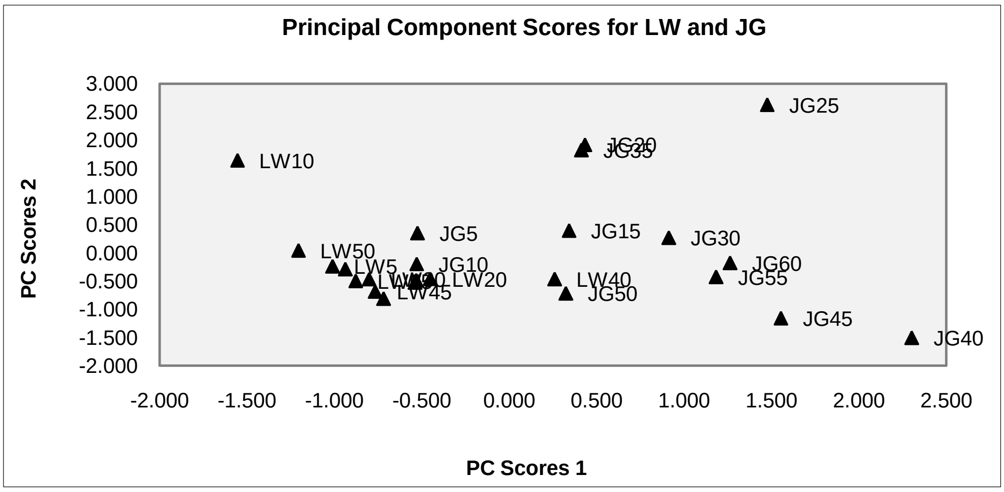

Figure 6.4: Varimax rotated principal component scores (PC1 versus PC2) of Chinese herbal formulae

HCA was applied to the systematized elemental concentrations and a pictorial description of formulae LW and JG was obtained using Ward's method as an amalgamation rule and the squared Euclidean distance as a measure of the closeness between samples (**Figure 6.5**). The resulting dendrogram gave five concise clusters. The first cluster involved formula LW at 10, 15, 25, 30, 35, 45, 50 and 55 min, as the amount of Ca, Fe, Mg, Mn, Na and K were low in formula LW when compared to JG. The second cluster involved LW at 5, 20 and 40 min and JG at 5, 10 and 50 min as all elements except Zn in LW and except Fe in JG followed the same pattern at those given intervals (**Figure 6.1** and **Figure 6.2** respectively). In addition, Ca, Fe, Mg, Mn and K were highest at 40 min in LW. For JG, comparing the metals at 5 and 10 min, Ca, Mg, Mn, Na, K and Zn were higher at 5 min than at 10 min. The metals leached more at 5 min than at 10 min for the formula JG. The third cluster involved JG at 25 and 40 min. This is because in JG: Ca, Mg and Zn were highest at 25 min and Fe, Mn and K were highest at 40 min. Fourth cluster involved JG at 30, 35, 45, 55 and 60 min because all the elements except Zn were higher in JG compared to LW. Fifth cluster involved JG at 15 and 20 min as it followed the same pattern of rise in all elements from 15 to 20 min (**Figure 6.2**).

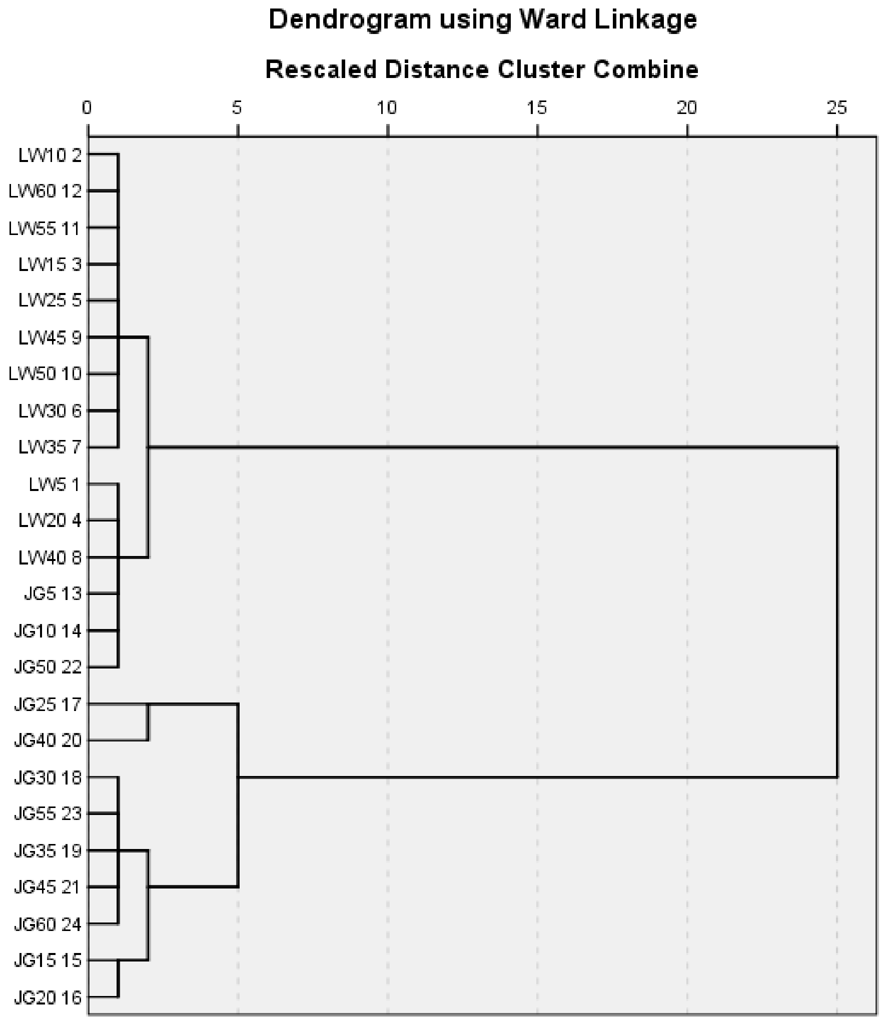

Figure 6.5: Dendrogram of hierarchical cluster analysis for the formulae LW and JG

Fisher's LDA analysis was performed using the leave-one-out method for model establishment and prediction. The discriminate function revealed a significant association between the formulae and predictors, accounting for 69.05% of between group variability. The cross validated function showed that overall 91.7% of original grouped cases correctly classified.

The variation of the metal contents with different decoction times is due to the combination of various herbs in the formula. The herbs in combination contributed to the synergistic effect that varies the percolation of the metals with time. Özcan analyzed the mineral contents of Turkish herbal tea at different infusion periods (3, 5, 7, 9 and 11 min). He showed that the mineral contents at different time intervals are not consistent and varied widely depending on the infusion periods [159]. Considering the nutritional properties, he proposed the first period of infusion may be taken as the optimum time for the Turkish herbal tea. One of the primary advantages of decoction is that minerals are rapidly absorbed by the body; its effects are strong and immediately perceived by the patient. It is not, however, without drawbacks: decoctions are relatively expensive, time consuming and also difficult to prepare. The elemental concentration of the herbal formulas LW and JG at different time intervals obtained in this study provides scientific evidence for their efficacy. This study can further assist Chinese health practitioners to determine the effectiveness of percolation of these metals with time that aids in the prescription of these decoctions to improve kidney function.

6.4.2 Metal intake from decoction of LW and JG

The two TCM formulae were examined for the contribution of each element on the adequate daily dietary intake (ADDIs). **Table 6.7** shows the results of the element concentration by cup (mg/100 mL formula). The percentage contribution of elements to ADDIs was shown in brackets (**Table 6.7**). It can be noticed that the highest contribution to ADDIs in the case of manganese is in agreement with the literature [193]. The next highest contribution to ADDIs was Fe followed by K in both the formulas LW and JG respectively. In chronic renal disease there is a decrease of manganese and iron levels in the kidney [235]. Manganese deficiency can have impact on major physiological processes. Excessive sweating and chronic liver disorders are also causes of manganese deficiency. In TCM's view, as the formula LW and JG are used to treat kidney and liver Yin deficiency (blood-deficiency) and kidney Yang deficiency (urinary difficulty), it can be considered from the elemental concentration approach that the highest contribution of ADDIs of the formulae LW and JG to Mn (8.25% and 12.5%) and Fe (7.2% and 11.2%) might be one of the reasons for using these formulae to improve kidney function. So to treat patients suffering from chronic liver disorders, blood deficiency and urinary difficulty decoction time of 40 min might be suggested for LW and JG.

Table 6.7: ADDI[a] for metals and average metal content by cup of formula

Metals	ADDI (mg d^{-1})[a]	Liu wei di huang wan (mg cup^{-1})[b]	Jin gui shen qi wan(mg cup^{-1})[b]
Ca	1000 - 1300	24.53 (2.45)	56.29 (5.62)
Fe	8 - 18	1.08 (7.2)	1.69 (11.2)
Mg	310 - 420	9.53 (2.72)	14.7 (4.2)
Mn	5 - 5.5	0.33 (8.25)	0.5 (12.5)
Na	460 - 920	4.09 (0.18)	8.5 (0.38)
K	2800 - 3800	169.12 (4.45)	237.2 (6.24)
Zn	15	0.09 (0.6)	0.103 (0.68)

[a]ADDIs of metals - range; Source:[42]

[b]In parenthesis, % of ADDI from a cup (100mL) of formulae

6.5 Conclusions

The concentration of mineral elements in TCM formulae LW and JG were determined at different decoction times by AAS. Formula JG contains higher amount of Ca, Fe, Mg, Mn, Na, K and Zn than formula LW. Information of element content in the decoctions of TCM is important to assess their beneficial effects for human health. The data were subjected to chemometrics in order to highlight the possible correlations between the elements and to recognize the patterns with the purpose of identifying the possible influences of soil and climate. Good correlation exists between the analyzed elements. PCA showed that in formula JG: Ca and Zn were highest at 25 min and Fe, Mn and K were highest at 40 min and, in formula LW: Ca, Fe, Mg, Mn, Na and K were highest at 40 min and Zn was highest at 10 min. HCA gave significant clusters that highlight 25 min and 40 min for JG. Fisher's LDA analyses indicated that 91.7% of the original grouped cases were correctly classified.

The percentage contribution of metals from the decoction of LW and JG showed that Mn had highest contribution to ADDIs.

7 EVALUATION OF SELECTED ELEMENTS IN TRADITIONAL CHINESE HERBAL FORMULAE: DA BU YIN WAN AND GUI LU ER XIAN JIAO WITH EMPIRICAL AND CHEMOMETRIC APPROACHES

7.1 ABSTRACT

Traditional Chinese herbal formulae Da bu yin wan (DB) is an effective tonifier for the Yin pill and Gui lu er xian jiao (GL) comprising tortoise shell and deer antler syrup were analyzed for Ca, Fe, Mg, Mn, Na, K and Zn at different decoction intervals by AAS. All elements except K were higher in formula GL than formula DB. K (1715.23 mg L^{-1}) followed by Ca (593.42 mg L^{-1}) were highest in DB. Ca (2123.54 mg L^{-1}) was highest in formula GL. Chemometric techniques such as PCA, HCA and LDA were applied to analyze the data and to understand the association between the elements. The metal intake related to the consumption of decoction has also been studied. Fe and Ca made the highest contribution to average daily dietary intakes from the formulae.

7.2 INTRODUCTION

CHM has a history of more than 2000 years. It has been extensively used in Asia both as a form of dietary therapy to maintain good health, and a major modality to treat disease. With the invasion of East Asian immigrants and rising demand for Chinese medicine outside Asia, the use of Chinese herbs has spread to the Western countries and become part of the multibillion-dollar herbal industry [247, 248].

CHM is essentially different from Western herbalism and has its roots in antiquity. The patient is viewed as a whole entity, with a complex system of positive and negative affects known as Yin and Yang. Illness causes an imbalance in these affects and the treatment is aimed at restoring the whole body balance. The most effective component is Qi, the essential life force, resulting from the interaction of Yin and Yang. Any Qi imbalance results in illness that is deficient Qi or stagnant Qi. Certain 'deviant energies' such as wind, cold, heat, damp and dryness also cause imbalance and are countered by appropriate treatment. For example, if a person has a hot type imbalance such as liver-heat or stomach-heat, the Chinese herbs recommended must be cool or cold. Herbs that can purge liver-heat may not be able to rid stomach-heat. Therefore, different herbs have been assessed for their multiple affects on the function of different organs and the herbs are combined into traditional formulas, with each herb having a specific and often complex role. There are four classes of herb [249]:

- Emperor herb, that is the main therapeutic ingredient
- Minister herb, that supports the main herb
- Messenger herb, that helps direct the effect to a particular part of the body
- Helper, or harmonizer, herbs, that are auxiliary herbs dealing with secondary symptoms or for countering toxic effects from other ingredients

TCM views the kidneys as the root of the body's Yin and Yang, the origin of life. The kidneys store the Yin and Yang, which is the place where nutrients are stored and physiological functions take place. Essence, which is called the essential Qi or Jing, is stored in the kidneys and is responsible for human growth and development. Essence includes semen, breast milk and blood. This is the reason that earlier in Chinese medicine, kidney failure was recognized as an important cause of anaemia. Kidneys store essence that produces marrow. The marrow develops in the bone cavities and nourishes their growth and development. When the kidney essence is sufficient, the bone marrow has good source of production and the bones are well nourished, firm and hard. If the kidney essence is deficient, it will fail to nourish the bones leading to weakness of the lumbar region and knees, weakness of the feet and tooth loss [250].

Kidney essence deficiency can be diagnosed with such symptoms and signs as weakness and soreness of the lower back and legs, inability to stand for a prolonged period of time, and decreased bone density. Kidney essence deficiency is directly related to the western diagnosis of ageing including osteoporosis. GL is the formula of choice for the treatment of osteoporosis as it contains herbs that increase the utilization of calcium that has the effects to strengthen the bones, prevent fractures, and promote healing. From the Western medicine perspective, GL is rich in calcium, and have functions to increase adsorption of calcium into bone, and promote the growth and healing of bones. A clinical study in Taiwan showed that the use of the herbal formula GL increased bone density by an average of 3.4 % in one year [251]. The kidney tonifying herbs used in the formula GL such as lu jiao, gui ban and gou qi zi were clinically identified for influencing mesenchymal stem cells for bone repairing [252].

For human beings trace elements are essential nutrients with a gamut of functions. Trace elements play important role in biological processes, both as essential components and toxins [253]. The trace elements incorporate in the structures of the proteins, enzymes, and the complex carbohydrates to participate in biochemical reaction. Trace elements with enzymes, for example, are necessary for the functioning and maintenance of the immune system [234]. Anaemia is frequent consequence of progressive renal disease [254]. Decreased plasma levels of Zinc have been reported in patients with renal insufficiency [255]. There are other reports however regarding the normal or elevated levels of Zinc in uremic patients [256]. Majority of patients with renal disease have hypocalcemia because of nutritional and metabolic disorders such as magnesium and vitamin D deficiency [257]. Medicinal plants contain minerals and trace elements that are essential to human health [167, 238]. The tea drink or infusion prepared by seeping the herbs in near-boiling water may be a rich source of some essential dietary elements [258]. Owing to the great importance of the minerals present in tea, many studies have been carried out to determine their levels in tea leaves and their infusions [160, 195].

In this study, two traditional Chinese herbal formulae used to improve the kidney function have been analyzed: DB is a formula that nourish and tonify the Yin and GL is a formula that warm and tonify the Yang. DB is known as the great tonify the Yin pill, used for liver and kidney deficiency and used in treating disorders such as night sweats, steaming bone disorder with afternoon tidal fever, irritability and sensation of heat and pain in the knees and legs which is sometimes accompanied by weakness. GL is known as tortoise shell and deer antler syrup, used for kidney essence deficiency and used in treating disorders such as weakness and soreness of the lower back and legs, in-ability to stand for prolonged period of time, and decreased bone mass density [227]. The formulae were analyzed for Ca, Fe, Mg, Mn, Na, K and Zn at different decoction intervals by AAS.

7.3 MATERIALS AND METHODS

7.3.1 Solutions and reagents

Standard stock solutions of Ca, Fe, Mg, Mn, Na, K and Zn at a concentration of 1 gL^{-1} were obtained from Merck, Australia, and working solutions were prepared by dilution of stock solutions in 5% (v/v) HNO_3 (Trace SELECT, 69%) from Sigma-Aldrich, Australia. Ionization in AAS was controlled by adding a 5mL (1 g/L CsCl + 10 g/L La, Merck) buffer solution to all samples and standards and made up to final volume of 100 mL. Deionized water with a specific sensitivity of 18 mΩ obtained from Millipore- Milli-Q water purifier system (Milford, USA). All chemicals and reagents were of analytical grade.

7.3.2 Sample preparation procedure

Samples of dried Chinese herbs were purchased from local importers of Chinese herbs. Animal products such as Plastrum Testudinis and Cornu Cervi used in this experiment were not from the category of endangered species. The herbs involved in the formulae and the amount of herb used that was generally prescribed by herbalist were listed in **Table 7.1**. Chinese standard method of decoction was followed for the preparation of decoction of the formula [43]. All herbs involved in the formula were kept in a glass beaker with a lid and 200 mL of water was added. The herbs were soaked for 20 min. The mixture was first heated to boiling 100º C and then kept at 60º C for different intervals: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min. The decoction was allowed to cool to room temperature and then filtered with an ashless filter paper and transferred into 100 mL volumetric flask. All analyses were done in triplicate.

Table 7.1: List of the traditional Chinese herbal formulae

Formula name	Chinese herbs	Botanical name	Amount used (g)
Da bu yin wan	shu di huang	*Radix Rehmanniae Glutinosae Conquitae*	18
	gui ban	*Plastrum Testudinis*	18
	huang bai	*Cortex Phellodendri*	12
	zhi mu	*Radix Anemarrhenae Asphodeloidis*	12
Gui lu er xian jiao	lu jiao	*Cornu Cervi*	12
	gui ban	*Plastrum Testudinis*	6.25
	gou qi zi	*Fructus Lycii*	3
	ren shen	*Radix Ginseng*	1.25

7.3.3 Instrumentation

FAAS analysis analyses of decoctions for the determination of Ca, Fe, Mg, Mn, Na, K and Zn were carried out using a Varian spectroAA-400 AAS (Varian Inc., Mulgrave, Australia) under optimized measurement conditions using hollow cathode lamps and a deuterium background correction system. The operating conditions for the working concentrations of elements were set as recommended by the manufacturer, given in **Table 7.2.**

Table 7.2: The operating parameters for working elements

Elements	Wavelength (nm)	Lamp current (mA)	Slit (nm)
Ca	422.7	10	0.5
Fe	248.3	5	0.2
Mg	285.2	4	0.5
Mn	279.5	5	0.2
K	404.4	5	0.5
Na	330.3	5	0.5
Zn	213.9	5	1

7.3.4 Data analysis

The statistical data analysis was done using the SPSS for windows PASW SPSS 18 (Release 18.0.0, Jul 30, 2009) and Microsoft Excel 2007 with add-in XL-STAT 2010. To get good information about the element content relationship and to get the insight of elemental patterns, statistical techniques such as Pearson correlation analysis and PCA were used. Classification of data was obtained by HCA and LDA.

7.4 RESULTS AND DISCUSSION

7.4.1 Mineral content of the formulae

Most of the Chinese formulas are heated for about 20-30 min, following the standard Chinese procedure for making decoctions. However, the formulas that contain rich, cloying substances should be heated for 45-60 min. The metal contents of the formulas DB and GL at different decoction times with 5 min interval from 5-60 min are given in **Table 7.3** and plotted in **Figure 7.1** and **Figure 7.2** respectively. Ca, Fe, Mg, Mn, Na and Zn were higher in formula GL than DB and K was higher in formula DB than GL. It can be seen from **Figure 7.1** that Ca, Fe, Mg and Mn were higher at 45 min. One-way ANOVA was done to determine the significant differences between the mean values of the elements in the formulae. The results showed that significant differences ($p<0.05$) exist between DB and GL for Ca (593.42 – 2123.54 mg L^{-1}), Fe (14.77 – 45.42 mg L^{-1}), Mg (129.1 – 288.02 mg L^{-1}), Mn (1.39 – 5.17 mg L^{-1}), Na (372.45 – 1227.13 mg L^{-1}), K (1715.23 – 1050 mg L^{-1}) and Zn (0.88 – 4.1 mg L^{-1}). In formula DB, K (1715.23 mg L^{-1}) followed by Ca (593.42 mg L^{-1}) were highest and Zn (0.88 mg L^{-1}) was lowest when compared to other elements. In formula GL, Ca (2123.54 mg L^{-1}) was highest and Zn (4.1 mg L^{-1}) was lowest when compared to other elements.

Table 7.3: Metal content (mg L^{-1}) of the analyzed formulae (mean ± S.D)

Formula name	Time (min)	Ca	Fe	Mg	Mn	Na	K	Zn
Da bu yin wan	5	486.66 ± 5.35	12.9 ± .25	104.52 ± 3.34	1.15 ± 0.06	310.44 ± 3.23	1395.23 ± 4.32	1.04 ± .02
	10	595.7 ± 3.11	13.12 ± .09	127.36 ± 2.75	1.30 ± .09	360.16 ± 1.44	1753.14 ± 4.31	1.20 ±.05
	15	597.25 ± 2.87	14.23 ± 1.03	121.33 ± 3.22	1.40 ± .10	406.91 ± 2.34	1768.22 ± 1.42	0.89 ± .01
	20	626.97 ± 1.97	17.41 ± .34	135.81 ± 3.09	1.57 ± .2	418.32 ± 2.05	1648.98 ± 1.62	0.81 ± .07
	25	678.58 ± 1.89	19.08 ± 1.6	137.93 ± 3.36	1.63 ± .28	334 ± 7.78	1732.9 ± 5.18	1.10 ± .06
	30	541.38 ± 4.15	15.02 ± .87	122.28 ± 5.63	1.37 ± .13	345.72 ± 3.93	1817.57 ± 5.70	0.87 ± .70
	35	567.09 ± 8.32	13.77 ± .35	129.01 ± 4.25	1.29 ± .05	362.98 ± 5.45	1843.51 ± 7.68	0.86 ± .55
	40	660.64 ± 4.49	12.12 ± .8	137.2 ± 3.31	1.40 ± .09	375.46 ± 4.95	1863.59 ± 4.20	0.76 ± .09
	45	733.73 ± 5.97	20.87 ± 3.36	148.25 ± 3.54	1.81 ± .24	390.46 ± 3.18	1823.95 ± 6.35	1.04 ± .15
	50	539.2 ± 4.28	10.87 ± .76	132.81 ± 5.46	1.16 ± .14	408.37 ± 6.37	1648.62 ± 3.80	0.71 ± .35
	55	522.85 ± 2.11	12.32 ± 1.36	121.63 ± 3.36	1.22 ± .14	368.47 ± 1.96	1578.94 ± 5.14	0.60 ± .46
	60	571.03 ± 7.09	15.45 ± 1.51	131.12 ± 7.16	1.38 ± .02	388.16 ± 4.74	1708.16 ± 2.89	0.66 ± .41
Mean		593.42	14.77	129.1	1.39	372.45	1715.23	0.88
Gui lu er xian jiao	5	1656.86 ± 8.18	30.1 ± 4.96	191.7 ± 5.93	4.19 ± .47	784.12 ± 7.0	437.68 ± 3.13	3.77 ± .24
	10	2080.51 ± 3.32	34.83 ± 1.61	225.42 ± 1.25	4.47 ± .54	917.63 ± 1.78	683.45 ± 4.83	3.99 ± .19
	15	2108.38 ± 1.29	43.72 ± 2.23	279.46 ± 4.14	4.54 ± .52	1158.57 ± 3.45	909.61 ± 4.08	4.21 ± .76
	20	2235.63 ± 3.57	50.63 ± 4.65	331.72 ± 6.03	5.43 ± .62	1369.02 ± 5.31	1055.99 ± 4.85	4.56± .79
	25	2387.03 ± 7.76	52.61 ± 4.47	260.49 ± 6.83	4.33 ± .68	981 ± 7.31	841.03 ± 6.15	3.83± .53
	30	1775.66 ± 4.85	41.62 ± 2.55	250.22 ± 6.45	4.6 ± .37	1026.29 ± 3.5	862.47 ± 7.9	3.54 ± .4
	35	1778.3 ± 4.88	38.84 ± 1.7	253.77 ± 5.27	4.87 ± .23	1119.8 ± 2.61	984.2 ± 3.5	3.33 ± .65
	40	1795.24 ± 7.04	42.76 ± 2.98	260.49 ± 6.38	4.91 ± .28	1215.29 ± 4.44	1089.08 ± 6.87	4.13 ± .84
	45	2234.61 ± 4.22	46.26 ± 2.72	311.67 ± 6.85	5.41 ± 1.11	1342.63 ± 5.29	1122.03 ± 7.10	4.39 ± .63
	50	2581.45 ± 6.21	54.81 ± 3.75	372.12 ± 2.74	6.15 ± 1.48	1718.65 ± 7.70	1541.12 ± 6.46	4.55± .52
	55	2346.39 ± 5.58	52.44 ± 4.09	342.88 ± 6.93	6.32 ± 1.10	1524.92 ± 5.48	1530.67 ± 6.34	4.09 ± 1.57
	60	2502.36 ± 6.97	56.42 ± 2.61	376.29 ± 6.48	6.64 ± .43	1567.68 ± 2.52	1552.04 ± 5.85	4.79 ± 1.13
Mean		2123.54	45.42	288.02	5.17	1227.13	1050.78	4.1

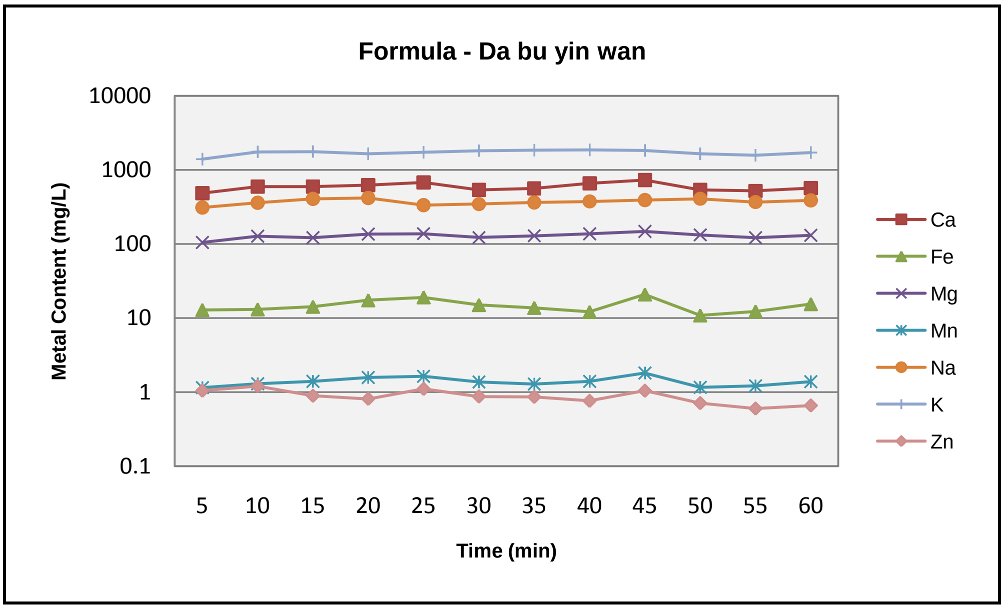

Figure 7.1: Metal content (mg L^{-1}) of formula DB

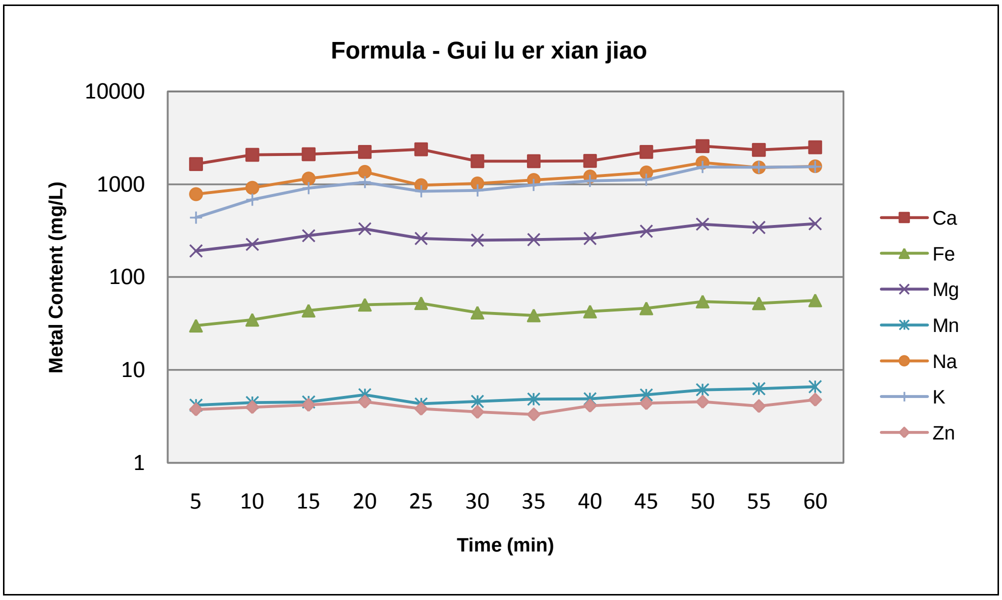

Figure 7.2: Metal content (mg L^{-1}) of formula GL

The association between two variables is measured by correlation matrix. It can be seen from **Table 7.4** that good correlation exists between all the elements except K. K is negatively correlated.

Table 7.4: Correlation matrix of elements (Pearson correlation)

Variables	Ca	Fe	Mg	Mn	Na	K	Zn
Ca	1						
Fe	0.981	1					
Mg	0.960	0.973	1				
Mn	0.976	0.973	0.975	1			
Na	0.957	0.964	0.991	0.981	1		
K	-0.650	-0.573	-0.461	-0.601	-0.497	1	
Zn	0.980	0.957	0.936	0.974	0.945	-0.717	1

PCA was performed to get the elemental patterns that are consistent. The principal components (PC) with eigenvalues greater than one were extracted and subjected to Varimax rotation. The percentages of variances after the Varimax rotation for the first two components PC1 and PC2 were 74.61 % and 24.14 %. The results after Varimax rotation: first and second factor loadings for elements were presented in **Table 7.5** and plotted in **Figure 7.3.** It can be seen from **Figure 7.3** that K appears to be away from rest of the elements as it had negative values for both the principal components PC1 and PC2 loadings (**Table 7.5**). The scores of the two PC's for formula DB and GL at different decoction times were given in **Table 7.6** and plotted in **Figure 7.4**. **Table 7.5** shows the larger loadings for PC1: Ca, Fe, Mg, Mn, Na and Zn and also Zn for PC2. It can be seen that from **Table 7.5**, loadings for PC2: Zn had slightly higher score than 0.5, which is 0.504 and hence PC2 component is not considered. From the loadings and scores of the PC1 (**Table 7.5** and **Table 7.6** respectively), it could be interpreted that for GL: Fe, Mg, Mn and Zn were highest at 60 min as GL60 had the first highest score 2.101 (**Table 7.6**) and, Ca and Na were highest at 50 min as GL50 had the second highest score 2.098 (**Table 7.6**), and as these scores are very close, GL50 and GL60 appear overlapped in the graph, **Figure 7.4**.

Table 7.5: Varimax rotated factor loadings of the first two principal components for elements

Element	PC1	PC2
Ca	0.898	0.422
Fe	0.931	0.330
Mg	0.978	0.199
Mn	0.925	0.360
Na	0.964	0.238
K	-0.275	-0.961
Zn	0.857	0.504

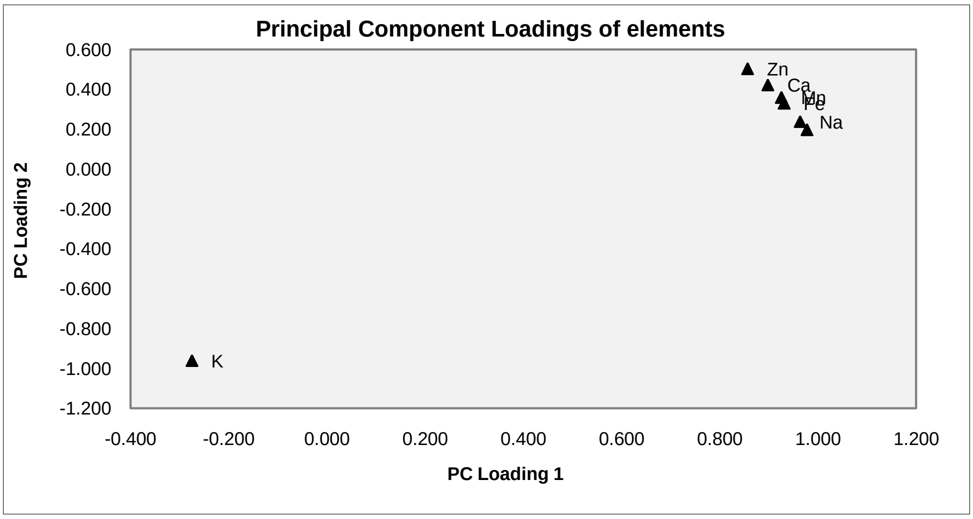

Figure 7.3: Varimax rotated principal component (PC1 versus PC2) loadings for elements

Table 7.6: Varimax rotated scores of the first two principal components of DB and GL

Formulae	PC1	PC2
DB5	-1.251	0.311
DB10	-0.782	-0.641
DB15	-0.755	-0.721
DB20	-0.752	-0.490
DB25	-0.666	-0.640
DB30	-0.746	-0.832
DB35	-0.711	-0.916
DB40	-0.658	-0.993
DB45	-0.474	-0.931
DB50	-0.909	-0.466
DB55	-1.016	-0.265
DB60	-0.792	-0.624
GL5	-0.702	2.574
GL10	-0.124	1.851
GL15	0.523	1.030
GL20	1.154	0.447
GL25	0.421	1.275
GL30	0.203	1.185
GL35	0.349	0.817
GL40	0.616	0.576
GL45	1.067	0.347
GL50	2.098	-1.016
GL55	1.804	-0.894
GL60	2.101	-0.984

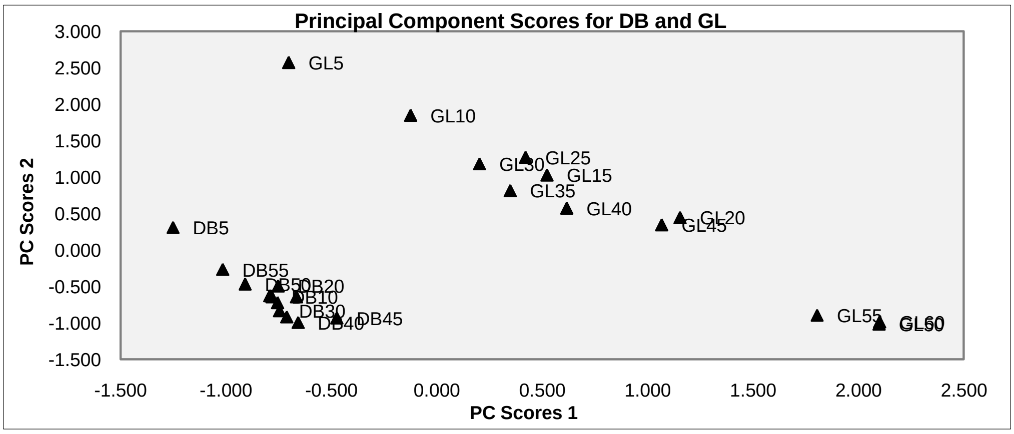

Figure 7.4: Varimax rotated principal component scores (PC1 versus PC2) of Chinese herbal formulae

HCA was applied to the elemental concentrations. The clusters formed by HCA for formulae DB and GL were given in **Figure 7.5**. Ward's method was used as an amalgamation rule and the squared Euclidean distance as a measure of proximity between samples. The resulting dendrogram gave three concise clusters. The first cluster involved formula DB at different time intervals from 5 to 60 min. This is because K was higher in formula DB than GL. The second cluster involved formula GL at 50, 55 and 60 min as all the elements followed the same pattern decreased at 55 min and increased at 60 min at these given intervals and also were highest at either 50 or 60 min for GL. Ca and Na were highest in GL at 50 min and Fe, Mg, Mn, K and Zn were highest in GL at 60 min (**Figure 7.2**). The third cluster involves GL from intervals 5 to 40 min as all the elements were lower at 5 min and increased comparatively at 40 min.

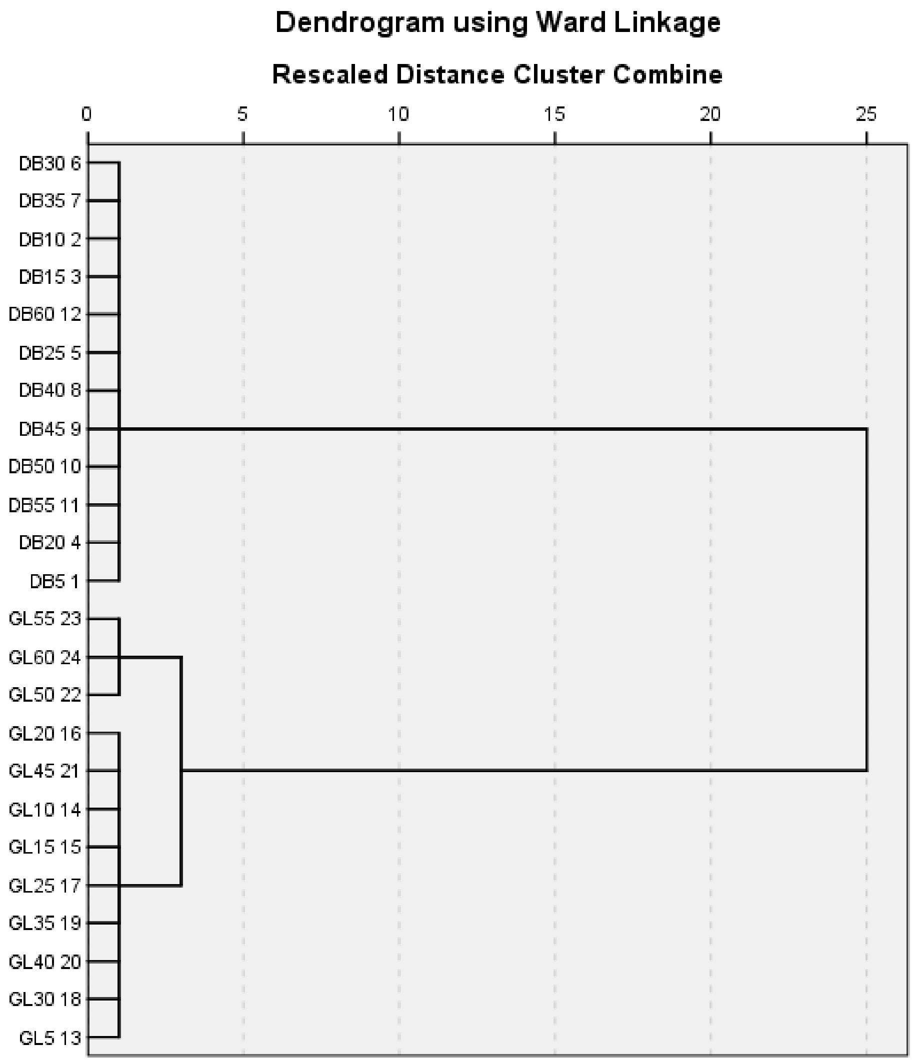

Figure 7.5: Dendrogram of hierarchical cluster analysis for the formulae DB and GL

Fisher's LDA analysis was performed using the leave-one-out method for model establishment and prediction. The discriminate function showed a significant association between the formulae and predictors, accounting for 79.95 % of between group variability. The cross validated function showed that overall 100 % of original grouped cases correctly classified.

It is interesting to note that the Pearson correlation matrix and PCA loadings for elements gave the same groupings and also scored positive values that are closed to 1 for Ca, Fe, Mg, Mn, Na and Zn, and negative values for K. First group involved Ca, Fe, Mg, Mn, Na and Zn as they were higher in formula GL when compared to DB and second group involved K was K was higher in DB than in GL.

The variation of the metal contents of TCM's DB and GL with different decoction times is due to the compatibility of various herbs in the formula. Wang *et al.* analyzed the TCM BHT for selected mineral elements by ICP-MS and FAAS. It was reported by that compatibility could significantly affect the dissolution of selected elements Mg, Fe, Mn, Cu, Zn, Al, and Co and fruit of *Oryza sativa* L. (Gramineae) acted as solubilising agent in this formula for most of the analyzed elements except for Co. [184].

7.4.2 Metal intake from decoction of DB and GL

The two formulae were examined for the contribution of each element on the ADDIs. **Table 7.7** shows the results of the element concentration by cup (mg/100 mL formula). The percentage contribution of elements to ADDIs is shown in parenthesis (**Table 7.7**). It can be noticed that the highest contribution from DB and GL to ADDIs is Fe followed by Ca. TCM views kidney essence deficiency leads to anaemia and bone loss. According to the western diagnosis, kidney essence deficiency is diagnosed as early ageing and includes disease such as osteoporosis. Referring back to the literature, GL is the formula used to increase the bone density. GL also used for kidney Yin and Yang deficiency accompanied by insufficiency of essence and blood in the conception and governing vessels that leads to weakness of the lower back and knees. DB is used for kidney Yin deficiency that leads to steaming bone syndrome with afternoon tidal fever, night sweats and weakness in knees and legs etc. So, from the elemental concentration approach, it can be considered that the highest contribution of Fe (9.84% and 30.2%) and Ca (5.93% and 21.23%) in formulae DB and GL to ADDIs might be one of the reasons for using them to improve kidney function. So to treat patients suffering from anaemia, boneloss, weakness of lower back and knees, decoction time of 45 min for DB, and 50 to 60 minutes for GL might be suggested. Synergistic effect of various herbs in GL not only provides Ca but also reveals the effect of other beneficial elements such as Fe, Mg, Mn, Na, K and Zn that contribute to the treatment of kidney deficiency pattern: which may be linked with osteoporosis. This result may be advantageous over calcium products that only supply calcium.

Table 7.7: ADDI[a] for metals and average metal content by cup of formula

Metals	ADDI ($mg\ d^{-1}$)[a]	Da bu yin wan($mg\ cup^{-1}$)[b]	Gui lu er xian jiao($mg\ cup^{-1}$)[b]
Ca	1000 - 1300	59.34 (5.93)	212.35 (21.23)
Fe	8 - 18	1.47 (9.84)	4.54 (30.2)
Mg	310 - 420	12.9 (3.68)	28.8 (8.2)
Mn	5 - 5.5	0.13 (3.47)	0.51 (12.9)
Na	460 - 920	37.2 (1.69)	122.7 (5.57)
K	2800 - 3800	171.52 (4.51)	105 (2.76)
Zn	15	0.08 (0.58)	0.41 (2.73)

[a]ADDIs of metals - range; Source:[42]

[b]In parenthesis, % of ADDI from a cup (100mL) of formulae

7.5 Conclusions

The concentration of mineral elements in the herbal formulae DB and GL were determined at different decoction times by AAS. Formula GL contains higher amounts of Ca, Fe, Mg, Mn, Na and Zn than formula DB. K was higher in formula DB than GL. In formula DB: Ca, Fe, Mg and Mn were higher at 45 min. Chemometrics were used to highlight the correlation between elements and to understand the elemental patterns. Good correlations exist between all the elements except K, which is negatively correlated. CA and PCA grouped the elements according to their concentrations into a group: Ca, Fe, Mg, Mn, Na and Zn as they were highest in formula GL. PCA highlighted that for formula GL: Fe, Mg, Mn and Zn were highest at 60 min and, Ca and Na were highest at 50 min. HCA gave concise clusters that highlighted GL at 50, 55 and 60 min as all the elements were highest at either 50 or 60 min for GL. Fisher's LDA analyses indicated that 100 % of original grouped cases were correctly classified. The percentage contributions of metals to ADDIs were high for Fe and Ca when compared to other metals from the decoctions of DB and GL.

8 EVALUATION AND COMPARISON OF MINERAL ELEMENTS IN CHINESE HERBAL KIDNEY AND LIVER TONIFYING FORMULAE: ER ZHI WAN AND QI BAO MEI RAN DAN AT DIFFERENT DECOCTION TIMES

8.1 ABSTRACT

Traditional Chinese herbal formulae EZW and Qi bao mei ran dan (QB) were analyzed for Ca, Fe, Mg, Mn, Na, K and Zn at different decoction intervals byAAS. EZW and QB are used to improve the kidney function. Ca, Mg, Na and K were higher in EZW than QB. K (9802.71 – 2168.14 mg L^{-1}) was highest in both formulae EZW and QB. Chemometrics such asPCA, HCA and LDA were applied to classify the data and to understand the relation between the elements. Metal intake related to the consumption of decoction has also been studied. Potassium in EZW and Magnesium in QB made the highest contribution to average daily dietary intakes.

8.2 INTRODUCTION

TCM is used in medicines as well as in daily dietary supplements in Asia. According to the law of compatibility of TCM, a single herbal medicine usually exerts a limited therapeutic action. When several herbal medicines are mixed in a certain proportion, they will display their superiority over a single drug in the treatment of a disease. Historically, TCMs are prepared as decoctions by a unique methodology with a specific combination of different herbs as formulas [259]. Therefore, for more than a millennium, traditional Chinese formulations have been extensively used, apparently safely and effectively, in Asian countries, especially in China, Japan and Korea, to alleviate various symptoms of diseases [260-264].

EZW, a famous traditional Chinese formulation firstly recorded in “Yi Bian” written in the Ming Dynasty, possess the actions of tonifying the liver and kidney yin, nourishing body's essential fluid, and arresting hemorrhage [265]. It is widely used in China to treat kidney and Alzheimer's diseases, invigorate the body, and modulate the immune system in clinical applications [266]. EZW has favourable potency to develop a new anti-osteoporotic agent in clinics [267]. QB, a TCM formulation enriches the kidney yin and nourishes the liver blood. Traditionally, this formula has been considered an effective remedy for the following syndromes: general kidney and liver insufficiencies, early appearance of grey hair, loosening of teeth, nocturnal emissions and weakness of lower back and knees [43]. QB with minor modifications according to the symptoms of the disease was also used to treat osteoporosis, aplastic anaemia, male infertility, chronic persisting hepatitis and menopausal syndrome [268-272].

The TCM theory believes that the kidney deficiency can cause osteoporosis. The kidney governs the bone system, that is to say, the development and functions of the bones depend on the kidney-essence, which contains the kidney yin and kidney yang. The kidney stores essence and the essence can transform into bone marrow to nourish the bones, promote the growth, repair the skeleton and strengthen the skeleton. The deficiency of the kidney-essence can affect the production of bone marrow, leading to flaccidity of skeleton.

In this study we have analyzed and compared the TCM formulae EZW and QB used to improve the kidney function that nourish and tonify the yin. EZW is known as the two-ultimate pill and QB is known as the seven-treasure special pill for beautiful whiskers [137]. In this study, the formulae were analyzed for Ca, Fe, Mg, Mn, Na, K and Zn at different decoction intervals by AAS.

8.3 MATERIALS AND METHODS

8.3.1 Solutions and reagents

Standard stock solutions of Ca, Fe, Mg, Mn, Na, K and Zn at a concentration of 1 gL^{-1} were obtained from Merck, Australia, and working solutions were prepared by dilution of stock solutions in 5% (v/v) HNO_3 (*Trace* SELECT, 69%) from Sigma-Aldrich, Australia. Ionization in AAS was controlled by adding a 5mL (1 g/L CsCl + 10 g/L La, Merck) buffer solution to all samples and standards and made up to final volume of 100 mL. Deionized water with a specific sensitivity of 18 mΩ obtained from Millipore-Milli-Q water purifier system (Milford, USA). All chemicals and reagents were of analytical grade.

8.3.2 Sample preparation procedure

Samples of dried Chinese herbs were purchased from local importers of Chinese herbs. The herbs involved in the formulae and the amount of herb used that was generally prescribed by herbalist are listed in Table 8.1. Chinese standard method of decoction was followed for the preparation of decoction of the formula [43]. All herbs involved in the formula were kept in a glass beaker with a lid and 200 mL of water was added. The herbs were soaked for 20 min. The mixture was first heated to boiling (100º C) and then kept at 60º C for different intervals: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min. The decoction was allowed to cool to room temperature and then filtered with an ashless filter paper and transferred into 100 mL volumetric flask. All analyses were done in triplicate.

Table 8.1: List of the traditional Chinese herbal formulae

Formula name	Chinese herbs	Botanical name	Amount used (g)
Er zhi wan	nu zhen zi	*Fructus Ligustri Lucidi*	12
	han lian cao	*Herba Ecliptae Prostratae*	12
Qi bao mei ran dan	he shou wu	*Radix Polygoni Multiflori*	30
	fu ling	*Sclerotium Poriae Cocos*	15
	niu xi	*Radix Achyranthi Bidentatae*	15
	dang gui	*Radix Angelicae Sinesis*	15
	gou qi zi	*Fructus Lycii*	15
	Hei zhi ma	*Sesamum Indicum L. (Pedaliaceae)*	5
	bu gu zi	*Fructus Psoraleae Corylifoliae*	12

8.3.3 Instrumentation

Analyses of decoctions for the determination of Ca, Fe, Mg, Mn, Na, K and Zn were carried out using a Varian spectroAA-400 AAS (Varian Inc., Mulgrave, Australia) under optimised measurement conditions using hollow cathode lamps and a deuterium background correction system. The operating conditions for the working concentrations of elements were set as recommended by the manufacturer, given in **Table 8.2**.

Table 8.2: The operating parameters for working elements

Elements	Wavelength (nm)	Lamp current (mA)	Slit (nm)
Ca	422.7	10	0.5
Fe	248.3	5	0.2
Mg	285.2	4	0.5
Mn	279.5	5	0.2
K	404.4	5	0.5
Na	330.3	5	0.5
Zn	213.9	5	1

8.3.4 Data analysis

The statistical data analysis was made using the SPSS for windows PASW SPSS 18 (Release 18.0.0, Jul 30, 2009) and Microsoft Excel 2007 with add-in XL-STAT 2010. To get good information about the element content relationship and to get the insight of elemental patterns, statistical techniques such as Pearson correlation analysis and PCA were used. Classification of data was obtained by HCA and LDA.

8.4 RESULTS AND DISCUSSION

8.4.1 Metal Content in Formulae

According to the Chinese standard procedure of making decoctions, most formulas are heated for 20-30 min. The formulas that contain rich, cloying substances should be heated for 45-60 min. The metal contents of the two formulae EZW and QB at different decoction times with 5 min interval from 5 to 60 min were given in **Table 8.3** and plotted in **Figure 8.1** and **Figure 8.2** respectively. From **Figure 8.1** it can be seen that in formula EZW at 45 min: Fe, Mn and Zn were highest and at 35 min: Ca, Mg and K were highest. It can also be seen from **Figure 8.2** that in formula QB at 45 min: Zn was highest and at 50 min: Fe, Mg, Mn and Na were highest. Ca, Mg, Na and K were higher in formula EZW than QB. Independent sample t-test was done to determine the significant differences between the mean values of the elements in the formulae. The results showed that, except for Zn (0.97 – 0.95 mg L^{-1}), significant differences ($p<0.05$) exist between EZW and QB for Ca (556.51 – 95.43 mg L^{-1}), Fe (3.65 – 11.43 mg L^{-1}), Mg (555.82 – 158.43 mg L^{-1}), Mn (6.46 – 2.74 mg L^{-1}), Na (606.17 – 145.23 mg L^{-1}) and K (9802.71 – 2168.14 mg L^{-1}). K was highest in both formulae EZW and QB.

Table 8.3: Metal content (mg L^{-1}) of the analyzed formulae (mean ± S.D)

Formula name	Time (min)	Ca	Fe	Mg	Mn	Na	K	Zn
Er zhi wan	5	376.2 ± 2.9	2.52 ± 0.59	429.55 ± 2.33	5.29 ± 1.1	549.1 ± 3.09	8485.34 ± 2.5	0.68 ± 0.85
	10	631.2 ± 3.2	2.76 ± 0.62	592.56 ± 2.9	6.71 ± 0.4	763.27 ± 3.5	11561.76 ± 5.3	0.85 ± 0.56
	15	445.63 ± 3.33	2.38 ± 0.75	420.21 ± 3.6	5.17 ± 1.13	538.59 ± 3.41	8694.58 ± 4.7	0.56 ± 0.71
	20	486.55 ± 3.54	2.98 ± 0.33	545.23 ± 3.64	5.9 ± 0.5	562.67 ± 4.8	9974.5 ± 5.02	0.91 ± 0.72
	25	528.11 ± 2.9	1.89 ± 0.61	632.31 ± 2.92	6.89 ± 0.31	578.84 ± 1.76	10083.36 ± 4.30	1.21 ± 0.66
	30	728.58 ± 4.03	4.37 ± 0.68	578.21 ± 2.21	6.27 ± 0.83	653.04 ± 2.31	9854.58 ± 3.4	1.04 ± 0.16
	35	836.65 ± 4.25	4.21 ± 1.24	740.52 ± 3.64	7.49 ± 0.63	721.07 ± 4.17	12342.2 ± 5.09	1.29 ± 0.79
	40	635.81 ± 2.7	4.31 ± 1.17	524.74 ± 3.26	5.97 ± 0.36	546.32 ± 3.2	9211.24 ± 4.6	0.93 ± 0.71
	45	767.9 ± 4.52	6.08 ± 1.31	662.11 ± 3.3	8.21 ± 0.97	632.61 ± 3.32	10304.95 ± 5.19	1.42 ± 0.88
	50	488.72 ± 2.5	3.78 ± 0.63	430.72 ± 3.2	5.69 ± 0.76	544.46 ± 3.45	9037.49 ± 5.72	0.83 ± 0.81
	55	397.49 ± 2.75	5.92 ± 0.6	688.55 ± 3.46	7.45 ± 0.92	641.98 ± 4.7	9782.98 ± 4.57	1.16 ± 0.91
	60	355.24 ± 3.54	2.51 ± 0.79	425.12 ± 3.5	6.47 ± 1.04	542.03 ± 5.2	8299.51 ± 4.43	0.76 ± 0.65
Mean		556.51	3.65	555.82	6.46	606.17	9802.71	0.97
Qi bao mei ran dan	5	91.16 ± 2.53	9.24 ± 1.0	145.38 ± 3.03	2.43 ± 0.95	136.35 ± 2.7	1961.57 ± 4.7	0.77 ± 0.78
	10	112.01 ± 2.56	10.17 ± 1.75	146.31 ± 2.78	2.81 ± 0.83	120.65 ± 2.84	2163.51 ± 4.82	1.06 ± 0.78
	15	93.46 ± 1.56	8.97 ± 0.57	142.54 ± 2.39	1.9 ± 0.60	119.63 ± 2.63	1894.15 ± 3.65	0.67 ± 0.92
	20	104.85 ± 3.39	12.64 ± 0.94	171.84 ± 3.25	2.74 ± 0.74	142.62 ± 2.98	2246.45 ± 2.35	0.91 ± 0.72
	25	94.85 ± 2.07	9.94 ± 0.59	139.89 ± 2.75	2.5 ± 0.90	118.97 ± 3.4	1955.02 ± 4.07	0.82 ± 0.82
	30	85.34 ± 1.34	12.04 ± 0.76	143.48 ± 1.51	2.88 ± 0.39	141.25 ± 2.21	2118.33 ± 3.76	1.08 ± 0.82
	35	127.37 ± 1.37	10.96 ± 0.71	174.78 ± 2.5	2.76 ± 0.48	171.42 ± 2.14	2500.94 ± 2.85	1.03 ± 0.90
	40	93.27 ± 2.18	10.58 ± 0.75	190.47 ± 2.9	3.06 ± 0.84	158.83 ± 1.78	2445.08 ± 3.95	1.15 ± 0.82
	45	100.92 ± 2.79	15.4 ± 1.11	173.54 ± 3.4	3.26 ± 0.95	180.61 ± 3.01	2468.25 ± 4.03	1.27 ± 0.83
	50	81.64 ± 2.35	16.74 ± 0.77	203.36 ± 3.02	3.77 ± 0.24	196.35 ± 2.8	2452.12 ± 5.33	1.18 ± 0.85
	55	80.34 ± 1.86	11.46 ± 0.83	138.75 ± 1.15	2.44 ± 0.71	126.06 ± 2.6	1912.66 ± 5.0	0.67 ± 0.92
	60	79.92 ± 1.72	8.94 ± 0.21	131.5 ± 2.3	2.3 ± 1.18	130.04 ± 2.07	1899.64 ± 3.46	0.69 ± 0.91
Mean		95.43	11.43	158.49	2.74	145.23	2168.14	0.95

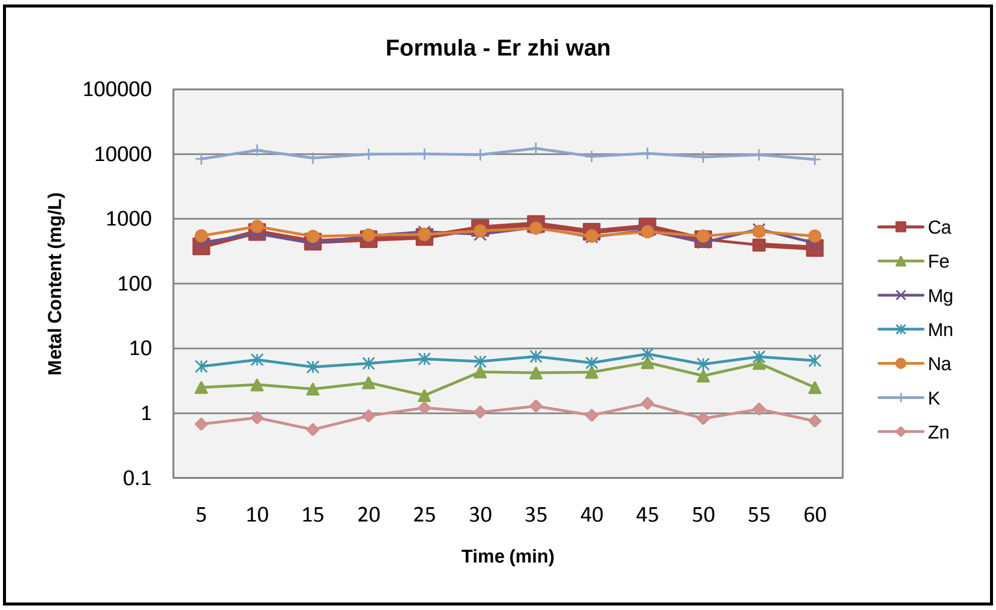

Figure 8.1: Metal content (mg L^{-1}) of formula EZW

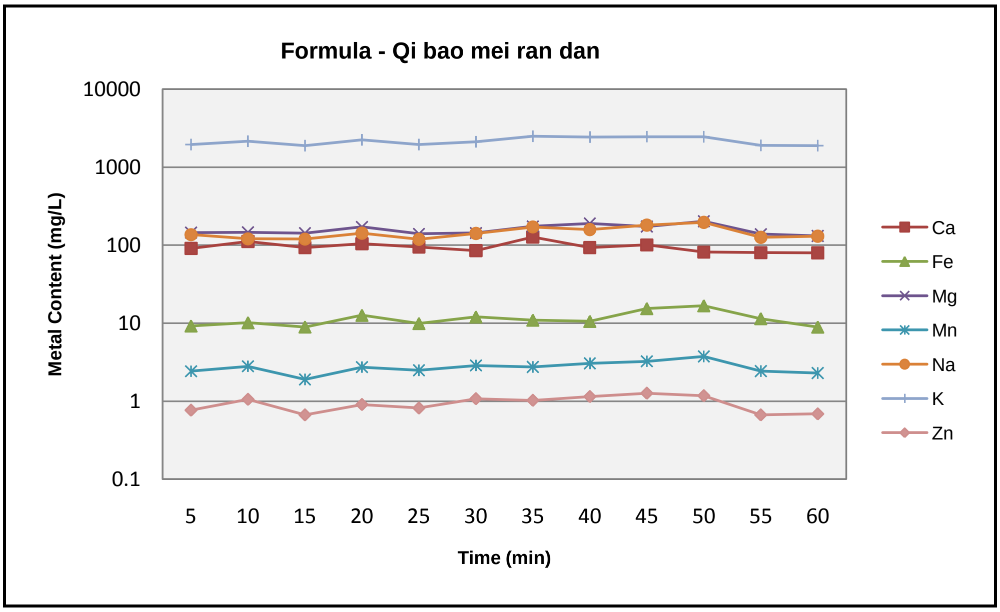

Figure 8.2: Metal content (mg L^{-1}) of formula QB

Correlation is a measure of association between two variables. The correlation matrix of the elements was presented in **Table 8.4**. It can be seen that good correlation exists between Ca, Mg, Mn, Na and K.

Table 8.4: Correlation matrix of elements (Pearson correlation)

Variables	Ca	Fe	Mg	Mn	Na	K	Zn
Ca	1						
Fe	-0.770	1					
Mg	0.940	-0.772	1				
Mn	0.916	-0.739	0.976	1			
Na	0.939	-0.835	0.967	0.961	1		
K	0.950	-0.854	0.974	0.960	0.994	1	
Zn	0.278	0.223	0.338	0.370	0.175	0.185	1

In order to assimilate the elemental patterns that are meaningful, PCA was performed. The principal components (PC) with eigenvalues greater than one were extracted and subjected to Varimax rotation. The percentages of variances after the Varimax rotation for the first two components were 78.65% and 18.0% respectively. The Varimax rotated factor loadings for PC1 and PC2 of elements were presented in **Table 8.5** and plotted in **Figure 8.3.** The varimax factor scores of the two PC's for formula EZW and QB at different decoction times were given in **Table 8.6** and were plotted in **Figure 8.4. Table 8.5** shows the larger loadings for PC1: Ca, Mg, Mn, Na and K and also Zn for PC2. When the larger loadings and higher scores in **Table 8.5** and **Table 8.6** were interpreted, first considering PC1, it can be seen that Ca, Mg and K were highest in EZW at 35 min as EZW35 had first highest score 1.486 (**Table 8.6**), Na was highest in EZW at 10 min as EZW10 had the next highest score 1.356 (**Table 8.6**) and, Fe and Mn were highest in EZW at 45 min as EZW45 had the next highest score 1.154 (**Table 8.6**). When PC2 was interpreted from loadings and scores of elements and formulae, it can be seen that Zn was highest in both formulae EZW and QB at 45 min as EZW45 has highest score 1.960 and QB45 has highest score 1.586 (**Table 8.6**).

Table 8.5: Varimax rotated factor loadings of the first two principal components for elements

Element	PC1	PC2
Ca	0.948	0.172
Fe	-0.894	0.370
Mg	0.964	0.229
Mn	0.948	0.267
Na	0.988	0.067
K	0.994	0.070
Zn	0.121	0.984

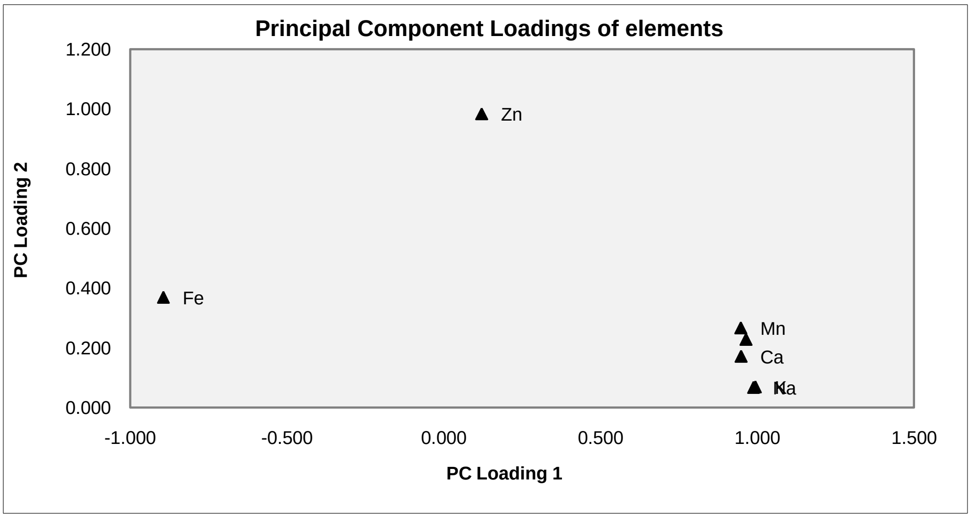

Figure 8.3: Varimax rotated principal component (PC1 versus PC2) loadings for elements

Table 8.6: Varimax rotated scores of the first two principal components of EZW and QB

Formulae	PC1	PC2
EZW5	0.699	-1.364
EZW10	1.356	-0.538
EZW15	0.768	-1.804
EZW20	0.905	-0.364
EZW25	1.067	0.710
EZW30	1.085	0.278
EZW35	1.486	1.335
EZW40	0.879	-0.169
EZW45	1.154	1.960
EZW50	0.729	-0.653
EZW55	0.931	0.903
EZW60	0.739	-0.977
QB5	-0.891	-0.765
QB10	-0.967	0.355
QB15	-0.909	-1.193
QB20	-1.014	0.078
QB25	-0.947	-0.519
QB30	-1.059	0.587
QB35	-0.915	0.343
QB40	-0.928	0.762
QB45	-1.155	1.586
QB50	-1.131	1.438
QB55	-0.990	-0.910
QB60	-0.893	-1.079

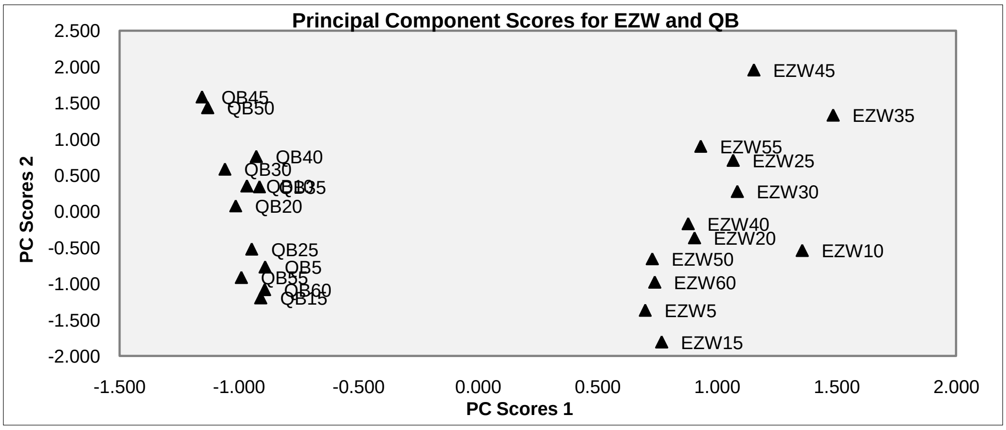

Figure 8.4: Varimax rotated principal component scores (PC1 versus PC2) of Chinese Herbal Formulae

HCA was applied to the elemental concentrations. The clusters formed by HCA for formulae EZW and QB were given in **Figure 8.5**. Ward's method was used as an amalgamation rule and the squared Euclidean distance as a measure of proximity between samples. The resulting dendrogram gave two clusters without any groups. This may be due to the response of pattern of elements in both of these groups not being similar and hence HCA did not generate groups.

Fisher's LDA analysis was performed using the leave-one-out method for model establishment and prediction. The discriminate function showed a significant association between the formulae and predictors, accounting for 79.55 % of between group variability. The cross validated function showed that overall 100 % of original grouped cases correctly classified.

The variation of the metal contents of TCM's EZW and QB with different decoction times is due to the combination of various herbs in the formula. Chinese herbal tea samples of 30 commercially available teas were analyzed by Xie *et al* at different infusion periods (1, 3, 5, and 10 mins). They reported that at 10 min the contents of K, Rb, Mn, Ni and Zn increased when compared to Ca and Fe [190].

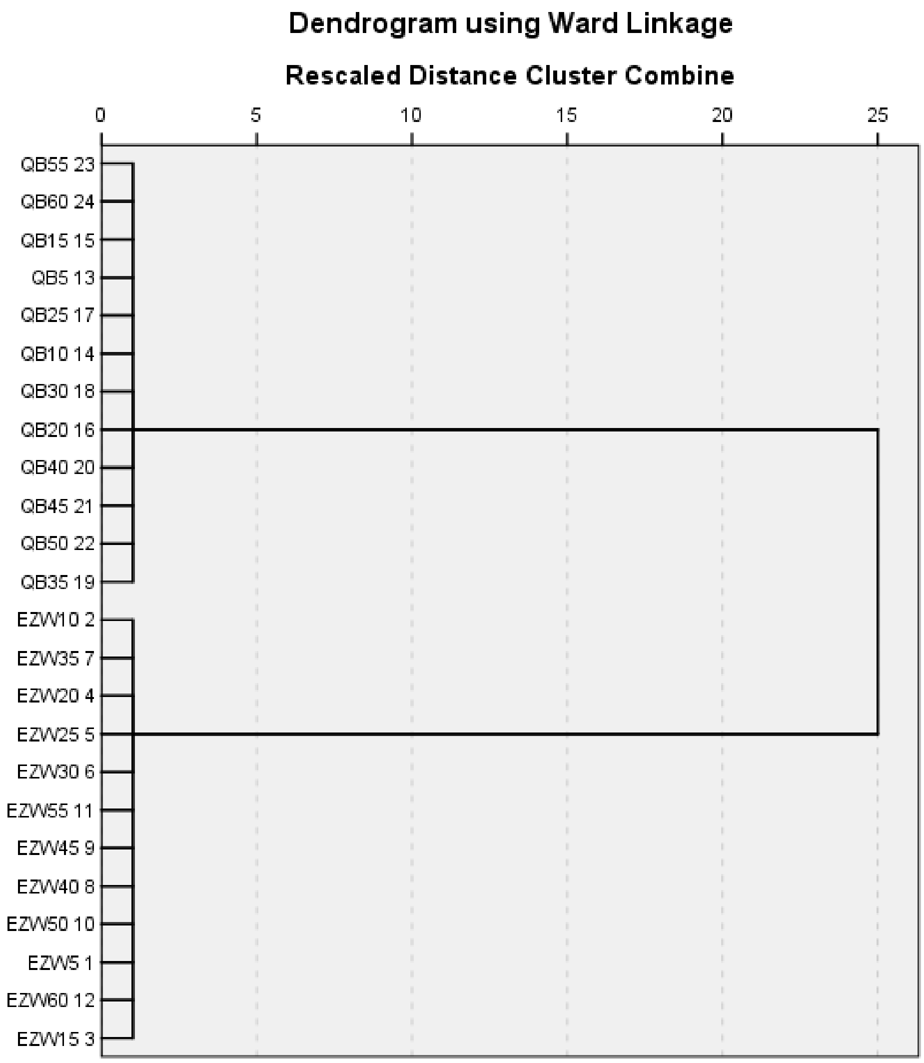

Figure 8.5: Dendrogram of hierarchical cluster analysis for the formulae QB and EZW

8.4.2 Metal intake from decoction of EZW and QB

The two formulae were examined for the contribution of each element on the ADDIs. Table 8.7 shows the results of the element concentration by cup (mg/100 mL formula). The percentage contribution of elements to ADDIs is shown in parenthesis (Table 8.7). It can be noticed that the highest contribution from EZW and QB to ADDIs is K and Mg respectively. The kidneys execute the major share of removing the dietary potassium. About 90% of the daily ingested potassium is excreted in an average amount of 1.5 L of urine [80]. Augmented intestinal potassium excretion becomes a relevant quantitative phenomenon in ESRD, where the colon is able to partially substitute for the reduced renal potassium excretory capacity [81-83]. There is a positive link between a diet rich in potassium and bone health [273] as EZW also has favourable potency to develop a new anti-osteoporotic agent [139] . An adequate serum magnesium concentration may be necessary to maintain renal function and protect the kidneys from damage. The kidney is a major regulator of total body magnesium homeostasis. So, from the elemental concentration approach, it can be considered that the highest contribution of K (25.7%) from EZW and Mg (10.2%) from QB to ADDIs might be one of the reasons for using them to improve kidney function. So to treat patients suffering from bone health, decoction time of 35 min for EZW and for patients suffering from impaired renal function, decoction time of 50 min for QB might be suggested.

Table 8.7: ADDI[a] for metals and average metal content by cup of formula

Metals	ADDI (mg d^{-1})[a]	Er zhi wan(mg cup^{-1})[b]	Qi bao mei ran dan (mg cup^{-1})[b]
Ca	1000 - 1300	55.65 (5.56)	9.54 (0.95)
Fe	8 - 18	0.36 (2.43)	1.14 (7.6)
Mg	310 - 420	55.58 (15.8)	36.03 (10.2)
Mn	5 - 5.5	0.64 (16.1)	0.27 (6.8)
Na	460 - 920	60.6 (2.75)	14.5 (0.6)
K	2800 - 3800	980.2 (25.7)	216.8 (5.7)
Zn	15	0.097 (0.64)	0.095 (0.63)

[a]ADDIs of metals - range; Source:[42]

[b]In parenthesis, % of ADDI from a cup (100mL) of formulae

8.5 Conclusions

The concentration of mineral elements in the herbal formulae EZW and QB were determined at different decoction times by AAS. Formula EZW contains higher amounts of Ca, Mg, Na and K than formula QB. K was highest in both formulae EZW and QB. Chemometrics were used to highlight the correlation between elements and to understand the elemental patterns. Good correlations exist between Ca, Mg, Mn, Na and K. PCA highlighted that Zn was highest at 45 min in both formulae EZW and QB. PCA also highlighted that in formula EZW: Ca, Mg and K were highest at 35 min, Na was highest at 10 min and, Fe and Mn were highest at 45 min. HCA gave only 2 clusters without highlighting any groups of similar elemental patterns. Fisher's

LDA analyses gave that 100 % of original grouped cases correctly classified. The percentage contributions of metals to ADDIs were high for K and Mg when compared to other metals from the decoctions of EZW and QB respectively.

9 DETERMINATION AND COMPARISON OF MINERAL ELEMENTS IN CHINESE HERBAL KIDNEY TONIFYING FORMULAE: YOU GUI WAN AND ZOU GUI WAN AT DIFFERENT DECOCTION TIMES

9.1 ABSTRACT

Traditional Chinese herbal formulae YGW: restore the right kidney pill and ZGW: restore the left kidney pill were analyzed for Ca, Fe, Mg, Mn, Na, K and Zn at different decoction intervals by AAS. YGW and ZGW were used to improve the kidney function. Fe, Mg, Mn, Na and K were higher in ZGW than YGW. K (2574.85 – 4206.23 mg L^{-1}) was highest in both formulae ZGW and YGW. Chemometrics such as PCA, HCA and LDA were applied to classify the data and to understand the relation between the elements. Metal intake related to the consumption of decoction has also been studied. Fe followed by Manganese made the highest contribution to average daily dietary intakes from the formulae.

9.2 INTRODUCTION

In Asia, preparations of TCM are used as conventional clinical treatments rather than as CAM in western countries. Due to its natural features and low side effects, TM has drawn considerable attention and the worldwide consumption of it is increasing rapidly over the recent years [23, 183].

Plants have always allured the attention of scientists, as our green planet is becoming less green every day. In order to protect the environment and as a final goal, human health searching for new solutions should provide a better insight into fundamental processes of plant physiology. Numerous processes are affected or regulated by minerals that are assigned as essential elements. Medicinal plants are of special importance taking into account their role in health protection or preventive or supportive therapy for numerous diseases and disorders [274, 275]. Generally, the main characteristics of essential elements depend on the regulatory mechanisms, which are able to keep the elements at the nutrition level.

In this study, comparison of two TCM formulae used to improve the kidney function: ZGW, formula that nourish and tonify the Yin and YGW, formula that warm and tonify the Yang. Kidney yang deficiency leads to heavy menstrual bleeding, metrorrhagia or complete ceasing of menstruation, soreness and weakness of lower back and knees and cold limbs [251]. ZGW, known as restore the left kidney pill has the effect to invigorate the kidney and is widely used for deficiency of body fluid, night sweat, sore waist, nocturnal emission, mental fatigue and dry mouth. YGW, known as restore the right kidney pill is used to treat exhaustion from long term illness, aversion to cold, impotence, spermatorrhea, and aching and weakness of the lower back and knees [43]. A study conducted by Yao et al. showed that YGW improves the immune function even in the serious immunosuppressive condition [145]. In this study, the formulae were analyzed for Ca, Fe, Mg, Mn, Na, K and Zn at different decoction intervals by AAS.

9.3 MATERIALS AND METHODS

9.3.1 Solutions and reagents

Standard stock solutions of Ca, Fe, Mg, Mn, Na, K and Zn at a concentration of 1 gL^{-1} were obtained from Merck, Australia, and working solutions were prepared by dilution of stock solutions in 5% (v/v) HNO_3 (*Trace* SELECT, 69%) from Sigma-Aldrich, Australia. Ionization in AAS was controlled by adding a 5mL (1 g/L CsCl + 10 g/L La, Merck) buffer solution to all samples and standards and made up to final volume of 100 mL. Deionized water with a specific sensitivity of 18 mΩ obtained from Millipore-Milli-Q water purifier system (Milford, USA). All chemicals and reagents were of analytical grade.

9.3.2 Sample preparation procedure

The metal content was analyzed in two TCM formulae: YGW and ZGW. The herbs involved in the formulae and the amount of each herb used that was generally prescribed by herbalist were listed in **Table 9.1**.

Table 9.1: List of the traditional Chinese herbal formulae

Formula name	Chinese herbs	Botanical name	Amount (g)
You gui wan	fu zi	*Radix Lateralis Aconiti Carmichaeli Praeparata*	6
	rou gui	*Cortex Cinnamomi Cassiae*	6
	lu jiao jiao	*Colla Cornu Cervi*	12
	shu di huang	*Radix Rehmanniae Glutinosae Conquitae*	24
	shan zhu yu	*Fructus Corni officinalis*	9
	shan yao	*Radix Dioscoreae Oppositae*	12
	gou qi zi	*Fructus Lycii*	12
	tu si zi	*Semen Cuscutae Chinesis*	12
	du zhong	*Cortex Eucommiae Ulmoidis*	12
	dang gui	*Radix Angelicae Sinesis*	9
Zou gui wan	shu di huang	*Radix Rehmanniae Glutinosae Conquitae*	24
	shan yao	*Radix Dioscoreae Oppositae*	12
	gou qi zi	*Fructus Lycii*	12
	shan zhu yu	*Fructus Corni officinalis*	12
	chuan niu xi	*Radix Cyathulae Officinalis*	9
	tu si zi	*Semen Cuscutae Chinesis*	12
	lu jiao jiao	*Colla Cornu Cervi*	12
	gui jiao	*Colla Plastrum Testudinis*	12

The method of preparation of decoction was done according to the Chinese standard procedure [43]. All the herbs involved in the formula were kept in a glass beaker with a lid and 200 mL of water was added and allowed to soak for 20 min. The mixture was first heated to boiling: 100º C and then kept at 60º C for different time intervals: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min. The decoction was allowed to cool to room temperature and then filtered with an ashless filter paper and transferred into 100 mL volumetric flask. All analyses were done in triplicate.

9.3.3 Instrumentation

The determination of Ca, Fe, Mg, Mn, Na, K and Zn was done on a Varian spectroAA-400 AAS (Varian Inc., Mulgrave, Australia) under optimized measurement conditions using hollow cathode lamps. The operating conditions for working concentrations of elements were set as recommended by the manufacturer, given in **Table 9.2**.

Table 9.2: The operating parameters for working elements

Elements	Wavelength (nm)	Lamp current (mA)	Slit (nm)
Ca	422.7	10	0.5
Fe	248.3	5	0.2
Mg	285.2	4	0.5
Mn	279.5	5	0.2
K	404.4	5	0.5
Na	330.3	5	0.5
Zn	213.9	5	1

9.3.4 Data analysis

The statistical data analysis was made using the SPSS for windows PASW SPSS 18 (Release 18.0.0, Jul 30, 2009) and Microsoft Excel 2007 with add-in XL-STAT 2010. To obtain more reliable information about the element content relationship, Pearson correlation analysis technique was used. To identify the elemental patterns in the data set, PCA was used. In order to classify the data, HCA: unsupervised pattern recognition technique and LDA: supervised pattern recognition techniques were used.

9.4 RESULTS AND DISCUSSION

9.4.1 Metal content in formulae

According to the Chinese standard procedure of making decoctions, most formulas are heated for 20-30 min. The formulas that contain rich, cloying substances should be heated for 45-60 min. The metal contents of the two formulae YGW and ZGW at different decoction times with 5 min interval from 5-60 min were given in **Table 9.3** and plotted in **Figure 9.1** and **Figure 9.2** respectively. Fe, Mg, Mn, Na and K were higher in formula ZGW than YGW. Independent sample t-test was done to determine the significant differences between the mean values of the elements in the formulae. The results showed that, significant differences ($p<0.5$) exist between YGW and ZGW for Ca (762.84 – 552.38 mg L^{-1}), Fe (22.76 – 37.98 mg L^{-1}), Mg (213.83 – 360.03 mg L^{-1}), Mn (5.09 – 7.76 mg L^{-1}), Na (362.98 – 613.2 mg L^{-1}), K (2574.85 – 4206.23 mg L^{-1}) and Zn (2.55 – 0.43 mg L^{-1}). K was highest in both formulae YGW and ZGW.

Table 9.3: Metal content (mg L^{-1}) of the analyzed formulae (mean ± S.D)

Formula name	Time (min)	Ca	Fe	Mg	Mn	Na	K	Zn
You gui wan	5	418.28 ± 2.88	9.24 ± 1.13	124.65 ± 2.1	2.88 ± .54	236.72 ± 2.53	1789.4 ± 3.02	1.11 ± .95
	10	442.19 ± 2.24	16.54 ± .81	156.59 ± 2.3	4.60 ± .76	284.61 ± 4.03	1860.3 ± 2.86	2.48 ± 1.06
	15	685.63 ± 4.02	20.05 ± 1.75	202.51 ± 2.92	4.83 ± .81	376.31 ± 3.46	2678.51 ± 4.43	2.44 ± .75
	20	745.12 ± 3.9	22.13 ± 1.7	192.64 ± 1.83	4.32 ± 1.09	352.93 ± 3.02	2298.24 ± 4.52	2.30 ± .94
	25	815.17 ± 3.16	23.36 ± 1.14	233.83 ± 2.7	5.71 ± .81	394.7 ± 4.12	2886.17 ± 3.23	3.31 ± .89
	30	452.12 ± 2.56	17.80 ± .8	158.68 ± 1.48	3.94 ± .73	281.25 ± 3.5	2136.4 ± 4.2	1.42 ± .86
	35	459.05 ± 2.94	21.51 ± 1.52	187.02 ± 2.27	5.86 ± .65	311.99 ± 2.4	2378.25 ± 4.38	2.05 ± 1.12
	40	1279.28 ± 3.23	26.88 ± 1.24	229.33 ± 2.7	4.76 ± .86	365.87 ± 2.05	2602.93 ± 4.54	2.22 ± 1.22
	45	1269.7 ± 4.0	29.44 ± 1.42	292.25 ± 2.19	7.41 ± .73	482.56 ± 2.89	3311.93 ± 3.1	4.01 ± 1.02
	50	1294.56 ± 3.31	33.26 ± 2.56	298.49 ± 2.8	5.86 ± .79	467.34 ± 2.10	3214.82 ± 4.2	3.95 ± .7
	55	536.86 ± 2.4	26.18 ± 1.73	219.27 ± 2.72	4.97 ± .16	383.74 ± 2.71	2659.43 ± 2.8	2.46 ± .83
	60	756.13 ± 2.9	26.70 ± 1.23	270.68 ± 2.79	5.82 ± .82	417.78 ± 2.73	3081.76 ± 3.5	2.77 ± .65
Mean		762.84	22.76	213.83	5.09	362.98	2574.85	2.55
Zuo gui wan	5	479.79 ± 2.1	21.89 ± 1.45	270.04 ± 2.55	6.46 ± 1.07	432.56 ± 2.58	2195.32 ± 3.39	0.06 ± .01
	10	495.24 ± 2.74	29.42 ± 1.43	304.69 ± 2.24	7.98 ± .67	557.32 ± 2.18	3228.12 ± 3.06	0.15 ± .02
	15	485.67 ± 2.79	28.14 ± 1.54	317.24 ± 2.03	7.94 ± .21	561.36 ± 2.75	3595.94 ± 4.15	0.10 ± .01
	20	555.65 ± 3.16	33.73 ± 1.02	283.16 ± 3.34	7.38 ± .77	566.26 ± 2.46	3292.8 ± 2.48	0.39 ± .06
	25	551.34 ± 3.1	36.05 ± 1.54	432.77 ± 2.97	9.33 ± .56	651.55 ± 2.39	5052.7 ± 3.19	0.42 ± .07
	30	610.31 ± 3.5	41.05 ± 1.05	430.02 ± 3.66	10.34 ± 1.08	734.37 ± 2.9	5093.5 ± 4.27	0.51 ± .09
	35	748.79 ± 2.6	60.48 ± 1.78	527.6 ± 2.24	6.99 ± .6	648.85 ± 2.74	5117.15 ± 3.63	0.76 ± .02
	40	647.88 ± 2.75	51.53 ± 2.09	332.82 ± 3.29	9.70 ± .73	672.12 ± 2.97	5293.65 ± 4.32	0.67 ± .05
	45	501.97 ± 2.7	46.9 ± 2.27	434.16 ± 2.82	7.63 ± .34	727.18 ± 1.9	5956.16 ± 4.68	0.74 ± .05
	50	576.58 ± 2.78	52.65 ± 1.24	403.01 ± 3.35	6.64 ± .47	663.96 ± 2.48	4552.5 ± 3.64	0.18 ± .01
	55	483.47 ± 2.47	26.0 ± 1.38	307.77 ± 1.42	6.34 ± .63	578.55 ± 2.63	3730.3 ± 3.65	0.32 ± .07
	60	491.82 ± 3.18	27.75 ± .72	280.31 ± 3.46	6.31 ± 1.17	564.34 ± 2.87	3366.56 ± 4.26	0.75 ± .06
Mean		552.38	37.98	360.3	7.76	613.2	4206.23	0.43

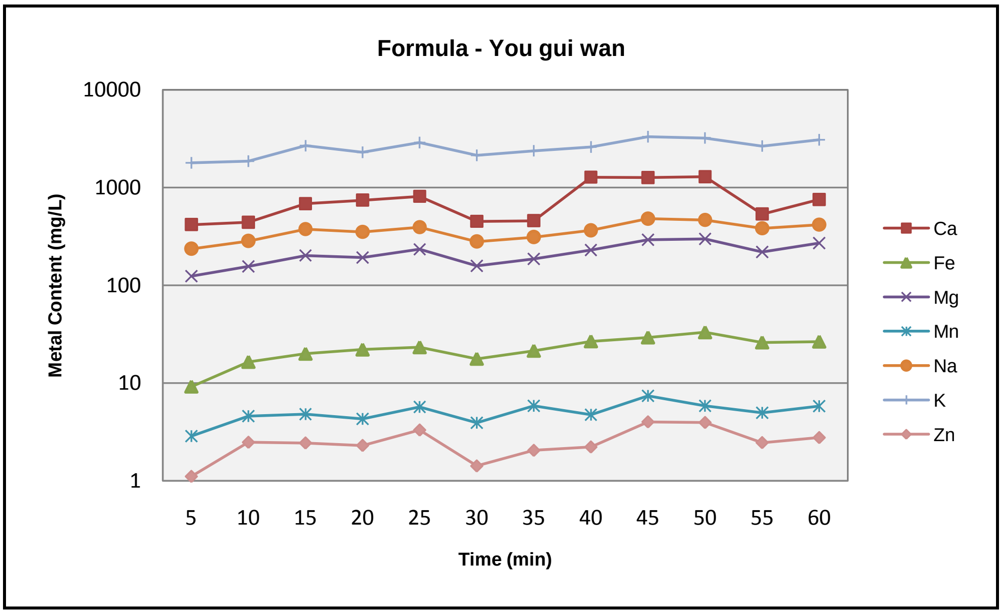

Figure 9.1: Metal content (mg L^{-1}) of formula YGW

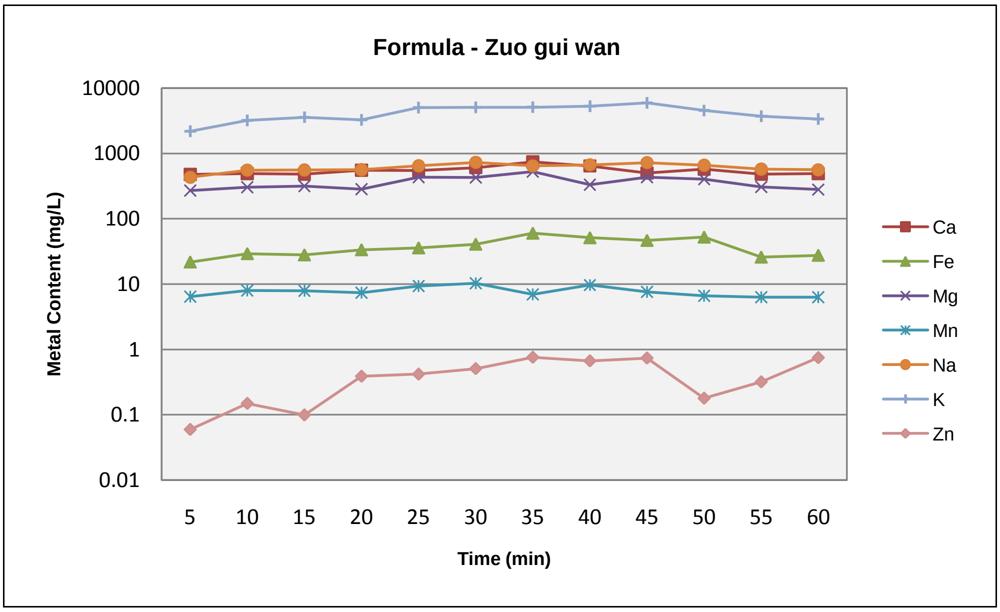

Figure 9.2: Metal content (mg L^{-1}) of formula ZGW

Correlation is a measure of association between two variables. The correlation matrix of the elements was presented in **Table 9.4.** It can be seen that good correlation exists between Ca and Zn, and Fe, Mg, Mn, Na and K.

Table 9.4: Correlation matrix of elements (Pearson correlation)

Variables	Ca	Fe	Mg	Mn	Na	K	Zn
Ca	1						
Fe	0.125	1					
Mg	0.057	0.897	1				
Mn	-0.037	0.675	0.769	1			
Na	-0.058	0.850	0.918	0.870	1		
K	-0.009	0.891	0.917	0.802	0.938	1	
Zn	0.679	-0.344	-0.440	-0.446	-0.549	-0.423	1

In order to assimilate the elemental patterns that are meaningful, PCA was performed. The principal components with eigenvalues greater than one were extracted and subjected to Varimax rotation. The percentages of variances after the Varimax rotation for the first two components were 64.8% and 24.5% respectively. The results after Varimax rotation: first and second factor loadings of elements were presented in **Table 9.5** and plotted in **Figure 9.3.** The score values of the two principal components PC1 and PC2 for each formula at different decoction times were given in **Table 9.6** and plotted in **Figure 9.4.** **Table 9.5** shows the larger loadings for PC1: Fe, Mg, Mn, Na and K and, Zn and Ca for PC2. When the larger loadings and higher scores of PC1 in **Table 9.5** and **Table 9.6** were interpreted, it can be seen that for YGW: Na and K were highest at 45 min as YGW45 had first highest score 0.313 (**Table 9.6**) and Ca was highest in YGW at 50 min as YGW50 had the next highest score 0.209 (**Table 9.6**). It can also be interpreted for formula ZGW: Fe and Mg were highest at 35 min as ZGW35 had highest score 1.732 (**Table 9.6**), Mn and Na were highest at 30 min as ZGW30 had the second highest score 1.565 (**Table 9.6**) and, K was highest at 45 min as ZGW45 had the third highest score 1.503 (**Table 9.6**). When the larger loadings and higher scores of second principal component PC2 in **Table 9.5** and **Table 9.6** were interpreted, it can be seen that for YGW: Fe, Mg and Mn were highest at 50 min as YGW50 had the first highest score 2.493 (**Table 9.6**) and, Zn was highest at 45 min as YGW45 had the second highest score 2.403 (**Table 9.6**). It can also be interpreted for formula ZGW: Ca and Zn were highest at 35 min as ZGW35 had the highest score 0.302 (**Table 9.6**).

Table 9.5: Varimax rotated factor loadings of the first two principal components of elements

Element	PC1	PC2
Ca	0.123	0.951
Fe	0.929	0.037
Mg	0.960	-0.060
Mn	0.858	-0.161
Na	0.961	-0.196
K	0.961	-0.095
Zn	-0.397	0.857

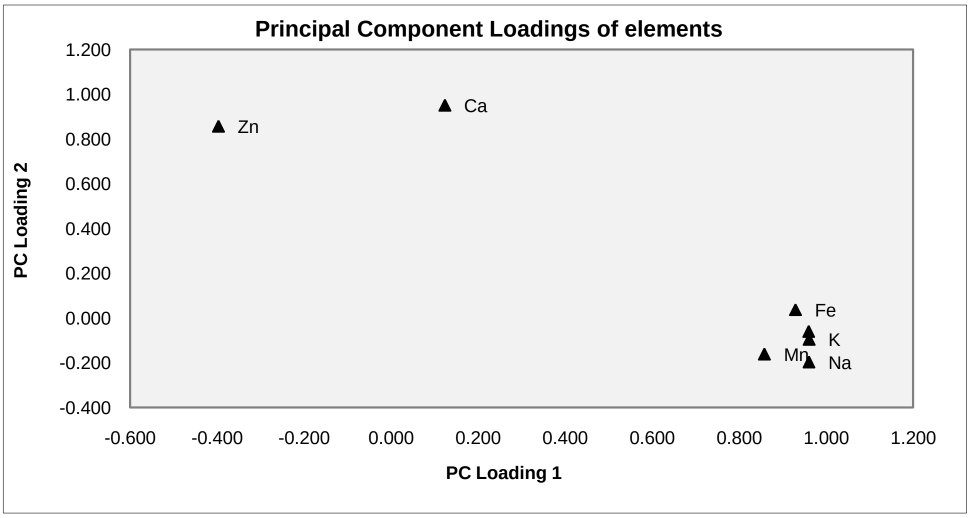

Figure 9.3:Varimax rotated principal component (PC1 versus PC2) loadings for elements

Table 9.6: Varimax rotated scores of the first two principal components for YGW and ZGW

Formulae	PC1	PC2
YGW5	-1.857	-0.905
YGW10	-1.403	-0.251
YGW15	-0.825	0.343
YGW20	-0.937	0.442
YGW25	-0.495	1.026
YGW30	-1.379	-0.623
YGW35	-0.969	-0.334
YGW40	-0.413	1.698
YGW45	0.313	2.403
YGW50	0.209	2.493
YGW55	-0.722	0.068
YGW60	-0.293	0.719
ZGW5	-0.547	-1.077
ZGW10	0.195	-0.944
ZGW15	0.265	-0.986
ZGW20	0.212	-0.675
ZGW25	1.228	-0.585
ZGW30	1.565	-0.398
ZGW35	1.732	0.302
ZGW40	1.440	-0.174
ZGW45	1.503	-0.447
ZGW50	1.127	-0.471
ZGW55	0.086	-0.907
ZGW60	-0.035	-0.717

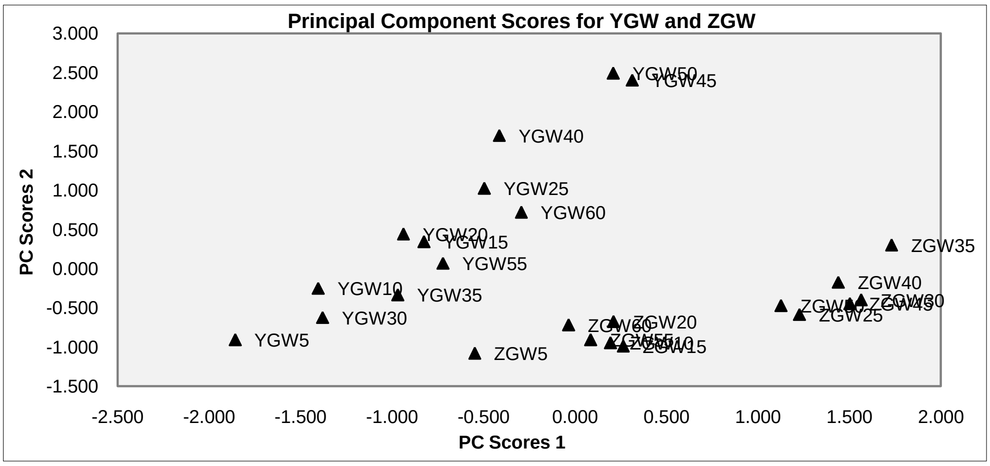

Figure 9.4:Varimax rotated principal component scores (PC1 versus PC2) of Chinese Herbal Formulae

HCA was applied to the systematized elemental concentrations and a pictorial description of formulae YGW and ZGW was obtained using Ward's method as an amalgamation rule and the squared Euclidean distance as a measure of the closeness between samples (**Figure 9.5**). The resulting dendrogram gave four concise clusters. The first cluster involved formula ZGW at 10, 15, 20, 55 and 60 min, as the amount of Ca, Fe and Zn followed the same pattern of rise from 10 to 20 min and 55 to 60 min, and the amount of Mg, Mn and K followed the same pattern of fall from 10 to 20 min and 55 to 60 min (**Figure 9.2**). The second cluster involved YGW at 15, 25, 40, 45, 50, 55 and 60 min, as Ca, Fe, Mg, Mn, Na, K and Zn increased from 15 to 25 min and followed the same pattern of rise from 40 to 60 min (**Figure 9.1**). In addition, in formula YGW, Ca, Fe, Mg and Mn were highest at 50 min and Na, K and Zn were highest at 45 min. The third cluster involved YGW at 5, 10, 20, 30 and 35 min and ZGW at 5 min. This is because in YGW, Ca, Fe, Mg, Mn, Na, K and Zn increased from 5 to 10 min and decreased from 20 to 30 min and increased from 30 to 35 min (**Figure 9.1**). In ZGW at 5 min, Ca, Fe, Mg, Mn, Na, K and Zn were lowest compared to other decoction times (**Figure 9.2**). Fourth cluster involved ZGW at 25, 30, 35, 40, 45 and 50 min because Ca, Fe; Mg, Mn; and Na, K and Zn followed the same patterns of rise and fall at those given intervals (**Figure 9.2**).

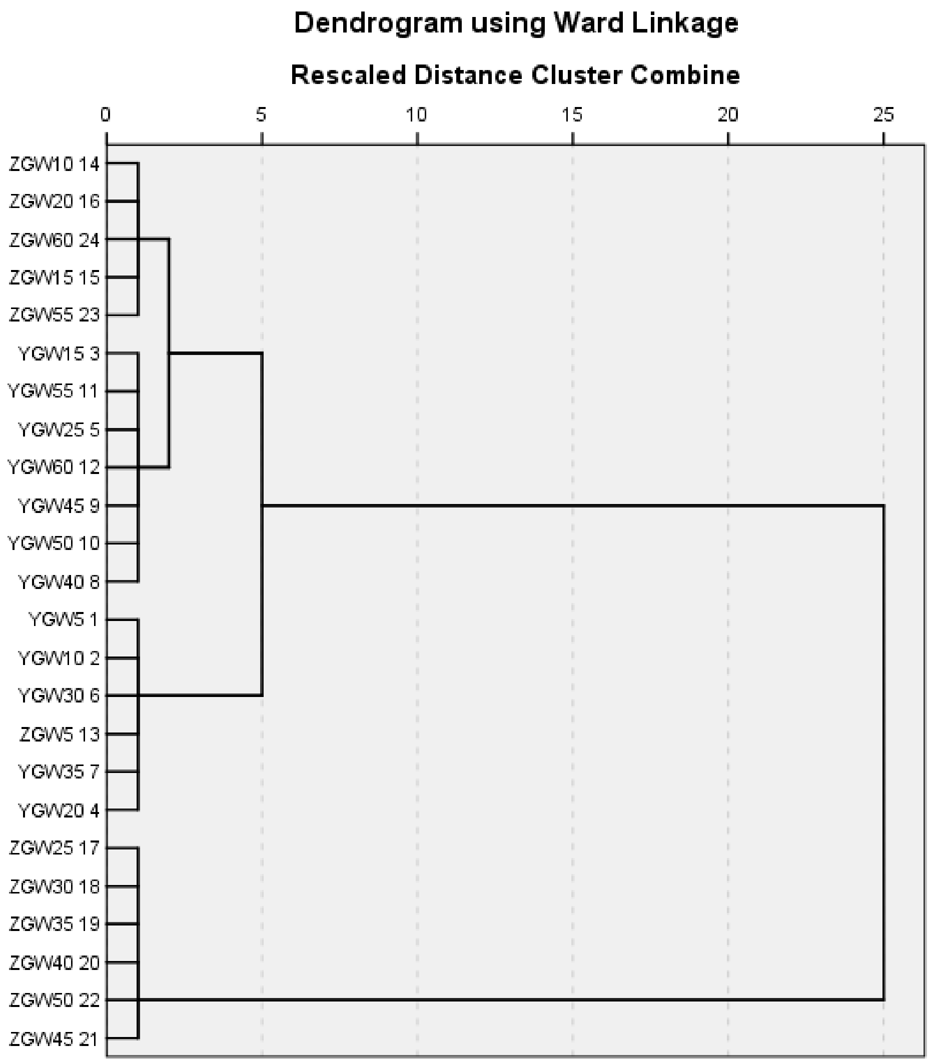

Figure 9.5: Dendrogram of hierarchical cluster analysis for the formulae YGW and ZGW

Fisher's LDA analysis was performed using the leave-one-out method for model establishment and prediction. The discriminate function revealed a significant association between the formulae and predictors, accounting for 79.07% of between group variability. The cross validated function showed that overall 100% of original grouped cases correctly classified.

The variation of the metal contents of TCM's YGW and ZGW with different decoction times is due to the combination of various herbs in the formula. Metal contents of black and green teas were studies at 3 and 5 min infusion times were studied by Fernandez *et al.* The results were analyzed by pattern recognition techniques such as principal components analysis and LDA. They reported that infusions prepared with black teas contained high levels of Mn, Mg, Al, Ca, K. Zn, Mn, and Ca present higher concentrations for green teas and Mg, Al, Ca and K for black teas prepared with the 5min extraction [193].

9.4.2 Metal intake from decoction of YGW and ZGW

The two TCM formulae were examined for the contribution of each element on the ADDIs. Table 9.7 shows the results of the element concentration by cup (mg/100 mL formula). The percentage contribution of elements to ADDIs was shown in brackets (Table 9.7). It can be noticed that high contribution from YGW and ZGW to ADDIs is Fe followed by Mn. Referring back to the literature, kidney yang deficiency leads to heavy menstrual bleeding and weakness of lower back and knees, spermatorrhea and night sweats. Kidney dominates water metabolism, so kidney yang deficiency causes a dysfunction of urinary bladder restriction manifesting enuresis Manganese plays an important role in a number of physiological processes and also helps to maintain sex hormone production. In TCM's view, the formula YGW and ZGW are used to treat kidney yang deficiency. It can be considered from the elemental concentration approach that the highest contribution of ADDIs to Fe (15.1% and 25.32%) and Mn (12.7% and 19.4%) in formulae YGW and ZGW might be one of the reasons for using these formulae to improve kidney function. So to treat patients suffering from heavy menstrual bleeding and weakness of lower back and knees, decoction time of 35 min for ZGW and 50 min for YGW might be suggested. For patients suffering from urinary bladder disorders, decoction time of 30 min for ZGW and 50 min for YGW might be suggested.

Table 9.7: ADDI[a] for metals and average metal content by cup of formula

Metals	ADDI (mg d^{-1})[a]	You gui wan(mg cup^{-1})[b]	Zou gui wan(mg cup^{-1})[b]
Ca	1000 - 1300	76.28 (7.62)	55.23 (5.52)
Fe	8 - 18	2.27 (15.1)	3.79 (25.32)
Mg	310 - 420	21.38 (6.1)	36.03 (10.2)
Mn	5 - 5.5	0.5 (12.7)	0.77 (19.4)
Na	460 - 920	36.29 (1.64)	61.3 (2.78)
K	2800 - 3800	257.48 (6.7)	420.6 (11)
Zn	15	0.25 (1.7)	0.04 (0.28)

[a]ADDIs of metals – range ; Source:[42]

[b]In parenthesis, % of ADDI from a cup (100mL) of formulae

9.5 Conclusions

The concentration of mineral elements in TCM formulae YGW and ZGW were determined at different decoction times by AAS. Formula ZGW contains higher amount of Fe, Mg, Mn, Na and K than formula YGW. Information of element content in the decoctions of TCM is important to assess their beneficial effects for human health. The data were subjected to chemometrics in order to highlight the possible correlations between the elements and to recognize the patterns with the purpose of identifying the possible influences of soil and climate. Good correlation exists between the analyzed elements. PCA showed that for formula YGW: Ca, Fe, Mg, Mn were highest at 50 min and, Na, K and Zn were highest at 45 min. PCA also highlighted that for formula ZGW: Ca, Fe, Mg and Zn were highest at 35 min, and Mn and Na were highest at 30 min. HCA highlighted similar clusters to that of PCA. Fisher's LDA analyses gave that 100% of original grouped cases correctly classified. The percentage contribution of metals from the decoction of YGW and ZGW showed that Fe followed by Mn had highest contribution to ADDIs.

10 SUMMARY OF RESULTS AND FUTURE RESEARCH DIRECTIONS

10.1 SUMMARY OF RESULTS

Preliminary study of elements, Zn and Pb on various parts of the Chinese herbs such as leaves, flowers, seeds, roots and fruits was undertaken using NIRS and AAS as the reference method. The concentration of Pb by AAS was higher in leaves for the herbs Bo He, He Ye, Sang Ye and Zi Su Ye compared to other parts of the herbs used. Also leaves: Lian Zi Xin and He Ye had higher concentration of Zn compared to other herbs. MLR and PLS methods were applied between predicted and laboratory values for the metals studied and correlations were obtained. This study shows that NIRS may be a promising rapid and feasible spectroscopy method for prediction of metal content in Chinese herbal plants.

Fifty Chinese herbs were analyzed for the following metals by AAS: Ca, Fe, Mg, Mn, K, Na and Zn. These herbs are used to improve kidney function in Chinese medicine. Ca was found to be highest in Fossils (Bie Jia and Long Gu). Fe and Zn were highest in herbs Yin Chen and Deng Xin Cao respectively. K and Mn were highest in Tong Cao. Mg was highest in root Chuan Niu Xi and Na was highest in fruit Gou Qi Zi. Of all the elements Ca and K were found to be high in all the herbs compared to other analyzed elements. Analyses of elements were done by Chemometrics such as ANOVA, CA, PCA and HCA. ANOVA showed that significant differenced exist between various samples analyzed for Ca whereas concentration of Fe, K, Mg, Mn, Na and Zn were not significantly different. CA showed that all elements are positively correlated with each other except that Ca is negatively correlated with Zn. Two significant groups of samples were obtained from PCA and HCA. Group 1 contained samples Tong Cao, high in Mn and K and Yin Chen, high in Fe and Group 2 contained samples Bie Jia and Long Gu that were high in Ca.

TCM formulae that are used to improve kidney function were analyzed for mineral elements such as Ca, Fe, Mg, Mn, K, Na and Zn by AAS. All the TCM decoctions used in this study were prepared at 5 min interval time from 5 to 60 min and data were subjected to chemometrics in order to highlight the possible correlations between the elements. LW, formula that nourish and tonify the kidney Yin and JG, formula that warm and tonify the kidney Yang were analyzed and compared. JG was higher in all elements compared to LW and K was highest in both the formulae. At 40 min the concentration of Fe, Mn and K was highest in both formulae. CA showed that good correlation exists between the analyzed elements. PCA and HCA showed similar pattern where for JG, PCA gave Ca and Zn as highest at 25min and Fe, Mn and K were highest at 40 min. For LW: Ca, Fe, Mg, Mn, Na and K were highest at 40 min. HCA gave significant clusters that highlights 25 and 40 min for JG. Fisher's LDA analysis showed that 91.7% of groups were correctly classified. Elements obtained from decoctions of LW and JG showed the highest contribution of Mn (8.25% and 12.5%) and Fe (7.2% and 11.2%) to ADDIs. So to treat patients suffering from chronic liver disorders, blood deficiency and urinary difficulty decoction time of 40 min might be suggested for LW and JG.

DB, formula that nourish and tonify the kidney Yin and GL, formula that warm and tonify the kidney Yang were analyzed and compared for the elements. Ca, Fe, Mg, Mn, Na and Zn were higher in formula GL than DB. In formula DB: Ca, Fe, Mg and Mn were higher at 45 min. ANOVA gave that significant differences exist between DB and GL. In formula DB, K was highest and Zn was lowest and in formula GL, Ca was highest and Zn was lowest. CA showed that good correlation exists between the analyzed elements except for K as K is negatively correlated. PCA showed that Fe, Mg, Mn and Zn were higher in formula GL at 60 min, and Ca and Na were highest at 50 min. HCA highlighted GL at 50, 55 and 60 min as all the elements were highest at either 50 or 60 min for GL. Fisher's LDA analysis showed that 100 % of original grouped cases were correctly classified. Contribution of elements obtained from decoctions of DB and GL showed highest contribution of Fe (9.84% and 30.2%) and followed by Ca (5.93% and 21.23%). So to treat patients suffering from anaemia, boneloss, weakness of lower back and knees, decoction time of 45 min for DB, and 50 to 60 min for GL might be suggested.

EZW, formula that tonify the kidney Yin and QB that enrich the kidney Yin were analyzed for the elements. Results showed that Ca, Mg, Na and K were higher in formula EZW than QB. K was highest in both formulae EZW and QB. CA showed that good correlation exists between the elements Ca, Mg, Mn, Na and K. PCA showed that in formula EZW at 45 min: Fe, Mn and Zn were highest and at 35 min: Ca, Mg and K were highest. PCA also showed that Zn was highest in both formulae EZW and QB at 45 min. HCA did not disclose any significant clusters. This may be due to the response of pattern of elements in both the groups not being similar. Fisher's LDA analysis showed that 100 % of original grouped cases were correctly classified. Contribution of elements obtained from decoctions of DB and GL showed highest contribution of K (25.7%) and Mg (10.2%). So to treat patients suffering from bone health, decoction time of 35 min for EZW and for patients suffering from impaired renal function, decoction time of 50 min for QB might be suggested.

ZGW, formula that nourish and tonify the Yin and YGW, formula that warm and tonify the Yang were analyzed for the elements. Results showed that Fe, Mg, Mn, Na and K were higher in formula ZGW than YGW and K was highest in both formulae YGW and ZGW. CA showed that good correlation exists between Ca and Zn, and Fe, Mg, Mn, Na and K. PCA showed that in formula YGW: Ca, Fe, Mg, Mn were highest at 50 min and in formula ZGW: Ca, Fe, Mg and Zn were highest at 35 min. HCA highlighted similar clusters to that of PCA. Fisher's LDA analysis showed that 100 % of original grouped cases were correctly classified. Contribution of elements obtained from decoctions of DB and GL showed highest contribution of Fe (15.1% and 25.32%) and followed by Mn (12.7% and 19.4%). So to treat patients suffering from heavy menstrual bleeding and weakness of lower back and knees, decoction time of 35 min for ZGW and 50 min for YGW might be suggested. For patients suffering from urinary bladder disorders, decoction time of 30 min for ZGW and 50 min for YGW might be suggested.

Papers in the literature shows that abnormalities in trace elements occur in patients with renal diseases. For human beings, trace elements are indispensable components of many enzymes, so they have some regulatory functions, and they may affect immune reactions and free radical generations. Trace elements accumulated in medicinal plants have the healing power in numerous ailments and disorders. The data on the elemental contents in Chinese medicinal kidney tonifying herbs and decoctions studies provide scientific evidence to Chinese theories. In Australia, there are at least three million AUD TCM consultations occurring each year and this represents an annual turnover of eighty four million AUD. Since 1992, there is a four-fold increase in imports of CHM. For the first time, leaching studies of the metals with different heating times on kidney tonifying herbal decoctions has been done in this study. The time intervals chosen in this study from 5 to 60 min at 5 min interval has not been reported earlier. This information will allow Chinese medicinal practitioners more precision in advising their patients on decoction heating times to target particular metal/metals that are more beneficial for a particular kidney problem.

10.2 FUTURE RESEARCH DIRECTIONS

Further work could be done on expanding the analysis of metals in different plant parts by using ICP as the reference method for the NIRS. For the calibrations to be robust hundreds of Chinese herbs would need to be analyzed and then NIR could be used as the screening process. The work reported here illustrated the principle of NIR detecting the metals in different plant parts but was not continued as the direction of the thesis changed.

The dry kidney tonifying herbs used in this study together with wider range of Chinese herbs that are used for curing other diseases could be analyzed by ICP in order to develop robust calibration for metal screening. In addition these kidney tonifying herbs, both dry and herbal decoctions could be analyzed for bio-availability and metal speciation.

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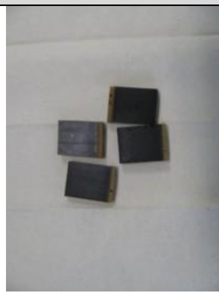
APPENDIX – IMAGES OF CHINESE HERBS

Fossil

Bie Jia	Gu Jia Jiao	Long Gu	Lu Jiao Jiao	Zi He Che
				

Fruits

Bu Gu Zi	Di Fu Zi	Guo Qui Zi	Long Yan Rou
			
Nu Zhen Zi	Sang Shen Zi	Shan Zhu Yu	
			

Flowers

Jin Yin Hua	Ju Hua	Mei Gui Hua	Mo Li Hua
			

Fungus

Dong Chong Xia Cao	Fulingpi	Ling Zhi	Zhu Ling
			

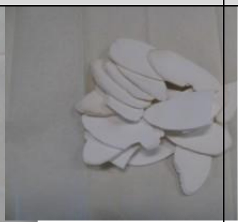
Leaves

Bo He	He Ye	Lian Zi Xin	Sang Ye	She Wei
				
Yin Yang Huo	Zi Su Ye			
				

Peel

Dong Gua Pi


Roots

Ba Ji Tian	Bi Xie	Chuan Niu Xi	Gan Cao	He Shou Wu
				
Hong Da Ji	Ren Shen	Mu Dan Pi	Shan Yao	Shu Di Huang
				
Xi Yang Shen	Xu Duan			
				

Seeds

Bai Guo	Che Qian Zi	Chi Xiao Dou	Dong Gua Ren	Hei Zhi Ma
				
Jue Ming Zi	Mu Hu Die	Pang Da Hai	Sha Yuan Zi	Tu Si Zi
				
Yi Yi Ren	Yi Zhi Ren			
				

Whole plant/Stem/Twig/Bark

Bian Xu Cao	Che Qian Cao	Deng Xin Cao	Du Zhong	Gu Sui Bu
				
Hai Jin Sha	Han Lian Cao	Huang Jing	Jin Qian	Mu Tong
				
Suo Yang	Tong Cao	Xian Mao	Yin Chen	Ze Xie