IN SILICO APPROACHES IN THE STUDY OF TRADITIONAL CHINESE HERBAL MEDICINE

UNG CHOONG YONG

(*B.Sc, University of Malaya, Malaysia; MSc, National University of Singapore, Singapore*)

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Summary

Recent development of Systems Biology in this "omics" era reinforced the therapeutic strategy of considering human systems as a whole. This "holistic" approach had been long practiced in traditional medicines such as traditional Chinese medicine (TCM). Multi-herb prescriptions have been routinely used in TCM formulated by using TCM-defined herbal properties (TCM-HPs) where the scientific basis is unclear. These multi-herb prescriptions often include special herb-pairs responsible in mutual enhancement, assistance, and restraint. Machine learning methods (MLMs) such as support vector machine (SVM) are used to explore the scientific basis of TCM prescription formulation. The studies reveal that MLMs are capable of classifying TCM prescriptions and herb pairs from those of random herb combinations showing that there is hidden scientific rule in the formulation of TCM prescriptions for which the molecular mechanisms are still unknown. Besides, a structural approach using inverse docking method (INVDOCK) is used to identify putative metastatic-related targets of Rhubarb anthraquinones such as emodin, aloe-emodin, and rhein from a protein structure database. Some targets identified by INVDOCK had been confirmed experimentally. The results implicate additive or synergistic effects of Rhubarb anthraquinones in anti-metastasis when used in combinations. In addition, current study of herbal synergism using literature-based approach reveals multiple mechanisms that involve either similar or distinct molecular targets as well as signaling pathways. In general, current *in silico* approaches used in this study covered both traditional and molecular aspects of TCM from top-down and bottom-up directions. More rigorous studies in the understanding of holistic

pharmacological mechanisms of TCM are needed and are believed to provide insight for incorporating Systems Biology in drug development.

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A. Publications relating to research work from the current thesis

1. **C.Y. Ung**, H. Li, Z.W. Cao, Y.X. Li and Y.Z. Chen (2007). Are Herb-Pairs of Traditional Chinese Medicine Distinguishable from Others? Pattern Analysis and Artificial Intelligence Classification Study of Traditionally-Defined Herbal Properties. *J. Enthopharmacol.* 112(2): 371-377.

2. **C. Y. Ung**, H. Li, C. Y. Kong, J. F. Wang and Y. Z. Chen (2006). Usefulness of Traditionally-Defined Herbal Properties for Distinguishing Prescriptions of Traditional Chinese Medicine from Non-Prescription Recipes. *J. Enthopharmacol.* 109 (1): 21-28.

3. C.J. Zheng, **C.Y. Ung**, H. Li, L.Y. Han, B. Xie, C.Y. Kong, C.W. Cao, and Y.Z. Chen (2007). Evidence from literature-described molecular interaction profiles supports the existence of synergistic effect in some herbal ingredients. (Submitted)

4. Chen X, **Ung CY**, Chen YZ. (2003). Can an in silico drug-target search method be used to probe potential mechanisms of medicinal plant ingredients? *Natural Product Reports* **20**(4):432-444.

5. Chen YZ, **Ung CY** (2002). Computer automated prediction of potential therapeutic and toxicity protein targets of bioactive compounds from Chinese medicinal plants. *American Journal of Chinese Medicine* **30**(1):139-154.

B. Publications from relevant projects not included in the current thesis

1. **C.Y. Ung**, H. Li, C. W. Yap and Y. Z. Chen (2007). *In Silico* Prediction of Pregnane X Receptor Activators by Machine Learning Approaches. *Mol. Pharmacol.* 71(1):158-168.

2. H. Li, C.W. Yap, **C.Y. Ung**, Y. Xue, Z.R. Li, L.Y. Han, H.H. Lin and Y.Z. Chen (2007) Machine Learning Approaches for Predicting Compounds That Interact with Therapeutic and ADMET Related Proteins. *J. Pharm. Sci.* (In press)

3. X. Chen, H. Li, C.W. Yap, **C.Y. Ung**, L. Jiang, Z.W. Cao, Y.X. Li and Y.Z. Chen (2007). Computer Prediction of Cardiovascular and Hematological Agents by Statistical Learning Methods. *Cardiovasc. Hematol. Agents Med. Chem.* 5(1): 11-19.

4. J. Cui, L.Y. Han, H. Li, **C.Y. Ung**, Z. Q. Tang, C. J. Zheng, Z. W. Cao, Y. Z. Chen (2007). Computer Prediction of Allergen Proteins from Sequence-Derived Protein Structural and Physicochemical Properties. *Mol. Immunol.* 44(4): 514-520.

5. X Chen, H Zhou, YB Liu, JF Wang, H Li, **CY Ung**, LY Han, ZW, Cao and YZ Chen. (2006) Database of traditional Chinese medicine and its application to studies of mechanism and to prescription validation. *Br. J. Pharmacol.* 149(8): 1092-1103.

6. H. Li, **C. Y. Ung**, C. W. Yap, Y. Xue, Z. R. Li and Y. Z. Chen (2006). Prediction of Estrogen Receptor Agonists and Characterization of Associated Molecular Descriptors by Statistical Learning Methods. *J. Mol. Graph. Mod.* 25 (3): 313-323.

7. H. Li, C. W. Yap, Y. Xue, Z. R. Li, **C. Y. Ung**, L. Y. Han, and Y. Z. Chen (2006). Statistical learning approach for predicting specific pharmacodynamic, pharmacokinetic or toxicological properties of pharmaceutical agents. *Drug Dev. Res.* 66 (4):245-259.

8. C. W. Yap, Y. Xue, H. Li, Z. R. Li, **C. Y. Ung**, L. Y. Han, C. J. Zheng, Z. W. Cao and Y. Z. Chen (2006). Prediction of Compounds with Specific Pharmacodynamic, Pharmacokinetic or Toxicological Property by Statistical Learning Methods. *Mini. Rev. Med. Chem.* 6(4):449-459.

9. Y. Xue, H. Li, **C.Y. Ung**, C.W. Yap and Y.Z. Chen (2006). Classification of a Diverse Set of *Tetrahymena Pyriformis* Toxicity Chemical Compounds from Molecular Descriptors by Statistical Learning Methods. *Chem Res Toxicol.* 19 (8): 1030-1039.

10. H. Li, C. W. Yap, **C. Y. Ung**,Y. Xue, Z. W. Cao, and Y. Z. Chen (2005). Effect of Selection of Molecular Descriptors on the Prediction of Blood-Brain Barrier Penetrating and Non-penetrating Agents by Statistical Learning Methods. *J. Chem. Inf. Model.* 45 (5): 1376-1384.

11. H. Li, **C. Y. Ung**, C. W. Yap, Y. Xue, Z. R. Li, Z. W. Cao, and Y. Z. Chen (2005). Prediction of Genotoxicity of Chemical Compounds by Statistical Learning Methods. *Chem Res Toxicol.*18(6):1071-1080.

List of Abbreviations

ADME — Absorption, distribution, metabolism, excretion

ACE — Angiotensin converting enzyme

CAM — Cell adhesion molecule

COX2 — Cyclooxygenase 2

CYP — Cytochrome

EGCG — Epigallocatechin gallate

EGFR — Epidermal growth factor receptor

ERα — Estrogen receptor α

GCG — (-)-Gallocatechin gallate

FN — False negatives

FP — False positives

hTERT — Human telomerase reverse transcriptase

INVDOCK — Inverse docking

k-NN — *k* nearest neighbour

LDL — Low density lipoprotein

LBD — Ligand binding domain

LR — Logistic regression

MLMs — Machine learning methods

PDB — Protein Data Bank

PNN — Probabilistic neural network

Q — Overall accuracy

QSAR — Quantitative structure activity relationship

SE — Sensitivity

SP — Specificity

SVM — Support vector machine

TCM — Traditional Chinese medicine

TCM-HPs — TCM-defined herbal properties

THR — Thyroid hormone receptor

Chapter 1 Introduction: Overview of Current Status in TCM Research and the Motivation in this Study

In the following subsections of this chapter the current status of drug discovery in this "omics" era and the rise of systems biology that reinforce "holistic" approach that subsequently lead us to face a paradigm shift in both philosphy and strategy in medicine are discribed. The reason why it is important to rethink our current approaches at this time in medicine and the necessity to revisit the study of traditional medicines such as traditional Chinese medicine (TCM) is explained. At the end of this chapter the motivation and approaches used in this study are presented.

1.1 The Need of Revisiting the Research on TCM

Plants have been used to treat diseases for more than a thousand of years. However, it was not until 1800s that pure compounds were isolated from plants, paving the way for modern pharmaceuticals. For instance, in 1805 morphine was isolated from the opium poppy (*Papaver somniferum*) by the German pharmacist Friedrich Serturner. Following the isolation of salicylic acid or aspirin from the bark of the willow tree (*Salix alba*), Felix Hoffmann synthesized aspirin in 1897. In the same year of first synthesis of aspirin, ephedrine was isolated from the Chinese herb *Ephedra* (*Ma Huang*) and became popular with American physicians in 1924 for its broncho-dilatating and decongestant properties [Fan *et al.* 2006]. In 1972 the antimalarial drug artemisinin was developed

from the Chinese herb *Artemisia annua L* (*Qing Hao*). All these examples illustrate the rich history of plant-based medicines.

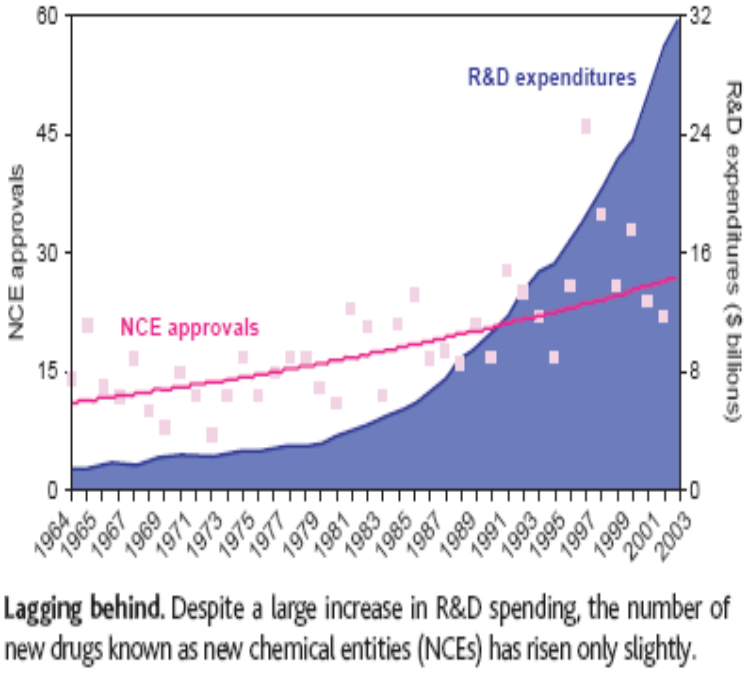
The proposal of the "Magic Bullet" theory by Paul Ehrlich in early 1900s [Winau *et al.* 2004] had directed pharmaceutical research towards target-directed drug discovery. Target-based screening was initially used to improve the drug-like properties of active compounds and their binding selectivity against pharmacological targets. This strategy was very successful when applied to well validated targets of known drugs. However, when the drug discovery process moved beyond these well validated targets it became apparent that the target-directed approach was flawed when testing on animal models and on human. This is due to the fact that most diseases such as diabetes, high blood pressure, high cholesterol levels and cancers are multi-factorial and that treating a single target provides only partial treatment. Although this awareness is not new it has been very difficult to find alternative routes due to the complexity of the living systems.

However, the revolution in the past decade in the "omics" areas such as genomics, proteomics, and metabolomics in biology has provided considerable support for a more holistic view on diagnosis and treatment. Besides, the issue of personalized medicine is now receiving considerable attention due to the new insights in pharmacogenomics [Wang *et al.* 2005e]. Although there are around 30,000 genes in the human genome only 600 to 1500 genes are estimated as potential drug targets [Hopkins *et al.* 2002]. Around 6000 marketed drugs interact with less than 120 molecular targets. Of particular relevance is the fact that 61% of the 877 drugs introduced between 1981 and 2002 are

obtained from natural products or their synthetic derivatives [Newman *et al.* 2003; Newman *et al.* 2007]. TCM herbs are rich sources of active compounds. For instance, numerous bioactive compounds have been found in Chinese medicinal plants for diabetes. These compounds include polysaccharides, terpenoids, flavonoids, sterols and alkaloids, and some of them have been developed as new drugs and are used in clinical treatment of diabetes in China [Li *et al.* 2004].

Recently there is indication showing that expenses spent in drug development increased dramatically while limited drugs are approved as shown in **Figure 1.1** [Service 2004]. Hence, we are now experiencing a paradigm shift from growing evidences of the multifactorial nature of diseases. Various evidences had showed that the therapeutic effects of a combined-drug therapy or drug cocktail not only produce higher therapeutic efficacy but the side effects are not necessarily additives but even less than the sum of toxicity from single-drug therapy. Combinatorial drug therapies had been used in cancer chemotherapies, HIV infection, and hypertension. More detail of combinatorial drug therapy will be presented in **Chapter 5**.

Figure 1.1 Costs spent and approved clinical drugs over years in the process of drug development. This figure is obtained from [Service 2004].



Lagging behind. Despite a large increase in R&D spending, the number of new drugs known as new chemical entities (NCEs) has risen only slightly.

Similar to Western combinatorial drug therapy, TCM uses mixtures of plant extracts to maximize efficacy and minimize adverse effects or toxicity. In fact, multitarget therapy has long been exploited in TCM in the form of multi-herb prescriptions. By its very nature, a TCM prescriptions works by attacking as well as modulating several targets simultaneously. However, a multitude of challenges exist in plant-based medicine. First, there are many active compounds present in each herb and the synergistic and antagonistic interactions between active compounds from different herbs are largely unknown. Furthermore individual active compounds are usually less potent than the total herbal extract from which they are isolated. In fact some herbal compounds are prodrugs and are active only after absorption and metabolism.

1.2 Brief Introduction of TCM Principles from Traditional Point of Views

In order to appreciate the holistic nature of TCM a brief description of theories used in TCM is presented. TCM has a long history dating back several thousands of years in China and is highly influenced by the development of Chinese culture. There are two main ideological ideas fundamental to TCM. The first is the homeostasis idea that focuses on the integrity of human body that emphasizes close relationship between human body and its social and natural environment (integrity between human and the cosmos). The second is the idea of dynamic balance that emphasizes the integrity of movement. Physiologically speaking TCM perceives human body in a cybernetic way. The important theories in TCM are the *Yin-Yang* theory, the *Wu Xing* (five elements) theory, as well as the *Zhang Fu* (meridians) theory that are used to explain the changes in the human body and to guide the diagnosis and treatment.

1.2.1 The Yin-Yang Theory

One of the most influential doctrines is the establishment of the *Yin-Yang* theory that had helped in the use of herbal materials for relieving illnesses. In *Yin-Yang* theory, the concept of *Yin-Yang* means “opposites” or “relatively speaking” such as hot vs cold, active vs inactive, healthy vs ill, etc [Zhang 2005a]. According to the *Yin-Yang* theory everything in the universe can be divided into *Yin* and *Yang* that are interchangeable as consistent with the modern scientific views of homeostasis where a biological system can

function to exert positive effect to one biological system but produce negative effect on another. In a more concise description, *Yin* and *Yang* are said to be complemented and rooted on each other [Zhang 2005a]. This produces mutual assistance, mutual conteraction, mutual suppression, and mutual antagonism of *Yin-Yang* actions.

Physiologically, *Yin* controls the internal, lower and front portions of the body, while *Yang* dominates the external, upper and back parts of the body. In addition, *Yin* also represents passiveness, coolness, static, downward, descending, and hypofunctional, while *Yang* represents activeness, hotness, dynamic, upward, ascending, and hyperfunctional. When the homeostasis of the autonomic nervous system is compared to the *Yin-Yang* theory, *Yang* seems to resemble the functions of sympathetic nervous system that mediates hyperactivities in the body while *Yin* resembles the functions of parasympathetic nervous system that mediates hypoactivities in the body. The use of acupuncture, moxibustion and herbal medicines in TCM thus aim to rectify the imbalance of physiological *Yin-Yang* states.

Recently, Ou *et al* defined the physical meaning of *Yin-Yang* in TCM by correlating it with biochemical processes. They proposed that *Yin-Yang* balance is correlated to antioxidation-oxidation balance with *Yin* representing antioxidation and *Yang* as oxidation. Their proposal is partially supported by the fact that the *Yin*-tonic traditional Chinese herbs on average have about six times more antioxidant activity and polyphenolic contents than the *Yang*-tonic herbs [Ou *et al.* 2003a]. More works to explore other biochemical espects of *Yin-Yang* are needed.

1.2.2 The Wu Xing theory (the theory of five elements)

Another important doctrine in TCM is the *Wu Xing* Theory (the theory of five elements). According to *Wu Xing* theory every process in this world is cyclic and is maintained in a kinetic balance that can be relatively categorized into five different stages that correspond to activation ("wood" stage), ascending ("fire" stage), transition from active to inactive ("soil" stage), declining ("metal" stage), inactive or dormant ("water" stage) and the cycle repeats to active ("wood" stage) again. There are two opposite cycles in the *Wu Xing* theory. One is generative (positive influence) and the other suppressive (negative influence). In the generating cycle, wood generates fire, fire generates earth, earth generates metal, metal generates water and water generates wood. In the suppressive cycle, wood suppresses earth, earth suppresses function of water, water suppresses fire generation, fire suppresses the function of metal, and metal suppresses generation of wood. In TCM, the physiological and psychological functions of the body are symbolically represented by five elementary components according to the *Wu Xing* theory.

1.2.3 The Zhang Fu theory (the theory of meridians)

The *Zhang Fu* theory explains the integrity of whole body by a cybernetic way as well as the pathophysiological states and locations of diseases. The words "*Zhang Fu*" in Chinese mean organs. However, in TCM it is more on body systems that are connected by meridians or "*Jing Luo*" in Chinese. For instance, when a TCM practitioner mentions the term "kidney" he does not means the organ kidney as mentioned in Western medicine

from the anatomical point of view but rather the meridian of "kidney" that can be composed of excretory and reproductory systems as well as part of symphatetic and parasympathetic nervous systems. Hence, the concept of "*Zhang Fu*" or meridians in TCM is "systemic" rather than "organic".

1.2.4 Diagnosis and treatment in TCM

In TCM the mind and the body are considered as one entity that involves signs and symptoms (or "*Zheng*" in Chinese) of illness rather than simply the cause of the disease. Practitioners of TCM diagnose the physiological *Yin-Yang* imbalance via "diagnosis of four" that comprises examining the patients by outlook, hearing the voice or breathing of patient, asking the illness states and the daily life style of patient, and examining the pulse. Each TCM herb has its own character and taste that corresponds to a particular *Yin-Yang* state. The TCM practitioner adjusts the imbalance of the *Yin-Yang* state in a patient by using multiple TCM herbs in a prescription or acupuncture as well as moxibustion.

In general, the treatments of TCM are "phenome-based" that rely on the whole-body response as revealed in signs and symptoms ("*Zheng*" in Chinese) display on patients irregardless of sources of infections. Each sign and symptom has the corresponding treatment which counterbalances the patient's disease states from normal health. Patients who show similar symptoms are relieved by the same treatment, even though the signs or symptoms may arise from different types of pathogens. Practitioners

of TCM select the appropriate treatment during the course of the disease as well as altering treatment to counterbalance the changes during disease development.

1.2.5 Pharmacological classification of TCM herbs

Each TCM herb possesses its own characters such as warm, hot, cool, or cold, tastes such as sweet, salty, pungent and the meridians such as heart or kidney where the herb exerts its therapeutic effect. The detailed descriptions of these properties of herb will be given in **Chapter 2** and **3**. In clinical application, TCM herbs are classified into 18 groups [Cheng 2000]. Some of these classes are similar to the therapeutic groups used in Western medicine. These classes are digestives, anthelmintics, purgatives (cathartics), diuretics, expectorants, and antitussives. However, some groups have no equivalent in Western medicine, such as herbs for relieving exterior syndrome, herbs for eliminating heat, herbs for eliminating wind dampness, herbs for dispelling dampness, herbs for warming the interior, and herbs for regulation of *Qi*.

Table 1.1 Pharmacological classifications of TCM Herbs. This table is derived from [Cheng 2000].

Class Number	TCM Pharmacological Class
1	Herbs for relieving exterior syndromes
2	Herbs for eliminating heat
3	Herbs for purgation
4	Herbs for eliminating wind dampness
5	Herbs for dispelling dampness
6	Diuretics
7	Herbs for warming the interior
8	Herbs for regulating *Qi*
9	Digestives
10	Anthelmintics
11	Hemostatics
12	Herbs for activating blood circulation and removing blood stasis
13	Dyspnea relieving herbs
14	Sedatives
15	Herbs for calming the liver and suppressing wind
16	Herbs for promoting resuscitation
17	Tonics
18	Astringents

1.3 TCM Research in the "Omics" Era

Recent developments in genomics, proteomics and metabolomics have advanced researches in life sciences including herbal medicines. In this "omics" era an unprecedented array of analytical tools has made a great forward leap in the understanding of the philosophy as well as scientific foundation of TCM prescriptions. Hence, reseaches on TCM in these days are not merely limited to animal testing from isolated active compounds, herbal extracts or multi-herb decoctions but to analyze

profiles as a whole in the disipline of systems biology in term of gene expression as well as proteome after taking these ingredients.

Microarray analysis has been applied to analyze gene expression on herbal recipes to study various diseases using animal models such as ischemic mice [Wang *et al.* 2004]. Besides, metabolome analysis that comprises of metabolite profiling is of growing importance in herbal medicine such as breeding, formulation, quality control and clinical trials [Kell 2004; Chan *et al.* 2007]. Methods that are currently being used in metabolomics are chromatography-based methods such as gas chromatography (GC), high performance liquid chromatography (HPLC); molecular weight-based methods such as mass spectrometry (MS); and physical characteristics-based methods such as NMR spectrometry.

1.4 *In Silico* Approaches in TCM Research

The development of "omics" research is not possible without the advancement of various *in silico* approaches used in genomics and bioinformatics. These *in silico* approaches include molecular docking, molecular dynamics simulation, machine learning methods, quantitative structure-activity relationship analysis (QSAR), local and global sequence alignment and comparison algorithms, data mining and pattern recognitions.

Recently some of these *in silico* methods have been used in TCM research. For instance, the Bayesian network has been used to deal with the information of symptoms and signs for syndrome differentiation [Zhu *et al.* 2006]. In addition, Bayesian network

was also used to model the relationship between quantitative features and diseases as extracted from tongue images [Pang *et al.* 2004]. Besides symptom recognition, computerized binary coding was used to decompose and reconstruct the *Yin-Yang* theory and was proven to be a successful simulation of the major ingredients of the theory further suggested the possibility of digitalization of the fundamental theories in TCM [Qin *et al.* 2004]. Recent work by Wang *et al* used machine learning approach to validate TCM herbal prescriptions [Wang *et al.* 2005b]. In addtion, the fingerprint analysis techniques used in the quality control of TCM via identifying characteristics and evaluating stability is set up with rapid development of instrumental analyses and computer pattern interpretation. Methods of computer pattern recognition in TCM figerprint include fuzzy information analysis, artificial neural networks and gray relational grade cluster [Su *et al.* 2001].

1.5 Motivation and In Silico Approaches Used in this Study

As discussed from previous subsections in this chapter, it is necessary to revisit the research on TCM and to rethink on current philosophy and strategy in medicine and drug development. The principal objective of the study of Chinese herbal formulations is to determine whether they may represent a platform for the development of novel therapeutics from the holistic point of views as currently reinforced in systems biology. Of course this is not a simple exercise of applying modern technologies and clinical designs to products that had been constantly used for some time. There are totally different philosophies of Western and Chinese medical practices towards human health.

Western medicine looks at the relationship between structure and function and target-driven drug design against pathogens and diseases. Chinese medicine, on the other hand, defines health in terms of balance and its medications are designed such that to restore health balance by interacting with a variety of targets where the mechanisms are largely unknown. However, there are arguments saying that the resulting medication effects of herbal prescriptions are due to placebo factors.

Although there are attempts of using *in silico* approaches to study TCM as described in **section 1.4**, the question of whether there is scientific basis of TCM practice is still not answered. Hence, the main motivation in this study is to explore the scientific basis of TCM. Two aspects of TCM are explored: from the traditional point of views described in TCM and from the level of molecular interactions. *In silico* approaches are used to study TCM in both traditional and molecular levels. *In silico* methods used in this study are supervised machine learning methods that include support vector machine (SVM), k-nearest neighbors (kNN) and probabilistic neural network (PNN), structural approach using inverse docking strategy (INVDOCK), and literature-based approach. The detail of these methods will be given in their respective chapters.

Chapter 2 and **3** discuss the work of using machine learning methods such as support vector machine (SVM) to study the pattern recognition in TCM prescriptions and herb pairs from traditional point of views, respectively. **Chapter 4** and **5** present the study of TCM at the molecular levels. In **Chapter 4**, structural approach using an inverse docking strategy was used to explore the metastasis-related targets of Rhubarb

anthraquinones such as emodin and aloe-emodin. In **Chapter 5**, a literature-based approach is conducted to search for the molecular mechanisms of herbal synergism. Finally, in the last chapter (**Chapter 6**) describes major findings and contributions of current work to the progress of using *in slico* approaches for studying TCM herbal medicine. Limitations and suggestions for future studies are also provided in this chapter.

Chapter 2 Use of Machine Learning Methods (MLMs) in the Study of TCM from the Traditional Point of Views

Traditional Chinese medicine (TCM) has been widely practiced and is considered as an attractive alternative to conventional medicine. The holistic approach of TCM is realized by multi-herbal prescriptions using TCM-defined herbal properties (TCM-HPs). However, the scientific basis of TCM-HPs is unclear. In this chapter, machine learning methods (MLMs) are used to "dig out" the hidden scientific rules within TCM-HPs used in the formulation of TCM prescriptions.

2.1 Introduction

Traditional medicines such as Traditional Chinese medicine (TCM) have been widely used for disease treatment and have been recognized as interesting alternatives to complement conventional medicine [Tang *et al.* 1992; Chan 1995b; Chen 1998; Yuan *et al.* 2000b; Ang-Lee *et al.* 2001a; Bhuiyan *et al.* 2003; Wang *et al.* 2003; Lazar 2004a]. These multi-herb recipes collectively exert therapeutic actions and modulating the pharmacological and toxicological effects of the chemical ingredients of the constituent herbs. The principle ingredients are believed to provide main therapeutic actions, secondary principle ingredients enhance or assist the effects of the principle ones, and the rest serve modulating roles such as treatment of accompanying symptoms, moderation of harshness and toxicity, enhancement of pharmacokinetic properties, and harmonization etc [Yuan *et al.* 2000b].

Multi-herb TCM prescriptions have been formulated by using traditional prescribing principles based on traditionally defined TCM herbal properties (TCM-HPs)[Li 2005], and by taking into such considerations as the disease stage and the conditions of an individual patient [Chan 1995b]. **Table 2.1** gives a complete list of TCM-HPs which include four fundamental characters (cold, cool, warm and hot), five fundamental tastes (salty, sour, bitter, sweet and pungent), four toxic states (toxic, non-toxic, very toxic, and slightly toxic), and 12 meridians (bladder, spleen, large intestine, stomach, small intestine, liver, cardiovascular, heart, kidney, gallbladder, xin bao or pericardium and san jiao). By using these TCM-HPs, herbs are combined for achieving mutual enhancement, mutual assistance, mutual restraint, mutual suppression, or mutual antagonism [Chan 1995b]. TCM prescription is a combination of "Master" (for principal diseases or symptoms), "Adviser" (for helping the "Master" and treating accompanying symptoms), "Soldier" (for modulating the effects of the "Master" and "Adviser" and restoring the body to pre-illness equilibrium) and "Guide" (for guiding active ingredients and harmonizing the actions of other herbs) herbs [Chan 1995b].

Table 2.1 List of Traditional Chinese Medicine herbal properties (TCM-HPs). These properties are classified into four classes, characters (Class C), tastes (Class T), meridians (Class M), and toxicity states (Class Tox). These are further divided into 5, 5, 2, and 2 sub-classes for C, T, M and Tox respectively, each of which include 11, 12, 12, and 4 TCM-HPs. The total number of unique TCM-HP vector for all TCM herbs is 11+12+12+4 = 39.

List of TCM herbal properties (TCM-HPs)							
Character Class (C)		**Taste Class (T)**		**Meridian Class (M)**		**Toxicity State Class (Tox)**	
Subclass (5 in total)	**TCM-HP (11 in total)**	**Subclass (5 in total)**	**TCM-HP (12 in total)**	**Subclass (2 in total)**	**TCM-HP (12 in total)**	**Subclass (2 in total)**	**TCM-HP (4 in total)**
CI: Cold	C1: Slightly cold	TI: Salty	T1: Slightly salty	MI: Yin Meridian	M1: Liver	ToxI: Toxic	Tox1: Severely toxic
	C2: Cold		T2: Salty		M2: Heart		Tox2: Toxic
					M3: Xin Bao		
	C3: Severely cold				M4: Spleen		Tox3: Slightly toxic
CII: Cool	C4: Slightly cool	TII: Sour	T3: Slightly sour		M5: Lung		
	C5: Cool		T4: Sour		M6: Kidney		
			T5: Severely sour				
CIII: Neutral	C6: Neutral	TIII: Bitter	T6: Slightly bitter	MII: Yang Meridian	M7: Bladder	ToxII: Non-toxic	Tox4: Non-toxic
			T7: Bitter		M8: Small intestine		
					M9: San Jiao		
CIV: Warm	C7: Slightly warm	TIV: Sweet	T8: Slightly sweet		M10: Stomach		
	C8: Warm		T9: Sweet		M11: Large intestine		
	C9: Severely warm		T10: Tasteless				
CV: Hot	C10: Hot	TV: Pungent	T11: Slightly pungent		M12:Gall bladder		
	C11: Severely hot		T12: Pungent				

The assumed usefulness of TCM-HPs for formulating TCM prescriptions is likely arising from the expected correlation between TCM-HPs and the physicochemical properties of the ingredients of the constituent herbs responsible for collectively producing the specific pharmacodynamic, pharmacokinetic, and toxicity-modulating effects. While details of this correlation remain to be determined, the usefulness of TCM-HPs can be studied by analyzing the statistical pattern of the collective TCM-HPs of the well established TCM prescriptions to find out whether the distribution show signs of synergy among the constituent herbs in each prescription. Moreover, previous work had showed that machine learning methods (MLMs) such as support vector machine (SVM) can be used to evaluate whether TCM-HPs are capable of distinguishing TCM prescriptions from non-TCM recipes [Wang *et al.* 2005a].

A TCM-HP digitization algorithm has been developed and used for computing digital TCM-HPs, which have been applied for clustering and classifying TCM prescriptions[Su 1997; Wang *et al.* 2005a]. However, by using this algorithm, the number of digital TCM-HPs for each recipe is dependent on the number of its constituent herbs, which gives feature vectors of un-equal components thereby introducing statistical noise to many MLM systems. Therefore, in this study, a new algorithm was introduced to derive digital TCM-HPs of fixed length independent of the number of constituent herbs in a recipe. Besides, a significantly higher number of TCM prescriptions and non-TCM recipes than those in other studies [Wang *et al.* 2005a] were used for training and testing the MLM systems. Moreover, two different MLMs were used, which were evaluated by

two separate testing methods, to adequately examine the usefulness of TCM-HPs in distinguishing between TCM prescriptions and non-recipes.

In addition to the assessment of the usefulness of TCM-HPs, the developed MLM classification systems may be potentially used for facilitating the validation of TCM prescriptions[Wang *et al.* 2005a]. This capability was tested by using a group of 48 recently published TCM prescriptions with both experimental and clinical data that are not used in developing the MLM classification systems. Formulation of TCM prescriptions often relies on practitioner's experience and intuition as well as one's knowledge of TCM herbal properties and the formulation principles. This task is further complicated by the personalized nature of TCM prescriptions. A particular difficulty is the validation of a newly constructed TCM prescription to answer questions such as whether it strictly conforms to the TCM formulating principles. Such a task may be facilitated by the TCM prescription classification systems developed by using MLMs.

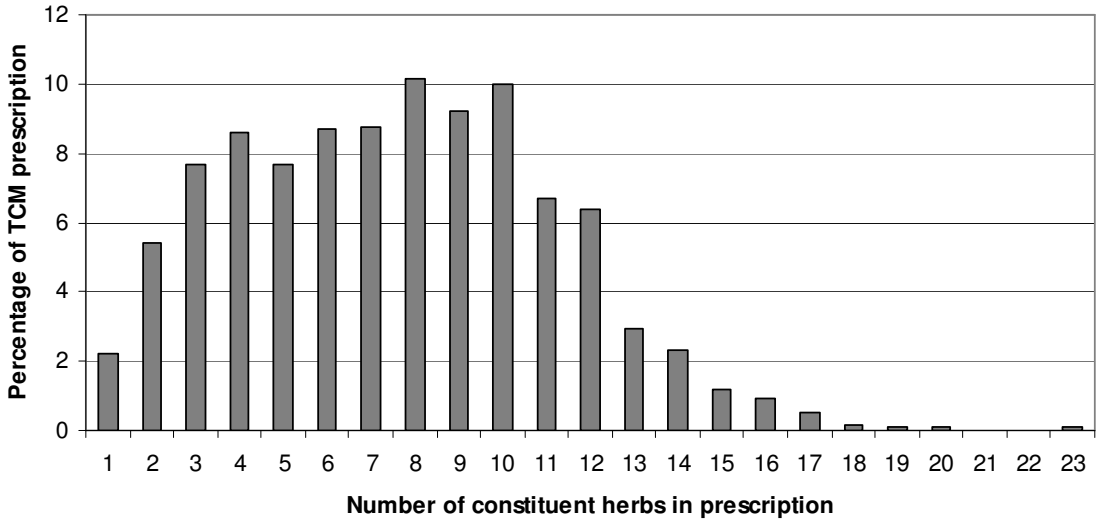
2.2 Methods

2.2.1 Selection of TCM prescriptions and non-TCM recipes

TCM prescriptions were selected from authoritative TCM prescription books and TCM commercial product handbooks[Yang 2001; Zhang 2005b; Sun 2006; Chen 1998; Zhang 1998a; Zhang 1998b]. The quality of the selected TCM prescriptions is maintained by the requirement that they satisfy at least one of the following three

conditions: (1) well known for many years of clinical application, (2) a commercial product, (3) published in an established TCM journal. These TCM prescriptions were subsequently screened to remove those with incomplete knowledge about the TCM-HPs of constituent herbs to ensure that the selected prescriptions can be studied in this work. A total of 1,161 established TCM prescriptions (which satisfy the first condition), 183 additional TCM prescriptions from a TCM book[Chen *et al.* 2002a], and 48 new TCM prescriptions from recently published journals were selected from this procedure. **Figure 2.1** shows the distribution of the 1,161 established TCM prescriptions with respect to the number of constituent herbs. It is found that most of the prescriptions (82.9%) are composed of 2~12 herbs, with 6-, 7-, 8-, 9- and 10-herb prescription groups (8.7%, 8.8%, 10.2%, 9.2%, and 10.0%) constituting the groups with the largest number of prescriptions.

Figure 2.1 Distribution of Traditional Chinese Medicine (TCM) prescriptions with respect to the number of constituent herbs. The distribution (in percentage) of 1161 TCM prescriptions in relation to the number of constituent herbs used in training set in this study is shown. Most of these TCM prescriptions contain 2 to 12 constituent herbs with 82.9% overall distribution.



Non-TCM recipes were generated by random combination of multiple herbs and by modification of existing TCM prescriptions. In the first approach, a total of 635 commonly-used TCM herbs were divided into 13 traditionally-defined therapeutic classes described in the TCM literature[Hou 2001]. For each class, two herbs with TCM-HPs closest to the average values of the other herbs were selected as the representative for that class. These representative herbs were then randomly combined and subsequently checked to remove hits of known TCM prescriptions to ensure that the generated non-TCM recipes are most likely to be true non-TCM recipes. This process gives rise to 10,936 non-TCM recipes each of which contains 2 to up to 13 herbs. In the second approach, existing TCM prescriptions with knowledge of their "Master" herbs were modified by one of the following three methods to generate 266 non-TCM recipes. The first is the removal of the "Master" and in some cases the "Adviser" as well. The second is to replace the "Master" with those possessing the opposite TCM-HPs to completely disrupt the expected synergy between the original "Master" and the other herbs in the prescription. The third is to add a specific herb to form a disallowed or un-favored herb-pair to convert the prescription into an invalid one. These "modified" recipes were subsequently checked to remove hits of known TCM prescriptions to ensure that the generated non-TCM recipes are most likely true non-TCM recipes. Overall, a total of 11,202 non-TCM recipes were generated by using these two approaches.

2.2.2 Digital representation of herbs and multi-herb recipes

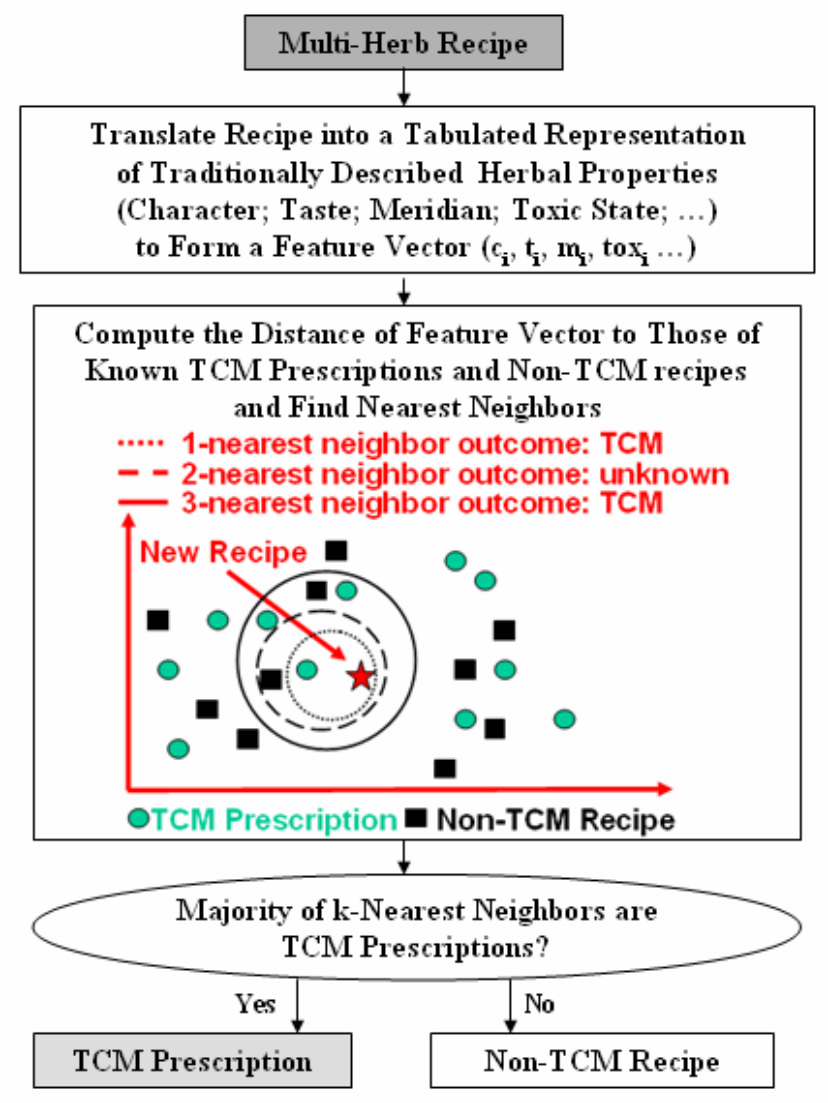
As shown in **Table 2.1**, TCM-HPs are divided into 4 classes: character (C), taste (T), meridian (M), and toxicity level (Tox), which further divided into 5, 5, 12, and 2 subclasses, and 11, 12, 12 and 4 TCM-HPs respectively. The total number possible of TCM-HP combinations is C×T×M×Tox = 11×12×12×4 = 6,336. The value of a TCM-HP is 1 or 0 if the herb possesses or does not possess the corresponding property. For instance, the herb *Herba Ephedrae* (*Ma Huang*) has warm character, pungent taste, meridians for lung and bladder, and is non-toxic. The TCM-HPs for *Herba Ephedrae* (*Ma Huang*) are C = (C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11) = (0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0), T = (T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12) = (0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0), M = (M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12) = (0, 0, 0, 0, 1, 0, 1, 0, 0, 0, 0, 0), Tox = (T1, T2, T3, T4)=(0, 0, 0, 1). This herb can then be digitally represented by using a feature vector $\boldsymbol{x}$ = C×T×M×Tox. Likewise, a multi-herb recipe can be digitally represented by a feature vector which is the sum of the feature vectors of the constituent herbs.

2.2.3 Machine Learning Methods (MLMs)

2.2.3.1 k-Nearest Neighbor (kNN)

Figure 2.2 illustrates the computational process of kNN for determining whether a multi-herb recipe is a TCM prescription from the TCM-HPs of constituent herbs of multi-herb recipes. The Euclidean distance between an unclassified vector $\boldsymbol{x}$ (feature vector of a new multi-herb recipe) and each individual vector $\boldsymbol{x}_i$ in the training set (feature vector of each known TCM prescription or non-TCM recipe) is measured[Johnson *et al.* 1982] by using the following formula: $D = \sqrt{\|\mathbf{x} - \mathbf{x}_i\|^2}$. A total of *k* number of vectors or k known TCM prescriptions and non-TCM recipes nearest to the unclassified vector $\boldsymbol{x}$ are used to determine the class (TCM prescription class or non-TCM recipe class) of that unclassified vector (new recipe). The class of the majority of the k nearest neighbors is chosen as the predicted class of the unclassified vector $\boldsymbol{x}$.

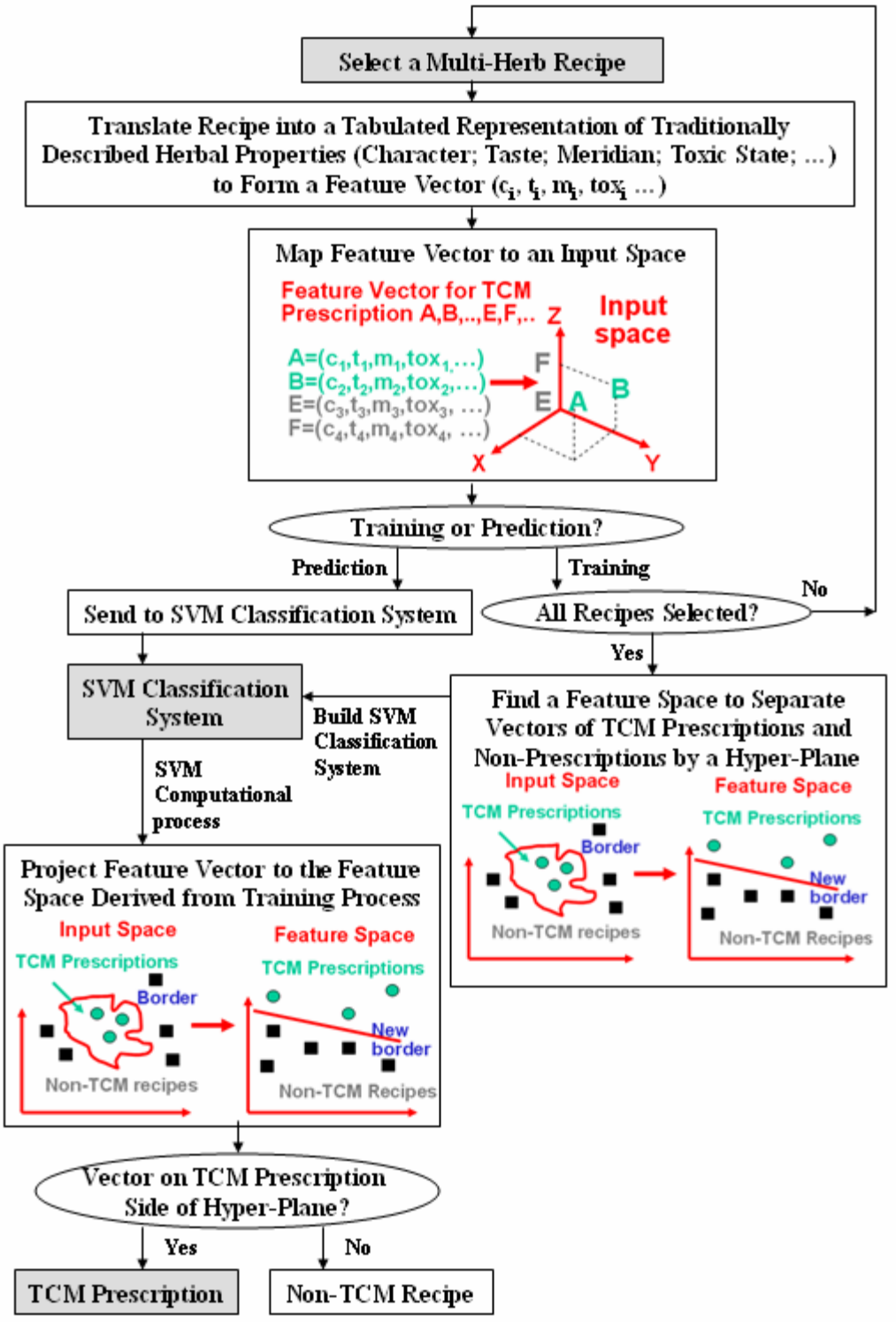
Figure 2.2 Schematic diagram illustrating the process of the prediction of Traditional Chinese Medicine (TCM) prescription from the traditionally described herbal properties of constituent herbs of a multi-herb recipe by using a machine learning method – k-nearest neighbors.



2.2.3.2 Support Vector Machine (SVM)

Figure 2.3 illustrates the process of the prediction of TCM prescription by using SVM from the TCM-HPs of constituent herbs of multi-herb recipes. Known TCM prescriptions and non-TCM recipes in a training set, represented by their TCM-HPs, are projected onto a hyperspace where they are separated by a hyperplane whose parameters are adjusted by using a separate testing set of TCM prescriptions and non-TCM recipes. By projecting the feature vector of a new multi-herb recipe onto this same hyperspace, this SVM system can be used to determine whether it is a TCM prescription based on its location with respect to the hyperplane.

Figure 2.3 Schematic diagram illustrating the process of the prediction of Traditional Chinese Medicine (TCM) prescription from the traditionally described herbal properties of constituent herbs of a multi-herb recipe by using a machine learning method - support vector machines. A, B: feature vectors of TCM prescriptions; E, F: feature vectors of non-TCM recipes; filled circles, TCM prescriptions; filled squares, non-TCM recipes.



2.2.4 Determination of generalization ability of MLM classification systems

To adequately assess the classification ability of the SVM and kNN classification systems developed in this work, two different evaluation methods are used. One is the 3-fold cross validation method, which as a special case of the N-fold cross validation method is one of the widely used methods for measuring the accuracies of MLM classification systems[Li *et al.* 2005a]. The other one is the use of external independent validation sets, which has been shown to be equally useful for evaluating the classification ability of MLM classification systems [Li *et al.* 2005a; Wang *et al.* 2005c].

In using the 3-fold cross validation method, 1161 TCM prescriptions and 11,202 non-TCM recipes were randomly divided into three subsets, with 389 TCM prescriptions and 3719 non-TCM recipes in subset 1, 384 and 3734 in subset 2, and 388 and 3749 in subset 3 respectively. Validation study can be conducted by using two subsets as the training set and the other as the testing set. Three distinct combinations of training-testing sets can be generated from these three subsets, which were used to conduct three iterations of validation studies. For the independent validation method, all the 1161 TCM prescriptions and 11,202 non-TCM recipes were used as the training set, and 183 TCM prescriptions from a TCM book[Chen *et al.* 2002a] were used as an independent validation set. An additional independent validation set that consists of 48 TCM prescriptions from recently published literature was used to further test the outperformed MLM classification system.

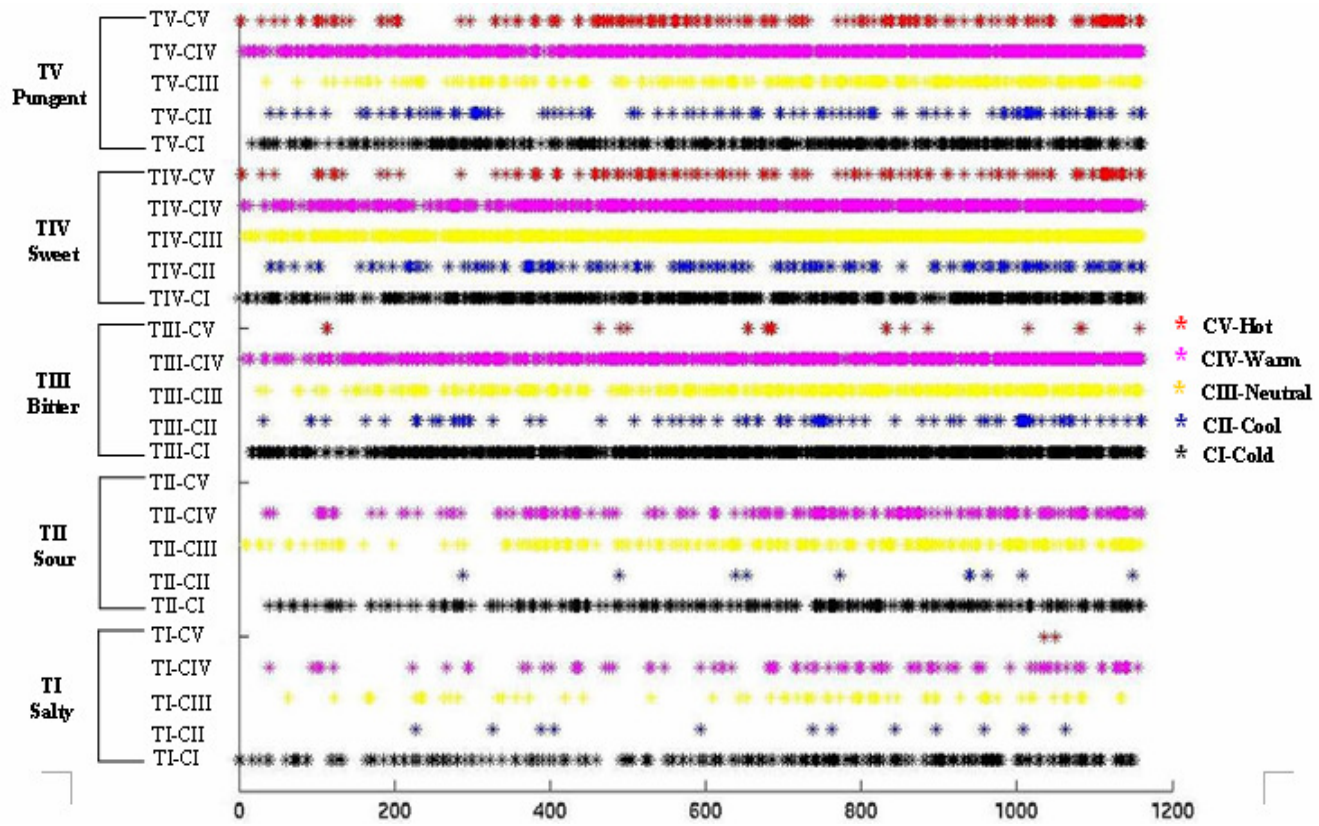
The performance of the kNN and SVM classification systems can be measured by the positive, negative and overall classification accuracies $P_+ = TP/(TP+FN)$, $P_- = TN/(TN+FP)$ and $P=(TP+TN)/N$, which correspond to the accuracies for TCM prescriptions, non-TCM recipes, and all recipes respectively. Here TP, TN, FP, and FN are the number of true positive (correctly classified TCM prescription), true negative (correctly classified non-TCM recipe), false positive (TCM prescription falsely classified as non-TCM recipe), and false negative (non-TCM recipe falsely classified as TCM prescription) respectively, and N is the total number of recipes.

2.3 Results

2.3.1 Distribution pattern of TCM-HPs and characteristics of TCM prescriptions

Figure 2.4 shows the distribution pattern of the TCM-HPs of the 1161 established TCM prescriptions. These prescriptions are aligned along the x-axis from left to right in the order of the number of constituent herbs from 1-herb to up to 23-herb prescriptions. Each individual dot represents the TCM-HP of an individual herb in a TCM prescription. The TCM-HPs of all of the herbs in a TCM prescription are grouped into taste-character subclasses and they are aligned along the y-axis from bottom to top in the order of Salty-Cold, Salty-Cool, Salty-Neutral, Salty-Warm, Salty-Hot, Sour-Cold, Sour-Cool, Sour-Neutral, Sour-Warm, Sour-Hot, Bitter-Cold, Bitter-Cool, Bitter-Neutral, Bitter-Warm, Bitter-Hot, Sweet-Cold, Sweet-Cool, Sweet-Neutral, Sweet-Warm, Sweet-Hot, Pungent-Cold, Pungent-Cool, Pungent-Neutral, Pungent-Warm, and Pungent-Hot.

Figure 2.4 Distribution pattern of the Traditional Chinese Medicine herbal properties (TCM-HPs) of 1161 TCM prescriptions. TCM prescriptions are aligned along the x-axis from left to right in the order of the number of constituent herbs from 1-herb to up to 23-23-herb prescriptions. Each individual dot represents the TCM-HP of an individual herb in a TCM prescription. The TCM-HPs of all of the herbs in a TCM prescription are grouped into taste-character subclasses and they are aligned along the y-axis from bottom to top in the order of TI-CI, TI-CII, TI-CIII, TI-CIV, TI-CV, TII-CI, TII-CII, TII-CIII, TII-CIV, TII-CV, TIII-CI, TIII-CII, TIII-CIII, TIII-CIV, TIII-CV, TIV-CI, TIV-CII, TIV-CIII, TIV-CIV, TIV-CV, TV-CI, TV-CII, TV-CIII, TV-CIV, TV-CV. The definition of TI, TII, TIII, TIV, TV, CI, CII, CIII, CIV, CV are given in Table 3.1.



2.3.2 Usefulness of TCM-HPs for distinguishing TCM prescriptions from non-prescription recipes

The usefulness of TCM-HPs for distinguishing TCM prescriptions from non-TCM recipes can be assessed by examining whether MLM methods can use TCM-HPs to separate them Two MLM methods, kNN and SVM, were used for conducting this test. **Table 2.2** gives the computed accuracies of these two methods by using 3-fold cross validation where 84.4% and 91.3% of the TCM prescriptions and 98.9% and 98.6% of the non-TCM recipes are correctly classified by kNN and SVM respectively. Testing results using the independent evaluation set shows that 83.1% and 97.3% of the TCM prescriptions are correctly classified by kNN and SVM respectively. Both validation methods consistently show that these two MLM methods are capable of using TCM-HPs to correctly separate TCM prescriptions from non-TCM recipes. It is possible that the distinguished characteristics of TCM-HPs of TCM prescriptions are exploited by the MLM methods for separating them from non-TCM recipes. As both validation methods show that SVM outperforms kNN, SVM is further tested by using the independent set of 48 newly published TCM prescriptions with proven therapeutic effects in both experimental and clinical data. All of these 48 TCM prescriptions are correctly classified by our SVM system.

Table 2.2 Traditional Chinese Medicine (TCM) prescription and non-TCM recipe classification accuracies of the machine learning classification systems, k Nearest Neighbor (kNN) and Support Vector Machine (SVM), evaluated by 3-fold cross validation study.

Classification system: kNN									
Cross Validation	**Training Set**		**Testing Set**						
	TCM prescriptions	Non-TCM recipes	TP	FN	P+ %	TN	FP	P- %	P %
1	773	7453	341	47	87.9	3706	43	98.8	97.8
2	777	7468	314	70	81.8	3699	35	99.1	97.4
3	772	7483	325	64	83.6	3670	49	98.7	97.3
Average Accuracy					**84.4**			**98.9**	**97.5**
SD					3.13			0.21	0.26
Classification system: SVM									
Cross Validation	**Training Set**		**Testing Set**						
	TCM prescriptions	Non-TCM recipes	TP	FN	P+ %	TN	FP	P- %	P %
1	773	7453	360	28	92.8	3692	57	98.4	97.9
2	777	7468	342	42	89.1	3684	50	98.6	97.7
3	772	7483	358	31	92.0	3670	49	98.7	98.1
Average Accuracy					**91.3**			**98.6**	**97.9**
SD					1.95			0.15	0.2

P+, P- and P represent the classification accuracy for TCM prescriptions, non-TCM recipes and all recipes. TP, TN, FP, and FN are the number of true positive (correctly classified TCM prescriptions), true negative (correctly classified non-TCM recipes), false positive (TCM prescriptions falsely classified as non-TCM recipes), and false negative (non-TCM recipes falsely classified as TCM prescriptions) respectively, and N is the total number of recipes. Statistical significance is indicated by SD (standard deviation).

2.3.3 Misclassified TCM prescriptions

There are 5 TCM prescriptions in the first independent set from Chen and Li [Chen *et al.* 2002a] incorrectly predicted by SVM method. The 5 misclassified TCM prescriptions are 11f13 (*Natrii sulfas* or Mang Xiao, *Indigo Naturalis* or Qing Dai, vinegar or Cu), 12f1 (*Radix Glycyrrhizae* or *Gan Cao*, *Radix Kansui* or *Gan Sui*), 12f12 (*Semen Arecae* or *Bing Lang*, *Fructus Evodiae* or *Wu Zhu Yu*), 15f7 (*Rhizoma Smilacis Glabrae* or *Tu Fu Ling*, *Radix Glycyrrhizae* or *Gan Cao*), and 18f64 (*Cordyceps sinensis* or *Dong Chong Xia Cao*, *Radix Adenophorae* or *Nan Sha Shen*, *Fructus Schisandrae* or *Wu Wei Zi*, *Radix Ophiopogonis* or *Mai Dong*). Prescription 12f1 was incorrectly predicted as a non-TCM recipe possibly because it contains an unfavorable herb pair of *Radix Glycyrrhizae* (*Gan Cao*) and *Radix Kansui* (*Gan Sui*). One possible reason for the misclassification of prescription 11f13 as a non-TCM recipe is that vinegar (*Cu*) counters the effect of *Natrii sulfas* (*Mang Xiao*) and *Indigo Naturalis* (*Qing Dai*). The reason for the misclassification of the other 3 TCM prescriptions (12f12, 15f7, 18f64) remained to be elucidated.

2.4 Discussion and Conclusion

The TCM-HP distribution pattern shows apparent internal correlation among the constituent herbs in a prescription particularly for those containing more than 6 herbs. For instance, herbs in the class of sweet-neutral, pungent-warm, sweet-warm, bitter-warm, and bitter-cold simultaneously appear in most of the prescriptions. At a slightly less level,

herbs in the class of sweet-cold and pungent-cold are also simultaneously present in these prescriptions. In these prescriptions, a general theme of herbal synergy seems to be exhibited by the TCM-HPs of their constituent herbs, and this theme is consistent with the TCM prescribing theory [Li 2005]. For instance, herbs in the class of bitter-cold have frequently been used for treating "hot" symptoms such as fever and inflammation. Herbs in the class of pungent-warm or bitter-warm have frequently been used for treating cold symptoms such as weak pulse, tiredness, favor of hot drink, whitish tongue, etc. Herbs in the class of sweet-warm and sweet-neutral have frequently been used for modulating the therapeutic and tonic activity as well as reducing the toxic effect of other constituent herbs. Overall, it appears that these prescriptions have been constructed on the basis of a delicate balance between herbs of warm characters and those of cold characters, with the warm effects of the warm herbs to various degrees counter-balanced by the cold effects of the cold herbs, and vice versa. This ensures the collective character of a prescription is not too biased towards the warm or cold side to overly correct the Ying-Yang state of a patient. Understanding the molecular mechanism of these collective actions enables advancement of systems biology and facilitates the design "soft drug cocktail" that targets and simultaneously modulates multiple systems in human.

Our study suggests that TCM-HPs are useful for distinguishing TCM prescriptions from non-TCM recipes. This supports the long held view in TCM theory that TCM-HPs can fully characterize TCM herbs and they can be used to formulate TCM prescriptions [Li 2005]. Moreover, these TCM-HPs are expected to be associated with the physicochemical properties of herbal ingredients responsible for producing the effects of

specific herb and the collective effects of specific TCM prescription. For instance, fluorometric analysis has shown that herbs with cold characters produce large amounts of superoxide, and herbs with hot characters have scavenging activities[Lin *et al.* 1995]. Specific group of "*Yang*-invigorating" herbs, which tend to have warm or hot characters, have been found to enhance mitochondrial ATP generation in addition to immunomodulatory activities[Yim *et al.* 2002; Ko *et al.* 2004; Siu *et al.* 2004]. Heat clearing herbs, which tend to have cold characters, have been found to produce some combination of anti-microbial, anti-toxic, anti-inflammatory, antipyretic, platelet aggregation inhibition, sedative, immunomodulatory, and hepatoprotective activities[Jiang 2005]. These studies suggest the merit for further studying the scientific basis of and for exploring TCM-HPs in developing multi-herb based therapeutics.

Several other factors might contribute to the misclassification of TCM prescriptions by MLMs. One is the possible inadequate representation of some of the TCM prescriptions in the training set. Some prescriptions have unique features not found in other prescriptions. Examples of such prescriptions are *Liu Shen Wan*, *Tong Guan San*, and *Yun Nan Bai Yao*. Their exclusion in the training set tends to make it difficult for an AI method to classify them into the correct class. The lack of information about the TCM-HPs for some herbs makes it impossible for classifying a substantial number of TCM prescriptions. So far, we were able to digitize only 1,161 of the 1,588 TCM prescriptions we have collected. These problems can be alleviated along with the availability of more TCM-HP data and TCM prescriptions.

Our study also raises an interesting possibility of using MLM methods to validate TCM prescriptions by determining whether a multi-herb recipe is a valid TCM prescription from the TCM-defined herbal property. To further assess this potential, the performance of one of the MLM methods, SVM, for classifying TCM prescriptions was evaluated by using the additional independent evaluation set of 48 TCM prescriptions published in recent years. All of them were correctly classified as TCM prescriptions. Further improvement in the coverage of herbs and prescriptions, refinement of digital representation of recipes, and improvement of MLM algorithms may enable the development of MLMs into a useful tool for facilitating the evaluation of TCM prescriptions.

Chapter 3 Pattern Analysis of TCM Herb Pairs Using Machine Learning Methods from the Traditional Point of Views

Multi-herbal prescriptions of traditional Chinese medicine (TCM) often include special herb-pairs for mutual enhancement, assistance, and restraint. These TCM herb-pairs have been assembled and interpreted based on traditionally-defined herbal properties (TCM-HPs) without knowledge of mechanism of their assumed synergy. While these mechanisms are yet to be determined, properties of TCM herb-pairs can be investigated to determine if they exhibit features consistent with their claimed unique synergistic combinations. In this chapter we analyzed distribution patterns of TCM-HPs of TCM herb-pairs to detect signs indicative of possible synergy and used machine learning methods (MLMs) to examine whether combination of their TCM-HPs are distinguishable from those of non-TCM herb-pairs assembled by random combinations and by modification of known TCM herb-pairs.

3.1 Introduction

Multi-herb recipes have frequently been used in TCM aimed at collectively exerting therapeutic actions and modulating the pharmacological and toxicological effects of the chemical ingredients of the constituent herbs. In formulating these TCM recipes, special herb pairs that are claimed to be unique combinations of the traditionally defined TCM herbal properties (TCM-HPs) [Li 2005] have been frequently used for achieving mutual enhancement, mutual assistance, mutual restraint, mutual suppression, or mutual antagonism [Chan 1995b].

The exact molecular mechanisms of these TCM herb-pairs are unclear. None-the-less, some possible modes of actions have been hypothesized [Yuan *et al.* 2000b]. Mutual enhancement can be achieved by using pair of herbs with ingredients of similar therapeutic actions, or by using a principal herb with main therapeutic actions and another one acting to enhance or assist the effects of the principle one. Mutual assistance can be achieved by using an herb that serves modulating roles such as treatment of accompanying symptoms, enhancement of pharmacokinetic properties, and harmonization etc. Mutual restraint, suppression and antagonism can be achieved by using an herb with ingredients capable of moderation of harshness and toxicity of the ingredients of the principal herb.

While it is not yet possible to determine their molecular mechanisms, properties of TCM herb-pairs can be investigated to determine if they exhibit features consistent with their claimed unique synergistic combinations. By analyzing distribution patterns of the combination of TCM-HPs of these TCM herb-pairs, one may detect signs of herb-pair correlations that are expected to exist if these TCM herb-pairs have some form of synergy believed to be manifested in their TCM-HPs by TCM practitioners. Moreover, machine learning methods (MLMs) can be used to examine whether combinations of TCM-HPs of these TCM herb-pairs are truly distinguishable from those of non-TCM herb-pairs assembled by random combination of herb-pairs and by modification of known TCM herb-pairs that disrupts the presumed synergy.

In this work, we analyzed the distribution profiles of the combination of TCM-HPs of the 394 known TCM herb-pairs described in TCM literatures [Wang 2004] by using the same method as that for studying the profiles of TCM prescriptions [Ung *et al.* 2006; Ung *et al.* 2007]. We also used three different MLMs to determine if the derived MLM classification systems can consistently distinguish TCM herb-pairs from the non-TCM herb-pairs based on their TCM-HPs. Digitization of TCM-HPs was conducted by using the algorithm developed for clustering and classifying TCM prescriptions [Su 1997; Wang *et al.* 2005a]. The accuracy of these MLM classification systems was evaluated by using two popularly used validation methods, 10-fold cross validation and independent evaluation sets.

3.2 Methods

3.2.1 Digital representation of herbs and herb pairs

TCM-HPs include five fundamental characters (cold, cool, neutral, warm and hot), five fundamental tastes (salty, sour, bitter, sweet and pungent), four toxic states (toxic, non-toxic, very toxic, and slightly toxic), and 12 meridians (Gui Jing) (bladder, spleen, large intestine, stomach, small intestine, liver, cardiovascular, heart, kidney, gallbladder, xin bao or pericardium and san jiao). TCM-HPs are thus divided into 4 classes: character (C), taste (T), meridian (M), and toxicity level (Tox) [Chan 1995b]. The class of C, T, M, and Tox can be further divided into 5, 5, 12, and 2 subclasses respectively, which along with the TCM-HPs in each subs-class are given in **Table 3.1**. For instance, the 12 meridians are categorized into *Yin* and *Yang* sub-classes. The *Yin* sub-class contains

meridians of liver, heart, *Xin Bao*, spleen, lung, and kidney and the *Yang* sub-class includes meridians of bladder, small intestine, San Jiao, stomach, large intestine, and gall bladder.

As given in **Table 2.3** from previous chapter, there are 11, 12, 12 and 4 TCM-HPs in the class of C, T, M and Tox respectively. These TCM-HPs form a vector $\boldsymbol{h}$=(C, T, M, Tox) with a total of 11+12+12+4 =39 features to represent each herb. The value of a specific TCM-HP is 1 if the herb possesses the corresponding property, and it is 0 if the herb does not possess the property [Ung *et al.* 2006]. For a herb-pair composed of herb A and B, two separate vectors $\boldsymbol{h_{AB}}$ = (C_A, T_A, M_A, Tox_A, C_B, T_B, M_B, Tox_B) and $\boldsymbol{h_{BA}}$ = (C_B, T_B, M_B, Tox_B, C_A, T_A, M_A, Tox_A) of dimension 78 can be formed, both of which were used to represent that herb-pair.

For instance, the herb *Ma Huang* (*Herba Ephedrae*) has warm character, pungent and slight bitter taste, meridians for lung and bladder, and is non-toxic. The TCM-HPs for Ma Huang (*Herba Ephedrae*) are C_A = (C_{A1}, C_{A2}, C_{A3}, C_{A4}, C_{A5}, C_{A6}, C_{A7}, C_{A8}, C_{A9}, C_{A10}, C_{A11}) = (0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0), T_A = (T_{A1}, T_{A2}, T_{A3}, T_{A4}, T_{A5}, T_{A6}, T_{A7}, T_{A8}, T_{A9}, T_{A10}, T_{A11}, T_{A12}) = (1, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0), M_A = (M_{A1}, M_{A2}, M_{A3}, M_{A4}, M_{A5}, M_{A6}, M_{A7}, M_{A8}, M_{A9}, M_{A10}, M_{A11}, M_{A12}) = (1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0), Tox_A = (T_{A1}, T_{A2}, T_{A3}, T_{A4})=(0, 0, 0, 1). Herb *Gui Zhi* (*Ramulus Cinnamomi*) has warm character, pungent and sweet tastes, meridians for heart, lung and bladder, and is non-toxic. The TCM-HPs for Gui Zhi (*Ramulus Cinnamomi*) are C_B = (C_{B1}, C_{B2}, C_{B3}, C_{B4}, C_{B5}, C_{B6}, C_{B7}, C_{B8}, C_{B9}, C_{B10}, C_{B11}) = (0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0), T_B = (T_{B1}, T_{B2}, T_{B3}, T_{B4}, T_{B5}, T_{B6},

T_{B7}, T_{B8}, T_{B9}, T_{B10}, T_{B11}, T_{B12}) = (1, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0), M_B = (M_{B1}, M_{B2}, M_{B3}, M_{B4}, M_{B5}, M_{B6}, M_{B7}, M_{B8}, M_{B9}, M_{B10}, M_{B11}, M_{B12}) = (1, 1, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0), Tox_B = (T_{B1}, T_{B2}, T_{B3}, T_{B4})=(0, 0, 0, 1). The herb pair composed of these two herbs can then be digitally represented by using two feature vectors $\boldsymbol{h_{AB}}$ = (C_A, T_A, M_A, Tox_A, C_B, T_B, M_B, Tox_B) and $\boldsymbol{h_{BA}}$ = (C_B, T_B, M_B, Tox_B, C_A, T_A, M_A, Tox_A) with 78 dimensions each.

Table 3.1 List of Traditional Chinese Medicine herbal properties (TCM-HPs). These properties are classified into four classes, characters (Class C), tastes (Class T), meridians (Class M), and toxicity states (Class Tox). These are further divided into 5, 5, 2, and 2 sub-classes for C, T, M and Tox respectively, each of which include 11, 12, 12, and 4 TCM-HPs. The total number of unique TCM-HP vector for all TCM herbs is 11+12+12+4 = 39.

List of TCM herbal properties (TCM-HPs)							
Character Class (C)		**Taste Class (T)**		**Meridian Class (M)**		**Toxicity State Class (Tox)**	
Subclass (5 in total)	**TCM-HP (11 in total)**	**Subclass (5 in total)**	**TCM-HP (12 in total)**	**Subclass (2 in total)**	**TCM-HP (12 in total)**	**Subclass (2 in total)**	**TCM-HP (4 in total)**
CI: Cold	C1: Slightly cold	TI: Salty	T1: Slightly salty	MI: Yin Meridian	M1: Liver	ToxI: Toxic	Tox1: Severely toxic
	C2: Cold		T2: Salty		M2: Heart		Tox2: Toxic
					M3: Xin Bao		
	C3: Severely cold				M4: Spleen		Tox3: Slightly toxic
CII: Cool	C4: Slightly cool	TII: Sour	T3: Slightly sour		M5: Lung		
	C5: Cool		T4: Sour		M6: Kidney		
			T5: Severely sour				
CIII: Neutral	C6: Neutral	TIII: Bitter	T6: Slightly bitter	MII: Yang Meridian	M7: Bladder	ToxII: Non-toxic	Tox4: Non-toxic
			T7: Bitter		M8: Small intestine		
					M9: San Jiao		
CIV: Warm	C7: Slightly warm	TIV: Sweet	T8: Slightly sweet		M10: Stomach		
	C8: Warm		T9: Sweet		M11: Large intestine		
	C9: Severely warm		T10: Tasteless				
CV: Hot	C10: Hot	TV: Pungent	T11: Slightly pungent		M12:Gall bladder		
	C11: Severely hot		T12: Pungent				

3.2.2 Selection of TCM herb-pairs and construction of non-TCM herb-pairs

A total of 394 well established TCM herb-pairs were collected from a reputable TCM literature [Wang 2004], which are composed of 264 TCM herbs. The non-TCM herb-pairs were generated by two approaches, random combination of TCM herbs and modification of known TCM herb-pairs with disruption of their assumed synergy. In the first approach, a total of 635 commonly-used TCM herbs were divided into 23 traditionally-defined therapeutic classes described in the TCM literature [Zhang 1998a]. For each therapeutic class, three herbs with TCM-HPs closest to the average values of herbs in the corresponding class were selected as the representative herbs for that class. These representative herbs were then randomly combined into pairs and subsequently checked to remove those matching the known TCM herb-pairs. This gave rise to 2,346 herb-pairs which were tentatively used as non-TCM herb-pairs. In the second approach, existing TCM herb-pairs with knowledge of their "Master" herbs were modified by replacing the "Master" herbs with those possessing the opposite TCM-HPs to completely disrupt the expected synergy between the original "Master" herbs and the companion herb. Overall, a total of 2,470 non-TCM herb pairs were generated by using these two approaches.

3.2.3 Machine Learning Methods (MLMs)

The detail algorithms of MLMs are described in **Chapter 2 section 2.1.2.3**. Only content relevant to the study of TCM herb pairs are described in further detail.

3.2.3.1 Probabilistic Neural Network method (PNN)

PNN is a form of neural network that uses Bayes optimal decision rule for classification [Specht 1990]. Traditional neural networks such as feed-forward back-propagation neural network rely on multiple parameters and network architectures to be optimized. In contrast, PNN only has a single adjustable parameter, a smoothing factor σ for the radial basis function in the Parzen's nonparameteric estimator. Thus the training process of PNN is usually orders of magnitude faster than those of the traditional neural networks.

3.2.3.2 k Nearest Neighbor (kNN)

For kNN a total of k vectors (representing k number of known TCM herb pairs and non-TCM herb pairs) nearest to the unclassified vector $\boldsymbol{x}$ are used to determine the class (TCM herb-pair class or non-TCM herb-pair class) of that unclassified vector (new herb pair). The class of the majority of the k nearest neighbors is chosen as the predicted class of the unclassified vector $\boldsymbol{x}$.

3.2.3.3 Support Vector Machine (SVM)

For SVM known TCM herb pairs and non-TCM herb pairs in a training set, represented by their TCM-HPs, are projected onto a hyperspace where they are separated by a hyperplane whose parameters are adjusted by using a separate testing set of TCM herb pairs and non-TCM herb pairs. By projecting the feature vector of a new multi-herb recipe onto this same hyperspace, this SVM system can be used to determine whether it is a valid TCM herb pairs based on its location with respect to the hyperplane.

3.2.3.4 Methods for validating MLM classification systems

To adequately assess the classification capability of the PNN, kNN and SVM classification systems developed in this work, two different evaluation methods were used. One is the 10-fold cross validation method, N-fold cross validation is one of the widely used methods for measuring the accuracies of MLM classification systems [Xue *et al.* 2004; Li *et al.* 2005a]. The other one is the use of external independent validation sets, which has been shown to be equally useful for evaluating the classification ability of MLM classification systems [Cai *et al.* 2003; Ung *et al.* 2006].

In using the 10-fold cross validation method, 394 TCM herb-pairs and 2470 non-TCM herb-pairs were randomly divided into 10 subsets. Validation study can be conducted by using nine subsets as the training set and the remaining one as the testing set. Ten distinct combinations of training-testing sets can be generated from these 10 subsets, and 10 iterations of validation studies were conducted. In using the independent

validation method, the 27 known forbidden herb-pairs from the 18-opposing-pairs ("*shi ba fan*") and the 19-dislike-pairs ("*shi jiu wei*") of TCM [Wang 2004] were used to test whether they can be predicted as non TCM herb-pairs. Moreover, a total of 1,065,100 possible herb-pairs were generated from the 1,462 TCM herbs described in TCM literatures [Zhang 1998b; Hou 2001; Chen *et al.* 2002a] after elimination of the known TCM herb-pairs and the non-TCM herb-pairs used in our study, which were also used to test whether they can be predicted as non-TCM herb-pairs.

3.2.3.5 Evaluating the Prediction Performance of Machine Learning Methods (MLMs)

As in the case of all discriminative methods, the performance of MLMs can be evaluated by the quantity of true positive or TP (correctly classified TCM herb-pairs), true negative or TN (correctly classified non-TCM herb-pairs), false positive or FP (non-TCM herb-pairs falsely classified as TCM herb-pairs), and false negative or FN (TCM herb-pairs falsely classified as non-TCM herb-pairs) respectively. Sensitivity (P_+), $SE=TP/(TP+FN)$ and specificity (P_-), $SP=TN/(TN+FP)$ are the prediction accuracy for TCM herb pairs and non-TCM herb pairs, respectively. The overall prediction accuracy (Q) and Matthews correlation coefficient (C) are used to measure the overall prediction performance:

$$Q = \frac{TP + TN}{TP + TN + FP + FN} \times 100\% \qquad (3\text{-}1)$$

$$C = \frac{TP * TN - FN * FP}{\sqrt{(TP + FN)(TP + FP)(TN + FN)(TN + FP)}} \qquad (3\text{-}2)$$

3.3 Results and Discussion

3.3.1 Distribution patterns of TCM-HPs of TCM herb-pairs and their characteristics

Based on the combination of their TCM-HPs, the 394 known TCM herb-pairs can be divided into three classes of predominantly warm, cold, and neutral characters respectively, which are given in **Table 3.2**. The "warm" class contains 6 groups of hot-hot, warm-hot, warm-warm, hot-neutral, warm-neutral and warm-cool taste combinations. The "cold" class contains 7 classes of cold-cold, cold-cool, cool-cool, cold-neutral, cool-neutral, cold-warm and cold-hot taste combinations. The "neutral" class contains 1 group of neutral-neutral taste combinations. **Figure 3.1, Figure 3.1**, and **Figure 3.3** show the distribution patterns of the combination of TCM-HPs of the TCM herb-pairs in each of these classes. There a total of 25 possible combinations of character-character groups. Apart from the 14 groups described above, no known TCM herb-pairs belong to the other 11 groups.

Table 3.2 Distribution of 394 known TCM herb-pairs in different classes and groups defined by the combination of their TCM-HPs.

TCM Herb-Pair Class	*TCM Herb-Pair Group*	*Number of Herb Pairs*
Predominantly warm	Hot-Hot	4
	Hot-Warm	23
	Warm-Warm	89
	Hot-Neutral	3
	Warm-Neutral	69
	Warm -Cool	21
Predominantly cold	Cold-Cold	34
	Cold-Cool	34
	Cool-Cool	10
	Cold-Neutral	19
	Cool-Neutral	17
	Cold-Warm	47
	Cold-Hot	4
Predominantly neutral	Neutral-Neutral	20

Figure 3.1 Distribution patterns of combinations of traditionally-defined herbal properties of TCM herb-pairs with predominantly warm characters. These herb-pairs are divided into hot-hot, warm-hot, warm-warm, hot-neutral, warm-neutral and warm-cool groups in decreasing order of warmness. The "warmer" pairs (hot-hot, warm-hot) primarily involve pungent-pungent, pungent-sweet, and sweet-sweet taste combinations. The "warm" pairs (warm-warm) primarily involve pungent-pungent and pungent-bitter combinations. The "less warm" pairs (hot-neutral, warm-neutral and warm-cool) primarily involve sweet-sweet, sweet-pungent, and pungent-pungent combinations.

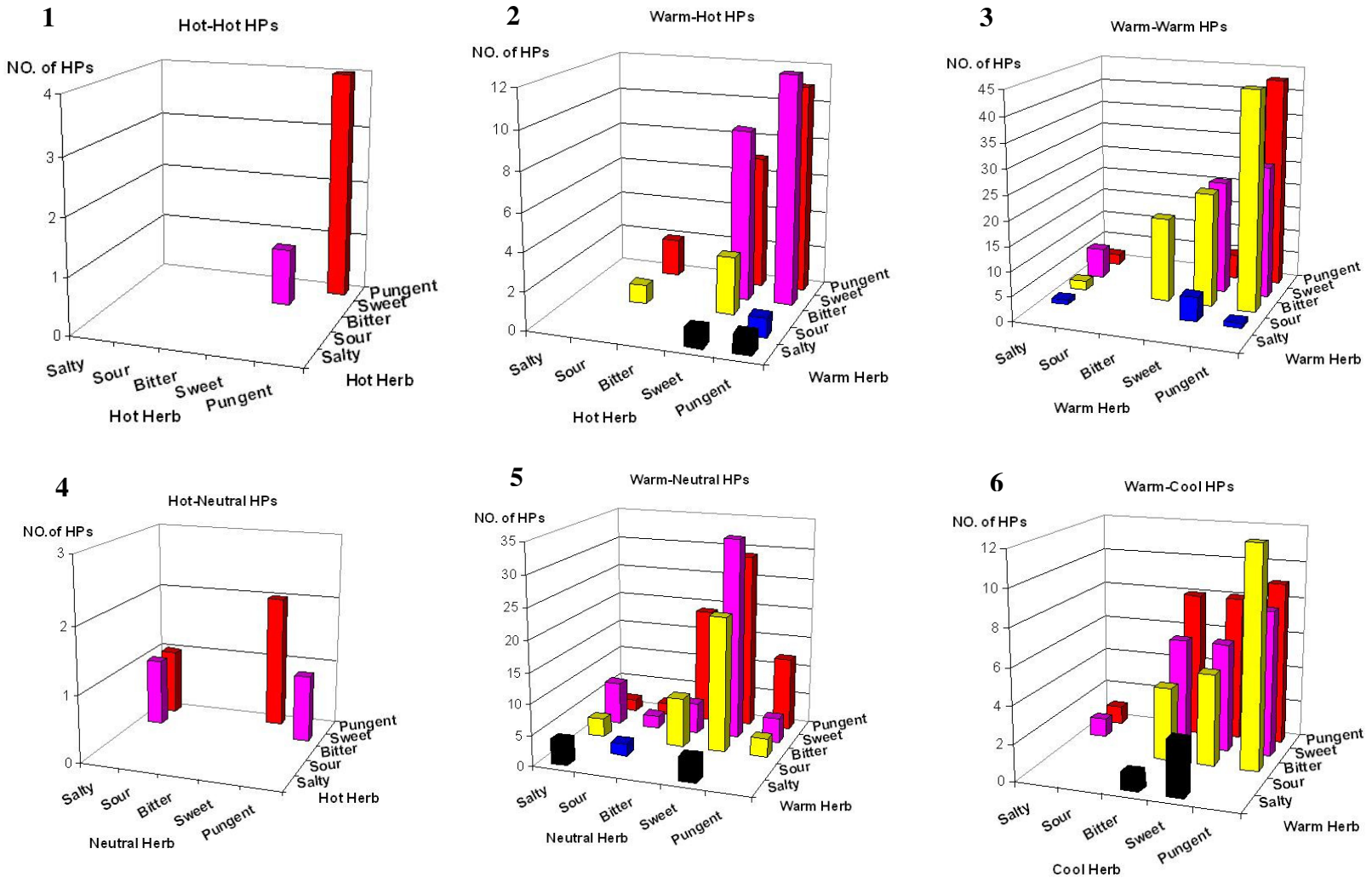


Figure 3.2 Distribution patterns of combinations of traditionally-defined herbal properties of TCM herb-pairs with predominantly cold characters. These herb-pairs are divided into cold-cold, cold-cool, cool-cool, cold-neutral, cool-neutral, cold-warm and cold-hot groups in decreasing order of coldness. The "extremely cold" pairs (cold-cold and cold-cool) primarily involve bitter-bitter taste combinations. The "colder" pairs (cool-cool) primarily involve bitter-sweet, bitter-pungent, bitter-bitter, and pungent-sweet combinations. The "somewhat cold" pairs (neutral-cold and neutral-cool) primarily involve bitter-sweet and sweet-sweet combinations. The "slightly cold" pairs (cold-warm and cold-hot) primarily involve bitter-pungent, bitter-sweet, and bitter-bitter combinations.

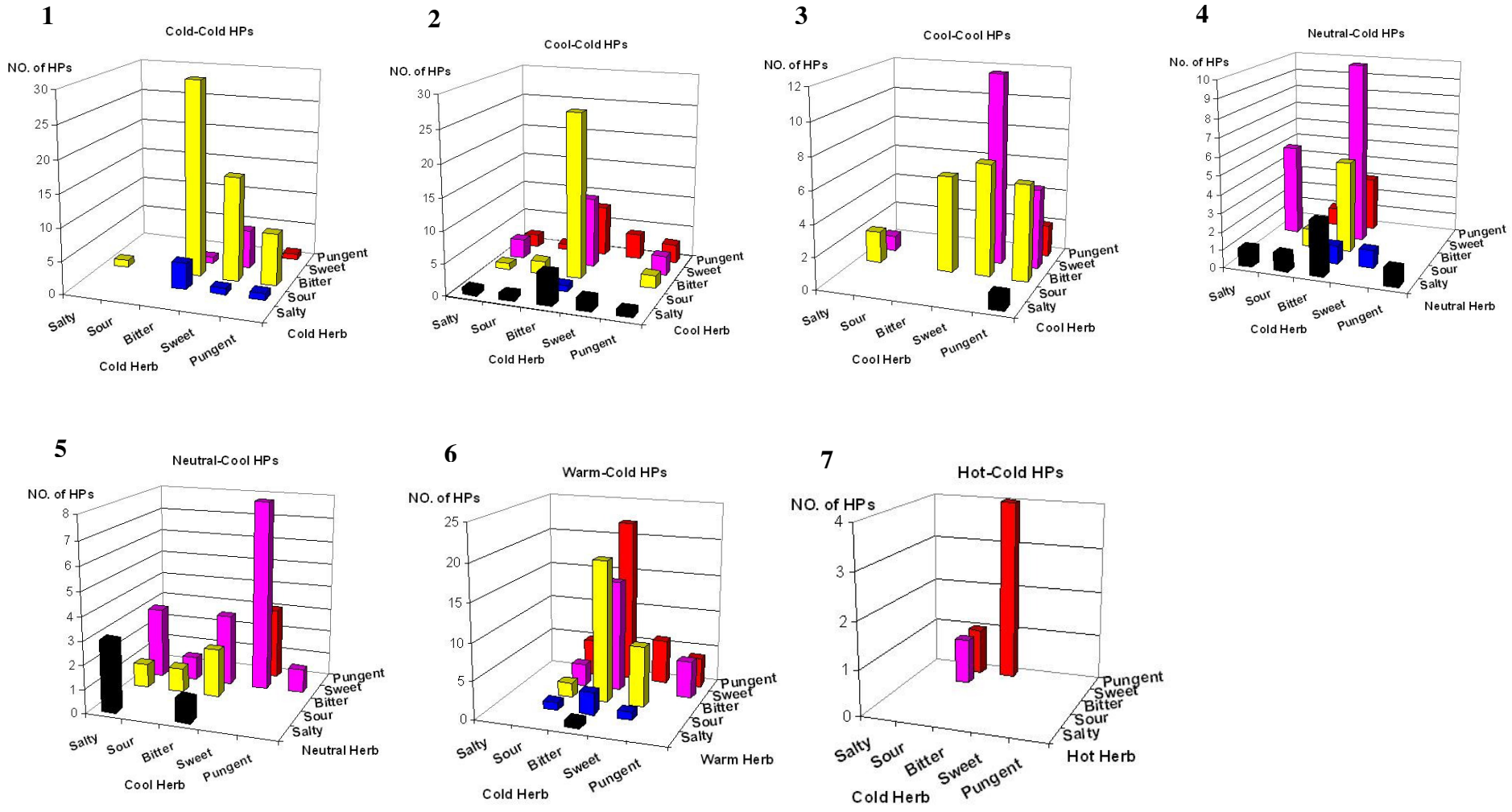
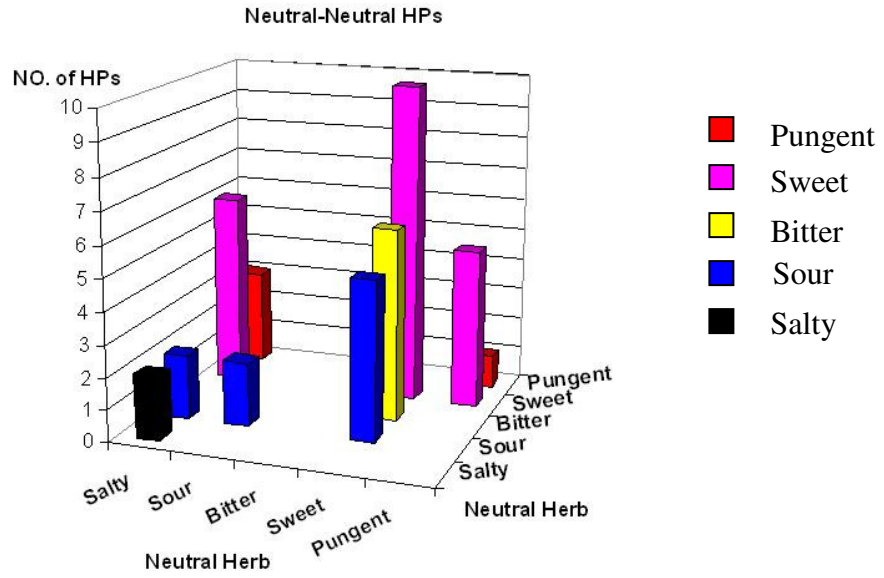


Figure 3.3 Distribution patterns of combinations of traditionally-defined herbal properties of TCM herb-pairs with predominantly neutral characters. The neutral-neutral pairs primarily involve sweet-sweet, sweet-salty, sweet-bitter, and sweet-sour taste combinations.



3.2.3.2 Usefulness of TCM-HPs for distinguishing TCM herb-pairs from non-TCM herb-pairs

A 10-fold cross validation study was conducted to determine whether the three machine learning methods (PNN, kNN and SVM) are able to separate TCM herb-pairs from non-TCM herb-pairs based on their TCM-HPs. The accuracies for predicting TCM herb-pairs are in the range of 72.1~87.9%, and those for predicting non-TCM herb-pairs are in the range of 91.6~97.6% respectively. The overall prediction accuracies range from 91.1% to 94.9% and the Matthews correlation coefficients range from 0.694 to 0.781. These results suggest that TCM herb-pairs and non-TCM herb-pairs can be separated by MLMs based on their TCM-HPs.

The developed MLM classification systems can be further tested to determine to what extent they are able to predict the following two groups of herb-pairs as non-TCM herb-pairs. One group contains the known non-TCM herb-pairs and the other includes vast number of the other possible herb-pairs not used as TCM herb-pairs and not used in our 10-fold cross validation studies. We found that 96.3% of the 27 known non-TCM herb pairs, and 99.7% of the other 1,065,100 possible herb-pairs were predicted as non-TCM herb-pairs by the best performing MLM system SVM. These results suggest that our MLM systems are capable of dividing known TCM herb-pairs from known non-TCM herb-pairs and the vast number of other herb-pairs, and the TCM-HPs of the known TCM herb-pairs contain useful information for distinguishing them from the other herb-pairs.

3.4 Discussion and Conclusion

The distribution patterns of combinations of the TCM-HPs of the majority of the 394 herb-pairs appear to show some level of internal correlation. As shown in **Figure 3.1**, the "warmer" pairs (hot-hot, warm-hot) primarily involve pungent-pungent, pungent-sweet, and sweet-sweet taste combinations. The "warm" pairs (warm-warm) primarily involve pungent-pungent and pungent-bitter combinations. The "less warm" pairs (hot-neutral, warm-neutral and warm-cool) primarily involve sweet-sweet, sweet-pungent, and pungent-pungent combination. As shown in **Figure 3.2**, the "extremely cold" pairs (cold-cold and cold-cool) primarily involve bitter-bitter taste combinations. The "colder" pairs (cool-cool) primarily involve bitter-sweet, bitter-pungent, bitter-bitter, and pungent-sweet combinations. The "somewhat cold" pairs (neutral-cold and neutral-cool) primarily involve bitter-sweet and sweet-sweet combinations. The "slightly cold" pairs (cold-warm and cold-hot) primarily involve bitter-pungent, bitter-sweet, and bitter-bitter combinations. From **Figure 3.3**, the neutral-neutral pairs were found to primarily involve sweet-sweet, sweet-salty, sweet-bitter, and sweet-sour taste combinations.

These patterns of combinations have clinical relevance in prescribing TCM multi-herb recipes. For instance, warm-warm herb-pairs such as *Ma Huang* (*Herba Ephehrae*) and *Gui Zhi* (*Ramulus Cinnamomi*), *Gui Zhi* (*Ramulus Cinnamomi*) and *Chuan Xiong* (*Rhizoma Chuanxiong*) have diaphoretic properties [Wang 2004] and have been used for treating exterior syndrome due to cold, promoting blood circulation, and tonics for internal warming [Wang 2004]. These symptoms have been described in TCM literatures

to be treated by herbs with warm characters and pungent and bitter tastes [Wang 2004]. Cold-cold herb-pairs such as *Shi Gao* (*Gypsum Fibrosum*) and *Zhi Mu* (*Rhizoma Anemarrhenae*) have been used for heat clearance, a symptom that requires herbs with cold characters and bitter tastes according to TCM theory [Li 2005]. Neutral-neutral herb-pairs such as *Dang Shen* (*Radix Codonopsis*) and *Fu Ling* (*Poria*) have been used as tonic in such mild conditions as diuresis and excreting dampness [Li 2005]. Herbs with neutral characters and sweet tastes are one of the main classes of herbs for treating these conditions in TCM literatures [Wang 2004].

Herbs with opposite characters (such as warm-cold pair *Gui Zhi* (*Ramulus Cinnamomi*) and *Shao Yao* (*Radix Paeoniae Alba*), and hot-cold pair *Fu Zi* (*Radix Aconiti Lateralis Preparata*) and *Da Huang* (*Radix et Rhizoma Rhei*)) have been used for modulating the harsh effect of the companion herb or to complement its action. For instance, *Fu Zi* (*Radix Aconiti Lateralis Preparata*) is an herb with hot character and pungent taste that has been used for interior warming to cure serious *Yang*-deficient syndromes. *Da Huang* (*Radix et Rhizoma Rhei*), on the other hand, is an herb with cold character and bitter taste that has been used as a purgative herb for treating constipation associated to hotness symptoms and those exhibiting coldness in both hands and legs. When used together, both herbs not only modulate the harsh effects of each other but produce other synergistic effects. *Fu Zi* (*Radix Aconiti Lateralis Preparata*) produces lifting actions in the body to promote Qi and blood circulation. *Da Huang* (*Radix et Rhizoma Rhei*) produces descending actions to clear the hotness trapped in the gastrointestinal tracts.

Both distribution patterns of TCM-HPs and MLM classification studies show that TCM herb-pairs can be distinguished from non-TCM herb-pairs assembled by random combination of herbs and by modification of known TCM herb-pairs. Future work for elucidating the molecular mechanism of the synergistic actions of TCM herb-pairs is required. Knowledge of the molecular mechanism of these synergistic actions can facilitate the understanding of some aspects of the regulation of human physiological systems and the application of the principles of synergistic combinations of TCM herbs for developing novel cocktail therapeutic approaches to modulate and harmonize the functions of multiple systems, which provide leads to more efficient treatment and recovery approaches.

Chapter 4 Identification of Metastatic-Related Targets of Rhubarb Anthraquinones by an Inverse Docking Approach

Metastasis is the fatal step in tumorigenesis. Proteins such as receptor tyrosine kinases, matrix metalloproteinases, cell adhesion molecules, small GTPases are identified as important mediators in cell invasion and motility in the process of metastasis. Previous experimental and proteomic works indicated that Rhubarb anthraquinones exhibit anti-proliferative and anti-metastatic activity. However, the molecular targets and mechanism of anti-metastatic activity of these anthraquinones are poorly understood. In this chapter, an in silico inverse docking approach is utilized to identify putative metastatic-related molecular targets of Rhubarb anthraquinones emodin, aloe-emodin, and rhein from a protein structure database. The identified molecular targets are then compared with reported bioactivity of these anthraquinones.

4.1 Introduction

Rhubarb root (*Da Huang*) is one of the commonly used Chinese herbs. The most commonly used species is *Rheum palmatum* or *Rheum officinale Baill* of the *Polygonaceae* family. Rhubarb is traditionally used as a laxative, in treatment of constipation jaundice, gastro-intestinal hemorrhage, and ulcers. The main bioactive constituents of rhubarb are anthraquinone derivatives, including emodin, aloe-emodin, rhein, chrysophanol, physcion, and danthron. In addition, catechins, gallic acid, and cinnamic acid are also present in rhubarb.

A number of studies have demonstrated that the main anthraquinones of rhubarb, emodin, aloe-emodin, and rhein, inhibit the growth and proliferation of various cancer cells. For instance, emodin has been reported to inhibit proliferation in breast cancer cells [Zhang *et al.* 1995; Zhang *et al.* 1999], lung [Su *et al.* 2005], cervical [Srinivas *et al.* 2003], and prostate cancers cells [Cha *et al.* 2005]. Aloe-emodin was also able to inhibit cell growth in several tumor cells, including human lung carcinoma [Lee *et al.* 2006], hepatoma [Kuo *et al.* 2002], and leukemia cell lines [Chen *et al.* 2004]. Rhein, another anthraquinone derivative of rhubarb, has also been reported to display inhibitory effect on the proliferation of human breast, colon, lung, CNS, and glioma cancer cells [Cichewicz *et al.* 2004]. Effect of the main rhubarb anthraquinones, emodin and aloe-emodin on G2/M cell cycle have been demonstrated on various cancer cells, including v-ras-transformed cells, hepatoma, leukemia, and neuroectodermal cells [Chan *et al.* 1993; Chen *et al.* 2004; Xiao *et al.* 2007].

In addition to anti-proliferative activity, a number of studies have demonstrated that emodin is capable of inducing apoptotic cell death in various cancer cells [Shieh *et al.* 2004]. Several studies indicate that emodin-induced apoptosis is mediated by ROS generated from the semiquinone. Similar to emodin, apoptosis can be induced by aloe-emodin in various tumor cells. Aloe-emodin can induce sub-G1 peak (one of the biomarkers of apoptosis) at 48 hr following G2/M arrest at 24 hr in neuroectodermal tumor cells. Aloe-emodin was also found to induce apoptosis in many other tumor cells derived from hepatoma, lung, bladder carcinoma, and leukemia. Similar to emodin, aloe-

emodin can induce DNA damage through oxidative stress, and later initiate apoptosis [Lin *et al.* 2006]. In addition, aloe-emodin-induced apoptosis in HepG2 cells was found to be ROS-dependent [Lu *et al.* 2007]. Rhein, another Rhubarb anthraquinone was shown to induce apoptosis in HL-60 cells through the mitochondrial death pathway by causing the loss of mitochondrial membrane potential, cytochrome c release, and cleavage of *Bid* protein [Lin *et al.* 2003]. Taken together, these results suggest that anthraquinones (emodin, aloe-emodin, and rhein) activate apoptotic cell death in different tumor cells and the mitochondrial-dependent pathway was suggested to be the main apoptotic process.

The lethality of cancers is caused by the metastatic spread of tumor cells from their original site. The metastasis events include angiogenesis, cell adhesion, cell invasion (extracellular matrix degradation and cell migration), and cell proliferation. One critical aspect of the anti-cancer activity of Rhubarb anthraquinones is their inhibitory effect on cancer metastasis. Emodin inhibits TNF-α-induced expression of cell surface adhesion proteins (ICAM-1, VCAM-1, ELAM-1), which is essential in cell adhesion, migration, and inflammation [Kumar *et al.* 1998] that is mainly due to suppression of emodin on NF-kB activation [Li *et al.* 2005b]. Emodin was found to exhibit significant inhibition on the adhesion of various cancer cells [Kim *et al.* 2005; Huang *et al.* 2006c]. Emodin reduces cholesterol content in the membrane lipid rafts, preventing rafts clustering and subsequent colocalization of integrin β1 and focal adhesion complex proteins, and eventually disrupting the lipid rafts-associated integrin-signaling pathway [Huang *et al.* 2006c]. In addition, emodin inhibits EGF-induced cell migration in various cancer cells. PI3K was found to be the molecular target for emodin and significantly suppressed the

PI3K-mediated Cdc42 and Rac1 activation and subsequent cytoskeletal changes, including filopodia and lamellipodia formation [Huang *et al.* 2006b]. Emodin also inhibits the invasion of human cancer cells by suppressing MMP-9 expression via inhibiting AP-1 and NF-kB signaling pathways [Kim *et al.* 2005]. Less study was conducted on rhein, some related reports implicated that rhein might have certain inhibiting effects in the cascade of tumor metastasis such as via the suppression of IL-1α-induced production of pro-MMPs [Sanchez *et al.* 2003].

Recent proteomic study by Lu and coworkers suggests that up-regulation of nucleoside diphosphate kinase A or nm23 (a metastasis suppressor gene) by aloe-emodin decreased cell migration [Lu *et al.* 2007]. Due to the difference in anti-metastatic activity of Rhubarb anthraquinones emodin and aloe-emodin, it is necessary to identify their potential metastatic-related targets and to unravel their mechanism. Understanding the molecular mechanisms of the anti-metastatic activities of these compounds can help to further refine not only molecular specificity but inhibition of metastasis process at different stages. Besides, the metastatic-related molecular targets of rhein are also worth exploring.

In this study, an *in silico* approach inverse docking method (INVDOCK) has been used to search the potential metastatic-related targets of Rhubarb anthraquinones emodin, aloe-emodin, and rhein. As opposed to conventional docking strategy where one protein target is searched against a compound library for potential drug candidates, INVDOCK was designed to facilitate identification of molecular targets of a compound from a protein structure database. INVDOCK had been shown to be able to identify known

protein targets of some well known pharmaceutics such as aspirin and tamoxifen [Chen *et al.* 2002b; Chen *et al.* 2003]. In addition, INVDOCK had been applied to identify therapeutic and toxicity targets of some herbal compounds from previous studies [Chen *et al.* 2002b; Chen *et al.* 2003].

4.2 Methods

4.2.1 The Algorithm of INVDOCK

For a conventional docking approach, a protein is searched against compounds from a chemical library to identify potential ligands of the protein. However, INVDOCK is based on a protein-ligand inverse docking strategy such that a compound is docked to known ligand-binding pockets of each protein in a protein 3D structural database such as the Protein Data Bank (PDB). A protein is considered as a potential target for a compound if that compound can be docked into the protein and the binding satisfies a molecular-mechanics based criterion for chemical complementarity.

To facilitate a high-speed search of potential protein targets of a compound, a protein cavity database has been developed from the corresponding protein 3D structures in the PDB. Each cavity entry is composed of a sphere cluster filling the cavity [Shoichet *et al.* 1991]. Each PDB entry has a corresponding cavity entry in cavity database. Docking of a compound is performed against each cavity entry in the cavity database to identify cavities that can be successfully docked to a compound.

Docking of a particular compound to a cavity is by the following steps. The compound is first aligned within the selected site by matching the position of each atom of the compound with the center of the spheres. Because of the relatively low-resolution nature of the conformation sampling of the compound, a certain degree of structural clash is allowed at this stage. Optimization is then conducted by a limited torsion space sampling of rotatable bonds in the structure and those in the side-chain of the receptor amino acid residues at the binding site. Each rotatable bond is sampled in the range of ±15 degree followed by 50 iterations of Cartesian coordinate energy minimization on all chemical and protein atoms at the binding site to further optimize the protein-ligand complex. The steepest descent method is used for energy minimization. In both torsion optimization and energy minimization, AMBER force fields [Case *et al.* 2005] are used for terms of covalent bond, bond angle, torsion, and non-bonded van der Waals and electrostatic interactions. Morse potential which is a function of donor-acceptor distance is used to represent hydrogen bond terms.

The overall energy function is as below:

$$V = 1/2\sum_{bonds} K_r (R - R_{eq})^2 + 1/2\sum_{angles} K_\theta (\theta - \theta_{eq})^2 +$$
$$1/2\sum_{torsions} V_n [1 - \cos(n(\phi - \phi_{eq}))] +$$
$$\sum_{H\quad bonds} [V_0 (1 - e^{-a(r-r_0)})^2 - V_0] +$$
$$\sum_{non\quad bonded} [A_{ij} / r_{ij}^{12} - B_{ij} / r_{ij}^{6} + q_i q_j / \varepsilon_r r_{ij}]$$

In this function, R, θ, and ϕ denote bond length, bond angle, and torsion angle respectively; values for R_{eq}, θ_{eq} and ϕ_{eq} are from the original PDB structure and the structure of the drug and taken as equilibrium bond length, bond angle, and torsion angle,

respectively. K_r and K_θ are covalent and bond angle bending force constant, respectively. V_n and n are torsion parameters; and r is the distance of hydrogen bond donor-acceptor. V_o, a, and r_o are parameters for hydrogen bond potential. Scoring of docked molecules is based on a protein-ligand interaction energy function ΔE_{LP} composed of the same hydrogen bond and non-bonded terms as used for structure optimization. Analysis of a large number of PDB ligand-protein complexes shows that the computed ΔE_{LP} is generally below $\Delta E_{Threshold} = \alpha N$ kcal mol^{-1}, where N is the number of ligand atoms and α is a constant ~1.0. The exact value of α can be determined by fitting the linear equation $\Delta E_{Threshold} = -\alpha N$ to the computed ΔE_{LP} for a large set of PDB structures. The statistically derived energy value can be used empirically as a threshold to screen for potential ligands. A polynomial form of $\Delta E_{Threshold}$ involving more parameters can be introduced to derive an energy threshold. ΔE_{LP} can be required to be lower than $\Delta E_{Threshold}$ when selecting successfully docked structures.

Since drug binding is competitive in nature, a drug is unlikely to be effective if it binds weakly to a protein target compared to its natural ligand. Ligands in PDB structures are known binders. Therefore PDB ligands bound to the same receptor site as that of a docked molecule may be considered as "competitors" of that molecule. To select a putative protein target using INVDOCK, the computed ΔE_{LP} is not only evaluated against $\Delta E_{Threshold}$ but also compared to the protein-ligand interaction energy of the corresponding PDB ligands that bind to the same cavity in this or other relevant PDB entries. The protein-ligand interaction energy for the relevant PDB structures is computed by the same energy functions as that for the docked molecule. In addition**,** the value of ΔE_{LP} of a

docked molecule is required to be lower (more stable) than a "competitor" energy threshold $\Delta E_{\text{Competitor}}$ when selecting a putative target. $\Delta E_{\text{Competitor}}$ can be taken as the highest ligand-protein interaction energy of the corresponding PDB ligands multiplied by a factor β. In order to be able to find weak binders as well as strong binders, a factor $\beta \leq 1$ is introduced to scale the protein-ligand interaction energy of PDB ligand since a weak binder may have slightly higher interaction energy than that of a PDB binder. No experimental data have been found to determine the value of β. Hence β has been tentatively determined by an analysis of the computed energy for a number of compounds. A value of 0.8 for β statistically is enough to produce reasonable results.

4.2.2 Validation of INVDOCK Results on Targets of Rhubarb Anthraquinones

The validation for INVDOCK prediction on the targets of synthetic chemicals had been conducted from a recent study on a number of clinical agents [Chen *et al.* 2002b; Chen *et al.* 2003]. In this study, the targets of Rhubarb anthraquinones such as emodin, aloe-emodin, and rhein identified by INVDOCK were validated with known reported targets identified from various experimental works. The INVDOCK-generated "docked" structures of Rhubarb anthraquinones were generated for visual inspection to identify putative binding residues around the binding sites. These "key residues" may serve for future mutagenesis studies to further explore the binding modes and functionality of Rhubarb anthraquinones.

4.3 Results

4.3.1 Targets Identified from INVDOCK and Comparison to Known Targets of Rhubarb Anthraquinones

Molecular targets of Rhubarb anthraquinones emodin, aloe-emodin, and rhein identified from various experimental works are used to validate the efficiency of the ability of INVDOCK to pick up known targets from structural-based criteria. **Table 4.1**, **Table 4.2**, and **Table 4.3** show the comparison of experimental verified targets and INVDOCK-identified targets denoted in PDB ID for emodin, aloe-emodin, and rhein, respectively. Since emodin is well studied than aloe-emodin and rhein, more known targets are available to verify the efficiency of INVDOCK for this compound. Overall, INVDOCK is able to identify a number of known targets of Rhubarb anthraquinones from a protein structure database. **Figures 4.1** to **Figure 4.4** show some representatives of INVDOCK-generated docked anthraquinone-protein complexes for kinases that involved in metastatic-related signaling pathways and metastatic-related targets that are involved in cell adhesion, cytoskeleton, and cell motility for emodin and aloe-emodin.

Table 4.1 Targets of emodin from biochemical studies and from INVDOCK denoted in PDB ID. Binding energy in kcal/mol for each target is given.

Targets of emodin	Effects of emodin on the targets	Publication with PubMed ID	PDB entries identified by INVDOCK
Focal Adhesion Kinase (FAK), a key regulator of cell adhesion and migration	Inhibit the association of FAK with integrin β1 and decrease in the phosphorylation level of FAK-Tyr397 in a time-dependent manner	Huang et al, 2006 (16740720)	1mp8 [-41.1] (human)
Phosphatidylinositol 3-Kinase (PI3K), involves in cell mobility	Suppress PI3K-Cdc42/Rac1 pathway leading to decreased cell mobility	Huang et al, 2005 (15928809)	2a98 [-39.4] (human), 1w2d [-46.6] (human)
Cdc42, involves in cell mobility	Interfere the formation of the Cdc42/Rac1 and PAK1 complex	Huang et al, 2005 (15928809)	1u4d [-45.4] (human), 1u54 [-48.2] (human)
Rac1, involves in cell mobility	Interfere the formation of the Cdc42/Rac1 and PAK1 complex	Huang et al, 2005 (15928809)	None
NF-κB, an important transcription factor involves in MMP-9 expression	Inhibit the binding to DNA in a dose-dependent manner	Huang et al, 2004 (15194008)	1lei [-56.7] (mouse)
Activator Protein-1 (AP-1), an important transcription factor involves in MMP-9 expression	Reduce transcription activity of AP-1	Huang et al, 2004 (15194008)	None
Extracellular Signal-Regulated Protein Kinase (ERK)	Suppress phosphorylation	Huang et al, 2004 (15194008)	None
c-Jun N-Terminal Kinase (JNK)	Suppress phosphorylation	Huang et al, 2004 (15194008)	None
Epidermal Growth Factor Receptor (EGFR)	Inhibits epidermal growth factor (EGF)-induced migration in various human cancer cell lines	Huang et al, 2005 (15928809)	None
Protein Tyrosine Protein Kinases		Zhang and Hung, 1996; Chan et al, 1994	1bbz [-39.2] (human ABL tyrosine kinase), 1qpj [-41.5] (human LCK tyrosine kinase)
$p56^{lck}$		Jayasuriya et al, 1992	1qpe (human), 1qpj (human), 1qpc (human)
Cyclin, Cyclin-Dependent Protein	Suppressing cyclin D1 and E expression and	Kwak et al, 2006	1dm2 [-45.0] (human), 1unh [-45.0] (human),

Kinase 2 and 5 (Implicated)	retinoblastoma protein phosphorylation		1unl [-42.6] (human)
SARS-CoV spike (S) protein	blocks the SARS-CoV spike (S) protein and ACE2 interaction in a dose-dependent manner	Ho et al, 2006 (16730806)	1q2w [-44.4]
Angiotensin-Converting Enzyme (ACE)	blocks the SARS-CoV spike (S) protein and ACE2 interaction in a dose-dependent manner	Ho et al, 2006 (16730806)	1o86 [-40.1] (human)
DNA	Interacts with DNA by intercalating into the double helix of DNA	Wang et al, 2006	324d [-52.4]
K-ATP Channel, Na+-K+-ATPase	Inhibit the activity of KATP channel in the guinea pig taenia coli smooth muscle cells; Emodin enhances the function of small intestinal peristalsis of mice by mechanisms of promoting secretion of motilin, lowering the content of somatostatin and inhibiting Na+-K+-ATPase activity of small intestinal mucosa	Li et al, 1998 (12016996); Zhang ey al, 2005 (15918207)	None
Caspase-3, Caspase-9 (Implicated)	Induces apoptosis in human promyeloleukemic HL-60 cells accompanied by activation of caspase 3 cascade; Emodin generated reactive oxygen species (ROS) in these cells which brought about a reduction of the intracellular mitochondrial transmembrane potential (DeltaPsim), followed by the activation of caspase-9 and caspase-3, leading to DNA fragmentation and apoptosis	Chen et al, 2002 (12445860); Jing et al, 2002 (12716464); Srinivas, 2003 (12892828)	1nme [-43.2] (human), 1rhm [-41.7] (human)
Protein Kinase C, CREB	Suppressing PKC activation and CREB phosphorylation	Chan et al, 2003	None
Androgen Receptor	Decreased the association of androgen receptor (AR) and heat shock protein 90 and increased the association	Cha et al, 2005	1t74 [-40.0] (Chimpanzee),

	of AR and MDM2		
Fatty Acid Synthase (FAS)	Emodin inhibits cell differentiation into adipocyte and is a potential to serve as a fat-reducing drug	Zhang et al, 2002 (12150738)	None
Receptor-2 (KDR/Flk-1)	Inhibitory effect of emodin toward VEGF-A-induced angiogenesis in vitro and responsible for its potent anti-angiogenic in vivo	Kwak et al, 2006 (16388516)	None

Table 4.2 Targets of aloe-emodin from biochemical studies and from INVDOCK denoted in PDB ID. Binding energy in kcal/mol for each target is given.

Targets of aloe-emodin	Effects of aloe-emodin on the targets	Publication with PubMed ID	PDB entries identified by INVDOCK
H(1) receptor	Aloe emodin is proposed to bind to H(1) receptor and stimulates mast cells within the colonic mucosa to release histamine	Kai et al, 2002 (12499649)	None
cAMP-Dependent Protein Kinase	Aloe-emodin induces apoptosis of H460 cells involves modulation of cAMP-dependent protein kinase, protein kinase C, Bcl-2, caspase-3 and p38 protein expression	Yeh et al, 2003 (12794753)	1stc [-48.8] (bovine)
Protein Kinase C	Aloe-emodin induces apoptosis of H460 cells involves modulation of cAMP-dependent protein kinase, protein kinase C, Bcl-2, caspase-3 and p38 protein expression	Yeh et al, 2003 (12794753)	None
Bcl-2	Aloe-emodin induces apoptosis of H460 cells involves modulation of cAMP-dependent protein kinase, protein kinase C, Bcl-2, caspase-3 and p38 protein expression	Yeh et al, 2003 (12794753)	None
Caspase-3	Aloe-emodin induces apoptosis of H460 cells involves modulation of cAMP-dependent protein kinase, protein kinase C, Bcl-2, caspase-3 and p38 protein expression	Yeh et al, 2003 (12794753); Chen et al, 2004 (15207375); Lian et al, 2005 (15910415)	1nme [-46.0] (human)
p38	Aloe-emodin induces apoptosis of H460 cells involves modulation of cAMP-dependent protein kinase, protein kinase C, Bcl-2, caspase-3 and p38 protein expression	Yeh et al, 2003 (12794753)	1bl7 [-61.6], 1di9 [-49.7], 1kv1 [-40.3] (human)
Arylamine N-Acetyltransferase (NAT)	Aloe-emodin displays a dose-dependent inhibition to cytosolic NAT activity and intact mice leukemia cells	Chung et al, 2003 (12804642); Lin et al, 2005 (16314733)	None
DNA	Binds to DNA and induces apoptosis	Pecere et al, 2003 (12918060)	None
Inducible Nitric Oxide	Aloe-emodin modulates	Mijatovic et al, 2004	4nos [-44.4]

Synthase (NOS)	survival of mouse L929 fibrosarcoma and rat C6 astrocytoma cells through interference with the activation of inducible nitric oxide synthase (NOS) and subsequent production of tumoricidal free radical NO	(15241556)	(human)
Extracellular Signal-Regulated Kinases 1 and 2 (ERK1/2)	Aloe-emodin inhibits the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) in C6 cells	Mijatovic et al, 2005 (15747063, 15905960)	4erk [-55.0] (rat)

Table 4.3 Targets of Rhein from biochemical studies and from INVDOCK denoted in PDB ID.

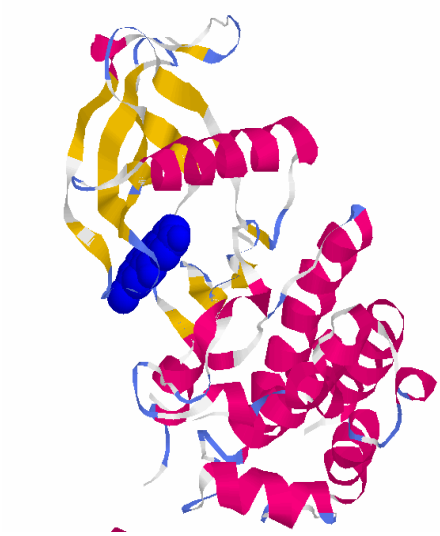
Targets of rhein	Effects of rhein	Publication with PubMed ID	PDB entries identified by INVDOCK
IL-1beta	potent inhibitor of IL-1beta induced NO production by chondrocytes and cartilage	Pelletier et al, 1998 (9858439); Yaron et al, 1999 (10329302)	1rwk [-40.8], 1ibc [-38.6] (human)
Matrix metalloproteinases	down-regulates the gene-expression and production of proMMPs and up-regulates the tissue inhibitors of metalloproteinase-1 (TIMP-1) production	Tamura et al, 2001 (11300749)	1jap [-59.9], 1g49 [-59.2], 966c [-62.6] (human)
GLUT1	Down-regulates the expression of glucose transporter-1 (GLUT1) mRNA and glucose uptake in mesangial cells	Zhang et al, 1999 (11721442)	None
TGF-beta1	hepatoprotective via antioxidant and anti-inflammatory activity	Guo et al, 2002 (12147197)	None
Nuclear factor-kappaB	inhibits nuclear factor-kappaB activation and the expression of nuclear factor-kappaB-dependent genes, such as the inducible nitric oxide synthase gene,that leads to antiosteoarthritic and antiinflammatory effects	Mendes et al, 2002 (12193257)	1lei [-58.9] (mouse)
Multidrug resistance pumps (MDRs),	Anti-bacterial by inhibiting MDRs	Tegos et al, 2002 (12234835)	1mv5 [-53.2], 2bow [-39.0] (bacterial)
Inducible nitric oxide synthase	Inhibitor of inducible nitric oxide synthase leading to anti-inflammation effects	Wang et al, 2002 (12391547)	4nos [-51.6] (human)
Activator protein-1 (AP-1)	anti-carcinogenesis	Lin et al, 2003 (12632075)	None
c-Jun	inhibits the phosphorylation and abundance of c-Jun protein, c-Jun NH2-terminal kinase (JNK) phosphorylation	Lin et al, 2003 (12632075)	None
Endothelial plasminogen activator inhibitor-1 (PAI-1)	inhibits the overexpression of PAI-1 for the treatment of	Zhu et al, 2003 (12781036)	None

	vascular diseases		
TGFbeta1	inhibits the proliferation of human mesangial cells (HMCs) may through inhibiting the bioactivities of TGFbeta1 and p38 MAPK	Tan et al, 2004 (15696926)	None
p38	inhibits the proliferation of human mesangial cells (HMCs) may through inhibiting the bioactivities of TGFbeta1 and p38 MAPK	Tan et al, 2004 (15696926)	1bl7 [-50.0], 1di9 [-46.3] (human)
Human serum albumin (HSA)		Li et al, 2007 (17174615)	1bj5 [-44.0] (human)

Figure 4.1 Examples of INVDOCK-generated binding of emodin to kinases involve in metastatic-related signaling pathways. The molecule of emodin is represented as space-filled model. The binding energy in kcal/mol is given.

(a) Binding of emodin to human focal adhesion kinase 1 (PDB ID: 1mp8 [-41.1])

(b) Binding of emodin to human FGF Receptor 1 (PDB ID: 1fgi [-47.6])



(c) Binding of emodin to human LCK kinase (PDB ID: 1qpe [-39.8])

(d) Binding of emodin to human activated CDC42 kinase 1 (PDB ID: 1u54 [-48.2])

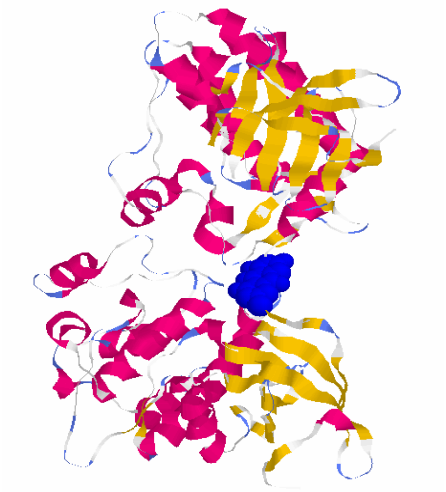
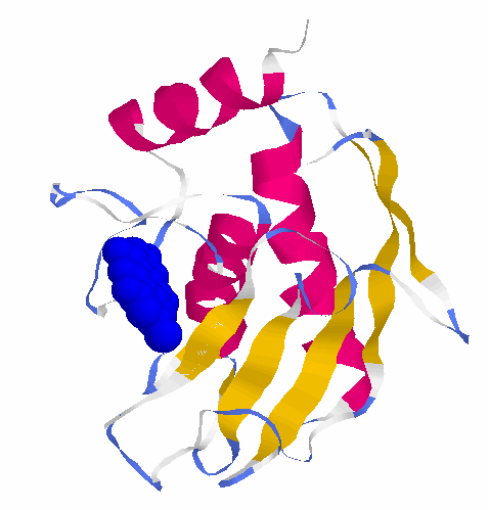
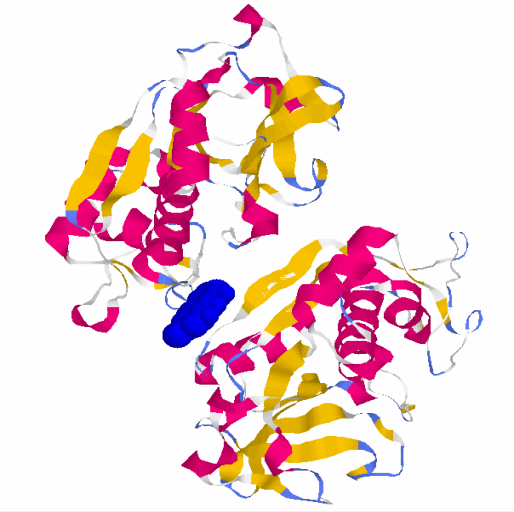


Figure 4.2 Examples of INVDOCK-generated binding of emodin to metastasic-related targets that involve in cell adhesion, cytoskeleton, and cell motility. The molecule of emodin is represented as space-filled model. The binding energy in kcal/mol is given.

(a) Binding of emodin to human MMP-1 (PDB ID: 966c [-67.9])



(b) Binding of emodin to human cathepsin F (PDB ID: 1m6d [-42.7])



(c) Binding of emodin to human actin (PDB ID: 1nm1 [-41.3])

(d) Binding of emodin to human angiotensin converting enzyme (PDB ID: 1o86 [-40.1])

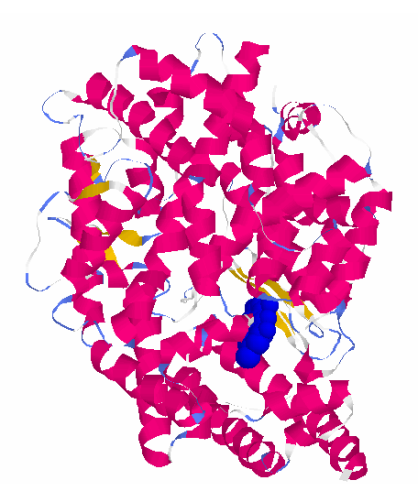
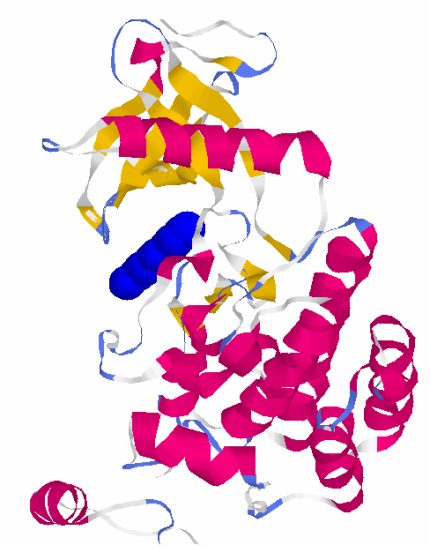


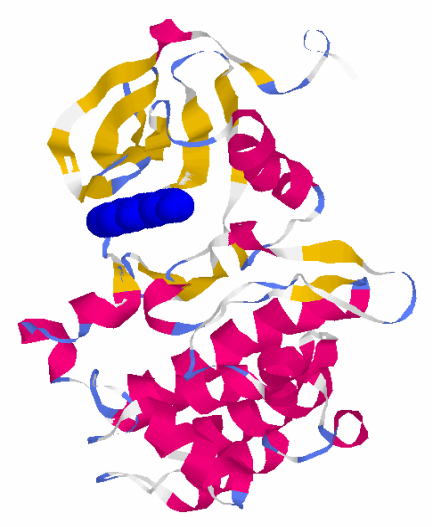
Figure 4.3 Examples of INVDOCK-generated binding of aloe-emodin to kinases involve in metastatic-related signaling pathways. The molecule of aloe-emodin is represented as space-filled model. The binding energy in kcal/mol is given.

(a) Binding of aloe-emodin to human proto-oncogene tyrosine-protein kinase Src (PDB ID: 1yol [-48.9])

(b) Binding of aloe-emodin to human FGF receptor 1 (PDB ID: 1agw [-45.2])



(c) Binding of aloe-emodin to human LCK kinase (PDB ID: 1qpe [-40.7])



(d) Binding of aloe-emodin to rat extracellular regulated kinase 2 (PDB ID: 4erk [-55.0])

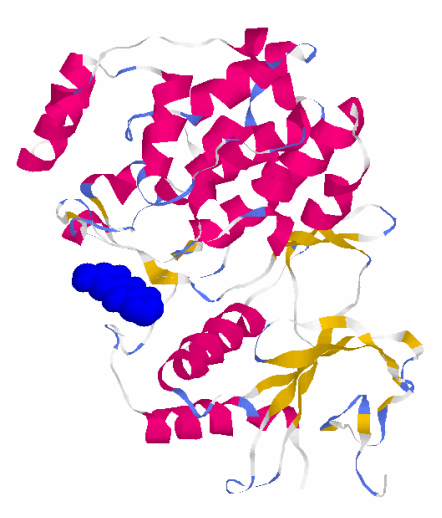
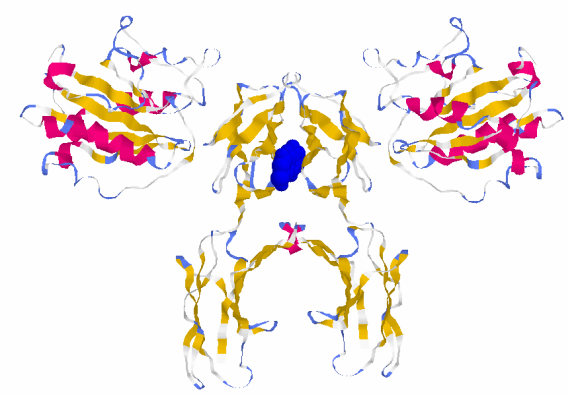


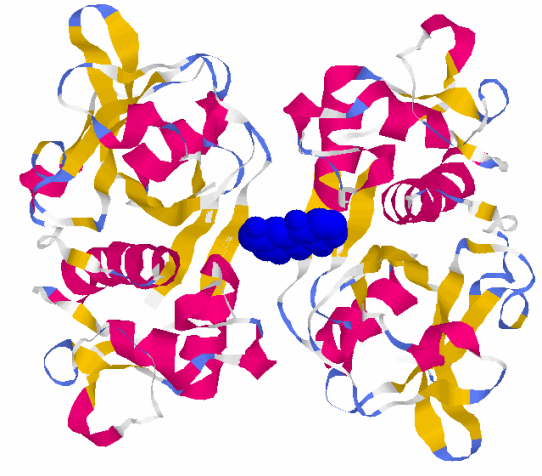
Figure 4.4 Examples of INVDOCK-generated binding of aloe-emodin to metastasic-related targets that involve in cell adhesion, cytoskeleton, and cell motility. The molecule of aloe-emodin is represented as space-filled model.

(a) Binding of aloe-emodin to human intercellular adhesion molecule-1 (PDB ID: 1mq8 [-39.5])

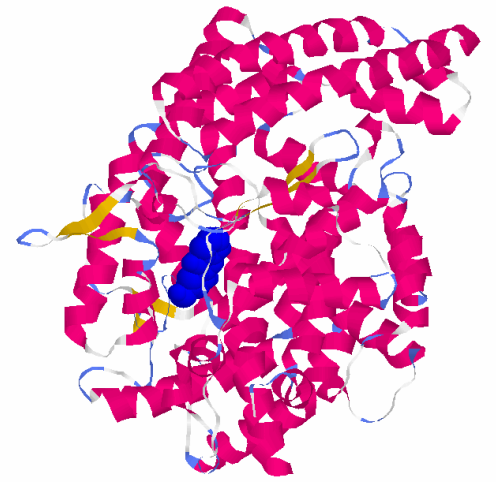


(b) Binding of aloe-emodin to human matrix metallo proteinase-8 (PDB ID: 1jap [-51.1])

(c) Binding of aloe-emodin to human cathepsin S (PDB ID: 2f1g [-45.2])



(d) Binding of aloe-emodin to human angiotensin converting enzyme (PDB ID: 1o86 [-45.2])



4.3.2 Metastatic-Related Targets of Emodin, Aloe-Emodin, and Rhein Identified by INVDOCK

Metastasis is a wide range process that involves cell-cell adhesion, regulation of cytoskeleton, cell signaling in survival and proliferation, angiogenesis, and cell migration. Hence, a wide range of important proteins mediate in these processes play an important role in regulating the fidelity of metastagenecity. These proteins include receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) that serve as in initiating the early event of metastatic-related pathways, small GTPases such as RhoA and Rac1 that regulate formation of cytoskeleton, proteinases such as matrix metalloproteinases (MMPs) that involve in detachment of cell adhesion and migration, angiotensin-converting enzymes (ACE), cell adhesion molecules (CAM) that mediate cell-cell adhesion, and prostaglandin synthesizing enzymes such as cyclooxygenase 2 (COX2).

The metastatic-related targets of emodin, aloe-emodin, and rhein identified from INVDOCK search were listed and compared with previous experimental works in **Table 4.4**. Results from both experimental works and INVDOCK implicate that emodin and aloe-emodin are more potent in their anti-metastasis activity than rhein. Although less works had been done on rhein, less metastatic-related targets identified from INVDOCK search implicates that rhein is less effective in inhibiting metastasis process. However, as shown in **Table 4.4**, rhein may be most effective in the stage of reducing cell detachment as it inhibits the activity of MMPs. **Table 4.4** shows that both emodin and aloe-emodin exhibit the anti-metastatic activity at various stages of metastasis process.

Table 4.4 Involvement of selected Rhubarb anthraquinones (emodin, aloe-emodin, rhein) in different stages of anti-metastatic processe as compared from both experimental findings as denoted in Pubmed ID and INVDOCK results as denoted in PDB ID. Exp: Experimental, INV: INVDOCK results, None: No report on a particular target for a given Rhubarb anthraquinone implicates insufficient studies performed or no result from INVDOCK.

Stages and Signaling Pathways of Metastasis	**Involvement of selected Rhubarb anthraquinones in different stages of metastatic process**					
	Emodin		**Aloe-Emodin**		**Rhein**	
	Exp	INV	Exp	INV	Exp	INV
Angiogenesis Angiotensin-Converting Enzymes (ACE)	16730806	1o86 (human)	None	1o86, 1r42 (human)	None	None
Interleukins	16433064	2b5i (human)	16900781	2b5i (human)	9858439	1rwk, 1ibc, 2b5i (human)
Receptor-2 (KDR/Flk-1)	16388516	None	None	None	None	None
Cell Adhesion Focal Adhesion Kinase (FAK)	16740720	1mp8 (human)	None	None	None	1mp8 (human)
Intercellular adhesion molecule-1	None	None	None	1mq8 (human)	None	1mq8 (human)
Cell Invasion (Extracellular matrix degradation and cell migration) Matrix metalloproteinases	16077936	1jap, 1jj9, 830c, 966c (human)	None	1jap, 1g49 (human)	11300749	1jap, 1g49, 966c (human)
14-3-3 proteins	None	1qja, 2c63, 2c74, 2btp (human)	None	1ywt, 1qja, 2btp, 2c74 (human)	None	None
Cathepsins	None	1m6d, 1sp4, 1kyn (human)	None	1cte (rat), 2f1g (human)	None	1cte (rat)
Actin	None	1nm1 (human)	12076313	1nm1 (human)	None	None

Catenins	None	1l7c, 1dow (human)	None	1h6g (human)	None	None
Metastatic-Related Signaling Molecules						
Extracellular Signal-Regulated Protein Kinase (ERK)	15194008	4erk (rat ERK2), 1gol (rat ERK2)	15747063	4erk (rat)	14527176	None
Epidermal Growth Factor Receptor (EGFR)	15928809	1ivo (human)	None	None	None	None
Protein Tyrosine Protein Kinases	8744799, 1517743	1a07 (human c-src tyrosine kinase), 1bbz (human ABL tyrosine kinase), 1qpj (human LCK tyrosine kinase)	None	2src, 1a1c (human c-src), 1bbz (human ABL tyrosine kinase), 1qpe, 1qpj (human LCK tyrosine kinase	None	None
p38	17074319	1bl7 (human)	12794753	1bl7, 1di9, 1kv1 (human)	15696926	1bl7, 1di9 (human)
Rho-associated protein kinase 1	None	None	None	2eto (human)	None	None
RhoA	None	None	None	1cc0 (human)	None	None
Phosphatidylinositol 3-Kinase (PI3K)	15928809	2a98, 1w2d (human)	None	None	None	1e8z (human)
Cdc42	15928809	1u4d , 1u54 (human)	None	1u4d (human)	None	None
Rac1	15928809	None	None	None	None	None
NF-κB	15194008	1lei (mouse)	None	1lei (mouse)	12193257	1lei (mouse)
Activator Protein-1 (AP-1)	15194008	None	None	None	12632075	None
Cyclooxygenases (COX)	12391547	4cox, 5cox, 6cox (mouse)	None	4cox (mouse)	None	None

Recent experimental works suggested that aloe-emodin is more cytotoxic and more effective in inducing apoptosis than emodin [Lu *et al.* 2007]. In addition, proteomic study showed that aloe-emodin was able to decrease cell migration via up-regulating nm23 a metastasis suppressor gene [Lu *et al.* 2007]. Experimental works indicate that nuclear receptors such as retinoic acid receptor (RAR), estrogen receptor (ER), and glucocorticoid receptor (GR) up-regulate and nuclear receptor such as thyroid hormone receptor (THR) down-regulate the expression of nm23 (**Table 4.5**). So far there is no experimental works done to explore the effect of Rhubarb anthraquinones on nuclear receptors towards nm23 expression and activity. Results from INVDOCK suggest that both emodin and aloe-emodin bind to these nuclear receptors implicating that these anthraquinones affect gene expression regulated by these nuclear receptor. In contrast, rhein only binds to estrogen receptor as suggested from INVDOCK search.

A ligand that binds competitively to the enzyme active site is most likely behaving as a competitive inhibitor of the enzyme. However, the ability of Rhubarb anthraquinones to bind to the ligand binding domain (LBD) of a nuclear receptor does not directly implicate their agonism or antagonism towards this receptor. Both agonists and antagonists are known to bind to LBD of nuclear receptors but at different binding modes culminating in recruiting coactivators or coreprressors, respectively. Crystallography works by Brzozowski and coworkers on estrogen receptor α (ERα) showed that the antagonist raloxifene induced repositioning of helix 12 of ERα that involved rotation of 130 degree combined with a 10-angstrom rigid-body shift towards N-terminus of the LBD as compared to the conformation induced by the agonist 17β-estradiol [Brzozowski

et al. 1997]. Thus, the agonism or antagonism of Rhubarb anthraquinones to these nuclear receptors to the expression of nm23 as identified from INVDOCK search remain for further experimental validation. Indeed, emodin was shown to act as a phytoestrogen while aloe-emodin acts as weaker phytoestrogen that competes the binding with natural estrogen 17β-estradiol for LBD of estrogen receptor [Matsuda *et al.* 2001] suggesting the capability of these anthraquinones in elevating the expression of nm23. Besides, emodin was shown to inhibit the growth of prostate cancer cells via down-regulating androgen receptor [Cha *et al.* 2005]. As shown in **Table 4.5** the capability of emodin and aloe-emodin in regulating the expression of nm23 via androgen receptor remain for future works.

Among these nuclear receptors, thyroid hormone receptor (THR) was shown to reduce the expression of nm23 [Lin *et al.* 2000]. Only anthraquinone aloe-emodin binds to LBD of THR as identified from INVDOCK search. As the cellular work on the human hepatoma HepG2 suggested that aloe-emodin but not emodin induce expression of nm23 [Lu *et al.* 2007] the antagonism of aloe-emodin towards THR on HepG2 cell remain for further investigations.

Table 4.5 Involvement of selected Rhubarb anthraquinones (emodin, aloe-emodin, rhein) in regulating the expression and activity of metastatic suppressor gene nm23 via binding to nuclear receptors.

Nuclear Receptors	Effect of receptor agonists to nm23 activity or expression	Status of INVDOCK-generated results		
		Emodin	Aloe-Emodin	Rhein
Orphan nuclear receptor retinoid Z receptor β (RZRβ)	Interacts with nm23-2 [8858107]	None	None	None
Retinoic acid related orphan receptor α (RORα)	Interacts with nm23-2 [8858107]	None	None	None
Retinoic acid receptor (RAR)	Elevates nm23 expression [12848344]	1fm6, 2nll (human), 1xdk (mouse)	2nll (human)	None
Estrogen receptor (ER)	Elevates nm23 expression [12848344]	1a52, 1gwq, 1r5k (human)	1err, 1gwr (human)	1err, 1gwr (human)
Glucocorticoid receptor (GR)	Elevates nm23 expression [12848344]	1nhz (human)	None	None
Thyroid hormone receptor (THR)	Reduces the elevation of nm23 [10875256]	None	1bsx (human)	None
Androgen receptor (AR)	Remain for further investigations	1t74 (chimpanzee)	1t76 (chimpanzee)	None
Bile acid receptor	Remain for further investigations	1ot7 (rat)	1ot7, 1osv (rat)	None
Vitamin D receptor	Remain for further investigations	1db1, 1txi (human)	1rkg (rat)	None
Retinoid X receptor (RXR)	Remain for further investigations	1mzn (human)	1mzn (human)	None

4.4 Discussion

4.4.1 Metastatic-related targets of Rhubarb Anthraquinone Emodin, Aloe-Emodin, and Rhein identified from INVDOCK search

The lethality of metastatic spread of tumor cells from their original site include angiogenesis, cell adhesion, cell invasion (extracellular matrix degradation and cell

migration), and cell proliferation. These processes involve myriad of cell signaling pathways, regulation of cytoskeleton and cell motility. Recent work from Massague's group using microarray data showed that EGFR, epiregulin, MMP1, MMP2, and COX2 are essential to mediate breast cancer metastasis in lung [Minn *et al.* 2005]. Their subsequent work using different combinations of drugs that target these proteins showed either additive or synergistic effects to inhibit the spread of metastatic breast cancer cells in mice [Gupta *et al.* 2007]. As Rhubarb anthraquinones target to EGFR, MMP1, MMP2, and COX2 as implicated from INVDOCK search (**Table 4.4**), this implicates that Rhubarb anthraquinones such as emodin, aloe-emodin, and rhein possess either additive or synergistic effects in inhibiting metastasis of cancer cells when used in combinations. The clinical values of these anthraquinones in anti-metastasis remain for future investigations.

Aloe-emodin but not emodin was shown to up-regulate a metastasis suppressor gene nm23 in human HepG2 carcinoma cells. However, the dependence of nm23 up-regulation by Rhubarb anthraquinones on the aspect of cellular context remained for further works. Agents that are known to elevate nm23 expression interact with nuclear hormone receptors including RAR, ER, and GR [Ouatas *et al.* 2003]. The estrogen E2 acts through estrogen receptor α (ERα) via a positive estrogen-responsive element (ERE) in the promoter region of the nm23-H1 gene [Lin *et al.* 2002]. However, binding of thyroid hormone receptor (THR) by thyroid hormone reduces the elevation of nm23 [Lin *et al.* 2000]. The thyroid hormone T3 acts through THR inhibiting the transcription of nm23-H1 via binding to a negative regulatory element in the promoter region of the gene

[Lin *et al.* 2000]. This suggests that T3 promotes tumor metastasis by down-regulating the expression of metastasis suppressor gene nm23-H1. INVDOCK results suggest that only aloe-emodin binds to THR (**Table 4.5**) showing the difference of emodin and aloe-emodin in their anti-metastatic activities. However, the antagonism of aloe-emodin on THR in HepG2 cells remains to work out.

4.4.2 Limitations and future improvement of INVDOCK and plans to incorporate INVDOCK results with experimental works

The discrepancy between the INVDOCK results and available experimental data arises due to a number of reasons. It is not expected that exhaustive experiments have been performed to determine all protein targets of Rhubarb anthraquinones. The lack of sufficient experimental data is likely to be an important factor for the discrepancy. Another source of discrepancy is the lack of relevant protein structures. A large number of known membrane-bound therapeutic targets especially G protein-coupled receptors (GPCRs) are not available. In addition, some entries in protein structure database may be of little relevance such as entries containing incomplete sections or chains. “False hits” may thus be generated if these irrelevant structures are selected by INVDOCK. Progress in structural genomics is expected to provide a more diverse set of relevant structures. Besides, studies of protein function facilitate the selection of potential protein targets related to a particular cellular or physiological condition. In addition, incorporation of proteomics data in term of cellular context and gene expression profiles is expected to improve the performance of INVDOCK.

Exhaustive search to find optimum binding modes of Rhubarb anthraquinones to their targets are not conducted due to heavy computational load as all protein cavities in the PDB are used. However, the docked structures from INVDOCK are sufficient to identify putative binding residues. Alanine scanning can then be applied to protein regions that cover the contacting residues for Rhubarb anthraquinones to further explore their binding affinities and activities.

Chapter 5 The Study of Molecular Mechanism of Synergistic Effects in Herbal Ingredients

There have been different views about the therapeutic efficacy of herbal supplements and medicinal herbs. Their use in therapeutics is primarily based on the hypothesis that multiple ingredients are better than one. The reported efficacy of certain herbs at apparently lower doses of active constituents suggests a need for molecular study to determine whether the efficacy is due to placebo or synergistic effects. Knowledge of synergism in some herbs, if confirmed, may be further explored for developing novel cock-tail therapeutics. This chapter provides in-depth and comprehensive analyses of lessons learned from rigorous molecular study of clinical combinations of drug-drug interactions to exhibit molecular interaction profiles likely contributing to herbal synergism. Current study of herbal synergism including literature reported synergistic events, analysis methods in comparison with those of drug combination studies, clinical trial or in vivo test results, known commercial explorations, and the molecular interaction profiles of active ingredients supporting the proclaimed synergism.

5.1 Introduction

5.1.1 Needs of evaluating synergistic mechanisms of herbal recipes

There are conflicting views to the mechanism of reported therapeutic efficacies of herbs. One view argues that the reported efficacies of herbs can be due to placebo effects [Tausk 1998; Beyerstein 2001; Kaptchuk 2002; Lewith *et al.* 2002], and the other

attributes them to synergistic actions of the active ingredients in the corresponding herbs [Stermitz *et al.* 2000; Williamson 2001; Spinella 2002; Gilbert *et al.* 2003]. While their exact mechanism is still unclear, herbs have been widely explored as supplements and for therapeutic applications particularly in traditional medicines [Eisenberg *et al.* 1998]. Herb-based therapeutic approaches have been recognized as a valuable alternative to conventional medicine [Chan 1995a; Cheng 2000; Yuan *et al.* 2000a; Ang-Lee *et al.* 2001b; Lazar 2004b]. Approximately 12% of the US population [Eisenberg *et al.* 1998] and 22%~23% of the preoperative and ambulatory patients [Kaye *et al.* 2000; Tsen *et al.* 2000] have used herbal products in surveys conducted in the 1990s. For instance, positive outcomes have been obtained in rigorous clinical trials of a number of herbal extracts (e.g. *Ginkgo biloba* EGb761 [Mazza *et al.* 2006], St John's wort [Fava *et al.* 2005; Kasper *et al.* 2006], north American ginseng [Predy *et al.* 2005], *Isatis tinctoria* [Heinemann *et al.* 2004], and others [Kupfersztain *et al.* 2003; McKay *et al.* 2003; Kennedy *et al.* 2004; Kucera *et al.* 2004; Collene *et al.* 2005; Lopatkin *et al.* 2005; Tang *et al.* 2005; Winther *et al.* 2005; Huseini *et al.* 2006; Kemmerich *et al.* 2006], individual herbs [Bub *et al.* 200; Bradwejn *et al.* 2000; Simpson *et al.* 2001; Rein *et al.* 2004; Cheng *et al.* 2005; Jayawardena *et al.* 2005; Dowdy *et al.* 2006], and multi-herb recipes (e.g. Zemaphyte [Sheehan *et al.* 1992], PC-SPES [Oh *et al.* 2004] and others [Andersen *et al.* 2001; Hioki *et al.* 2004; Saxena *et al.* 2004; Chang *et al.* 2005; Hsu *et al.* 2005; Huseini *et al.* 2005; Kaiho *et al.* 2005; Morin *et al.* 2005; Naser *et al.* 2005; Park *et al.* 2005; Satoh *et al.* 2005; Suehiro *et al.* 2005; Ushiroyama *et al.* 2005; Wen *et al.* 2005; Zhong *et al.* 2005; Chan *et al.* 2006; Elder *et al.* 2006; Odaguchi *et al.* 2006; Wang *et al.* 2006; Wcw *et al.* 2006]. Because of their extensive applications, there is a need to scientifically evaluate the

therapeutic efficacy of herbs and multi-herb recipes and to determine their true mechanism of actions.

Significant progress has been made in probing and analyzing bioactive ingredients of herbs [Sutter *et al.* 1993; Zhu *et al.* 1996; Li *et al.* 1998; Gong *et al.* 1999; Lee 1999]. In fact, approximately one third of the top-selling drugs currently in the market were derived from plant ingredients [Strohl 2000]. Natural products continue to be used as valuable sources for deriving new drug leads [Harvey 2000]. In spite of the wealth of information about their ingredients, the molecular mechanism and clinical pharmacology is known for only a small percentage of herbs [Sutter *et al.* 1993; Gong *et al.* 1999]. This hinders efforts for standardizing and scientifically evaluating herb-based therapeutics. A lack of the knowledge of mechanism also makes it difficult for more extensively exploring the therapeutic potential of some of the useful herbs and herbal recipes. Therefore more research is needed to fully understand the molecular physiological effects and therapeutic implications resulting from the interactions of herbal ingredients with human body and disease processes.

It has been found that the contents of active ingredients in some herbs are lower than those needed to produce the expected therapeutic effects [Schmid *et al.* 2001; Williamson 2001; Danz *et al.* 2002], which has led to the skepticism about herbal medicines and the suggestions that therapeutic efficacies of herbs are due primarily to placebo effects[Tausk 1998; Beyerstein 2001; Kaptchuk 2002; Lewith *et al.* 2002]. On the other hand, there have been reports of the total contents of some herb showing a

significantly better effect than an equivalent dose of a single isolated active compound [Williamson 2001; Leonard *et al.* 2002; O'Byrne *et al.* 2002; Carpinella *et al.* 2003], and some herbal combinations being more effective than the constituent herb used alone [Scholey *et al.* 2002]. There are observations that in some cases alternative medicines are not necessarily associated with their positive or negative effects [Lewith *et al.* 2002]. These findings suggest that, while placebo effects may play important roles in some herb-based therapies, it is possible for some of the herbs or herbal products to produce their therapeutic effects by synergistic, additive, or potentiative actions of their active ingredients [Stermitz *et al.* 2000; Williamson 2001; Spinella 2002; Gilbert *et al.* 2003]. Additive actions are also capable of enhancing therapeutic effects albeit at a less significant level per amounts of compounds than those of synergstic and potentiative actions. However, current work only focuses on the synergistic and potentiative actions to literature described synergistic effects of active herbal ingredients.

5.1.2 Lessons from investigations of molecular mechanism of additive, synergistic, antagonistic, potentiative, and reductive effects of clinical drug-drug interactions

Knowledge and tools generated from the extensive studies of additive, synergistic, antagonist, potentiative, and reductive effects are mostly from combinations of clinical drugs [Peters *et al.* 2000; Barrera *et al.* 2005; Jonker *et al.* 2005; Chou 2006; Tallarida 2007]. The combinatorial effects of clinical drugs can be applied to investigate the effects and molecular mechanism of the interaction between herbal ingredients.

When two drugs produce similar effects, their combination collectively produces the same effect of various magnitudes in comparison to the summed response of the individual drugs. A combination is pharmacodynamically additive, synergistic, or antagonistic if the effect is equal to, greater than, or less than the summed response of the participating drugs. Drug combinations may also produce pharmacokinetically potentiative or reductive effects such that the therapeutic activity of one drug is enhanced or reduced by the action of the other drug via regulation of its metabolism, transport, permeation, distribution or localization [Chou 2006]. Synergism of drug combinations have been explored for therapeutic applications to achieve one or more of the following favorable outcomes: enhanced therapeutic efficacies, decreased dosage with the same or increased level of efficacy for reducing side-effects, reduced or delayed development of drug resistance, simultaneous enhancement of actions against a target and reduction of unwanted actions (efficacy synergism plus toxicity antagonism) [Chou 2006].

Synergism is hence expected to occur if two or more herbal ingredients mutually enhance each other's therapeutic effects more significantly than simple sum of these ingredients [Phillipson 1999; Williamson 2001; Spinella 2002; Gilbert *et al.* 2003]. Signficant enhancement effects may occur via pharmacokinetic actions such as the modulation of bioavailability of active ingredients by others [Stermitz *et al.* 2000; Spinella 2002]. In fact, combination of different herbs may eliminate problematic side effects associated with some of the ingredients [Morre *et al.* 2003b; Shao *et al.* 2004]. These might be the main reasons why many of the proclaimed effective herbal products have been provided as sets of multi-component herbal extracts or multiple herbs, and why

the reported therapeutic efficacy of these herbal products [Williamson 2001; Spinella 2002; Gilbert *et al.* 2003] and traditional medicines [Cheng 2000; Yuan *et al.* 2000a] are believed to arise from collective effects of herbal ingredients. Although synergistic effects are considered to be positive and beneficial in general, there are also instances that show negative and adverse reactions that tend to be more apparent with combinations of herbs than with prescribed synthetic medicines [Williamson 2001]. Therefore, comprehensive studies at the molecular level and clinical analyses are needed to conclusively demonstrate the existence of herbal synergism and their real contribution to the therapeutic and toxicological effects of herbal products.

5.2 Methods

5.2.1 Literature search method for cases of drug-drug interactions

A number of computational analysis methods have been developed and extensively used for accurately studying the effect of drug combinations, which are particularly useful for differentiating synergistic and additive effects. Examples of the popular methods are checkerboard method, combination index, fractional effect analysis, isobolographic analysis, interaction index method, median drug effect analysis, and response surface approach [Peters *et al.* 2000; Barrera *et al.* 2005; Jonker *et al.* 2005; Chou 2006; Tallarida 2007]. Other methods such as Valeriote & Lin's comparative analysis method [Primeau *et al.* 2003], Yonetani & Theorell plot [Cruchaga *et al.* 2005] and 3D model of drug-drug interactions[Prichard *et al.* 1990] have also been used for studying drug combinations.

Insights into how drug combinations contribute to their additive, synergistic, antagonistic, potentiative, or reductive activities can be obtained from analysis of reported cases of known mechanisms of actions as well as assessments of combinatorial effects by established drug effect analysis methods. Relevant literatures were searched in Medline [Wheeler *et al.* 2004] by using combinations of keywords such as "drug combination", "drug-drug interaction", "additive", "synergism", "synergy", "synergistic", "antagonism", "antagonistic", "potentiation", "potentiate", "potentiative", "reductive", "reduction", and "reductive" to identify 12, 35, and 8 cases of pharmacodynamically additive, synergistic, and antagonistic drug combinations and 18 and 7 cases of pharmcokinetically potentiative and reductive drug combinations published in 2000-2006 where the molecular mechanism of combination effects have been determined by established synergism/additive/antagonism analysis.

5.2.2 Literature search method for cases of herbal synergism

Combinations of keywords of "herb", "herbs', "herbal", "plant", or "plants' together with those of "synergy", "synergistic", and "synergism" were used to search the Medline database [Wheeler *et al.* 2004] to find literature reported cases of synergism of herbs and herbal products, which were subsequently checked for the availability of the information about the active ingredients of the herbs involved. The information of these active ingredients were searched from authoritative books of medicinal herbs [Xinquan *et al.* 2002] and Medline database [Wheeler *et al.* 2004] by using combinations of herb names and the keywords "ingredient", "constituent", or "component". Some of the

identified herbs or herbal extracts, and thus their reported synergistic effect, have been explored in commercial products. The relevant information was searched by using the combinations of herb names and the keywords “commercial”, “product”, or “company” via Google.

5.3 Results

5.3.1 Pharmacodynamically additive, synergistic, and antagonistic combinations of clinical drugs

The reported cases of these pharmacodynamic and pharmacokinetic drug combinations are summarized in **Table 5.1** and **Table 5.2**, respectively. As shown in **Table 5.1** there are 5, 5, 1 and 1 of the 12 cases of pharmacodynamically additive drug combinations involve drug actions at the same target, different targets of related pathways, different targets of the same pathway, and different targets of unrelated pathways, respectively. Many of these additive drug combinations are associated with overlapping drug actions at the same target that might produce mutually substitutable or redundant effects. For instance, combination of doxorubicin and Ecteinascidin-743 produces additive anticancer effect via redundant action of DNA intercalation and covalent guanine adduct formation at specific sites in DNA minor groove [Meco *et al.* 2003].

In 3 out of 5 cases of additive drug combinations acting on different targets of same pathway, additive effects appear to occur when the corresponding actions are

significantly overlapping such that they produce focused actions on the activity and expression level of a specific target and its closely associated molecules. For instance, retinoic acid and trichostatin A additively inhibit cell proliferation by overlapping actions in up-regulating retinoic acid receptor beta and reactivation of its messenger RNA (mRNA) expression [Touma *et al.* 2005]. There are 2 cases of additive drug combinations acting on different targets of different pathways, in which the corresponding actions appear to be independent of each other. For instance, CP55940 (cannabinoid analog) and morphine produce additive antinociceptive effects in some cases via independent actions of cannabinoid antagonism and mu opioid receptor agonism [Tham *et al.* 2005].

There are 12, 15, and 5 of the 32 cases of synergistic drug combinations that involve drug actions at different targets of the same pathway, different targets of related pathways, and different binding sites or different sections of the same site of the same target, respectively. Non-competitive inhibitors, antagonists, and blockers are potential candidates for finding synergistic combinations that interact with the same target. Synergistic drug combinations in these 32 cases appear to primarily arise from complementary (19 cases), facilitating (4 cases), negative effect reducing (3 cases), derivative which derives new effect (1 case), and partially overlapping (5 case) actions. The remaining 4 cases involve overlapping or independent actions and it is unclear how these combinations lead to synergism. One possible reason for these cases is that additional interactions contributing to the observed synergism have not been exposed. An example of complementary actions against different targets of the same pathway is

cisplatin-topotecan combination for producing synergistic cytotoxic activity [van Waardenburg *et al.* 2004]. Cisplatin forms inter- and intra-DNA strand adducts that inhibites DNA polymerization. On the other hand, topotecan targets DNA polymerase I by reversibly stabilizing covalent enzyme-DNA intermediate, which stops mutagenic translesional bypass replication across cisplatin-DNA adducts and subsequently produces complementary effects [Lin *et al.* 1999; Rhee *et al.* 2002].

An example of negative effect reducing combinations is tamoxifen-trastuzumat combination that synergistically inhibits the growth of estrogen receptor positive, HER-2/neu-overexpressing BT-474 breast tumor cells [Argiris *et al.* 2004]. Tamoxifen is an estrogen receptor antagonist and rastuzumab is an anti-HER-2/neu antibody that interacts with different targets of related pathways. Trastuzumab stops HER-2/neu induced activation of estrogen receptor and coactivator AIB1 (amplified in breast cancer-1), while tamoxifen stops estrogen receptor induced activation of growth factor pathways, thereby reducing the negative effects of each other [Osborne *et al.* 2003].

There are 5 and 2 of the 7 cases of antagonistic drug combinations that involve drug actions at the same target and different targets of related pathways, respectively. While the exact mechanism of antagonism has not been explicitly described for the cases involving drug actions at the same target, one possible mechanism is mutual interference at the same target site, which can be illustrated by the carboplatin- paclitaxel combination [Guminski *et al.* 2001]. Carboplatin is a cisplatin anolog that forms covalent adduct with DNA and alters its local conformation. Paclitaxel is an external DNA binder that partially

stabilizes DNA helix without altering its B-conformation. Such a binding conformation is incompatible with that of carboplatin, which may be an important factor for the antagonism.

Combination of other mutually interfering pairs of twin competitive inhibitors, antagonists, blockers, activators, or agonists may produce pharmacodynamically antagonistic effects. Likewise, inhibitor-activator, antagonist-agonist, or blocker-substrate pairs that bind to the same site may also produce mutually antagonistic effect. A possible mechanism for antagonistic drug combinations against different targets of related pathways is the counteractive actions that make it unfavorable for a partner drug to perform its normal actions, which can be illustrated by the Ara-C and 17-AAG combination [Pelicano *et al.* 2006]. Ara-C is a DNA binder, and 17-AAG is a heat-shock protein antagonist that abrogates Akt survival pathway. 17-AAG antagonizes the cytotoxic activity of ara-C, due in part to the induction of G1 arrest, thereby preventing ara-C incorporation into cellular DNA.

5.3.2 Pharmacokinetically potentiative and reductive combinations

As shown in **Table 5.2** there are 9, 8 and 5 of the 22 cases of potentiative drug combinations that involve modulation of drug transport/permeation, distribution/localization, and metabolism, respectively. The primary mechanisms of potentiative modulations of drug transport/permeation are enhancement of drug absorption via disruption of transport barrier, delay of barrier recovery, or reduction of first-pass excretion via inhibition of drug efflux. The main mode of actions of

potentiative modulation of drug distribution/localization is to increase drug concentration in plasma or specific tissue by blocking drug uptake and inhibiting metabolic processes that convert drugs into excretable forms. Potentiative metabolism modulation is primarily associated with stimuation of the metabolism of drugs into active forms, and inhibition the metabolism of drugs into inactive forms. Typical potentiative effects can be illustrated by the example of cyclosporine enhancement of the actions of rosuvastatin [Simonson *et al.* 2004]. Rosuvastatin decreases levels of atherogenic lipoproteins in patients with or at high risk of cardiovascular disease via HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) inhibition. As a substrate of OATP-C (organic anion transporting polypeptide-C), systemic exposure of rosuvastatin is decreased by OATP-C mediated uptake process. Inhibition of this process by cyclosporine therefore enables an increase of rosuvastatin concentration.

There are 2, 2 and 3 of the 7 cases of reductive drug combinations that involve modulation of drug transport/permeation, distribution/localization and metabolism, respectively. Reductive modulation of drug transport/permeation typically involves the blocking drug absorption or promotion of first-pass excretion by such actions as drug-drug aggregation to reduce permeability and inhibition of drug transport into plasma or target site. Reductive modulation of drug distribution/localization is associated with the decrease of drug concentration in plasma or specific tissue, which typically involves stimuation of metabolic processes for converting drugs into excretable forms and inhibition of metabolic processes for increasing drug concentation. In addition, drug

activity can also be reduced by metabolism modulation to covert drugs into inactive forms.

Table 5.1 Literature reported pharmacodynamically additive, synergistic, and antagonistic drug combinations in 2000-2006, where reported action has been determined by well established synergy/additive analysis methods and its molecular mechanism has been revealed.

Class of drug-combination effect	Type of target relations	Mode of actions	Drug combinations reported to have the effect arising from this mode of actions [reference]	Example of drug-combination and reported mode of actions [reference]
Pharmacodynamically additive combination	Same target	Mutually substitutable actions	Ampicillin-Imipenem[Rand *et al.* 2004]; Diazoxide-Sodium nitroprusside[Alves *et al.* 2004]; Diazoxide-Dibutyryl-cGMP[Alves *et al.* 2004]; Propofol –Sevoflurane[Harris *et al.* 2006];	Ampicillin blocked penicillin-binding protein 2A and thus bacterial cell wall synthesis. Imipenem inhibited penicillin-binding protein -1A, -1B, -2, -4 and -5. These produce mutually substitutable actions on penicillin-binding protein 2A, producing additive antibacterial effect[Rand *et al.* 2004]
		Overlapping actions on different binding sites	Doxorubicin-ET-743[Meco *et al.* 2003];	Doxorubicin intercalated DNA. ET-743 formed covalent guanine adduct at specific sites in DNA minor grove and interacted with DNA repair system, producing additive anticancer effect[Meco *et al.* 2003]
	Different targets of the same pathway	Overlapping actions on targets of the same pathway	Retinoic acid -Trichostatin A[Touma *et al.* 2005];	Retinoic acid up-regulated retinoic acid receptor beta. Trichostatin A, a histone deacetylase inhibitor, reactivated retinoic acid receptor beta mRNA expression. They produce overlapping action by interacting with the up- and down- stream targets of the same pathway, resulting in additive inhibition of cell prolifer ation[Touma *et al.* 2005]
	Different targets of related pathways	Independent actions on targets of related pathways	Anidulafungin- Amphotericin B [Karlowsky *et al.* 2006]; Artemisinin-Curcumin[Nandakumar *et al.* 2006]; CP55940-Dexmedetomidine[Tham *et al.* 2005]; CP55940-Morphine[Tham *et al.* 2005];	Anidulafungin inhibited beta-(1,3)-dglucan synthase, an essential component of fungal cell wall. Amphotericin B formed ion channels in fungal membranes. They independently targeted different targets of related pathways, producing additive antifungal effect[Karlowsky *et al.* 2006]
		Complementary actions on targets of related pathways	Azithromycin-Imipenem[Fernandez-Cuenca *et al.* 2003];	Azithromycin hindered bacterial protein synthesis by binding to 50S component of 70S ribosomal subunit. Imipenem inhibited penicillin-binding protein -1A, -1B, -2, -4 and -5. The former reduces penicillin-binding proteins to complement the later's inhibition of these proteins, producing additive antibacterial effect[Fernandez-Cuenca *et al.* 2003]

	Different targets of un-related pathways	Independent actions on targets of un-related pathways	Citicoline-Nimodipine[Sobrado *et al.* 2003];	Citicoline increased BCL-2 expression. Nimodipine blocked L-type voltage sensitive calcium channel. They act independently on targets of un-related pathways, producing additive neuroprotective effect[Sobrado *et al.* 2003]
Pharmacodynamically synergistic combinations	Same target	negative effect reducing actions	AZT- NNRTI[Cruchaga *et al.* 2005];	AZT is a HIV-1 reverse transcriptase inhibitor, NNRTI (Non-nucleoside HIV-1 reverse transcriptase inhibitor) inhibited RT catalyzed phosphorolysis, thereby reduced AZT resistance, resulting in antiviral synergism[Cruchaga *et al.* 2005]
		Partially overlapping actions	Cisplatin-Yondelis[D'Incalci *et al.* 2003]; Mycophenolate mofetil – Mizoribine[Shimmura *et al.* 2006]; Paclitaxel-Discodermolide[Honore *et al.* 2004]; Paclitaxel-Ecteinascidin 743 [Takahashi *et al.* 2002];	Cisplatin formed DNA inter- and intra- strand adduct, Yondelis interacted with DNA and DNA repair systems in a way different from cisplatin, leading to synergistic antitumor activity[D'Incalci *et al.* 2003]
	Different targets of the same pathway	negative effect reducing actions	Gefitinib - CPT-11[Koizumi *et al.* 2004];	Gefitinib inhibited EGFR tyrosine kinase inhibitor. CPT-11, a DNA topoisomerase I inhibitor, increased EGFR phosphorylation in Lovo & WiDR cells. EFFR tyrosine kinase inhibitor offsets negative effect of increased EGFR phosphorylation by CPT-11, producing synergistic inhibitory effect on colorectal cancer Lovo & WiDR cells[Koizumi *et al.* 2004]
		Complementary actions	5-AZA-2'-deoxycytidine – Depsipeptide[Primeau *et al.* 2003]; Candestartan-cilexetil –Ramipril[Raasch *et al.* 2004]; Celecoxib – Emodin[Lai *et al.* 2003]; Cisplatin-Topotecan[Lin *et al.* 1999; Rhee *et al.* 2002; van Waardenburg *et al.* 2004]; Gefitinib - PD98-59[Normanno *et al.* 2006]; Oxaliplatin - CPT-11[Tanaka *et al.* 2005]; Paclitaxel-Lonafarnib[Shi *et al.* 2000]; Paclitaxel-SCH66336 [Marcus *et al.* 2005]; Paclitaxel –Tubacin[Marcus *et al.* 2005]; Paclitaxel – Trichostatin[Dowdy *et al.* 2006];	5-AZA-2'-deoxycytidine inhibited DNA methyltransferase -1 and -3B. Depsipeptide, a histone deacetylase inhibitor, induced tumor suppressor genes. The former stops silencing of tumor suppressor genes in cancer cells to complement the later's induction of tumor suppressor genes, producing synergistic antineoplastic effect[Primeau *et al.* 2003]
		partially overlapping	17-AAG - Arsenic trioxide[Pelicano *et al.* 2006];	17-AAG, a heat-shock protein antagonist, abrogated Akt survival pathway. Arsenic trioxide degraded

		actions		aberrant PML-retinoic acid receptor alpha fusion protein and affected intracellular signal transduction pathways. They possiblly produce partially overlapping actions on Akt survival pathway, resulting in synergistic effect on long-term cell survival[Pelicano *et al.* 2006]
	Different targets of related pathways	Complementary actions	5-FU - RPR-115135 [Russo *et al.* 2002]; Azithromycin-Ceftazidime[Fernandez-Cuenca *et al.* 2003]; Dexmedetomidine-ST-91[Graham *et al.* 2000; Philipp *et al.* 2002]; Dipropofol-Vancomycin[Ogata *et al.* 2005]; DL-cycloserine-Epigallocatechin gallate[Zhao *et al.* 2001]; Gefitinib-Taxane[Takabatake *et al.* 2007]; R115777 - Zoledronic acid[Caraglia *et al.* 2004]; Paclitaxel -NU6140[Pennati *et al.* 2005]; Bacterial protein inhibitor-Antisense drug targeting mRNA of bacterial protein[Dryselius *et al.* 2005];	5-FU stabilized P53 due to RNA-directed effects. RPR-115135, a farnesyl transferase inhibitor, inhibited Ras farnesylation. These produce joint tumor suppressive and antiproliferative activities, leading to synergistic cytotoxic effect[Russo *et al.* 2002].
		negative effect reducing actions	Tamoxifen-Trastuzumab[Osborne *et al.* 2003; Argiris *et al.* 2004];	Tamoxifen antagonized estrogen receptor. Trastuzumab (herceptin) is an anti-HER-2/neu antibody. Trastuzumab stops HER-2/neu induced activation of estrogen receptor and coactivator AIB1, tamoxifen stops estrogen receptor induced activation of growth factor pathways, leading to synergistic growth inhibition in ER- positive, HER-2/neu -overexpressing BT-474 breast tumor cells[Osborne *et al.* 2003; Argiris *et al.* 2004]
		facilitating actions	Ampicillin-Daptomycin[Rand *et al.* 2004]; CP55940-Dexmedetomidine; Daptomycin-Rifampicin[Rand *et al.* 2004]; Gentamicin-Vancomycin[Cottagnoud *et al.* 2003];	Ampicillin blocked penicillin-binding protein 2A and thus bacterial cell wall synthesis. Daptomycin disrupted bacterial membrane structure, which enhances ampicillin penetration, producing significant antibacterial synergy[Rand *et al.* 2004]
		Extra actions	BQ-123-Enalapril[Goddard *et al.* 2004];	BQ-123 antagonized endothelin A receptor. Enalapril, an angiotensin converting enzyme inhibitor, increased ETB mRNA expression. BQ-123 displaces endogenous ET-1 from the ETA onto upregulated ETB, which produces an extra ETB mediated vasodilation activity, resulting in synergistic endothelium-dependent vasodilation enhancing actions[Goddard *et al.* 2004]

pharmacodynamically antagonistic	Same target	Possible mutual interference	Aminophylline-Theophylline[Nickels *et al.* 2006]; Chloroquine-Mefloquine[Famin *et al.* 2002]; Chloroquine-Pyronaridine[Auparakkitanon *et al.* 2006]; Tramadol-Ondansetron[Riering *et al.* 2004; Dursteler *et al.* 2006]; Carboplatin-Paclitaxel[Guminski *et al.* 2001];	Aminophylline and Theophylline both antagonized adenosine receptor. Their combination produced antagonism of inhibitory adenosine autoreceptors and release of intracellular calcium[Nickels *et al.* 2006]
	Different targets of related pathways	Mutual interference	17-AAG - Ara-C[Pelicano *et al.* 2006]; Amphotericin B-Ravuconazole[Carrillo-Munoz *et al.* 2006; Meletiadis *et al.* 2006];	17-AAG, a heat-shock protein antagonist, abrogated Akt survival pathway. Ara-C is a DNA binder. 17-AAG antagonized the cytotoxic activity of ara-C, due in part to induction of G1 arrest, thus preventing ara-C incorporation into cellular DNA[Pelicano *et al.* 2006]

Table 5.2 Literature reported pharmacokinetically potentiative and reductive drug combinations in 2000-2006, where the reported effect has been determined by established methods and its molecular mechanism has been revealed

Class of drug-combination effect	Biochemical class of potentiative effect	Mode of actions	Drug combinations reported to have the effect arising from this mode of actions [reference]	Example of drug-combination and reported mode of actions [reference]
pharmacokinetically potentiative	Drug transport and permeation	Enabled drug permeation by disrupting absorption barrier	AZT – Cineole[Narishetty *et al.* 2004]; Low molecular weight heparin – Chitosan[Thanou *et al.* 2001]; Low molecular weight heparin - Sodium caprate[Tomita *et al.* 1995; Verhaeghe 1998; Motlekar *et al.* 2005]	AZT is a HIV-1 reverse transcriptase inhibitor. Cineole formed hydrogen bonds with lipid head groups of stratum corneum lipids. Cineole enhanced cross-skin permeation of AZT[Narishetty *et al.* 2004]
		Enabled drug absorption by avoiding its efflux	Doxorubicin - HPMA copolymer[Duncan *et al.* 2005];	Doxorubicin produced anticancer effect by DNA intercalation. HPMA copolymer formed conjugate with anthracycline, which enabled bypass of multi-drug resistance[Duncan *et al.* 2005]
		Avoided drug excretion	Ciprofloxacin – Gatifloxacin[Pankey *et al.* 2005]; Fexofenadine-Probenecid[Nakajima *et al.* 2004];	Ciprofloxacin inhibited DNA gyrase, an enzyme specific and essential for all bacteria. Gatifloxacin inhibited efflux pump of ciprofloxacin, producing synergistic antibacterial action against pseudomonas aeruginosa via efflux pump inhibition[Pankey *et al.* 2005]
		Delayed recovering of drug transport barrier	Levodopa - Fatty acid synthesis inhibitor[Tsai *et al.* 1996; Koller *et al.* 1998; Babita *et al.* 2005];	Levodopa is a dopaminergic agent for treating Parkinson's disease. Fatty acid synthesis inhibitor selectively inhibited fatty acid synthesis, which delayed barrier recovery rates after barrier perturbation of drugs, thereby enhanced transcutaneous delivery of levodopa[Tsai *et al.* 1996; Koller *et al.* 1998; Babita *et al.* 2005]
	Drug distribution and localization	Enhanced level of drug in plasma by inhibiting drug metabolism or uptake	Cerivastatin – Gemfibrozil[Prueksaritanont *et al.* 2002; Fujino *et al.* 2003; Shitara *et al.* 2004]; 5-FU – Sorivudine[Kanamitsu *et al.* 2000]; Rosuvastatin-Cyclosporine[Simonson *et al.* 2004]; Rosuvastatin-Gemfibrozil[Schneck *et al.* 2004];	Cerivastatin produces cholesterol-lowering effect by inhibiting HMG-CoA reductase. Gemfibrozil inhibited OATP2 mediated uptake of cerivastatin and CYP2C8 mediated metabolism of statins, thereby increased plasma concentration of cerivastin and other statins [Prueksaritanont *et al.* 2002; Fujino *et al.* 2003; Shitara *et al.* 2004]

		Enhanced level of prodrug metabolite	HSV thymidine kinase gene and ganciclovir – Scopadulcio[Hayashi *et al.* 2006]; HSV thymidine kinase gene and ganciclovir – Ponicidin[Hayashi *et al.* 2000];	Ganciclovir in combination of HSV thymidine kinase gene therapy has been used for anticancer treatment. Scopadulcio stimulated HSV thymidine kinase activity, increased levels of ganciclovir metabolite. Improved efficacy of cancer gene therapy via enhanced activity and increased level of prodrug metabolite[Hayashi *et al.* 2006]
	Drug metabolism	Enhanced metabolism of prodrug into active metabolite	5-FU - 2'-deoxyinosine[Ciccolini *et al.* 2000]; Doxorubicin-Paclitaxel[Minotti *et al.* 2001];	5-FU is metabolized by thymidine phosphorylase and others, metabolite stabilized P53 due to RNA-directed effects, producing anticancer effect. 2'-deoxyinosine is a modulator that enhances thymidine phosphorylase activity, thereby enhanced antitumor activity of 5-fluorouracil in human colorectal cell lines and colon tumor xenografts[Ciccolini *et al.* 2000]
pharmacokinetically reductive	Drug transport and permeation	Reduced drug permeability and transport	Amphotericin B – Miltefosine[Menez *et al.* 2006];	Amphotericin B, an antileishmanial, formed aggregate with Miltefosine, an antileishmanial, thereby reduced miltefosine-induced paracellular permeability enhancement in Caco-2 cell monolayers, inhibited uptake of both drugs, decreased transepithelial transport of both drugs[Menez *et al.* 2006]
		Enhanced drug excretion	Gamma-hydroxybutyrate – Luteolin[Wang *et al.* 2007];	Gamma-hydroxybutyrate, a drug of abuse, increased dopamine concentration, its disposition and renal reabsorption is mediated by MCT1 transporter. Luteolin exhibited MCT1 transporter mediated uptake of gamma-hydroxybutyrate, which significantly increased renal and total clearances of gamma-hydroxybutyrate[Wang *et al.* 2007]
	Drug distribution and localization	Reduced level of drug in plasma by metabolism inhibition	Valproic acid – Carbapenem[Perucca 2002; Nakajima *et al.* 2004];	Valproic acid, an antiepileptic, is metabolized into valproic acid glucuronide. Carbapenem antibiotics inhibited the hydrolytic enzyme involved in the hydrolysis of valproic acid glucuronide to valproic acid, resulting in a decrease of plasma concentration of valproic acid, which caused seizures in epileptic patients due to lowered plasma levels of valproic acid[Perucca 2002; Nakajima *et al.* 2004]
		Reduced level of drug by complex formation	Cisplatin - Procainamide hydrochloride[Zicca *et al.* 2002];	Cisplatin is a DNA inter- and intra- strand adduct. Procainamide hydrochloride formed cisplatin-procainamide complex, thereby reduced cisplatin-induced hepatotoxicity via formation of less toxic platinum complex, leading to inactivation of cisplatin or its highly toxic metabolites and to a different subcellular distribution of platinum[Zicca *et al.* 2002]
	Drug metabolism	Enhanced metabolism of	Warfarin-Quinidine[Ngui *et al.* 2001];	Warfarin, an anticoagulant and antithrombotic, is metabolized by CYP3A4. Quinidine stimulated CYP3A4 mediated metabolism of

		active drug into inactive metabolite	Diclofenac-Quinidine[Ngui *et al.* 2000]; Mycophenolate mofetil – Rifampin[Kuypers *et al.* 2005];	warfarin, thereby reduced anticoagulanet effect of warfarin by stimulating its metabolism[Ngui *et al.* 2001]

5.3.3 Literature described cases of herbal synergism

In many cases, literature reported synergism is not specific enough to point to the clues about their possible molecular mechanism. Typical words or sentences of synergy descriptions in these reports are antioxidant synergism [Hwang *et al.* 2001], anti-HIV synergy [Mahmood *et al.*], synergy in spasm and pain relief [Huang *et al.*], antioxidant synergy between elements in leaf [Saada *et al.*], synergistically induced leukemia cell HL60 differentiation [Ariga *et al.*], antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin [Stermitz *et al.* 2000], camphor and 1,8-cineole produced antibacterial synergism [Viljoen *et al.* 2003], synergistic antibacterial effect of monomeric polyphenols epigallocatechin gallate (EGCG) and (-)-gallocatechin gallate (GCG) in the extract of oolong tea [Sasaki *et al.* 2004], and isoflavones extracts potentiate the antibacterial activity of alpha-linolenic acid [Morel *et al.* 2003].

In some reports, useful clues about the possible molecular mechanism of synergism are provided. For instance, it has been reported that revestratrol and catechin produced synergistic protection of PC12 cells from beta-AP toxicity of hydrogen peroxide [Conte *et al.* 2003b]. In another report, the *Ginkgo biloba* extract (EGb 761) and bilobalide have been found to provide synergistic protection against ischemia-induced neuronal death in vivo and glutamate-induced neuronal death in vitro involving anti-excitotoxicity, inhibition of free radical generation, scavenging of reactive oxygen species, and regulation of mitochondrial gene expression [Chandrasekaran *et al.* 2002]. There is also a report about phytoestrogen and ascorbic acid synergistically inhibiting low

density lipoprotein (LDL) oxidation [Hwang *et al.* 2000]. Moreover, Pd-Ia, pteryxin and Pra-C have been found to produce synergistic calcium antagonistic action for relaxing the smooth muscle of tracheas and pulmonary arteries [Zhao *et al.* 1999]. Other examples of the synergy descriptions are synergistic inhibition of low-density lipoprotein oxidation by rutin and gamma-terpinene [Milde *et al.* 2004], and the combined inhibition of estrogen-dependent human breast carcinoma by soy and tea bioactive components [Zhou *et al.* 2004].

5.3.4 Assessment of herbal synergism determination methods

The synergism determination methods used in the 19 cases of literature reported pharmacodynamic synergisms, which are given in **Table 5.3** and **Table 5.4**, can be divided into 3 groups. The first group includes 4 cases where synergism has been determined by an established drug combination analysis method such as isobolographic analysis [Mahmood *et al.* 1996], interaction index [Savelev *et al.* 2003], and ANOVA (Analysis of Variance) tools [Hsieh *et al.* 1999; Hostanska *et al.* 2003]. The second group consists of 4 cases where synergism has been determined by direct comparison of effective concentrations. Synergism is assumed to exist if the effective concentration of ingredients in combination is significantly reduced with respect to that of individual ingredient. A large enough margin of effective concentration reduction is usually needed to confidently distinguish synergism and additive effect. Two of the 4 cases appear to have large enough margin. In one case, the principal ingredients of tea extracts (-)-epicatechin, (-)-epigallocatechin-3-gallate, (-)-epicatechin-3-gallate, and theaflavins in combination are effective against AH109A cell proliferation at concentrations 10-fold smaler than in that of individual ingredient in separation [Zhang *et al.*]. In the second

case, resveratrol and catechin in combination protect PC12 cells from beta-AP (1-41) toxicity of hydrogen peroxide at concentrations 2~5 times smaler than that of individual ingredient in separation [Conte *et al.* 2003b]. The synergism detection results of the remaining 2 cases are difficult to judge.

The third group contains 11 cases where synergism has been determined by direct comparison of effects. Synergism is assumed to exist if the effect of ingredients in combination is significantly increased with respect to that of individual ingredient. Reliability of this approach depends on whether the effects of ingredients in combination are truly greater than individual alone combined. Based on the results described by the respective publications, 8 of these 11 cases appear to satisfy this criterion. For instance, capsicum-tea mixtures, which contain active ingredients (-)-epigallocatechin-3-ga llate, catechin, caffeine, and vanilloid capsaicin, have been found to produce 10-fold stronger anticancer activity than that of individual ingredient [Morre *et al.* 2003a]. Therefore the effect of these ingredients in combination is clearly greater than individual alone combined. In another example, the low-density lipoprotein oxidation inhibition activity of rutin and gamma-terpinen combination has been found to be better than the computed value of individual alone combined [Milde *et al.* 2004]. The synergism detection results of the remaining 3 cases are difficult to judge.

Overall, 74% of the 19 cases of reported synergism appear to give reliable prediction eventhough only a few of them are based on the established analysis methods on drug combination. None-the-less, the use of established drug combination analysis

methods is recommended because of their higher level of accuracy, efficiency, and conveniency in facilitating the determination of synergistic effects, particularly in distinguishing synergistic and additive effects.

5.3.5 Modes of putative molecular interactions contribute to synergism of herbal ingredients

Based on literature reported molecular analysis of additive, synergistic, antagonistic, potentiative, and reductive actions of drug combinations, it appears that the same molecular mechanisms play important roles in the postulated or proclaimed herbal synergism. For active ingredients that produce similar therapeutic actions, their combinations may lead to synergistic effects when they interact with different targets of the same pathway, or different targets of related pathways, different binding sites of the same target, or different section of the same site of the same target in such a way that their actions are complementary, or facilitating, or negative effect reducing, or derivative, or partially overlapping. Non-competivite inhibitors, antagonists, and blockers are potential candidates for finding synergistic combinations of ingredients interacting with the same target.

For active ingredients that produce different actions, e.g. one therapeutic and another non-therapeutic or irrelevant, the therapeutic effects of one ingerident may be potentiated by the second ingredient to produce a pharmacokinetically potentiative effect when the second ingredient modulates the transport/permeation, distribution/localization, or metabolism of the first ingredient in such a way that it disrupts transport barrier, delays

barrier recovery, inhibits efflux of the first ingredient, blocks uptake of the first ingredient, inhibits metabolic processes for converting the first ingredient into excretable form, stimulates the metabolism of the first ingredient into active form, or inhibits the metabolism of the first ingredient into inactive form.

While at a less significant level per amounts of compounds than those of synergistic combinations, combinations of active ingredients of similar therapeutic actions producing additive effects also lead to enhanced therapeutic effects of herbs and herbal products. Therefore, additive combinations may also need to be evaluated in studying the molecular mechanism of collective enhancement effects of herbal ingredients. For active ingredients that produce similar therapeutic actions, their combinations may lead to additive effects when they interact with completely independent targets, or the same target in such a way that their actions are mutually substitutable or redundant, or different targets of related pathways in such a way that their actions are focused on the activity and expression level of a specific target and closely associated molecules.

5.3.6 Literature reported molecular interaction profiles of herbal active ingredients

As part of the effort for studying the molecular mechanism of herbs and for screening new therapeutic drug leads from plant sources, a large number of biologically active ingredients have been extracted from herbs and other natural sources and many of these have been studied for their potential therapeutic effects [Sutter *et al.* 1993; Zhu *et al.* 1996; Li *et al.* 1998; Gong *et al.* 1999; Lee 1999]. The DNP (Dictionary of Natural

Products) module of the chemical database CCD [Chapman&Hall/CRC 2005] contains 40,000 chemicals extracted from plant sources. A traditional Chinese medicine database TCM-ID [Wang *et al.* 2005d] has a collection of 9852 ingredients isolated from herbs used in traditional Chinese medicine. These databases are therefore excellent sources for obtaining information about active herbal ingredients. Not all ingredients of an herb found from sources such as CCD [Chapman&Hall/CRC 2005] and TCM-ID [Wang *et al.* 2005d] databases are active ingredients. Additional literature study was conducted to select the active ingredients from the pool of all ingredients by the condition that they either have been explicitly reported to be an active constituent, or are of higher abundances in their host herb, or they have been reported to produce an effect consistent with a literature-described effect of their host herb.

The molecular interaction profile of an active ingredient describes its interaction with a specific biomolecule, or its interference with a biological pathway or biological process. These literature-reported profiles can be retrieved from the Medline database [Wheeler *et al.* 2004] by keyword search using the name or alternative names of an ingredient combined with the name or alternative names of each of the known proteins, pathways, and biological processes. Overall, at least one literature-described molecular interaction profile for ~50% of the ingredients can be found. The number of literature-described interacting partners of an active ingredient varies significantly, with some ingredient having more than a few dozen interacting partners. The significant variation in the number of the literature-described interaction partners likely arises partly from the intrinsic differences in the binding specificity of these ingredients and partly because of

the differences in the extent of research effort directed at these ingredients. However, statistically meaningful clues may still be obtained from these literature-described molecular interaction profiles.

A majority, about 40%, of our collected literature-described molecular interaction profiles of active herbal ingredients directly point to a specific biomolecule as the interaction partner and provide the information about the type of interaction in terms of inhibition, activation, and expression patterns. Typical examples of the relevant descriptions are genistein inhibited metmyoglobin-dependent LDL oxidation [Patel *et al.* 2001], daidzein increased catalase level by activating its promoter[Rohrdanz *et al.*], catechin gallate inhibited P-glycoprotein [Jodoin *et al.* 2002], resveratrol inhibited cytochrome P450 CYP1A2 [Chang *et al.*; Ursing *et al.*], and 2-Phenylethanol-O-(6-O-galloyl)-beta-D-glucopyranoside 8 interacted with viral protein gp120 and prevented its binding to CD4 (cluster of differentiation 4) [Mahmood *et al.*]. Hence, it is possible to determine both the pharmacodynamic synergism and pharmacokinetic potentiative effects of these herbal ingredients based on the expected effects of these interactions on the therapeutic, toxicological, or pharmacokinetic actions of some of these ingredients.

Although the relevant molecular target is not exactly specified, some of the literature-described molecular interaction profiles identify specific pathway or process as the interaction partner of an herbal ingredient and point out the pharmacodynamic or pharmacokinetic consequence of this interaction. For instance, (-)-epigallocatechin-3-gallate has been described to potently inhibit the tumor cellular proteasome activity,

which may contribute to the cancer-preventative effect of green tea [Kuhn *et al.*]. Caffeine is known to exert its antioxidant effect by quenching reactive species [Devasagayam *et al.* 1996] as well as by increasing the activity of superoxide dismutase [Rossowska *et al.* 1995]. Apparently, this kind of information is useful for finding the clues to both the pharmacodynamic and pharmacokinetic effects of these herbal ingredients

Table 5.3 List of medicinal herbs or herbal extracts whose active ingredients have been reported to produce synergistic efect. PD-ST, PD-SP, PD-RP, and PD-SP&RP refer to pharmacodynamic synergism of active ingredients that interact with the same target, different targets of the same pathway, different targets of related pathways, and different targets of the same and related pathways respectively. PK refers to pharmacokinetically potantiative effects.

Herb or Herbal Extract (principal ingredients) [Ref]	Reported synergistic effect [Ref]	Pharmacodynamic synergism determination method and reliability of synergism detection result	Literature reported clinical trial or in vivo test results of herb or herbal extract [Ref]	Clinical or commercial exploration [producer/ distributor]	Possible molecular mechanism of synergism [Ref]	Type of Synergism
Soy extract (genistein, daidzein, dihydrodaidzein, O-desmethylangolensin, equol)[Lampe 2003; Maubach *et al.* 2003]	Synergistic anticancer effect of the soy isoflavonoids in human, which are weakly active in separation but active in combination, peak isoflavone levels in urine, plasma and in breast milk are 60μM, 2μM and 0.2μM respectively [Franke *et al.* 1998]	Comparison of effects, weakly active individually and active in combination. Synergism detection result difficult to judge	Randomized trial of 24 prostate cancer patients in 3 groups each taking soy (high phytoestrogen), soy and linseed (high phytoestrogen), and wheat (low phytoestrogen) showed that high soy phytoestrogen diets reduce risk of prostate cancer development and progression[Dalais *et al.* 2004]	Iso-Rich Soy [Jarrow Formulas, USA]	Genistein is a tyrosine kinase inhibitor [Jing *et al.* 1995] and reduced the transcriptional activity of hTERT dose-dependently (5~50 mM) leading to anticancer activity [Ouchi *et al.* 2005]. Daidzein (6~100μM) reduced expression of hTERT mRNA which affected human nonhormone-dependent cervical cancer cells [Guo *et al.* 2004]. Expression of hTERT extends the life span of normal human cells [Kharbanda *et al.* 2000]. Effective concentration in combination (between 0.2μM and 2μM) is at least 3 fold smaller than that of invidual ingredient, indicative of pharmacodynamic	PD-SP

					synergism. Genistein and daidzein likely interact with different targets of the same pathway.	
Aloe vera (Lu-Hui) (aloin, barbaloin, 2'-O-p-coumaroylaloesin, 2'-O-feruloylaloesin, isorabaichromone)[Beppu *et al.*], [Yagi *et al.*]	Antioxidant synergy between elements in leaf [Saada *et al.*]; Significant amelioration in superoxide dismutase and catalase activities in lungs, kidneys and liver of rats days after exposure to radiation [Saada *et al.*]		Trial of 30 adult females with bilateral occupational dry skin with or without irritant contact dermatitis showed aloe vera gel uniformly improved skin integrity, decreased appearance of fine wrinkling, and decreased erythema in the management of occupational dry skin and irritant contact dermatitis[West *et al.* 2003]. Antioxidants are important for maintaining skin smoothness and for breaking the dry-skin cycle[Loden 2003]	Aloe Immune™ [Longevity Plus, USA]	Unknown	
Oolong tea extract (epigallocatechin gallate, gallocatechin gallate, catechin gallate, epicatechin, caffeine)[Zhang *et al.* 2004c]	Synergistic antibacterial effect of monomeric polyphenols epigallocatechin gallate and catechin gallate in the extract as observed in S. mutans cells[Sasaki *et al.* 2004]		Trial of 110 patients receiving tea tree oil regimen vs. 114 patients receiving a standard regimen for treatment of methicillin-resistant Staphylococcus aureus colonization showed that tea tree	Various Oolong tea products from Fujian China	Catechin gallate inhibited P-glytoprotein (30µg/ml)[Jodoin *et al.* 2002] and thus the eflux of epigallocatechin gallate (50µM)[Hong *et al.* 2002], thereby potentiating the antibacterial effect of later.	PK

			is effective, safe and well tolerated[Dryden *et al.* 2004] Trial of 104 patients with impetigo contagiosa showed antibacterial effect of tea liquor lotion[Sharquie *et al.* 2000]			
Tea ((-)-Epicatechin, (-)-epigallocatechin-3-gallate, (-)-epicatechin-3-gallate, and theaflavins) [Zhang *et al.*]	Tea extracts (-)-Epicatechin exhibited synergistic effects with (-)-epigallocatechin-3-gallate, (-)-epicatechin-3-gallate, and theaflavins against AH109A cell proliferation[Zhang *et al.*]	Comparison of effective concentrations, effective concentration in combination 10-fold smaler than in separation. Synergism detection result reliable.	Double-blind, placebo-controlled trial on the safety and efficacy of green tea catechins for cancer chemoprevention in 60 high-grade prostate intraepithelial neoplasia volunteers showed values of total prostate-specific antigen constantly lower with respect to placebo-treated ones, and improved international prostate symptom scores, without significant side effects or adverse effects[Bettuzzi *et al.* 2006]	Various green tea products	(-)-epigallocatechin-3-gallate inhibited proteasome activity[Kuhn *et al.*] , which induce apoptosis and show antitumor efficacy by stopping proteasome degradation of proteins crucial to apoptosis and cell cycle regulation. Epicatechin inhibited tyrosine kinase activity of PDGF-R producing anticancer effect [Sachinidis *et al.*] by stopping PDGF-R regulated multiple tumor-associated processes including stimulation of tumor cells, angiogenesis and recruitment and regulation of tumor fibroblasts[Ostman 2004]. They may produce pharmacodynamic synergism by targeting different targets of related	PD-RP

					pathways.	
Green tea (Polyphenols: (-)-epigallocatechin-3-ga llate, catechin, caffeine)[Morre *et al.* 2003a]	Synergy in augmenting and prolonging sympathetic stimulation of thermogenesis in Sprague-Dawley rats[Dulloo *et al.*]	Comparison of effects, effect 2.4-fold stronger in combination. Synergism detection result reliable	Randomized trials of 10 healthy men in 3 groups each taking green tea extract, caffeine, and placebo showed that green tea has thermogenic properties and promotes fat oxidation beyond that explained by its caffeine content per se[Dulloo *et al.* 1999]	Green tea extract product [SlimTone, UK]	Catechin-polyphenols inhibited catechol-O-methyl-transferase which degrades noradrenaline, caffeine inhibited trancellular phosphodiesterases which break down noradrenaline induced cAMP. They collectively stimulated thermogenesis by relieving different control points along the NA-cAMP axis[Dulloo *et al.*].	PD-SP
Osmitopsis asteriscoides (1,8-cineole, Camphor)[Viljoen *et al.* 2003]	Camphor and 1,8-cineole produced antibacterial synergism in *Staphylococcus aureus* infected cells [Stermitz *et al.* 2000]		No report found	Revulsan [Sopharma, USA]	Unknown	
Melia azedarach L. (Chuan-Lian-Zi) (Vanillin, 4-hydroxy-3-methoxycinnamaldehyde, (+/-)-pinoresinol)[Carpinella *et al.* 2003]	Synergistic antifungal effect against *Aspergillus flavus, Diaporthe phaseolorum var. meridionales, Fusarium oxysporum, Fusarium solani, Fusarium verticillioides*, and *Sclerotinia sclerotiorum* assays[Carpinella *et al.* 2003]		No report found	Infantile malnutrition TCM patent medicine [Jiangxi Nanchang Pharmaceuticals, China]	Unknown	
Ginkgo biloba extract (Flavonoids: Bilobalides; Ginkgolides: ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J)	Synergistic protection against ischemia-induced neuronal death in vivo and glutamate-induced neuronal death in vitro involving anti-excitotoxicity, inhibition of free radical generation, scavenging of reactive		Double-blind, placebo-controlled trial on neuropsychological functioning of 262 cognitively intact older adults showed those taking ginkgo	Various Ginkgo biloba extract products	Bilobalide (15~30μM) increased mRNA levels of COXIII subunit of cytochrome *c* oxidase and subunit 1 of NADH dehydrogenase (ND1) thus preserving brain energy metabolism and providing	PD-RP

	oxygen species, and regulation of mitochondrial gene expression[Chandrasekaran *et al.* 2002]		biloba extract EGb 761 daily for 6 weeks exhibited significantly more improvement on SRT tasks involving delayed free recall and recognition of noncontextual, auditory-verbal material, compared with the placebo controls. The EGb 761 group also demonstrated significantly greater improvement on the WMS-III FII subtest assessing delayed recognition of visual material (human faces), compared with the placebo group[Mix *et al.* 2002]		neuroprotection[Defeudis 2002]. Ginkgolide B blocked glycine-gated chloride channel (IC50 0.27µM) and glycine-activated responses in pyramidal hippocampal neurons leading to neural effect[Kondratskaya *et al.* 2004]. Thus, neuroprotective synergistic effects may arise from actions at two different points of neuronal cellular processes.	
Berberis (berberine, 5'-methoxyhydnocarpin) [Yesilada *et al.* 2002; Musumeci *et al.*]	Antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin [Stermitz *et al.* 2000]		No report found	Barberry Root Botanical [Viable Herbal Solutions, USA]	5'-methoxyhydnocarpin (1µg/ml) inhibited the berberine effluxing multidrug pump and thus increased berberine bioavailability. When combined with subinhibitory amounts of berberine, 5'-methoxyhydnocarpin caused complete inhibition of growth at a concentration of 1 µg/ml.	PK

					Berberine alone showed poor antimicrobial activity, and 5'-methoxyhydnocarpin alone had no antimicrobial activity at a concentration above 500µg/ml [Stermitz *et al.* 2000]. Hence, 5'-methoxyhydnocarpin potentiated the effects of berberine.	
Rosa damascene (Kaempferol, 2-Phenylethanol-O-(6-O-galloyl)-beta-D-glucopyranoside)[Mahmood *et al.*]	Herbal extracts produced anti-HIV synergy in HIV infected cell assays[Mahmood *et al.*]	Isobolographic analysis. Synergism detection result reliable.	No report found	One Face Toner Mist [Green Valley Aromatherapy, Canada]; Herbal facial toner [Herbal Luxuries, USA]	2-Phenylethanol-O-(6-O-galloyl)-beta-D-glucopyranoside 8 (EC_{50} =40 µg/mL) interacted with viral protein gp120 and prevented its binding to CD4 [Mahmood *et al.*], kaempferol inhibited viral protease (EC_{50} =2 µg/mL). They may produce pharmacodynamic synergism by interacting with different targets of related pathways	PD-RP
Salvia lavandulaefolia (1,8-cineole, camphor, alpha-pinene, beta-pinene, borneol, caryophyllene oxide, linalool and bornyl acetate)[Savelev *et al.*]	Synergistic and antagonistic interactions of anticholinesterase terpenoids in Salvia lavandulaefolia as tested in an in vitro assay[Savelev *et al.*]	Interaction index. Synergism detection result reliable.	Placebo-controlled, double-blind, balanced, crossover trial on mood and cognition modulating effect of this herb in 24 patients showed it is capable of acute modulation of mood and cognition in healthy young adults. *Salvia lavandulaefolia* has	Recall™ [Life Enhancement, USA]	1,8-cineole, alpha-pinene, and camphor inhibited acetylcholinesterase at IC50 values of 0.67 mM, 0.63 mM, and, >10mM[Perry *et al.*]. A 2-compound combination may produce synergism if each interacts at different site or different section of the same site of the same target. A 2-compound combination may produce	PD-ST

			been used as cholinergic and memory-enhancing agents[Tildesley *et al.* 2005].		antagonistic effect if they mutally interfere at the same binding site.	
Helichrysum pedunculatum (Linoleic acid, Oleic acid, foleic acid)[Dilika *et al.*]	Synergistic effect of linoleic acid and foleic acid, extracted from leaves of *Helichrysum pedunculatum*, against *Staphylococcus aureus* and *Micrococcus kristinae* with minimum inhibitory concentration 0.01~1.0mg/ml as tested in an in vitro assay[Dilika *et al.*]		No report found	Various body lotion products such as "Olive & Helichrysum" Body Lotion [Cheri's Country Cottage, Inc., USA]	Unknown	
Peucedanum praeruptorum Dunn. {Qian-Hu} (Praeruptorins, scopolin, pteryxin, peucedanocoumarins, nodakenetin, praerosides, 8-methoxy-psoralen)[Zhao *et al.* 1999]	Synergistic calcium antagonistic action for relaxing the smooth muscle of isolated rabbit tracheas and pulmonary arteries[Zhao *et al.* 1999]		*In vivo* test on isolated tracheas and pulmonary arteries of rabbits[Zhao *et al.* 1999]. Testing results described in third colomn.	Qian Hu, concentrated extract powder [Plum Flower®, Mayway Corp., USA]	(+/-)-praeruptorin A, pteryxin and (+)-praeruptorin A at 30μM all have calcium antagonistic action, 8-methoxy-psoralen has inhibitory effect on contraction induced by phenylephrine, these may produce a synergistic effect for relaxing the smooth muscle of tracheas and pulmonary arteries by interacting with different targets of related pathways[Zhao *et al.* 1999].	PD-RP
Schefflera bodinieri (Bodinone, Bodinone glycoside, D-sorbitol, trisaccharide, stigmasterol 3-O-glucoside, bodirin A) [Zhu *et al.* 1999]	Synergistic effects on the activities of central nervous system determined by bioassay-guided isolation in conjunction with receptor binding assays[Zhu *et al.*		No report found	None	Bodinone, bodinone glycoside and D-sorbitol selectively antagonized muscarine receptors, trisaccharide antagonized Ca2+ channel and 5HT-2	PD-RP

	1999]				receptors, stigmasterol 3-O-glucoside antagonized 5HT-2 receptors, and bodirin A antagonized dopamine-2 receptors [Zhu *et al.* 1999]. They may produce pharmacodynamic synergism on CNS by interacting with different targets of related pathways.	
St John's wort (Hyperforin, hypericin, pseudohypericin)[Hostanska *et al.* 2003]	Synergistic anticancer effect as tested on leukemia K562 and U937 cells[Hostanska *et al.* 2003]	ANOVA. Synergism detection result reliable.	No report found	MOVANA™ [Pharmaton Natural Health Products, USA]	Hyperforin activated a caspase cascade thus inducing cell death in the dark. Hypericin activated caspases-8 and -3 thus causing light-activated apoptosis in various tumor cells[Hostanska *et al.* 2003]. They collectively induce apoptosis by targeting different upstream signals of the caspase cascade.	PD-SP
Cranberries (Vaccinium macrocarpon Ait.) (Benzoic acid, proanthocyanidin, fructose, flavonol glycosides)[Seeram *et al.* 2004]	Synergistic or additive antiproliferative interactions of the anthocyanins, proanthocyanidins, and flavonol glycosides within the cranberry extract against human tumor cell lines[Seeram *et al.* 2004]		No report found	Foods Cranberry Concentrate (NOW, USA) Enzymatic Therapy ORGANICran (ORGANICran,USA)	Unknown	
Lupinus argenteus (Genistein, biochanin A, alpha-linolenic acid, gamma-linolenic acid)[Morel *et al.* 2003]	Isoflavones extracts potentiate the antibacterial activity of alpha-linolenic acid by increasing the uptake of berberine into *Staphylococcus aureus* cells[Morel *et al.* 2003]		No report found	United States Patent 6,846,494 (Nutricia,USA)	Isoflavones (10 µg/mL) may inhibit a multidrug resistance pump, which increase the potency of a weak antibacterial alpga-linolenic acid (MIC, 62.5 µg/mL)[Morel *et al.* 2003]	PK

					by maintaining its concentration, leading to pharmacokinetically potentiative effect.	

Table 5.4 List of pairs of herbs or herbal extracts reported to produce synergistic effects. PD-ST, PD-SP, PD-RP, and PD-SP&RP are defined in Table 5.3.

Herb 1 (principal ingredients) [Ref]	**Herb 2 (principal ingredients) [Ref]**	**Reported synergistic effect [Ref]**	**Pharmacodynamic synergism determination method and reliability of synergism detection result**	**Literature reported clinical trial or *in vivo* study of pair of herbs or extracts [Ref]**	**Clinical or commercial exploration [producer/ distributor]**	**Possible molecular mechanism of synergy [Ref]**	**Type of Synergism**
Soy extract (Phytoestrogens: genistein, daidzein, dihydrodaidzein, O-desmethylangolensin, equol)[Lampe 2003; Maubach *et al.* 2003]	Acerola cherry extract (Ascorbic acid)[Hwang *et al.* 2001]	Soy phytoestrogen extracts become potent low-density lipoprotein antioxidants in the presence of acerola cherry extract as tested on specific cell cultures[Hwang *et al.* 2001]	Comparison of effects, effect of combination grater than individual alone combined. Synergism detection result reliable	No report found	Protein Powder and Bio C Plus combination [Nutrilite, USA]; Ultra Bone Balance™ [Source Naturals, USA]	Genistein binds to metmyoglobin (IC50 ~40μM) and inhibited Mb-dependent LDL oxidation, leading to antioxidant effect[Patel *et al.* 2001]. Ascorbic acid (150μM) enabled metabolism of oxidant species H_2O_2 via binding to metmyoglobin[Galaris *et al.* 1997] and thus reduced metmyoglobin-dependent LDL oxidation. They may produce pharmacodynamic synergism by interacting with either different sites or different section of the same site of the same target.	PD-ST
Alfalfa extract (Phytoestrogens: formononetin, biochanin A, genistatine, daidzein)	Acerola cherry extract (Ascorbic acid)[Hwang *et al.* 2001]	Alfalfa phytoestrogen extracts become potent low-density lipoprotein antioxidants in the presence of acerola cherry extract as tested	Comparison of effects, effect of combination grater than individual alone combined. Synergism detection result reliable	No report found	Protein Powder and Bio C Plus combination [Nutrilite, USA]	Genistein binds to metmyoglobin (IC50 ~40μM) and inhibited Mb-dependent LDL oxidation, leading to antioxidant effect [Patel *et al.* 2001]. Ascorbic acid (150μM) enabled metabolism of oxidant species H_2O_2 via binding to metmyoglobin [Galaris *et al.* 1997] and thus reduced	PD-ST

		on specific cell cultures[Hwang *et al.* 2001]				metmyoglobin-dependent LDL oxidation. They may produce pharmacodynamic synergism by interacting with either different sites or different section of the same site of the same target.	
Soy extract (Phytoestrogens: genistein, daidzein, dihydrodaidzein, O-desmethylangolensin, equol)[Lampe 2003; Maubach *et al.* 2003]	Fruits (Ascorbic acid)[Proteggente *et al.* 2003]	Phytoestrogen and ascorbic acid synergistically inhibited LDL oxidation in an in vitro test[Hwang *et al.* 2000]	Comparison of effective concentrations, combination at lower concentrations producing higher effect than individual at higher concentration. Synergism detection result difficult to judge.	No report found	Vitamin C/Flavonoid complex products	Genistein binds to metmyoglobin (IC50 ~40μM) and inhibited Mb-dependent LDL oxidation, leading to antioxidant effect [Patel *et al.* 2001]. Ascorbic acid (150μM) enabled metabolism of oxidant species H_2O_2 via binding to metmyoglobin [Galaris *et al.* 1997] and thus reduced metmyoglobin-dependent LDL oxidation. They may produce pharmacodynamic synergism by interacting with either different sites or different section of the same site of the same target.	PD-ST
Green tea (Polyphenols: (-)-epigallocatechin-3-gallate, catechin, caffeine)[Morre *et al.* 2003a]	*Capsicum* (vanilloid capsaicin)[Morre *et al.* 2003a]	Capsicum-tea mixtures synergistically produced anticancer activity against cancer cells[Morre *et al.* 2003a]	Comparison of effects, growth inhibition 10-fold stronger in combination. Synergism detection result reliable	No report found	Capsibiol-T [Scientific Motive Systems, USA]	Capsaicin and (-)-epigallocatechin-3-gallate (EGCg) both blocked NADH oxidase activity of a cancer-specific cell surface protein tNOX at ED50 of ~5nM [Morre *et al.* 1995] and EC50 of 1 μM [Morre *et al.* 2000] and thus stopped the growth of tumour cells [Chueh *et al.* 2004] by inducing tNOX related apoptosis [Morre *et al.* 2006]. They may produce pharmacodynamic synergism	PD-ST

						by interacting with either different sites or different section of the same site of the same target.	
Green tea (Polyphenols, (-)-epigallocatechin-3-gallate, catechin, caffeine)[Morre *et al.* 2003a]	Soy extract (genistein, daidzein, dihydrodaidzein, O-desmethylangolensin, equol)[Lampe 2003; Maubach *et al.* 2003]	Combined inhibition of tumor angiogenesis, and reduction of estrogen receptor (ER)-alpha and serum levels of insulin-like growth factor (IGF)-I by soy and tea bioactive components leading to anti-cancer effect as tested in female SCID mices[Zhou *et al.* 2004]	Comparison of effects, significant effect in combination and no or low effect in separation. Synergism detection result likely reliable	Phase III trial being conducted on 154 patients by National Cancer Institute to test prostate cancer prevention activity of Soy isoflavones and green tea, outcome of this and earlier trials not reported[Green wald 2004]	Soy Fusion [American Soy Products, Inc. USA] Green Tea Extract with added soy [NOW, USA] Pearl Green Tea Organic Soymilk [Kikkoman International Inc.USA] Green Tea Soymilk [VITASOY, USA]	(-)-epigallocatechin-3-gallate (EGCg) blocked NADH oxidase activity of a cancer-specific cell surface protein tNOX at EC50 of 1 μM[Morre *et al.* 2000] and thus stopped the growth of tumor cells[Chueh *et al.* 2004] by inducing tNOX related apoptosis[Morre *et al.* 2006]. Genistein is a tyrosine kinase inhibito [Jing *et al.* 1995] and reduced the transcriptional activity of hTERT dose-dependently (5~50 mM) leading to anticancer activity[Ouchi *et al.* 2005]. Daidzein (6~100μM) reduced expression of hTERT mRNA which affected human nonhormone-dependent cervical cancer cells[Guo *et al.* 2004]. Expression of hTERT extends the life span of normal human cells[Kharbanda *et al.* 2000]. They may produce pharmacodynamic synergism by interacting with different targets of both the same and different pathways.	PD-SP&RP
Grapes and peanuts (Resveratrol)[Cal *et al.*]	Green tea (Polyphenols: (-)-epigallocatechin-3-gallate, catechin,	Resveratrol and catechin produced synergistic	Comparison of effective concentrations, effective	No report found	FastOne [Longevity, USA]	With peptide 1-17 of gastrin as substrate, resveratrol (25μM) inhibited PC12 particulate tyrosine kinases without	PD-ST

	caffeine)[Morre *et al.* 2003a]	protection of PC12 cells from beta-AP (1-41) toxicity of hydrogen peroxide[Conte *et al.* 2003b]	concentration in combination 2~5 times smaler than in separation. Synergism detection result likely reliable.			affecting soluble activity, catechin (100µM) inhibited particulate and increased soluble activity. When peptide 6-20 of cell division kinase p34cdc2 was utilized, catechin (100µM) inhibited PC12 soluble tyrosine kinase activity and increased particulate activity, resveratrol (25µM) inhibited both soluble and particulate activities[Conte *et al.*]. beta-AP induces toxicity by activating tyrosine kinase-based signaling response which produces neurotoxic secretory products, proinflammatory cytokines, and reactive oxygen species[Combs *et al.* 2001]. These active ingredients may produce pharmacodynamic synergism by interacting with either different sites or different section of the same site of the same target.	
Vitis vinifera (Resveratrol, catechin, epicatechin, gallocatechin, gallic acid, ellagic acid, beta-carotene, lutein, 8-hydroxylinalool)[Yilmaz *et al.* 2004]	*Coffea robusta* (Caffeine, 5-O-caffeoilquinic acid)	*Vitis vinifera* and a Coffea robusta powder exhibited a synergistic antioxidant efficiency in *in vitro* test[Shafiee *et al.* 2002]		No report found	NSI Grape Seed, Green Tea, & Red Wine [Nutraceutical Sciences Institute, USA]	Caffeine exerted antioxidant effect by increasing the activity of superoxide dismutase [Rossowska *et al.* 1995] and by quenching reactive species [Devasagayam *et al.* 1996]. Resveratrol (15.5µM) inhibited caffeine metabolizing enzyme cytochrome P450 CYP1A2 [Chang *et al.*; Ursing *et al.*],	PK

						thereby maintaining the concentration and thus potentiating the effects of caffeine.	
Onion (quercetin, myricetin, kaempferol, dipropenyl sulfide)[Perchellet *et al.*; Sellappan *et al.*]	Garlic (Sulphurous substances: diallyl disulfide, diallyl disulfide, allicin, alliin, ajoene, N-acetyl cysteine, S-allyl cysteine, S-ethyl cysteine, S-methyl cysteine, S-propyl cysteine)	Synergistic antioxidant activity in the inhibition of lipid peroxidation by lipoxygenase as tested in a lipid peroxidation assay[Shobana *et al.*]	Comparison of effects, effect of combination grater than individual alone combined. Synergism detection result reliable	Trial of 10 healthy persons to test the effect of garlic and onion on alimentary hyperlipemia induced by feeding 100g butter showed that garlic and onion have a significant protective action against fat-induced increases in serum cholesterol and plasma fibrinogen and decreases in coagulation time and fibrinolytic activity[Bordia *et al.* 1975]	Cyclone Cider [Kalyx.com, USA]	Quercetin inhibited 5-lipoxygenases (IC50 = 25 µM) [Prasad *et al.*; Teklu *et al.*], myricetin inhibited lipoxygenase[Robak *et al.*], kaempferol inhibited 15-lipoxygenase and phospholipase A2[Alcaraz *et al.*], diallyl sulfide inhibited superoxide production by xanthine oxidase[Ou *et al.*]. They may produce pharmacodynamic synergism by interacting with different targets of the same and related pathways.	PD-SP&RP
Onion (quercetin, myricetin, kaempferol, dipropenyl sulfide)[Perchellet *et al.*; Sellappan *et al.*]	Ginger (zingiberene ,ar-curcumene β-sesquiphellandrene, bisabolene, farnesen , [6]-gingerol, Zingerone)[Yamahar	Synergistic antioxidant activity in the inhibition of lipid peroxidation by lipoxygenase as tested in a lipid	Comparison of effects, effect of combination grater than individual alone combined. Synergism detection result	No report found	Cyclone Cider [Kalyx.com, USA]	Quercetin inhibited 5-lipoxygenases (IC50 = 25 µM) [Prasad *et al.*; Teklu *et al.*], myricetin inhibited lipoxygenase[Robak *et al.*], kaempferol inhibited 15-lipoxygenase and	PD-SP&RP

	a *et al.*]	peroxidation assay[Shobana *et al.*]	reliable			phospholipase A2[Alcaraz *et al.*], [6]-gingerol effectively suppressed peroxynitrite-induced oxidation of dichlorodihydrofluorescein[Ippoushi *et al.*]. They may produce pharmacodynamic synergism by interacting with different targets of the same and related pathways.	
Garlic oil (allicin, allyl-methyltrisulphide, diallyldisulphide, diallyltrisulphide, diallyltetrasulphide, allylpropyldisulphide, ajoene, 2-vinyl-4H-l, 3 dithiin, and alliin) [Ariga *et al.*]	Retinoic acid	Synergistically induced leukemia cell HL60 differentiation[Ariga *et al.*; Seki *et al.*]		No report found	None	Unknown	
Garlic (Sulphurous substances: diallyl disulfide, diallyl disulfide, allicin, alliin, ajoene, N-acetyl cysteine, S-allyl cysteine, S-ethyl cysteine, S-methyl cysteine, S-propyl cysteine)[Ariga *et al.*]	Ginger (zingiberene, ar-curcumene β-sesquiphellandrene, bisabolene, farnesen, [6]-gingerol, Zingerone)[Yamahara *et al.*]	Synergistic antioxidant activity in the inhibition of lipid peroxidation by lipoxygenase as tested in a lipid peroxidation assay[Shobana *et al.*]	Comparison of effects, effect of combination grater than individual alone combined. Synergism detection result reliable	No report found	Cyclone Cider [Kalyx.com, USA] Garlic + Ginger [Jarrow Formulas, USA]	Diallyl sulfide inhibited superoxide production by xanthine oxidase [Ou *et al.*], [6]-gingerol effectively suppressed NO production of lipopolysaccharide (LPS)-activated J774.1 macrophages iNOS (IC50 23.9 μM) [Ippoushi *et al.*]. They may produce pharmacodynamic synergism by interacting with different targets of related pathways.	PD-RP
Garlic (Sulphurous substances: diallyl disulfide, allicin, alliin, ajoene, N-acetyl cysteine, S-allyl	Tomato (Lycopene, beta-carotene, ascorbic acid, tocopherols,	Synergistic action of garlic and tomato in cancer preventive effect as tested in	Comparison of effects, effect of combination grater than individual. Synergism	*In vivo* test on cell proliferation inhibitory activity of garlic and tomato,	NSI Synergy Advanced Multi-Vitamin Version 3 [Nutraceutical Sciences	Diallyl disulfide inhibited RNA polymerase and nuclear RNA synthesis leading to possible anticancer effect[Yu *et al.*], Allicin caused a	PD-RP

cysteine, S-ethyl cysteine, S-methyl cysteine, S-propyl cysteine)[Ariga *et al.*]	phytoene, phytofluene, phytosterols)[Fuhrman *et al.*]	rats[Sengupta *et al.*]	detection result may be difficult to judge	individually and in combination, against azoxymethane induced colon carcinogenesis in Sprague-Dawley rats. The effect was observed on aberrant crypt foci, the preneoplastic lesion[Sengupta *et al.*]. Testing results described in third colomn.	Institute, USA]	transient drop in the intracellular glutathione level leading to growth inhibitory activity (IC_{50}=10-25μM)[Hirsch *et al.*], Lycopene (2-3μM) inhibited cell cycle progression via reduction of the cyclin D level and retention of p27 in cyclin E--cdk2[Nahum *et al.*], Gamma-tocopherol (25μM) down-regulated cyclins thus inhibited human cancer cell cycle progression and cell proliferation[Gysin *et al.*]. They may produce pharmacodynamic synergism by interacting with different targets of related pathways.	
Tomato extract (Lycopene)[Amir *et al.*]	Chloroform extract of the leaves of Tomato (1,25-dihydroxyvitamin D3)[Prema *et al.*]	Combination of low concentrations of lycopene with 1,25-dihydroxyvitamin D3 exhibited a synergistic effect on cell proliferation and differentiation in HL-60 promyelocytic leukemia cell line[Amir *et al.*]	Comparison of effective concentrations, combination at lower concentrations produces effect while individual at same concentration produces no effect. Synergism detection result may be difficult to judge	No report found		Lycopene (2-3μM) inhibited cell cycle progression via reduction of the cyclin D level and retention of p27 in cyclin E--cdk2[Nahum *et al.*], 1,25-dihydroxyvitamin D3 (0.1 μM) regulated mineral homeostasis and exhibited potent anti-proliferative, prodifferentiative, and immunomodulatory activities by binding to the vitamin D receptor[Lu *et al.*]. They may produce pharmacodynamic synergism by interacting with different targets of related pathways.	PD-RP
Carrot (Beta-carotene)[Livny *et al.*]	Fruits or vegetable extracts (Vitamins C;	Synergistic in prevention and		Randomized, controlled trials	Willow Lake Conditioner Dry	Unknown	

	Vitamins E)[Czernichow *et al.*]	treatment of nonmelanoma skin cancer and melanoma[Bialy *et al.*]		in adults taking combined supplementation of vitamin C, vitamin E, beta-carotene, selenium, and zinc reduced the rate of cancer by 31% in men but not in women[Huang *et al.* 2006a]	or Damaged Hair Vitamin E, Carrot Extract & Milk Protein [Alleghany Pharmacal Corp., USA] and various other body care products		
Radix Paeoniae Alba (Bai-Shao) (gallotannin, Paeoniflorin, liquiritin, formononetin, isoliquiritigenin, isoquiritin)[Sugishita *et al.*; Tan *et al.*; Goto *et al.*; Liu *et al.*]	*Radix Glycyrrhiza* (Gan-Cao) (glycyrrhizic acid, liquilitin apioside, glycyrrhizin)[Sugishita *et al.*; De Clercq; Kamei *et al.*]	Synergy in spasm and pain relief [Huang *et al.*]		A traditionally-defined herb pair frequently used in formulating traditional Chinese medicine recipes for pain relief and harmonizing liver[Ung *et al.* 2007]. It has been applied to patients without rigorous clinical test. It is thus classified into *in vivo* test category	EstroReviv [Hrbal Powers, USA] Trivestin™ Pain Relief [Covaxil Laboratories, USA]	None	
Rhizoma Anemarrhenae {Zhi-Mu} (Sarsasapogenin, Timosaponin A1, A2, A3, A4, B1, B2, A-I, A-III, Xanthone C-	*Radix Astragali* {Huang-Qi} (Osthole, calycosin, Astragaloside I, II, IV, Soyasaponin I, Formononetin,	Synergy in protecting cardiac dysfunction as tested in rats[Hu *et al.* 2002]		*In vivo* test of two-clib one kidney operation induced renal hypertension in rats. After 8	Yu Ye Tang TCM patent medicine [Guangdong Yifang Pharmaceutical	None	

glycoside, Mangiferin)	Isomucronulatol, Asparagine)			weeks, they were divided into groups and medicated for 6 weeks. Their heart rate, blood pressure, heart-weight index and left-ventricle-weight index were measured, and left ventricle was cannulated to estimate heart function[Hu *et al.* 2002]. Testing results described in third colomn.	Corp, China]		
Rhizoma Polygoni Cuspidati {Hu-Zhang} (emodin, physcion, chrysophanol, anthraglycoside A, fallacinol, citreorosein, questin, questinol)	*Radix Astragali* {Huang-Qi} (Osthole, calycosin, Astragaloside I, II, IV, Soyasaponin I, Formononetin, Isomucronulatol, Asparagine)	Synergistic antiviral effect as tested in HEp-2 cell system[Wang *et al.* 1999]		A trial of 60 chronic hepatitis C patients orally taking Bing Gan Ling liquid composed mainly of *Cornu Bubali*, *Rhizoma Polygoni Cuspidati*, *Radix Paeoniae Rubra*, and *Radix Astragali*, etc. The total effective rate is 86.7%, which is considerably better than that	Yu Ye Tang TCM patent medicine [Guangdong Yifang Pharmaceutical Corp, China]	Emodin binds to and disrupted membranes by inducing formation of hexagonal-H(II) phase which lead to antiviral and antimicrobial effects (MIC_{50}=2.2μM)[Alves *et al.*], Osthole suppressed the secretion of HBV by increasing the glycosylation of HBV surface antigen (20 μg/mL)[Huang *et al.*]. They may produce pharmacodynamic synergism by interacting with different targets of related pathways.	PD-RP

				of the control group[You *et al.* 1998]. This trial might implicate the synergistic antiviral effect of *Rhizoma Polygoni Cuspidati* and *Radix Astragali.*			
Uncaria rhynchophylla (Miq.) Jack {Gou-Teng} (Rhynchophylline, Isorhynchophylline, Corynoxeine, Isocorynoxeine, Corynantheine, dihydrocorynantheine, hirsutine, hirsuteine)[Ozaki 1989; Ozaki 1990; Zhang *et al.* 2004b]	*Gastrodia elata BI.* {Tian-Ma} (vanilline, vanillic acid, vanillyl alcohol, gastrodin)[Wu *et al.*; Hsieh *et al.* 2000]	Synergistic anticonvulsive effects as tested in male Sprague-Dawley rats[Hsieh *et al.* 1999]	ANOVA + Scheffe's test. Synergism detection result reliable	*In vivo* test of male Sprague-Dawley rats (6 in each group) that received intraperitoneal injection of kainic acid to induce epileptic seizures and generation of free radicals, with or without oral administration of *Uncaria rhynchophylla (Miq.) Jack* alone or comination of *Uncaria rhynchophylla (Miq.) Jack* and *Gastrodia elata BI.* Herb combination exhibited greater inhibition on the	Gastrodia & Uncaria Combination (Tianma Gouteng Yin) [balance healthcare ltd, UK]	Hirsuteine (300 nM-10 μM) blocked ion permeation through nicotinic receptor channel complexes[Watano *et al.* 1993] which might contribute to its anticonvulsive effect[Krampfl *et al.* 2000], Vanillyl alcohol (200 mg/kg) has free radical scavenging activities which may be responsible for its anticonvulsive propertics[Hsieh *et al.* 2000]. They may produce pharmacodynamic synergism by interacting with different targets of related pathways.	PD-RP

				onset time of wet dog shakes than UR alone[Hsieh *et al.* 1999]. Testing results described in third colomn. A traditionally-defined herb pair frequently used in formulating traditional Chinese medicine recipes for blocking wind and convulsion, and for soothing liver Yang[Ung *et al.* 2007]. It has been applied to patients without rigorous clinical test. It is thus classified into *in vivo* test category			
Scutellaria baicalensis (Huang-Qin) (Wogonin, Wogonoside, baicalein, baicalin, oroxylin A, skullcapflavone II) [Liao *et al.*;	Grape seed (Proanthocyanidins: catechin, epicatechin)	Acted synergistically to scavenge reactive oxygen species and potentially enhance their antioxidant efficacy as found		No report found	Biowhite® Skin Whitening Cream [Softuch Skin Care., AU]		

Wakabayashi; Wozniak *et al.*]		in an *in vitro* test[Shao *et al.* 2004]					
Aristolochia manshuriensis {Guan-Mu-Tong} (aristolochic acids, cause nephrotoxicity)[Liu *et al.*]	*Rhizoma Coptidis* {Huang-Lian} (palmatine, berberine, coptisine, epiberberine)[Yokozawa *et al.* 2004; Yokozawa *et al.* 2005]	Renal toxicity significantly reduced after using ethanol extract of R. coptidis to process HZ Aristolochia manshuriensis[Hu *et al.* 2004]		*In vivo* test of herb extracts fed to mice via gastric tube for 8 weeks. The blood was collected to assess liver and renal functions. The tissue samples of the liver, kidney and bladder were collected from executed animals for pathology examination[Hu *et al.* 2004]. Testing results described in third colomn.	None		
Buckweat (Rutin)[Milde *et al.* 2004]	Lemon oil (Gamma-terpinene)[Milde *et al.* 2004]	Synergistic inhibition of low-density lipoprotein oxidation by rutin and gamma-terpinene as shown by an *in vitro* test[Milde *et al.* 2004]	Comparison of effects, effect of combination grater than individual alone combined. Synergism detection result reliable	No report found	Vivatap® Water Improver [Brunel Healthcare Ltd, UK]	Rutin (500 µM) inhibited HUVEC nuclear condensation and fragmentation induced by Cu(2+)-oxidized LDL[Jeong *et al.*]. Gamma-terpinene efficiently slowing down the oxidation of LDL [Grassmann *et al.*]. They may produce synergism by interacting with different targets of sequentially related pathways	PD-RP
Dalea versicolor (4',6'-Dihydroxy-3',5'-	Berberis (Berberine)[Yesilada	4',6'-Dihydroxy-3',5'-dimethyl-2'-		No report found	None	4',6'-Dihydroxy-3',5'-dimethyl-2'-methoxychalcone	PK

dimethyl-2'-methoxychalcone; 3,5,4'-Trimethoxy-trans-stilbene)[Belofsky *et al.* 2004]	*et al.* 2002]	methoxychalcone and 3,5,4'-Trimethoxy-trans-stilbene potentiated the activity of berberine as shown by an *in vitro* test[Belofsky *et al.* 2004]				and 3,5,4'-Trimethoxy-trans-stilbene inhibited berberine effluxing multidrug-resistance pumps with MICs of 250 and 500 µg/mL, respectively , but they caused complete growth inhibition at very low concentrations (~3.3 µg/mL)[Belofsky *et al.* 2004]. Thus the effects of berberine are potentiated by maintaining its concentration.	

5.4 Discussion

5.4.1 Current opinions and investigations of herbal synergism

While herbal synergism has been reported in some herbs or herbal products [Stermitz *et al.* 2000; Williamson 2001; Spinella 2002; Gilbert *et al.* 2003], the underlying molecular mechanism is mostly unknown [Williamson 2001]. In general, synergism between herbal ingredients has been considered to be similar to those of synergism from drug combinations. For instance, pharmacodynamic synergism of herbs results from two or more active ingredients interacting with related therapeutic or toxicological targets, and in some cases with additional targets involved in the related biological pathways. These interactions collectively produce a therapeutic or toxicity-regulating effect that is more significant than the simple sum of effects of these ingredients. Pharmacokinetical potentiative effects result from one or more active ingredients interfering with the pharmacokinetic processes of other active ingredients. This interference affects the therapeutic and toxicological actions of the other ingredients by modulating their bioavailability. Interference with a pharmacokinetic process often occurs through binding to proteins responsible for absorption, distribution, metabolism, and excretion (ADME) of drugs and xenobiotics [Caldwell *et al.* 1995; Lee *et al.* 2004].

Thus, the pharmacokinetical potentiative and toxicity modulating effects of herbal combination is mediated by the interaction of active ingredients with specific biomolecules that either directly alters pharmacodynamical activities or modulates the therapeutic, toxicological, and ADME effects of other active ingredients. Investigation of

the molecular interaction profiles between active ingredients of herbs and the relevant biological entities can thus provide useful hint about the molecular mechanism of reported therapeutic efficacies and whether these are due to synergistic or placebo effects.

Knowledge of molecular interaction profiles between active ingredients of herbs has recently been used in several studies for explaining the synergistic effects of some herbal ingredients [Stermitz *et al.* 2000; Williamson 2001; Spinella 2002]. For instance, some active ingredients in St. John's wort have been found to separately inhibit catechol-omethyltransferase, monoamine receptor and monoamine reuptake, but their concentrations are at levels that they are individually sub-therapeutic for depression [Spinella 2002]. However, these ingredients combine to produce a pharmacodynamic synergism that contributes to the observed anti-depression effects of this herb[Spinella 2002]. In another example, an active ingredient in chaulmoogra oil has been found to potentiate the antimicrobial action of berberine in berberis plants by enhancing its bioavailability via inhibition of the berberine-removing multidrug pump, which is a typical example of pharmacokinetic potentiative effect between the two active ingredients [Stermitz *et al.* 2000].

5.4.2 Do literature-described molecular interactions of active herbal ingredients support the reported synergism in some herbs or herbal products?

As shown in **Table 5.3** and **Table 5.4**, there are 17 cases of single herb synergism and 22 cases of twin-herb synergism. We were able to find literature reported molecular interaction profiles for 12 and 16 of these cases respectively, which are summarized in

Table 5.3 and **Table 5.4**. These profiles may be potentially used for supporting, explaining and investigating the proclaimed synergism. It appears that literature described MI profiles may be used for supporting 11 cases of single-herb and 16 cases of twin-herb synergism. Analysis of the profiles of the 11 cases of single-herb synergism suggests that 3 cases may be attributed to pharmacokinetically potentiative effects, and 5, 3, 3, 1, and 1 cases may be attributable to pharmacodynamically synergistic effects arising from collective interaction with different targets of related pathways, different targets of the same pathway, different targets of both the same pathway and related pathways, and the same target respectively. Analysis of the molecular interaction profiles of the 16 cases of twin-herb synergism suggest that 2 cases may be attributed to pharmacokinetically potentiative effects, and 6, 5, and 3 cases may be attributable to pharmacodynamically synergistic effects arising from collective interaction with different targets of related pathways, the same target, and different targets of both the same pathway and related pathways, respectively. The molecular interaction profiles of these cases also suggest that there is no significant difference between the possible molecular mechanisms of synergism among the active ingredients within single-herb and between two herbs.

5.4.3 Cases of pharmacodynamic synergism interacting with different targets of the same pathway

In studying soy isoflavonoids' inhibition of neoplastic transformation in human, soy isoflavonoids that are weakly active in separation have been found to be active in combination [Franke *et al.* 1998]. A randomized trial has been conducted to test 24 prostate cancer patients, divided into 3 groups each taking soy (high phytoestrogen), soy

and linseed (high phytoestrogen), and wheat (low phytoestrogen), which has demonstrated that high soy phytoestrogen diets reduce risk of prostate cancer development and progression [Dalais *et al.* 2004]. The primary soy isoflavonoids are genistein, daidzein, dihydrodaidzein, O-desmethylangolensin, and equol [Lampe 2003; Maubach *et al.* 2003]. Genistein is a tyrosine kinase inhibitor [Jing *et al.* 1995] that reduces the transcriptional activity of human telomerase reverse transcriptase (hTERT) dose-dependently at 5~50mM leading to anticancer activity [Ouchi *et al.* 2005]. Daidzein at 6~100μM reduces the expression of hTERT mRNA which affected human nonhormone-dependent cervical cancer cells [Guo *et al.* 2004]. Expression of hTERT extends the life span of normal human cells [Kharbanda *et al.* 2000]. The peak isoflavone levels in urine, plasma and in breast milk in that study is 60μM, 2μM and 0.2μM respectively [Kharbanda *et al.* 2000]. These studies suggest that the effective concentration in combination (between 0.2μM and 2μM) is at least 3 fold smaller than that of invidual ingredient, indicative of pharmacodynamic synergism that arise from the interaction with different targets of the same pathway.

Polyphenol extracts of green tea has been found to produce synergism in augmenting and prolonging sympathetic stimulation of thermogenesis in Sprague-Dawley rats [Dulloo *et al.* 2000]. A randomized trial of 10 healthy men divided into 3 groups each taking green tea extract, caffeine, and placebo has shown that green tea has thermogenic properties and promotes fat oxidation beyond that explained by its caffeine content per se [Dulloo *et al.* 1999]. The principal polyphenols of green tea are (-)-epigallocatechin-3-gallate, catechin, and caffeine [Morre *et al.* 2003a]. Catechin-

polyphenols are known to inhibit catechol-O-methyl-transferase which degrades noradrenaline, and caffeine has been found to inhibit trancellular phosphodiesterases which break down noradrenaline induced cAMP. Thus these green tea ingredients collectively stimulate thermogenesis by relieving different control points along the NA-cAMP axis [Dulloo *et al.*].

5.4.4 Cases of pharmacodynamic synergism interacting with different targets of related pathways

Tea extract (-)-Epicatechin has been found to exhibit synergistic effects together with other extracts (-)-epigallocatechin-3-gallate, (-)-epicatechin-3-gallate, and theaflavins against AH109A cell proliferation [Zhang *et al.* 2000]. A double-blind, placebo-controlled trial has been conducted to test the safety and efficacy of green tea catechins for cancer chemoprevention in 60 high-grade prostate intraepithelial neoplasia volunteers, which have shown values of total prostate-specific antigen to be constantly lower than that of placebo-treated ones, and improved international prostate symptom scores, without significant side effects or adverse effects [Bettuzzi *et al.* 2006]. (-)-epigallocatechin-3-gallate inhibited proteasome activity [Kuhn *et al.* 2004], which induce apoptosis and show antitumor efficacy by stopping proteasome degradation of proteins crucial to apoptosis and cell cycle regulation. Epicatechin inhibited tyrosine kinase activity of PDGF-R producing anticancer effect [Sachinidis *et al.* 2000] by stopping PDGF-R regulated multiple tumor-associated processes including stimulation of tumor cells, angiogenesis and recruitment and regulation of tumor fibroblasts [Ostman 2004].

These studies suggest that these extracts produce pharmacodynamic synergism by targeting different targets of related pathways.

Active ingredients of *Ginkgo biloba* extract have been reported to produce synergistic protection against ischemia-induced neuronal death *in vivo* and glutamate-induced neuronal death *in vitro* involving anti-excitotoxicity, inhibition of free radical generation, scavenging of reactive oxygen species, and regulation of mitochondrial gene expression [Chandrasekaran *et al.* 2002]. A double-blind, placebo-controlled trial on neuropsychological functioning of 262 cognitively intact older adults has shown that those taking *Ginkgo biloba* extract EGb761 daily for 6 weeks exhibited significantly more improvement on SRT tasks involving delayed free recall and recognition of noncontextual, auditory-verbal material, compared with the placebo controls. The EGb761 group also demonstrated significantly greater improvement on the WMS-III FII subtest assessing delayed recognition of visual material (human faces), compared with the placebo group [Mix *et al.* 2002]. Bilobalide at 15~30μM increases mRNA levels of COXIII subunit of cytochrome *c* oxidase and subunit 1 of NADH dehydrogenase (ND1) thus preserving brain energy metabolism and providing neuroprotection [Defeudis 2002]. Ginkgolide B blocks glycine-gated chloride channel at IC50 0.27μM and thus glycine-activated responses in pyramidal hippocampal neurons leading to neural effect [Kondratskaya *et al.* 2004]. Therefore, neuroprotective synergistic effects arise from actions at two different points of neuronal cellular processes.

5.4.5 Cases of pharmacodynamic synergism interacting with different targets of both the same and related pathways

In an *in vivo* test on female SCID mices, a combination of soy and tea bioactive components has been found to inhibit tumor angiogenesis and reduce estrogen receptor (ER)-alpha and serum levels of insulin-like growth factor IGF-I leading to anti-cancer effect [Zhou *et al.* 2004]. It has been reported that the National Cancer Institute has been conducting Phase III trial on 154 patients to test prostate cancer prevention activity of soy isoflavones and green tea, even-though the outcome of this and earlier trials has not been made publically available [Greenwald 2004]. The main green tea polyphenols are (-)-epigallocatechin-3-ga llate, catechin, and caffeine [Morre *et al.* 2003a]. Soy extract primarily comprises genistein, daidzein, dihydrodaidzein, O-desmethylangolensin, and equol [Lampe 2003; Maubach *et al.* 2003]. (-)-epigallocatechin-3-gallate (EGCg) blocks NADH oxidase activity of a cancer-specific cell surface protein tNOX at EC50 of 1 μM 93 and thus stopped the growth of tumor cells [Chueh *et al.* 2004] by inducing tNOX related apoptosis [Morre *et al.* 2006]. Genistein is a tyrosine kinase inhibitor 66 and reduced the transcriptional activity of hTERT at 5~50 mM dose-dependently leading to anticancer activity [Ouchi *et al.* 2005]. Daidzein at 6~100μM reduced expression of hTERT mRNA which affected human nonhormone-dependent cervical cancer cells [Guo *et al.* 2004]. Expression of hTERT is known to extend the life span of normal human cells [Kharbanda *et al.* 2000]. These studies suggest that these active ingredients produce pharmacodynamic synergism by interacting with different targets of both the same pathway and different pathways.

5.4.6 Cases of pharmacodynamic synergism interacting with the same target

From an *in vitro* test, some combinations of terpenoids in *Salvia lavandulaefolia*, an herb used as cholinergic and memory-enhancing agent, have been reported to produce synergistic as well as antagonistic anticholinesterase activity [Savelev *et al.*]. A placebo-controlled, double-blind, balanced, crossover trial has been conducted on mood and cognition modulating effect of this herb in 24 patients, which shows that it is capable of acute modulation of mood and cognition in healthy young adults [Tildesley *et al.* 2005]. Three active ingredients, 1,8-cineole, alpha-pinene, and camphor, have been found to inhibit acetylcholinesterase at IC50 values of 0.67mM, 0.63mM, and >10mM respectively [Perry *et al.* 2000]. Collectively these inhibitors of weak IC50 values may produce sufficiently strong anticholinesterase activity to produce the observed synergism by mutual enhancement against the same target. Based on statistical analyis of the results from drug combination studies, combination of active ingredients interacting with the same target may produce synergism if each interacts at different site or different section of the same site. It may produce antagonistic effect if they mutally interfere at the same binding site. 1,8-cineole, alpha-pinene and camphor have been found to be uncompetitive reversible inhibitors [Perry *et al.* 2000]. Therefore, there is a much higher probability for them to mutually enhance than to mutually interfere with each other's inhibitory activity, resulting in a pharmacodynamically synergistic effect against the same target.

5.4.7 Cases of pharmacokinetically potentiative effect

There has been a report about the synergistic antibacterial effect of monomeric polyphenols epigallocatechin gallate and catechin gallate in the extract of Oolong tea as observed in an in vitro test on staphylococcus mutans cells [Sasaki *et al.* 2004]. Two clinical tests have been reported. In a trial of 110 patients receiving tea tree oil regimen vs. 114 patients receiving a standard regimen for treatment of methicillin-resistant staphylococcus aureus colonization, it has been showed that tea tree oil is effective, safe and well tolerated [Dryden *et al.* 2004]. Another trial of 104 patients with impetigo contagiosa has demonstrated antibacterial effect of tea liquor lotion [Sharquie *et al.* 2000]. Oolong tea extract contains epigallocatechin gallate, gallocatechin gallate, catechin gallate, epicatechin, and caffeine [Zhang *et al.* 2004c]. Catechin gallate is known to inhibit P-glycoprotein [Jodoin *et al.* 2002] which effluxes a major antibacterial component in the extract, epigallocatechin gallate [Hong *et al.* 2002]. Thus pharmacokinetic synergism of the Oolong tea extracts is likely produced by the capability of one of its active ingredient, catechin gallate, in effectively maintaining the level of its principal antibacterial ingredient, epigallocatechin gallate.

5.5 Conclusion

Current study raises a question whether literature-described molecular interaction profiles of active herbal ingredients can be explored for supporting, validating and investigating reported synergistic effects of herbs and herbal products. Knowledge of

herbal synergism from these studies may be further explored for developing novel cocktail therapeutic approaches to achieve synergistically enhanced therapeutic effect relatively free of the adverse effects produced by large doses of a single constituent. Molecular level study of herbal synergism is possible if the currently available literature-described molecular interaction profiles of active herbal ingredients are comprehensive enough to cover a diverse spectrum of herbs. Search of the Medline literature database[Wheeler *et al.* 2004] found that current available literatures contain information about experimentally indicated molecular interaction profiles for more than 1,876 active ingredients from more than 1,239 herbs. Overall these active ingredients are known to interact with at least 970 distinct proteins, many of which are therapeutic targets, toxicologically-related proteins and ADME-associated proteins. Therefore, current information seems to have reached a meaningful level for the preliminary study of molecular mechanism of some herbs.

A total of 39 such cases were collected from a search of Medline[Wheeler *et al.* 2004] literatures followed by the search of the active ingredients of the herbs involved. These cases can be further divided into 17 cases of single-herb synergism given in **Table 5.3**, and 22 cases of twin-herb synergism given in **Table 5.4**. The molecular interaction profiles of the active ingredients of these herbs were then retrieved from a search of Medline literatures. These profiles were subsequently studied to evaluate whether they support the reported synergism.

There is a need to evaluate the analysis methods used for identifying herbal synergism. As shown in **Table 5.3** and **Table 5.4**, herbal synergism has primarily been identified based upon the observation of either enhanced effects or reduced effective concentrations. While these approaches can be straightforwardly applied for identifying herbal synergism arising from pharmacokinetically potentiative effects, careful analysis is needed in applying them for identifying pharmacodynamic synergism, particularly for distinguishing synergistic and additive effects [Peters *et al.* 2000; Barrera *et al.* 2005; Jonker *et al.* 2005; Chou 2006; Tallarida 2007].

The majority of the reported cases of herbal synergism analyzed in this study appeared to be potentially supportable and explainable by the literature-described molecular interaction profiles of the relevant herbal ingredients. Most of these reported cases of synergism are of the pharmacodynamic type, in which multiple components of a disease or symptom-related process are inhibited or modulated by the ingredients in different constituent herbs of a multi-herb product. These are consistent with numerous experimental findings that simultaneous targeting of multiple components of a disease process can be more effective in the treatment of that disease. For instance, it is known that several mutations are required for the development of colorectal and other cancers [Kinzler *et al.* 1996] and the correction of these defective pathways require several interventions [Keith *et al.* 2005] which is the main reason for why oncological chemotherapeutic regiments most often involve the use of combination therapies such as CHOP (doxorubicin, cyclophosphamide vincristine, and prednisone) [Devita 1997].

Some of the reported cases of herbal synergism are of pharmacokinetic type, in which the efflux pump or metabolizing enzyme of one or more therapeutically active ingredient of an herb is inhibited or modulated by an ingredient of another herb. Inhibition of drug efflux pumps has been explored as one of the key strategies for reversing multi-drug resistance or maintaining the levels of drug concentration thereby enhancing the efficacy of drugs [Robert *et al.* 2003]. There are also clinical trials for combination drugs that contain a drug and the inhibitor of its metabolizing enzyme. For instance, a combination of NMDA antagonist dextromethorphan and the inhibitor of its metabolizing enzyme, cytochrome P450 CYP2D6, quinidine is currently undergoing Phase III trial [Adis International Limited 2005]. Therefore, it is not surprising that such kind of pharmacokinetic synergy has been empirically explored in herb-based products and medicines[Spinella 2002], and some of the natural product efflux pump and metabolizing enzyme inhibitors are being investigated for therapeutic applications [Zhang *et al.* 2004a].

The present study is focused on the molecular interaction profiles of the active ingredients most relevant to the literature-reported synergistic effect of the herbs and herbal products studied. Apart from the reported synergistic effects, other effects of these herbs and herbal products are not considered. The active ingredients of these herbs and herbal products are known to interact with other molecular entities than those described. A more complete picture of the overall effects of these herbs and herbal products needs to be given based on the analysis of all of these molecular interaction profiles.

Besides, molecular interaction profiles may also be potentially explored for facilitating the identification of herbal synergism not yet discovered by the current investigations. For instance, studies on *Isatis tinctoria* by Hamburger et al. have identified a number of anti-inflammatory ingredients that include tryptanthrin (dual inhibitor of COX-2 and 5-lipoxygenase), apha-linolic acid (COX-2 inhibitor), and indirubin (inhibitor of chemokine RANTES) [Mohn *et al.* 2007]. Newly developed dual COX-5-lipoxygenase (5-LOX) inhibitors share the anti-inflammatory effect and gastric safety of COX-2 inhibitors, but also inhibit COX-1-mediated platelet function and 5-LOX-mediated synthesis of gastrotoxic leukotrienes [de Gaetano *et al.* 2003]. Chemokine RANTES (regulated on activation normal T cell expressed and secreted) is expressed in several inflammatory diseases of the central nervous system and is a powerful stimulus for astrocyte production of proinflammatory mediators [Zhang *et al.* 2003]. Therefore, these active ingredients may produce synergistic effects by at least two main actions: one is efficacy synergism plus toxicity antagonism via dual inhibition of COX-2 and 5-lipoxygenase, and the other is inhibition of two targets in related pathways.

Investigation into the existence of herbal synergism can be enhanced with further accumulation of the information about active ingredients of herbs and their molecular interaction profiles, expansion of the knowledge of molecular networks in human and disease processes, and in the understanding of mechanism of actions of synergistic interactions. It is feasible to adopt bioinformatics approaches that integrate all of these relevant information to facilitate the identification and understanding of synergism in herbs and multi-herb recipes.

Chapter 6 Conclusion

This last chapter summarizes the major contributions and findings for the work described in Chapter 2 to Chapter 5. In addition, limitations of the present work and suggestions for future studies are also discussed.

6.1 Major Contributions and Findings

In this section major contributions and findings of using in silico approaches are discussed. As far as we know current study is the first attempt to use novel TCM digitalization algorithms in machine learning methods to address the scientific basis of formulating TCM prescriptions and herb pairs. In addition, the current study is also the first to use an inverse docking approach to explore the anti-metastasis mechanisms of Rhubarb anthraquinones such as emodin and aloe-emodin especially in the mechinism of inducing a metastatic suppressor gene nm23 via nuclear receptors. Besides, a comprehensive survey of the mechanisms of herbal synergism by literature-based approach was also conducted.

6.1.1 Merits of MLMs in the studies of TCM from traditional view point

Developing a novel TCM digitalization algorithm from TCM-HP is one of the merits in this study. A TCM-HP digitization algorithm has been developed and used for computing digital TCM-HPs, which have been applied for classifying TCM prescriptions

[Su 1997; Wang *et al.* 2005a]. However, by using this algorithm, the number of digital TCM-HPs for each recipe is dependent on the number of its constituent herbs, which gives feature vectors of unequal components thereby introducing statistical noise to many MLM classification systems. In this study, a new algorithm was introduced to derive digital TCM-HPs of fixed length independent of the number of constituent herbs in a recipe. The second merit in using TCM digitalization algorithm in study of TCM prescriptions is that a significantly higher number of TCM prescriptions and non-TCM recipes than those in other studies [Wang *et al.* 2005a] were used for training and testing the MLM systems. Moreover, two different MLM methods were used, which were evaluated by two separate testing methods, to adequately examine the usefulness of TCM-HPs in distinguishing between TCM prescriptions and non-recipes. Overall these MLM systems correctly classified 83.1%~97.3% of the TCM prescriptions, 90.8%~92.3% of the non-TCM recipes. Hence current study suggests that TCM-HPs are capable of separating TCM prescriptions from non-TCM recipes, which are useful for formulating TCM prescriptions and consistent with the expected correlation between TCM-HPs and the physicochemical properties of herbal ingredients responsible for producing the collective pharmacological and other effects of specific TCM prescriptions.

We further ultilize novel TCM digitalization algorithm in MLMs to analyze distribution patterns of TCM-HPs to detect signs of possible synergism in TCM herb pairs. Patterns of 394 known TCM herb-pairs were found to exhibit signs of herb-pair correlation. Overall these MLM systems correctly classified 72.1%~87.9% of 394 TCM herb-pairs and 91.6%~97.6% of 2,470 non-TCM herb-pairs. The best MLM system predicted 96.3%

of the 27 known non-TCM herb-pairs and 99.7% of the other 1,065,100 possible herb-pairs as non-TCM herb-pairs. Hence, our studies suggest that TCM-HPs of known TCM herb-pairs contain features distinguishable from those of non-TCM herb-pairs consistent with their claimed synergistic or modulating combinations.

6.1.2 Merits of using inverse docking approach in the study of anti-metastatic activities of Rhubarb Anthraquinones

Previous experimental and proteomic works indicated that Rhubarb anthraquinones exhibit anti-proliferative and anti-metastatic activity. However, the molecular targets and mechanism of anti-metastatic activity of these anthraquinones are poorly understood. The merit in this study is that an *in silico* inverse docking approach (INVDOCK) is utilized to perform "*in silico* screening" for putative metastatic-related molecular targets of Rhubarb anthraquinones emodin, aloe-emodin, and rhein from a protein structure database. Targets that are known to involve in metastasis processes such as matrix metalloproteinases, EGRF, tyrosine kinases are the INVDOCK-identified targets for these Rhubarb anthraquinones. Some of these targets had been verified experimentally as discussed in **Chapter 4**. Current study also identified nuclear receptors such as estrogen receptor, androgen receptor, and thyroid hormone receptor as targets of these anthraquinones. Since nuclear receptors regulate the expression of one of metastatic suppressor gene, nm23, as discussed in **Chapter 4** current study provides some insight of regulation of nm23 activities by Rhubarb anthraquinones. Thus the *in silico* results suggest the direction on future experimental tests on activity relationship of Rhubarb

anthraquinones with nuclear receptors and their ability to suppress the activity of metastasis cells.

6.1.3 Merits of using literature-based approach in the study of mechanism of herbal synergism

There have been different views about the therapeutic efficacy of herbal supplements and medicinal herbs. Their use in therapeutics is primarily based on the hypothesis that multiple ingredients are better than one. The reported efficacy of certain herbs at apparently lower doses of active constituents suggests a need for molecular study to determine whether the efficacy is due to placebo or synergistic effects. Knowledge of synergism in some herbs, if confirmed, may be further explored for developing novel cocktail therapeutics. Current study provides the first in-depth and comprehensive analysis of studies from rigorous molecular study of clinical drug combinations (non-herbal) to exhibit molecular interaction profiles that likely to contribute to herbal synergism. Overall, this study compiles the multiple mechanisms reported to be involved in herbal synergisms.

6.2 Limitations and Suggestions for Future Studies

The performance of machine learning methods critically depends on the diversity of data in training set. The dataset used in this works are not expected to fully represent all TCM prescriptions and herb pairs. More diverse pharmacological groups of TCM prescriptions and herb pairs are required to further improve the robustness and

performance of current MLM systems. Besides, other traditional aspects of TCM such as the correlation of TCM-HPs with disease syndromes and the molecular basis of TCM-HPs are not yet explored. All these works require us to redesign our current algorithm of TCM digitalization based on TCM-HPs and descriptions used to describe molecular properties.

For the structural strategy using INVDOCK the result validations depend heavily on how well a compound is studied from available experimental reports. The lack of sufficient experimental data is the major factor for INVDOCK validation. Since INVDOCK select a putative binding targets of a compound based on chemical complementality and energy score "false hits" may be generated if irrelevant structures that show certain degree of structural fitness. Currently we have no general rule to eliminate these false hits simply based on energy function. Inclusion of other criteria such as proteomics and gene expression profiles should enhance the performance of INVDOCK by eliminating some targets that are not actually present in a cell type.

In the literature-based approach using a set of appropriate combinations of keywords is improtant to mine relevant data. Expert manual reading on these literatures is required in order to accurately identify the relavent information. Similar to validation of INVDOCK results, literature-based approach is heavily limited by current available experimental works reported. However, current development of high throughput screening tools and analysis methods used in "omics" works such as clustering methods used in microarray analysis had helped the correlation analysis of huge data possible.

It is still a long way for us to fully understand the holistic pharmacological mechanisms of TCM. Current study uses some well established supervised *in silico* methods in the study of TCM. Other unsupervised *in silico* methods such as clustering methods that had been widely used in microarray gene analysis can be applied to explore correlation of herbal activities in both traditional and compound levels.

Bibliography

Adis International Limited (2005). Dextromethorphan/quinidine: AVP 923, dextromethorphan/cytochrome P450-2D6 inhibitor, quinidine/dextromethorphan. *Drugs R D* **6**(3):174-177.

Alcaraz MJ, Hoult JR (1985). Effects of hypolaetin-8-glucoside and related flavonoids on soybean lipoxygenase and snake venom phospholipase A2. *Archives Internationales de Pharmacodynamie et de Therapie* **278**(1):4-12.

Alves DS, Perez-Fons L, Estepa A, Micol V (2004). Membrane-related effects underlying the biological activity of the anthraquinones emodin and barbaloin. *Biochemical Pharmacology* **68**(3):549-561.

Amir H, Karas M, Giat J, Danilenko M, Levy R, Yermiahu T, Levy J, Sharoni Y (1999). Lycopene and 1,25-dihydroxyvitamin D3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. *Nutrition and Cancer* **33**(1):105-112.

Andersen T, Fogh J (2001). Weight loss and delayed gastric emptying following a South American herbal preparation in overweight patients. *Journal of human nutrition and dietetics: the official journal of the British Dietetic Association* **14**(3):243-250.

Ang-Lee M, Moss J, Yuan C (2001a). Herbal medicines and perioperative care. *JAMA* **286**(2):208-216.

Ang-Lee MK, Moss J, Yuan CS (2001b). Herbal medicines and perioperative care. *JAMA* **286**(2):208-216.

Argiris A, Wang CX, Whalen SG, DiGiovanna MP (2004). Synergistic interactions between tamoxifen and trastuzumab (Herceptin). *Clinical Cancer Research* **10**(4):1409-1420.

Ariga T, Tsuj K, Seki T, Moritomo T, Yamamoto JI (2000). Antithrombotic and antineoplastic effects of phyto-organosulfur compounds. *Biofactors* **13**(1-4):251-255.

Auparakkitanon S, Chapoomram S, Kuaha K, Chirachariyavej T, Wilairat P (2006). Targeting of hematin by the antimalarial pyronaridine. *Antimicrobial Agents and Chemotherapy* **50**(6):2197-2200.

Babita K, Tiwary AK (2005). Transcutaneous delivery of levodopa: enhancement by fatty acid synthesis inhibition. *Molecular Pharmacology* **2**(1):57-63.

Barrera NP, Morales B, Torres S, Villalon M (2005). Principles: mechanisms and modeling of synergism in cellular responses. *Trends in Pharmacological Sciences* **26**(10):526-532.

Belofsky G, Percivill D, Lewis K, Tegos GP, Ekart J (2004). Phenolic metabolites of Dalea versicolor that enhance antibiotic activity against model pathogenic bacteria. *J Nat Prod* **67**(3):481-484.

Beppu H, Koike T, Shimpo K, Chihara T, Hoshino M, Ida C, Kuzuya H (2003). Radical-scavenging effects of Aloe arborescens Miller on prevention of pancreatic islet B-cell destruction in rats. *Journal of Ethnopharmacology* **89**(1):37-45.

Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A (2006). Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a

preliminary report from a one-year proof-of-principle study. *Cancer Research* **66**(2):1234-1240.

Beyerstein BL (2001). Alternative medicine and common errors of reasoning. *Academic Medicine* **76**(3):230-237.

Bhuiyan M, Fant M, Dasgupta A (2003). Study on mechanism of action of Chinese medicine Chan Su: dose-dependent biphasic production of nitric oxide in trophoblastic BeWo cells. *Clinica Chimica Acta* **330**(1-2):179-184.

Bialy TL, Rothe MJ, Grant-Kels JM (2002). Dietary factors in the prevention and treatment of nonmelanoma skin cancer and melanoma. *Dermatologic Surgery* **28**(12):1143-1152.

Bordia A, Bansal HC, Arora SK, Singh SV (1975). Effect of the essential oils of garlic and onion on alimentary hyperlipemia. *Atherosclerosis* **21**(1):15-19.

Bradwejn J, Zhou Y, Koszycki D, Shlik J (2000). A double-blind, placebo-controlled study on the effects of Gotu Kola (Centella asiatica) on acoustic startle response in healthy subjects. *Journal of Clinical Psychopharmacology* **20**(6):680-684.

Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA, Carlquist M (1997). Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **389**(6652):753-758.

Bub S, Brinckmann J, Cicconetti G, Valentine B (200). Efficacy of an herbal dietary supplement (Smooth Move) in the management of constipation in nursing home residents: A randomized, double-blind, placebo-controlled study. *Journal of the American Medical Directors Association* **7**(9):556-561.

Cai CZ, Han LY, Ji ZL, Chen X, Chen YZ (2003). SVM-Prot: Web-based support vector machine software for functional classification of a protein from its primary sequence. *Nucleic Acids Research* **31**(13):3692-3697.

Cal C, Garban H, Jazirehi A, Yeh C, Mizutani Y, Bonavida B (2003). Resveratrol and cancer: chemoprevention, apoptosis, and chemo-immunosensitizing activities. *Curr Med Chem Anti-Canc Agents* **3**(2):77-93.

Caldwell J, Gardner I, Swales N (1995). An introduction to drug disposition: the basic principles of absorption, distribution, metabolism, and excretion. *Toxicologic Pathology* **23**(2):102-114.

Caraglia M, D'Alessandro AM, Marra M, Giuberti G, Vitale G, Viscomi C, Colao A, Prete SD, Tagliaferri P, Tassone P and others (2004). The farnesyl transferase inhibitor R115777 (Zarnestra) synergistically enhances growth inhibition and apoptosis induced on epidermoid cancer cells by Zoledronic acid (Zometa) and Pamidronate. *Oncogene* **23**(41):6900-6913.

Carpinella MC, Giorda LM, Ferrayoli CG, Palacios SM (2003). Antifungal effects of different organic extracts from Melia azedarach L. on phytopathogenic fungi and their isolated active components. *Journal of Agricultural and Food Chemistry* **51**(9):2506-2511.

Carrillo-Munoz AJ, Giusiano G, Ezkurra PA, Quindos G (2006). Antifungal agents: mode of action in yeast cells. *Revista Espanola de Quimioterapia* **19**(2):130-139.

Case DA, Cheatham TE, 3rd, Darden T, Gohlke H, Luo R, Merz KM, Jr., Onufriev A, Simmerling C, Wang B, Woods RJ (2005). The Amber biomolecular simulation programs. *Journal of Computational Chemistry* **26**(16):1668-1688.

Cha TL, Qiu L, Chen CT, Wen Y, Hung MC (2005). Emodin down-regulates androgen receptor and inhibits prostate cancer cell growth. *Cancer Research* **65**(6):2287-2295.

Chan CK, Kuo ML, Shen JJ, See LC, Chang HH, Huang JL (2006). Ding Chuan Tang, a Chinese herb decoction, could improve airway hyper-responsiveness in stabilized asthmatic children: a randomized, double-blind clinical trial. *Pediatric Allergy and Immunology* **17**(5):316-322.

Chan EC, Yap SL, Lau AJ, Leow PC, Toh DF, Koh HL (2007). Ultra-performance liquid chromatography/time-of-flight mass spectrometry based metabolomics of raw and steamed Panax notoginseng. *Rapid Communications in Mass Spectrometry* **21**(4):519-528.

Chan K (1995a). Progress in traditional Chinese medicine. *Trends in Pharmacological Sciences* **16**(6):182-187.

Chan K (1995b). Progress in traditional Chinese medicine. *Trends in Pharmacological Sciences* **16**:182-187.

Chan TC, Chang CJ, Koonchanok NM, Geahlen RL (1993). Selective inhibition of the growth of ras-transformed human bronchial epithelial cells by emodin, a protein-tyrosine kinase inhibitor. *Biochemical and Biophysical Research Communications* **193**(3):1152-1158.

Chandrasekaran K, Mehrabian Z, Spinnewyn B, Chinopoulos C, Drieu K, Fiskum G (2002). Bilobalide, a component of the Ginkgo biloba extract (EGb 761), protects against neuronal death in global brain ischemia and in glutamate-induced excitotoxicity. *Cell Mol Biol (Noisy-le-grand)* **48**(6):663-669.

Chang TK, Chen J, Lee WB (2001). Differential inhibition and inactivation of human CYP1 enzymes by trans-resveratrol: evidence for mechanism-based inactivation of CYP1A2. *Journal of Pharmacology and Experimental Therapeutics* **299**(3):874-882.

Chang YH, Lin HJ, Li WC (2005). Clinical evaluation of the traditional chinese prescription Chi-Ju-Di-Huang-Wan for dry eye. *Phytotherapy Research* **19**(4):349-354.

Chapman&Hall/CRC. 2005. Dictionary of Natural Products. Chapman &Hall/CRC.

Chen HC, Hsieh WT, Chang WC, Chung JG (2004). Aloe-emodin induced in vitro G2/M arrest of cell cycle in human promyelocytic leukemia HL-60 cells. *Food and Chemical Toxicology* **42**(8):1251-1257.

Chen Q. (1998). *Pharmacology and clinical studies of well-known Traditional Chinese medicinal recipes.* Beijing: People Health Pub. Co.

Chen RH, Li JG. (2002a). *Zhong Guo Ming Fang Quan Shu (Famous TCM Prescriptions in China).* Beijing: Scientific and Technical Documents Publishing House.

Chen X, Ung CY, Chen Y (2003). Can an in silico drug-target search method be used to probe potential mechanisms of medicinal plant ingredients? *Natural Product Reports* **20**(4):432-444.

Chen YZ, Ung CY (2002b). Computer automated prediction of potential therapeutic and toxicity protein targets of bioactive compounds from Chinese medicinal plants. *American Journal of Chinese Medicine* **30**(1):139-154.

Cheng CY, Chung WY, Szeto YT, Benzie IF (2005). Fasting plasma zeaxanthin response to Fructus barbarum L. (wolfberry; Kei Tze) in a food-based human supplementation trial. *British Journal of Nutrition* **93**(1):123-130.

Cheng JT (2000). Review: drug therapy in Chinese traditional medicine. *Journal of Clinical Pharmacology* **40**(5):445-450.

Chou TC (2006). Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacological Reviews* **58**(3):621-681.

Chueh PJ, Wu LY, Morre DM, Morre DJ (2004). tNOX is both necessary and sufficient as a cellular target for the anticancer actions of capsaicin and the green tea catechin (-)-epigallocatechin-3-gallate. *Biofactors* **20**(4):235-249.

Ciccolini J, Peillard L, Evrard A, Cuq P, Aubert C, Pelegrin A, Formento P, Milano G, Catalin J (2000). Enhanced antitumor activity of 5-fluorouracil in combination with 2'-deoxyinosine in human colorectal cell lines and human colon tumor xenografts. *Clinical Cancer Research* **6**(4):1529-1535.

Cichewicz RH, Zhang Y, Seeram NP, Nair MG (2004). Inhibition of human tumor cell proliferation by novel anthraquinones from daylilies. *Life Sciences* **74**(14):1791-1799.

Collene AL, Hertzler SR, Williams JA, Wolf BW (2005). Effects of a nutritional supplement containing Salacia oblonga extract and insulinogenic amino acids on postprandial glycemia, insulinemia, and breath hydrogen responses in healthy adults. *Nutrition* **21**(7-8):848-854.

Combs CK, Karlo JC, Kao SC, Landreth GE (2001). beta-Amyloid stimulation of microglia and monocytes results in TNFalpha-dependent expression of inducible nitric oxide synthase and neuronal apoptosis. *The Journal of neuroscience* **21**(4):1179-1188.

Conte A, Pellegrini S, Tagliazucchi D (2003a). Effect of resveratrol and catechin on PC12 tyrosine kinase activities and their synergistic protection from beta-amyloid toxicity. *Drugs under Experimental and Clinical Research* **29**(5-6):243-255.

Conte A, Pellegrini S, Tagliazucchi D (2003b). Synergistic protection of PC12 cells from beta-amyloid toxicity by resveratrol and catechin. *Brain Research Bulletin* **62**(1):29-38.

Cottagnoud P, Cottagnoud M, Tauber MG (2003). Vancomycin acts synergistically with gentamicin against penicillin-resistant pneumococci by increasing the intracellular penetration of gentamicin. *Antimicrobial Agents and Chemotherapy* **47**(1):144-147.

Cruchaga C, Odriozola L, Andreola M, Tarrago-Litvak L, Martinez-Irujo JJ (2005). Inhibition of phosphorolysis catalyzed by HIV-1 reverse transcriptase is responsible for the synergy found in combinations of 3'-azido-3'-deoxythymidine with nonnucleoside inhibitors. *Biochemistry* **44**(9):3535-3546.

Czernichow S, Blacher J, Hercberg S (2004). Antioxidant vitamins and blood pressure. *Curr Hypertens Rep* **6**(1):27-30.

D'Incalci M, Colombo T, Ubezio P, Nicoletti I, Giavazzi R, Erba E, Ferrarese L, Meco D, Riccardi R, Sessa C and others (2003). The combination of yondelis and cisplatin is synergistic against human tumor xenografts. *European Journal of Cancer* **39**(13):1920-1926.

Dalais FS, Meliala A, Wattanapenpaiboon N, Frydenberg M, Suter DA, Thomson WK, Wahlqvist ML (2004). Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer. *Urology* **64**(3):510-515.

Danz H, Baumann D, Hamburger M (2002). Quantitative determination of the dual COX-2/5-LOX inhibitor tryptanthrin in Isatis tinctoria by ESI-LC-MS. *Planta Medica* **68**(2):152-157.

De Clercq E (2000). Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. *Medicinal Research Reviews* **20**(5):323-349.

de Gaetano G, Donati MB, Cerletti C (2003). Prevention of thrombosis and vascular inflammation: benefits and limitations of selective or combined COX-1, COX-2 and 5-LOX inhibitors. *Trends in Pharmacological Sciences* **24**(5):245-252.

Defeudis FV (2002). Bilobalide and neuroprotection. *Pharmacological Research* **46**(6):565-568.

Devasagayam TP, Kamat JP, Mohan H, Kesavan PC (1996). Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. *Biochimica et Biophysica Acta* **1282**(1):63-70.

Devita VT. (1997). In: Devita VT, Hellman S, Rosenberg SA, editors. *Cancer: Principles and practice of Oncology*. Philadelphia: Lippincott-Raven. p 333-347.

Dilika F, Bremner PD, Meyer JJ (2000). Antibacterial activity of linoleic and oleic acids isolated from Helichrysum pedunculatum: a plant used during circumcision rites. *Fitoterapia* **71**(4):450-452.

Dowdy SC, Jiang S, Zhou XC, Hou X, Jin F, Podratz KC, Jiang SW (2006). Histone deacetylase inhibitors and paclitaxel cause synergistic effects on apoptosis and microtubule stabilization in papillary serous endometrial cancer cells. *Molecular cancer therapeutics* **5**(11):2767-2776.

Dryden MS, Dailly S, Crouch M (2004). A randomized, controlled trial of tea tree topical preparations versus a standard topical regimen for the clearance of MRSA colonization. *Journal of Hospital Infection* **56**(4):283-286.

Dryselius R, Nekhotiaeva N, Good L (2005). Antimicrobial synergy between mRNA- and protein-level inhibitors. *Journal of Antimicrobial Chemotherapy* **56**(1):97-103.

Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, Chantre P, Vandermander J (1999). Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *American Journal of Clinical Nutrition* **70**(6):1040-1045.

Dulloo AG, Seydoux J, Girardier L, Chantre P, Vandermander J (2000). Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *International Journal of Obesity and Related Metabolic Disorders* **24**(2):252-258.

Duncan R, Vicent MJ, Greco F, Nicholson RI (2005). Polymer-drug conjugates: towards a novel approach for the treatment of endrocine-related cancer. *Endocrine-related cancer* **Suppl**(1):S189-S199.

Dursteler C, Mases A, Fernandez V, Pol O, Puig MM (2006). Interaction between tramadol and two anti-emetics on nociception and gastrointestinal transit in mice. *European journal of pain* **10**(7):629-638.

Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC (1998). Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA* **280**(18):1569-1575.

Elder C, Aickin M, Bauer V, Cairns J, Vuckovic N (2006). Randomized trial of a whole-system ayurvedic protocol for type 2 diabetes. *Alternative Therapies in Health and Medicine* **12**(5):24-30.

Famin O, Ginsburg H (2002). Differential effects of 4-aminoquinoline-containing antimalarial drugs on hemoglobin digestion in Plasmodium falciparum-infected erythrocytes. *Biochemical Pharmacology* **63**(3):393-398.

Fan TP, Yeh JC, Leung KW, Yue PY, Wong RN (2006). Angiogenesis: from plants to blood vessels. *Trends in Pharmacological Sciences* **27**(6):297-309.

Fava M, Alpert J, Nierenberg AA, Mischoulon D, Otto MW, Zajecka J, Murck H, Rosenbaum JF (2005). A Double-blind, randomized trial of St John's wort, fluoxetine, and placebo in major depressive disorder. *Journal of Clinical Psychopharmacology* **25**(5):441-447.

Fernandez-Cuenca F, Martinez-Martinez L, Pascual A, Perea EJ (2003). In vitro activity of azithromycin in combination with amikacin, ceftazidime, ciprofloxacin or imipenem against clinical isolates of Acinobacter baumannii. *Chemotherapy* **49**(1-2):24-26.

Franke AA, Cooney RV, Custer LJ, Mordan LJ, Tanaka Y (1998). Inhibition of neoplastic transformation and bioavailability of dietary flavonoid agents. *Adv Exp Med Biol* **439**:237-248.

Fuhrman B, Volkova N, Rosenblat M, Aviram M (2000). Lycopene synergistically inhibits LDL oxidation in combination with vitamin E, glabridin, rosmarinic acid, carnosic acid, or garlic. *Antioxid Redox Signal* **2**(3):491-506.

Fujino H, Shimada S, Yamada I, Hirano M, Tsunenari Y, Kojima J (2003). Studies on the interaction between fibrates and statins using human hepatic microsomes. *Arzneimittel-Forschung* **53**(10):701-707.

Galaris D, Korantzopoulos P (1997). On the molecular mechanism of metmyoglobin-catalyzed reduction of hydrogen peroxide by ascorbate. *Free Radical Biology and Medicine* **22**(4):657-667.

Gilbert B, Alves LF (2003). Synergy in plant medicines. *Current Medicinal Chemistry* **10**(1):13-20.

Goddard J, Eckhart C, Johnston NR, Cumming AD, Rankin AJ, Webb DJ (2004). Endothelin A receptor antagonism and angiotensin-converting enzyme inhibition are synergistic via an endothelin B receptor-mediated and nitric oxide-dependent mechanism. *Journal of the American Society of Nephrology* **15**(10):2601-2610.

Gong X, Sucher NJ (1999). Stroke therapy in traditional Chinese medicine (TCM): prospects for drug discovery and development. *Trends in Pharmacological Sciences* **20**(5):191-196.

Goto H, Shimada Y, Akechi Y, Kohta K, Hattori M, Terasawa K (1996). Endothelium-dependent vasodilator effect of extract prepared from the roots of Paeonia lactiflora on isolated rat aorta. *Planta Medica* **62**(5):436-439.

Graham BA, Hammond DL, Proudfit HK (2000). Synergistic interactions between two alpha(2)-adrenoceptor agonists, dexmedetomidine and ST-91, in two substrains of Sprague-Dawley rats. *Pain* **85**(1-2):135-143.

Grassmann J, Schneider D, Weiser D, Elstner EF (2001). Antioxidative effects of lemon oil and its components on copper induced oxidation of low density lipoprotein. *Arzneimittel-Forschung* **51**(10):799-805.

Greenwald P (2004). Clinical trials in cancer prevention: current results and perspectives for the future. *The journal of nutrition* **134**(Suppl 12):3507S-3512S.

Guminski AD, Harnett PR, deFazio A (2001). Carboplatin and paclitaxel interact antagonistically in a megakaryoblast cell line--a potential mechanism for paclitaxel-mediated sparing of carboplatin-induced thrombocytopenia. *Cancer Chemotherapy and Pharmacology* **48**(3):229-234.

Guo JM, Kang GZ, Xiao BX, Liu DH, Zhang S (2004). Effect of daidzein on cell growth, cell cycle, and telomerase activity of human cervical cancer in vitro. *Int J Gynecol Cancer* **14**(5):882-888.

Gupta GP, Nguyen DX, Chiang AC, Bos PD, Kim JY, Nadal C, Gomis RR, Manova-Todorova K, Massague J (2007). Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. *Nature* **446**(7137):765-770.

Gysin R, Azzi A, Visarius T (2002). Gamma-tocopherol inhibits human cancer cell cycle progression and cell proliferation by down-regulation of cyclins. *FASEB Journal* **16**(14):1952-1954.

Harris RS, Lazar O, Johansen JW, Sebel PS (2006). Interaction of propofol and sevoflurane on loss of consciousness and movement to skin incision during general anesthesia. *Anesthesiology* **104**(6):1170-1175.

Harvey A (2000). Strategies for discovering drugs from previously unexplored natural products. *Drug Discov Today* **5**(7):294-300.

Hayashi K, Hayashi T, Sun HD, Takeda Y (2000). Potentiation of ganciclovir toxicity in the herpes simplex virus thymidine kinase/ganciclovir administration system by ponicidin. *Cancer Gene Therapy* **7**(1):45-52.

Hayashi K, Lee JB, Maitani Y, Toyooka N, Nemoto H, Hayashi T (2006). The role of a HSV thymidine kinase stimulating substance, scopadulciol, in improving the efficacy of cancer gene therapy. *The journal of gene medicine* **8**(8):1056-1067.

Heinemann C, Schliemann-Willers S, Oberthur C, Hamburger M, Elsner P (2004). Prevention of experimentally induced irritant contact dermatitis by extracts of Isatis tinctoria compared to pure tryptanthrin and its impact on UVB-induced erythema. *Planta Medica* **70**(5):385-390.

Hioki C, Yoshimoto K, Yoshida T (2004). Efficacy of bofu-tsusho-san, an oriental herbal medicine, in obese Japanese women with impaired glucose tolerance. *Clinical and Experimental Pharmacology and Physiology* **31**(9):614-619.

Hirsch K, Danilenko M, Giat J, Miron T, Rabinkov A, Wilchek M, Mirelman D, Levy J, Sharoni Y (2000). Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation. *Nutrition and Cancer* **38**(2):245-254.

Hong J, Lu H, Meng X, Ryu JH, Hara Y, Yang CS (2002). Stability, cellular uptake, biotransformation, and efflux of tea polyphenol (-)-epigallocatechin-3-gallate in HT-29 human colon adenocarcinoma cells. *Cancer Res* **62**(24):7241-7246.

Honore S, Kamath K, Braguer D, Horwitz SB, Wilson L, Briand C, Jordan MA (2004). Synergistic suppression of microtubule dynamics by discodermolide and paclitaxel in non-small cell lung carcinoma cells. *Cancer Research* **64**(14):4957-4964.

Hopkins AL, Groom CR (2002). The druggable genome. *Nature reviews. Drug discovery.* **1**(9):727-730.

Hostanska K, Reichling J, Bommer S, Weber M, Saller R (2003). Hyperforin a constituent of St John's wort (Hypericum perforatum L.) extract induces apoptosis by triggering activation of caspases and with hypericin synergistically exerts cytotoxicity towards human malignant cell lines. *European Journal of Pharmaceutics and Biopharmaceutics* **56**(1):121-132.

Hou SL. (2001). *Zhong Yao 800 Zhong Xiang Jie (The Detail Description of 800 TCM Herbs)*. Henan: He Nan Science and Technology Publiser.

Hsieh CL, Chang CH, Chiang SY, Li TC, Tang NY, Pon CZ, Hsieh CT, Lin JG (2000). Anticonvulsive and free radical scavenging activities of vanillyl alcohol in ferric chloride-induced epileptic seizures in Sprague-Dawley rats. *Life Sci* **67**(10):1185-1195.

Hsieh CL, Tang NY, Chiang SY, Hsieh CT, Lin JG (1999). Anticonvulsive and free radical scavenging actions of two herbs, Uncaria rhynchophylla (MIQ) Jack and Gastrodia elata Bl., in kainic acid-treated rats. *Life Sci* **65**(20):2071-2082.

Hsu CH, Lu CM, Chang TT (2005). Efficacy and safety of modified Mai-Men-Dong-Tang for treatment of allergic asthma. *Pediatric Allergy and Immunology* **16**(1):76-81.

Hu SL, Zhang HQ, Chan K, Mei QX (2004). Studies on the toxicity of Aristolochia manshuriensis (Guanmuton). *Toxicology* **198**(1-3):195-201.

Hu YC, Hou JY (2002). [Study on the effect of zhimu combined huangqi on improving renal hypertension rat's cardiac dysfunction]. *Zhongguo Zhong Yao Za Zhi* **27**(11):858-861, 877.

Huang HY, Caballero B, Chang S, Alberg AJ, Semba RD, Schneyer CR, Wilson RF, Cheng TY, Vassy J, Prokopowicz G and others (2006a). The efficacy and safety of multivitamin and mineral supplement use to prevent cancer and chronic disease in adults: a systematic review for a National Institutes of Health state-of-the-science conference. *Annals of Internal Medicine* **145**(5):372-385.

Huang L, Liu J, Li D, Wang Z, Ye W, Cai B, Liu M, Li M (1991). [A study on components and compound prescription of huangqin decoction]. *Zhongguo Zhong yao za zhi* **16**(3):177-181, back cover.

Huang Q, Lu G, Shen HM, Chung MC, Ong CN (2006b). Anti-cancer properties of anthraquinones from Rhubarb. *Medicinal Research Reviews*.

Huang Q, Shen HM, Shui G, Wenk MR, Ong CN (2006c). Emodin inhibits tumor cell adhesion through disruption of the membrane lipid Raft-associated integrin signaling pathway. *Cancer Research* **66**(11):5807-5815.

Huang RL, Chen CC, Huang YL, Hsieh DJ, Hu CP, Chen CF, Chang C (1996). Osthole increases glycosylation of hepatitis B surface antigen and suppresses the secretion of hepatitis B virus in vitro. *Hepatology* **24**(3):508-515.

Huseini HF, Alavian SM, Heshmat R, Heydari MR, Abolmaali K (2005). The efficacy of Liv-52 on liver cirrhotic patients: a randomized, double-blind, placebo-controlled first approach. *Phytomedicine* **12**(9):619-624.

Huseini HF, Larijani B, Heshmat R, Fakhrzadeh H, Radjabipour B, Toliat T, Raza M (2006). The efficacy of Silybum marianum (L.) Gaertn. (silymarin) in the

treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial. *Phytotherapy Research* **20**(12):1036-1039.

Hwang J, Hodis HN, Sevanian A (2001). Soy and alfalfa phytoestrogen extracts become potent low-density lipoprotein antioxidants in the presence of acerola cherry extract. *Journal of Agricultural and Food Chemistry* **49**(1):308-314.

Hwang J, Sevanian A, Hodis HN, Ursini F (2000). Synergistic inhibition of LDL oxidation by phytoestrogens and ascorbic acid. *Free Radic Biol Med* **29**(1):79-89.

Ippoushi K, Azuma K, Ito H, Horie H, Higashio H (2003). [6]-Gingerol inhibits nitric oxide synthesis in activated J774.1 mouse macrophages and prevents peroxynitrite-induced oxidation and nitration reactions. *Life Sciences* **73**(26):3427-3437.

Jayawardena MH, de Alwis NM, Hettigoda V, Fernando DJ (2005). A double blind randomised placebo controlled cross over study of a herbal preparation containing Salacia reticulata in the treatment of type 2 diabetes. *Journal of Ethnopharmacology* **97**(2):215-218.

Jeong YJ, Choi YJ, Kwon HM, Kang SW, Park HS, Lee M, Kang YH (2005). Differential inhibition of oxidized LDL-induced apoptosis in human endothelial cells treated with different flavonoids. *British Journal of Nutrition* **93**(5):581-591.

Jiang WY (2005). Therapeutic wisdom in traditional Chinese medicine: a perspective from modern science. *Trends in Pharmacological Sciences* **26**(11):558-563.

Jing Y, Waxman S (1995). Structural requirements for differentiation-induction and growth-inhibition of mouse erythroleukemia cells by isoflavones. *Anticancer Research* **15**(4):1147-1152.

Jodoin J, Demeule M, Beliveau R (2002). Inhibition of the multidrug resistance P-glycoprotein activity by green tea polyphenols. *Biochim Biophys Acta* **1542**(1-3):149-159.

Johnson R, Wichern D. (1982). *Applied multivariate statistical analysis*. Englewood Cliffs, NJ: Prentice Hall.

Jonker DM, Visser SA, van der Graaf PH, Voskuyl RA, Danhof M (2005). Towards a mechanism-based analysis of pharmacodynamic drug-drug interactions in vivo. *Pharmacology & Therapeutics* **106**(1):1-18.

Kaiho T, Tanaka T, Tsuchiya S, Yanagisawa S, Takeuchi O, Miura M, Saigusa N, Miyazaki M (2005). Effect of the herbal medicine Dai-kenchu-to for serum ammonia in hepatectomized patients. *Hepato-Gastroenterology* **52**(61):161-165.

Kamei J, Nakamura R, Ichiki H, Kubo M (2003). Antitussive principles of Glycyrrhizae radix, a main component of the Kampo preparations Bakumondo-to (Mai-men-dong-tang). *European Journal of Pharmacology* **469**(1-3):159-163.

Kanamitsu SI, Ito K, Okuda H, Ogura K, Watabe T, Muro K, Sugiyama Y (2000). Prediction of in vivo drug-drug interactions based on mechanism-based inhibition from in vitro data: inhibition of 5-fluorouracil metabolism by (E)-5-(2-Bromovinyl)uracil. *Drug Metabolism and Disposition* **28**(4):467-474.

Kaptchuk TJ (2002). The placebo effect in alternative medicine: can the performance of a healing ritual have clinical significance? *Annals of Internal Medicine* **136**(11):817-825.

Karlowsky JA, Hoban DJ, Zhanel GG, Goldstein BP (2006). In vitro interactions of anidulafungin with azole antifungals, amphotericin B and 5-fluorocytosine against Candida species. *International Journal of Antimicrobial Agents* **27**(2):174-177.

Kasper S, Anghelescu IG, Szegedi A, Dienel A, Kieser M (2006). Superior efficacy of St John's wort extract WS 5570 compared to placebo in patients with major depression: a randomized, double-blind, placebo-controlled, multi-center trial [ISRCTN77277298]. *BMC medicine* **4**:14.

Kaye AD, Clarke RC, Sabar R, Vig S, Dhawan KP, Hofbauer R, Kaye AM (2000). Herbal medicines: current trends in anesthesiology practice--a hospital survey. *Journal of Clinical Anesthesia* **12**(6):468-471.

Keith CT, Borisy AA, Stockwell BR (2005). Multicomponent therapeutics for networked systems. *Nat Rev Drug Discov* **4**(1):71-78.

Kell DB (2004). Metabolomics and systems biology: making sense of the soup. *Current Opinion in Microbiology* **7**(3):296-307.

Kemmerich B, Eberhardt R, Stammer H (2006). Efficacy and tolerability of a fluid extract combination of thyme herb and ivy leaves and matched placebo in adults suffering from acute bronchitis with productive cough. A prospective, double-blind, placebo-controlled clinical trial. *Arzneimittel-Forschung* **56**(9):652-660.

Kennedy DO, Haskell CF, Wesnes KA, Scholey AB (2004). Improved cognitive performance in human volunteers following administration of guarana (Paullinia cupana) extract: comparison and interaction with Panax ginseng. *Pharmacology, Biochemistry and Behavior* **79**(3):401-411.

Kharbanda S, Kumar V, Dhar S, Pandey P, Chen C, Majumder P, Yuan ZM, Whang Y, Strauss W, Pandita TK and others (2000). Regulation of the hTERT telomerase catalytic subunit by the c-Abl tyrosine kinase. *Current Biology* **10**(10):568-575.

Kim MS, Park MJ, Kim SJ, Lee CH, Yoo H, Shin SH, Song ES, Lee SH (2005). Emodin suppresses hyaluronic acid-induced MMP-9 secretion and invasion of glioma cells. *International Journal of Oncology* **27**(3):839-846.

Kinzler KW, Vogelstein B (1996). Lessons from hereditary colorectal cancer. *Cell* **87**(2):159-170.

Ko K, Mak D, Chiu P, Poon M (2004). Pharmacological basis of 'Yang-invigoration' in Chinese medicine. *Trends in Pharmacological Sciences* **25**(1):3-6.

Koizumi F, Kanzawa F, Ueda Y, Koh Y, Tsukiyama S, Taguchi F, Tamura T, Saijo N, Nishio K (2004). Synergistic interaction between the EGFR tyrosine kinase inhibitor gefitinib ("Iressa") and the DNA topoisomerase I inhibitor CPT-11 (irinotecan) in human colorectal cancer cells. *International Journal of Cancer* **108**(3):464-472.

Koller WC, Rueda MG (1998). Mechanism of action of dopaminergic agents in Parkinson's disease. *Neurology* **50**(Suppl 6):S11-S14; S44-S48.

Kondratskaya EL, Fisyunov AI, Chatterjee SS, Krishtal OA (2004). Ginkgolide B preferentially blocks chloride channels formed by heteromeric glycine receptors in hippocampal pyramidal neurons of rat. *Brain Research Bulletin* **63**(4):309-314.

Krampfl K, Schlesinger F, Dengler R, Bufler J (2000). Pentobarbital has curare-like effects on adult-type nicotinic acetylcholine receptor channel currents. *Anesthesia and Analgesia* **90**(4):970-974.

Kucera M, Barna M, Horacek O, Kovarikova J, Kucera A (2004). Efficacy and safety of topically applied Symphytum herb extract cream in the treatment of ankle distortion: results of a randomized controlled clinical double blind study. *Wiener Medizinische Wochenschrift* **154**(21-22):498-507.

Kuhn DJ, Burns AC, Kazi A, Dou QP (2004). Direct inhibition of the ubiquitin-proteasome pathway by ester bond-containing green tea polyphenols is associated with increased expression of sterol regulatory element-binding protein 2 and LDL receptor. *Biochimica et Biophysica Acta* **1682**(1-3):1-10.

Kumar A, Dhawan S, Aggarwal BB (1998). Emodin (3-methyl-1,6,8-trihydroxyanthraquinone) inhibits TNF-induced NF-kappaB activation, IkappaB degradation, and expression of cell surface adhesion proteins in human vascular endothelial cells. *Oncogene* **17**(7):913-918.

Kuo PL, Lin TC, Lin CC (2002). The antiproliferative activity of aloe-emodin is through p53-dependent and p21-dependent apoptotic pathway in human hepatoma cell lines. *Life Sciences* **71**(16):1879-1892.

Kupfersztain C, Rotem C, Fagot R, Kaplan B (2003). The immediate effect of natural plant extract, Angelica sinensis and Matricaria chamomilla (Climex) for the treatment of hot flushes during menopause. A preliminary report. *Clinical and experimental obstetrics & gynecology* **30**(4):203-206.

Kuypers DR, Verleden G, Naesens M, Vanrenterghem Y (2005). Drug interaction between mycophenolate mofetil and rifampin: possible induction of uridine diphosphate-glucuronosyltransferase. *Clinical Pharmacology and Therapeutics* **78**(1):81-88.

Lai GH, Zhang Z, Sirica AE (2003). Celecoxib acts in a cyclooxygenase-2-independent manner and in synergy with emodin to suppress rat cholangiocarcinoma growth in vitro through a mechanism involving enhanced Akt inactivation and increased activation of caspases-9 and -3. *Molecular cancer therapeutics* **2**(3):265-271.

Lampe JW (2003). Isoflavonoid and lignan phytoestrogens as dietary biomarkers. *J Nutr* **133 Suppl 3**:956S-964S.

Lazar M (2004a). East meets West: an herbal tea finds a receptor. *Journal of Clinical Investigation* **113**(1):23-25.

Lazar MA (2004b). East meets West: an herbal tea finds a receptor. *Journal of Clinical Investigation* **113**(1):23-25.

Lee HZ, Lin CJ, Yang WH, Leung WC, Chang SP (2006). Aloe-emodin induced DNA damage through generation of reactive oxygen species in human lung carcinoma cells. *Cancer Letters* **239**(1):55-63.

Lee KH (1999). Novel antitumor agents from higher plants. *Medicinal Research Reviews* **19**(6):569-596.

Lee W, Kim RB (2004). Transporters and renal drug elimination. *Annual Review of Pharmacology and Toxicology* **44**:137-166.

Leonard SS, Cutler D, Ding M, Vallyathan V, Castranova V, Shi X (2002). Antioxidant properties of fruit and vegetable juices: more to the story than ascorbic acid. *Annals of Clinical and Laboratory Science* **32**(2):193-200.

Lewith GT, Hyland ME, Shaw S (2002). Do attitudes toward and beliefs about complementary medicine affect treatment outcomes? *American Journal of Public Health* **92**(10):1604-1606.

Li F, Sun S, Wang J, Wang D (1998). Chromatography of medicinal plants and Chinese traditional medicines. *Biomedical Chromatography* **12**(2):78-85.

Li H, Yap C, Ung C, Xue Y, Cao Z, Chen Y (2005a). Effect of Selection of Molecular Descriptors on the Prediction of Blood-Brain Barrier Penetrating and Nonpenetrating Agents by Statistical Learning Methods. *Journal of Chemical Information and Modeling* **45**(5):1376-1384.

Li HL, Chen HL, Li H, Zhang KL, Chen XY, Wang XW, Kong QY, Liu J (2005b). Regulatory effects of emodin on NF-kappaB activation and inflammatory cytokine expression in RAW 264.7 macrophages. *International Journal of Molecular Medicine* **16**(1):41-47.

Li QY. (2005). *Zhong Yi Chu Fang Fang Fa Xue (Methodology in the Construction of TCM Prescriptions)*. Beijing: Ren Min Jun Yi Publisher.

Li WL, Zheng HC, Bukuru J, De Kimpe N (2004). Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *Journal of Ethnopharmacology* **92**(1):1-21.

Liao JF, Wang HH, Chen MC, Chen CC, Chen CF (1998). Benzodiazepine binding site-interactive flavones from Scutellaria baicalensis root. *Planta Medica* **64**(6):571-572.

Lin JG, Chen GW, Li TM, Chouh ST, Tan TW, Chung JG (2006). Aloe-emodin induces apoptosis in T24 human bladder cancer cells through the p53 dependent apoptotic pathway. *Journal of Urology* **175**(1):343-347.

Lin KH, Shieh HY, Hsu HC (2000). Negative regulation of the antimetastatic gene Nm23-H1 by thyroid hormone receptors. *Endocrinology* **141**(7):2540-2547.

Lin KH, Wang WJ, Wu YH, Cheng SY (2002). Activation of antimetastatic Nm23-H1 gene expression by estrogen and its alpha-receptor. *Endocrinology* **143**(2):467-475.

Lin S, Fujii M, Hou DX (2003). Rhein induces apoptosis in HL-60 cells via reactive oxygen species-independent mitochondrial death pathway. *Archives of Biochemistry and Biophysics* **418**(2):99-107.

Lin WS, Chan WC, Hew CS (1995). Superoxide and traditional Chinese medicines. *Journal of Ethnopharmacology* **48**(3):165-171.

Lin X, Kim HK, Howell SB (1999). The role of DNA mismatch repair in cisplatin mutagenicity. *Journal of Inorganic Biochemistry* **77**(1-2):89-93.

Liu J, Zhai Y, Du H (1997). [Quality criteria for bazhen shennongyin liquor]. *Zhongguo Zhong yao za zhi* **22**(1):28-31, 61.

Liu MC, Maruyama S, Mizuno M, Morita Y, Hanaki S, Yuzawa Y, Matsuo S (2003). The nephrotoxicity of Aristolochia manshuriensis in rats is attributable to its aristolochic acids. *Clin Exp Nephrol* **7**(3):186-194.

Livny O, Reifen R, Levy I, Madar Z, Faulks R, Southon S, Schwartz B (2003). Beta-carotene bioavailability from differently processed carrot meals in human ileostomy volunteers. *European Journal of Nutrition* **42**(6):338-345.

Loden M (2003). Do moisturizers work? *Journal of cosmetic dermatology* **2**(3-4):141-149.

Lopatkin N, Sivkov A, Walther C, Schlafke S, Medvedev A, Avdeichuk J, Golubev G, Melnik K, Elenberger N, Engelmann U (2005). Long-term efficacy and safety of a combination of sabal and urtica extract for lower urinary tract symptoms--a

placebo-controlled, double-blind, multicenter trial. *World Journal of Urology* **23**(2):139-146.

Lu GD, Shen HM, Ong CN, Chung CM (2007). Anticancer effects of aloe-emodin on HepG2 cells: Cellular and proteomic studies. *Proteomics* **1**(4):410-419.

Lu J, Goldstein KM, Chen P, Huang S, Gelbert LM, Nagpal S (2005). Transcriptional profiling of keratinocytes reveals a vitamin D-regulated epidermal differentiation network. *Journal of Investigative Dermatology* **124**(4):778-785.

Mahmood N, Piacente S, Pizza C, Burke A, Khan AI, Hay AJ (1996). The anti-HIV activity and mechanisms of action of pure compounds isolated from Rosa damascena. *Biochemical and Biophysical Research Communications* **229**(1):73-79.

Marcus AI, Zhou J, O'Brate A, Hamel E, Wong J, Nivens M, El-Naggar A, Yao TP, Khuri FR, Giannakakou P (2005). The synergistic combination of the farnesyl transferase inhibitor lonafarnib and paclitaxel enhances tubulin acetylation and requires a functional tubulin deacetylase. *Cancer Research* **65**(9):3883-3893.

Matsuda H, Shimoda H, Morikawa T, Yoshikawa M (2001). Phytoestrogens from the roots of *Polygonum cuspidatum* (*Polygonaceae*): structure-requirement of hydroxyanthraquinones for estrogenic activity. *Bioorganic & Medicinal Chemistry Letters* **11**(14):1839-1842.

Maubach J, Bracke ME, Heyerick A, Depypere HT, Serreyn RF, Mareel MM, De Keukeleire D (2003). Quantitation of soy-derived phytoestrogens in human breast tissue and biological fluids by high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* **784**(1):137-144.

Mazza M, Capuano A, Bria P, Mazza S (2006). *Ginkgo biloba* and donepezil: a comparison in the treatment of Alzheimer's dementia in a randomized placebo-controlled double-blind study. *European Journal of Neurology* **13**(9):981-985.

McKay L, Gemmell H, Jacobson B, Hayes B (2003). Effect of a Topical Herbal Cream on the Pain and Stiffness of Osteoarthritis: A Randomized Double-Blind, Placebo-Controlled Clinical Trial. *Journal of clinical rheumatology: practical reports on rheumatic & musculoskeletal diseases* **9**(3):164-169.

Meco D, Colombo T, Ubezio P, Zucchetti M, Zaffaroni M, Riccardi A, Faircloth G, Jose J, D'Incalci M, Riccardi R (2003). Effective combination of ET-743 and doxorubicin in sarcoma: preclinical studies. *Cancer Chemotherapy and Pharmacology* **52**(2):131-138.

Meletiadis J, Petraitis V, Petraitiene R, Lin P, Stergiopoulou T, Kelaher AM, Sein T, Schaufele RL, Bacher J, Walsh TJ (2006). Triazole-polyene antagonism in experimental invasive pulmonary aspergillosis: in vitro and in vivo correlation. *Journal of Infectious Diseases* **194**(7):1008-1018.

Menez C, Buyse M, Besnard M, Farinotti R, Loiseau PM, Barratt G (2006). Interaction between miltefosine and amphotericin B: consequences for their activities towards intestinal epithelial cells and Leishmania donovani promastigotes in vitro. *Antimicrobial Agents and Chemotherapy* **50**(11):3793-3800.

Milde J, Elstner EF, Grassmann J (2004). Synergistic inhibition of low-density lipoprotein oxidation by rutin, gamma-terpinene, and ascorbic acid. *Phytomedicine* **11**(2-3):105-113.

Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, Viale A, Olshen AB, Gerald WL, Massague J (2005). Genes that mediate breast cancer metastasis to lung. *Nature* **436**(7050):518-524.

Minotti G, Saponiero A, Licata S, Menna P, Calafiore AM, Teodori G, Gianni L (2001). Paclitaxel and docetaxel enhance the metabolism of doxorubicin to toxic species in human myocardium. *Clinical Cancer Research* **7**(6):1511-1515.

Mix JA, Crews WDJ (2002). A double-blind, placebo-controlled, randomized trial of Ginkgo biloba extract EGb 761 in a sample of cognitively intact older adults: neuropsychological findings. *Human psychopharmacology* **17**(6):267-277.

Mohn T, Potterat O, Hamburger M (2007). Quantification of Active Principles and Pigments in Leaf Extracts of Isatis tinctoria by HPLC/UV/MS. *Planta Medica*:DOI: 10.1055/s-2007-967105.

Morel C, Stermitz FR, Tegos G, Lewis K (2003). Isoflavones as potentiators of antibacterial activity. *J Agric Food Chem* **51**(19):5677-5679.

Morin CM, Koetter U, Bastien C, Ware JC, Wooten V (2005). Valerian-hops combination and diphenhydramine for treating insomnia: a randomized placebo-controlled clinical trial. *Sleep* **28**(11):1465-1471.

Morre DJ, Bridge A, Wu LY, Morre DM (2000). Preferential inhibition by (-)-epigallocatechin-3-gallate of the cell surface NADH oxidase and growth of transformed cells in culture. *Biochemical Pharmacology* **60**(7):937-946.

Morre DJ, Chueh PJ, Morre DM (1995). Capsaicin inhibits preferentially the NADH oxidase and growth of transformed cells in culture. *Proceedings of the National Academy of Sciences of the United States of America* **92**(6):1831-1835.

Morre DJ, Morre DM (2003a). Synergistic Capsicum-tea mixtures with anticancer activity. *Journal of Pharmacy and Pharmacology* **55**(7):987-994.

Morre DJ, Morre DM, Sun H, Cooper R, Chang J, Janle EM (2003b). Tea catechin synergies in inhibition of cancer cell proliferation and of a cancer specific cell surface oxidase (ECTO-NOX). *Pharmacology and Toxicology* **92**(5):234-241.

Morre DM, Morre DJ (2006). Catechin-vanilloid synergies with potential clinical applications in cancer. *Rejuvenation research* **9**(1):45-55.

Motlekar NA, Srivenugopal KS, Wachtel MS, Youan BB (2005). Oral delivery of low-molecular-weight heparin using sodium caprate as absorption enhancer reaches therapeutic levels. *Journal of Drug Targeting* **13**(10):573-583.

Musumeci R, Speciale A, Costanzo R, Annino A, Ragusa S, Rapisarda A, Pappalardo MS, Iauk L (2003). Berberis aetnensis C. Presl. extracts: antimicrobial properties and interaction with ciprofloxacin. *International Journal of Antimicrobial Agents* **22**(1):48-53.

Nahum A, Hirsch K, Danilenko M, Watts CK, Prall OW, Levy J, Sharoni Y (2001). Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27(Kip1) in the cyclin E-cdk2 complexes. *Oncogene* **20**(26):3428-3436.

Nakajima Y, Mizobuchi M, Nakamura M, Takagi H, Inagaki H, Kominami G, Koike M, Yamaguchi T (2004). Mechanism of the drug interaction between valproic acid and carbapenem antibiotics in monkeys and rats. *Drug Metabolism and Disposition* **32**(12):1383-1391.

Nandakumar DN, Nagaraj VA, Vathsala PG, Rangarajan P, Padmanaban G (2006). Curcumin-artemisinin combination therapy for malaria. *Antimicrobial Agents and Chemotherapy* **50**(5):1859-1860.

Narishetty ST, Panchagnula R (2004). Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. *Journal of Controlled Release* **95**(3):367-379.

Naser B, Lund B, Henneicke-von Zepelin HH, Kohler G, Lehmacher W, Scaglione F (2005). A randomized, double-blind, placebo-controlled, clinical dose-response trial of an extract of Baptisia, Echinacea and Thuja for the treatment of patients with common cold. *Phytomedicine* **12**(10):715-722.

Newman DJ, Cragg GM (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products* **70**(3):461-477.

Newman DJ, Cragg GM, Snader KM (2003). Natural products as sources of new drugs over the period 1981-2002. *Journal of Natural Products* **66**(7):1022-1037.

Ngui JS, Chen Q, Shou M, Wang RW, Stearns RA, Baillie TA, Tang W (2001). In vitro stimulation of warfarin metabolism by quinidine: increases in the formation of 4'- and 10-hydroxywarfarin. *Drug Metabolism and Disposition* **29**(6):877-886.

Ngui JS, Tang W, Stearns RA, Shou M, Miller RR, Zhang Y, Lin JH, Baillie TA (2000). Cytochrome P450 3A4-mediated interaction of diclofenac and quinidine. *Drug Metabolism and Disposition* **28**(9):1043-1050.

Nickels TJ, Schwartz AD, Blevins DE, Drummond JT, Reed GW, Wilson DF (2006). Effect of theophylline and aminophylline on transmitter release at the mammalian neuromuscular junction is not mediated by cAMP. *Clinical and Experimental Pharmacology and Physiology* **33**(5-6):465-470.

Normanno N, De Luca A, Maiello MR, Campiglio M, Napolitano M, Mancino M, Carotenuto A, Viglietto G, Menard S (2006). The MEK/MAPK pathway is involved in the resistance of breast cancer cells to the EGFR tyrosine kinase inhibitor gefitinib. *Journal of Cellular Physiology* **207**(2):420-427.

O'Byrne DJ, Devaraj S, Grundy SM, Jialal I (2002). Comparison of the antioxidant effects of Concord grape juice flavonoids alpha-tocopherol on markers of oxidative stress in healthy adults. *American Journal of Clinical Nutrition* **76**(6):1367-1374.

Odaguchi H, Wakasugi A, Ito H, Shoda H, Gono Y, Sakai F, Hanawa T (2006). The efficacy of goshuyuto, a typical Kampo (Japanese herbal medicine) formula, in preventing episodes of headache. *Current Medical Research and Opinion* **22**(8):1587-1597.

Ogata M, Kunikane T, Seki M, Oka K, Urano S, Seki S, Seki Y, Endo T (2005). Mechanism of action of dipropofol and synergistic action with other antibacterial agents in vitro. *Biological and Pharmaceutical Bulletin* **28**(9):1773-1775.

Oh WK, Kantoff PW, Weinberg V, Jones G, Rini BI, Derynck MK, Bok R, Smith MR, Bubley GJ, Rosen RT and others (2004). Prospective, multicenter, randomized phase II trial of the herbal supplement, PC-SPES, and diethylstilbestrol in patients with androgen-independent prostate cancer. *Journal of Clinical Oncology* **22**(18):3705-3712.

Osborne CK, Schiff R (2003). Growth factor receptor cross-talk with estrogen receptor as a mechanism for tamoxifen resistance in breast cancer. *Breast* **12**(6):362-367.

Ostman A (2004). PDGF receptors-mediators of autocrine tumor growth and regulators of tumor vasculature and stroma. *Cytokine and Growth Factor Reviews* **15**(4):275-286.

Ou B, Huang D, Hampsch-Woodill M, Flanagan JA (2003a). When east meets west: the relationship between yin-yang and antioxidation-oxidation. *FASEB Journal* **17**(2):127-129.

Ou CC, Tsao SM, Lin MC, Yin MC (2003b). Protective action on human LDL against oxidation and glycation by four organosulfur compounds derived from garlic. *Lipids* **38**(3):219-224.

Ouatas T, Salerno M, Palmieri D, Steeg PS (2003). Basic and translational advances in cancer metastasis: Nm23. *Journal of Bioenergetics and Biomembranes* **35**(1):73-79.

Ouchi H, Ishiguro H, Ikeda N, Hori M, Kubota Y, Uemura H (2005). Genistein induces cell growth inhibition in prostate cancer through the suppression of telomerase activity. *Int J Urol* **12**(1):73-80.

Ozaki Y (1989). [Pharmacological studies of indole alkaloids obtained from domestic plants, Uncaria rhynchophylla Miq. and Amsonia elliptica Roem. et Schult]. *Nippon Yakurigaku Zasshi* **94**(1):17-26.

Ozaki Y (1990). [Vasodilative effects of indole alkaloids obtained from domestic plants, Uncaria rhynchophylla Miq. and Amsonia elliptica Roem. et Schult]. *Nippon Yakurigaku Zasshi* **95**(2):47-54.

Pang B, Zhang D, Li N, Wang K (2004). Computerized tongue diagnosis based on Bayesian networks. *IEEE Transactions on Biomedical Engineering* **51**(10):1803-1810.

Pankey GA, Ashcraft DS (2005). In vitro synergy of ciprofloxacin and gatifloxacin against ciprofloxacin-resistant Pseudomonas aeruginosa. *Antimicrobial Agents and Chemotherapy* **49**(7):2959-2964.

Park E, Kang M, Oh JW, Jung M, Park C, Cho C, Kim C, Ji S, Lee Y, Choi H and others (2005). Yukmijihwang-tang derivatives enhance cognitive processing in normal young adults: a double-blinded, placebo-controlled trial. *American Journal of Medicine* **33**(1):107-115.

Patel RP, Boersma BJ, Crawford JH, Hogg N, Kirk M, Kalyanaraman B, Parks DA, Barnes S, Darley-Usmar V (2001). Antioxidant mechanisms of isoflavones in lipid systems: paradoxical effects of peroxyl radical scavenging. *Free Radic Biol Med* **31**(12):1570-1581.

Pelicano H, Carew JS, McQueen TJ, Andreeff M, Plunkett W, Keating MJ, Huang P (2006). Targeting Hsp90 by 17-AAG in leukemia cells: mechanisms for synergistic and antagonistic drug combinations with arsenic trioxide and Ara-C. *Leukemia* **20**(4):610-619.

Pennati M, Campbell AJ, Curto M, Binda M, Cheng Y, Wang LZ, Curtin N, Golding BT, Griffin RJ, Hardcastle IR and others (2005). Potentiation of paclitaxel-induced apoptosis by the novel cyclin-dependent kinase inhibitor NU6140: a possible role for survivin down-regulation. *Molecular cancer therapeutics* **4**(9):1328-1337.

Perchellet JP, Perchellet EM, Abney NL, Zirnstein JA, Belman S (1986). Effects of garlic and onion oils on glutathione peroxidase activity, the ratio of reduced/oxidized

glutathione and ornithine decarboxylase induction in isolated mouse epidermal cells treated with tumor promoters. *Cancer Biochemistry Biophysics* **8**(4):299-312.

Perry NS, Houghton PJ, Theobald A, Jenner P, Perry EK (2000). In-vitro inhibition of human erythrocyte acetylcholinesterase by salvia lavandulaefolia essential oil and constituent terpenes. *Journal of Pharmacy and Pharmacology* **52**(7):895-902.

Perucca E (2002). Pharmacological and therapeutic properties of valproate: a summary after 35 years of clinical experience. *CNS drugs* **16**(10):695-714.

Peters GJ, van der Wilt CL, van Moorsel CJ, Kroep JR, Bergman AM, Ackland SP (2000). Basis for effective combination cancer chemotherapy with antimetabolites. *Pharmacology & Therapeutics* **87**(2-3):227-253.

Philipp M, Brede M, Hein L (2002). Physiological significance of alpha(2)-adrenergic receptor subtype diversity: one receptor is not enough. *American journal of physiology. Regulatory, integrative and comparative physiology* **283**(2):R287-295.

Phillipson JD (1999). New drugs from nature--it could be yew. *Phytotherapy Research* **13**(1):2-8.

Prasad NS, Raghavendra R, Lokesh BR, Naidu KA (2004). Spice phenolics inhibit human PMNL 5-lipoxygenase. *Prostaglandins Leukotrienes and Essential Fatty Acids* **70**(6):521-528.

Predy GN, Goel V, Lovlin R, Donner A, Stitt L, Basu TK (2005). Efficacy of an extract of North American ginseng containing poly-furanosyl-pyranosyl-saccharides for preventing upper respiratory tract infections: a randomized controlled trial. *CMAJ* **173**(9):1043-1048.

Prema TP, Raghuramulu N (1996). Vitamin D3 and its metabolites in the tomato plant. *Phytochemistry* **42**(3):617-620.

Prichard MN, Shipman CJ (1990). A three-dimensional model to analyze drug-drug interactions. *Antiviral Research* **14**(4-5):181-205.

Primeau M, Gagnon J, Momparler RL (2003). Synergistic antineoplastic action of DNA methylation inhibitor 5-AZA-2'-deoxycytidine and histone deacetylase inhibitor depsipeptide on human breast carcinoma cells. *International Journal of Cancer* **103**(2):177-184.

Proteggente AR, Saija A, De Pasquale A, Rice-Evans CA (2003). The compositional characterisation and antioxidant activity of fresh juices from sicilian sweet orange (Citrus sinensis L. Osbeck) varieties. *Free Radic Res* **37**(6):681-687.

Prueksaritanont T, Tang C, Qiu Y, L. M, Subramanian R, Lin JH (2002). Effects of fibrates on metabolism of statins in human hepatocytes. *Drug Metabolism and Disposition* **30**(11):1280-1287.

Qin JZ, Chen BT (2004). [Digital model of the theory of Yin and Yang in traditional Chinese medicine]. *Di Yi Jun Yi Da Xue Xue Bao* **24**(8):933-934.

Raasch W, Johren O, Schwartz S, Gieselberg A, Dominiak P (2004). Combined blockade of AT1-receptors and ACE synergistically potentiates antihypertensive effects in SHR. *Journal of Hypertension* **22**(3):611-618.

Rand KH, Houck H (2004). Daptomycin synergy with rifampicin and ampicillin against vancomycin-resistant enterococci. *Journal of Antimicrobial Chemotherapy* **53**(3):530-532.

Rein E, Kharazmi A, Winther K (2004). A herbal remedy, Hyben Vital (stand. powder of a subspecies of Rosa canina fruits), reduces pain and improves general wellbeing

in patients with osteoarthritis--a double-blind, placebo-controlled, randomised trial. *Phytomedicine* **11**(5):383-391.

Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, Cui H, Feinberg AP, Lengauer C, Kinzler KW and others (2002). DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. *Nature* **416**(6880):552-556.

Riering K, Rewerts C, Zieglgansberger W (2004). Analgesic effects of 5-HT3 receptor antagonists. *Scandinavian Journal of Rheumatology. Supplement* **119**:19-23.

Robak J, Shridi F, Wolbis M, Krolikowska M (1988). Screening of the influence of flavonoids on lipoxygenase and cyclooxygenase activity, as well as on nonenzymic lipid oxidation. *Polish Journal of Pharmacology and Pharmacy* **40**(5):451-458.

Robert J, Jarry C (2003). Multidrug resistance reversal agents. *Journal of Medicinal Chemistry* **46**(23):4805-4817.

Rohrdanz E, Ohler S, Tran-Thi QH, Kahl R (2002). The phytoestrogen daidzein affects the antioxidant enzyme system of rat hepatoma H4IIE cells. *Journal of Nutrition* **132**(3):370-375.

Rossowska MJ, Ghanaei P, Nakamoto T (1995). Effect of dietary caffeine and zinc on the activity of antioxidant enzymes, zinc, and copper concentration of the heart and liver in fast-growing rats. *Biol Trace Elem Res* **50**(3):229-236.

Russo P, Malacarne D, Falugi C, Trombino S, O'Connor PM (2002). RPR-115135, a farnesyltransferase inhibitor, increases 5-FU- cytotoxicity in ten human colon cancer cell lines: role of p53. *International Journal of Cancer* **100**(3):266-275.

Saada HN, Ussama ZS, Mahdy AM (2003). Effectiveness of Aloe vera on the antioxidant status of different tissues in irradiated rats. *Pharmazie* **58**(12):929-931.

Sachinidis A, Seul C, Seewald S, Ahn H, Ko Y, Vetter H (2000). Green tea compounds inhibit tyrosine phosphorylation of PDGF beta-receptor and transformation of A172 human glioblastoma. *FEBS Letters* **471**(1):51-55.

Sanchez C, Mathy-Hartert M, Deberg MA, Ficheux H, Reginster JY, Henrotin YE (2003). Effects of rhein on human articular chondrocytes in alginate beads. *Biochemical Pharmacology* **65**(3):377-388.

Sasaki H, Matsumoto M, Tanaka T, Maeda M, Nakai M, Hamada S, Ooshima T (2004). Antibacterial activity of polyphenol components in oolong tea extract against Streptococcus mutans. *Caries Res* **38**(1):2-8.

Satoh N, Sakai S, Kogure T, Tahara E, Origasa H, Shimada Y, Kohoda K, Okubo T, Terasawa K (2005). A randomized double blind placebo-controlled clinical trial of Hochuekkito, a traditional herbal medicine, in the treatment of elderly patients with weakness N of one and responder restricted design. *Phytomedicine* **12**(8):549-554.

Savelev S, Okello E, Perry NS, Wilkins RM, Perry EK (2003). Synergistic and antagonistic interactions of anticholinesterase terpenoids in Salvia lavandulaefolia essential oil. *Pharmacology, Biochemistry and Behavior* **75**(3):661-668.

Saxena VS, Venkateshwarlu K, Nadig P, Barbhaiya HC, Bhatia N, Borkar DM, Gill RS, Jain RK, Katiyar SK, Nagendra Prasad KV and others (2004). Multicenter clinical trials on a novel polyherbal formulation in allergic rhinitis. *International Journal of Clinical Pharmacology Research* **24**(2-3):79-94.

Schmid B, Ludtke R, Selbmann HK, Kotter I, Tschirdewahn B, Schaffner W, Heide L (2001). Efficacy and tolerability of a standardized willow bark extract in patients with osteoarthritis: randomized placebo-controlled, double blind clinical trial. *Phytotherapy Research* **15**(4):344-350.

Schneck DW, Birmingham BK, Zalikowski JA, Mitchell PD, Wang Y, Martin PD, Lasseter KC, Brown CD, Windass AS, Raza A (2004). The effect of gemfibrozil on the pharmacokinetics of rosuvastatin. *Clinical Pharmacology and Therapeutics* **75**(5):455-463.

Scholey AB, Kennedy DO (2002). Acute, dose-dependent cognitive effects of Ginkgo biloba, Panax ginseng and their combination in healthy young volunteers: differential interactions with cognitive demand. *Human psychopharmacology* **17**(1):35-44.

Seeram NP, Adams LS, Hardy ML, Heber D (2004). Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. *Journal of Agricultural and Food Chemistry* **52**(9):2512-2517.

Seki T, Tsuji K, Hayato Y, Moritomo T, Ariga T (2000). Garlic and onion oils inhibit proliferation and induce differentiation of HL-60 cells. *Cancer Letters* **160**(1):29-35.

Sellappan S, Akoh CC (2002). Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. *Journal of Agricultural and Food Chemistry* **50**(19):5338-5342.

Sengupta A, Ghosh S, Das S (2004). Modulatory influence of garlic and tomato on cyclooxygenase-2 activity, cell proliferation and apoptosis during azoxymethane induced colon carcinogenesis in rat. *Cancer Letters* **208**(2):127-136.

Service RF (2004). Surviving the blockbuster syndrome. *Science* **303**(5665):1796-1799.

Shafiee M, Carbonneau MA, d'Huart JB, Descomps B, Leger CL (2002). Synergistic antioxidative properties of phenolics from natural origin toward low-density lipoproteins depend on the oxidation system. *J Med Food* **5**(2):69-78.

Shao ZH, Vanden Hoek TL, Li CQ, Schumacker PT, Becker LB, Chan KC, Qin Y, Yin JJ, Yuan CS (2004). Synergistic effect of Scutellaria baicalensis and grape seed proanthocyanidins on scavenging reactive oxygen species in vitro. *American Journal of Chinese Medicine* **32**(1):89-95.

Sharquie KE, al-Turfi IA, al-Salloum SM (2000). The antibacterial activity of tea in vitro and in vivo (in patients with impetigo contagiosa). *Journal of Dermatology* **27**(11):706-710.

Sheehan MP, Rustin MH, Atherton DJ, Buckley C, Harris DW, Brostoff J, Ostlere L, Dawson A (1992). Efficacy of traditional Chinese herbal therapy in adult atopic dermatitis. *Lancet* **340**(8810):13-17.

Shi B, Yaremko B, Hajian G, Terracina G, Bishop WR, Liu M, Nielsen LL (2000). The farnesyl protein transferase inhibitor SCH66336 synergizes with taxanes in vitro and enhances their antitumor activity in vivo. *Cancer Chemotherapy and Pharmacology* **46**(5):387-393.

Shieh DE, Chen YY, Yen MH, Chiang LC, Lin CC (2004). Emodin-induced apoptosis through p53-dependent pathway in human hepatoma cells. *Life Sciences* **74**(18):2279-2290.

Shimmura H, Tanabe K, Habiro K, Abe R, Toma H (2006). Combination effect of mycophenolate mofetil with mizoribine on cell proliferation assays and in a mouse heart transplantation model. *Transplantation* **82**(2):175-179.

Shitara Y, Hirano M, Sato H, Sugiyama Y (2004). Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. *Journal of Pharmacology and Experimental Therapeutics* **311**(1):228-236.

Shobana S, Naidu KA (2000). Antioxidant activity of selected Indian spices. *Prostaglandins Leukotrienes and Essential Fatty Acids* **62**(2):107-110.

Shoichet BK, Kuntz ID (1991). Protein docking and complementarity. *Journal of Molecular Biology* **221**(1):327-346.

Simonson SG, Raza A, Martin PD, Mitchell PD, Jarcho JA, Brown CD, Windass AS, Schneck DW (2004). Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine. *Clinical Pharmacology and Therapeutics* **76**(2):167-177.

Simpson M, Parsons M, Greenwood J, Wade K (2001). Raspberry leaf in pregnancy: its safety and efficacy in labor. *Journal of midwifery & women's health* **46**(2):51-59.

Siu KM, Mak DH, Chiu PY, Poon MK, Du Y, Ko KM (2004). Pharmacological basis of 'Yin-nourishing' and 'Yang-invigorating' actions of Cordyceps, a Chinese tonifying herb. *Life Sciences* **76**(4):385-395.

Sobrado M, Lopez MG, Carceller F, Garcia AG, Roda JM (2003). Combined nimodipine and citicoline reduce infarct size, attenuate apoptosis and increase bcl-2 expression after focal cerebral ischemia. *Neuroscience* **118**(1):107-113.

Specht DF (1990). Probabilistic neural networks. *Neural Networks* **3**(1):109-118.

Spinella M (2002). The importance of pharmacological synergy in psychoactive herbal medicines. *Alternative Medicine Review* **7**(2):130-137.

Srinivas G, Anto RJ, Srinivas P, Vidhyalakshmi S, Senan VP, Karunagaran D (2003). Emodin induces apoptosis of human cervical cancer cells through poly(ADP-ribose) polymerase cleavage and activation of caspase-9. *European Journal of Pharmacology* **473**(2-3):117-125.

Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K (2000). Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. *Proceedings of the National Academy of Sciences of the United States of America* **97**(4):1433-1437.

Strohl WR (2000). The role of natural products in a modern drug discovery program. *Drug Discov Today* **5**(2):39-41.

Su W, Wu Z, Quan J (2001). [Application of fingerprint analysis and computer pattern interpretation in identification and quality evaluation of traditional Chinese medicines]. *Zhong Yao Cai* **24**(4):295-298.

Su WW (1997). Computer-aided analysis of Traditional Chinese Medicine. *China Journal of Chinese Materia Medica* **22**:186-188.

Su YT, Chang HL, Shyue SK, Hsu SL (2005). Emodin induces apoptosis in human lung adenocarcinoma cells through a reactive oxygen species-dependent mitochondrial signaling pathway. *Biochemical Pharmacology* **70**(2):229-241.

Suehiro T, Matsumata T, Shikada Y, Sugimachi K (2005). The effect of the herbal medicines dai-kenchu-to and keishi-bukuryo-gan on bowel movement after colorectal surgery. *Hepato-Gastroenterology* **52**(61):97-100.

Sugishita E, Amagaya S, Ogihara Y (1984). Studies on the combination of Glycyrrhizae Radix in Shakuyakukanzo-To. *Journal of Pharmacobio-Dynamics* **7**(7):427-435.

Sun SM (2006). *Yao Wang Qian Jing Fang*. Hua Xia Publisher.

Sutter MC, Wang YX (1993). Recent cardiovascular drugs from Chinese medicinal plants. *Cardiovascular Research* **27**(11):1891-1901.

Takabatake D, Fujita T, Shien T, Kawasaki K, Taira N, Yoshitomi S, Takahashi H, Ishibe Y, Ogasawara Y, Doihara H (2007). Tumor inhibitory effect of gefitinib (ZD1839, Iressa) and taxane combination therapy in EGFR-overexpressing breast cancer cell lines (MCF7/ADR, MDA-MB-231). *International Journal of Cancer* **120**(1):181-188.

Takahashi N, Li W, Banerjee D, Guan Y, Wada-Takahashi Y, Brennan MF, Chou TC, Scotto KW, Bertino JR (2002). Sequence-dependent synergistic cytotoxicity of ecteinascidin-743 and paclitaxel in human breast cancer cell lines in vitro and in vivo. *Cancer Research* **62**(23):6909-6915.

Tallarida RJ (2007). Interactions between drugs and occupied receptors. *Pharmacology & Therapeutics* **113**(1):197-209.

Tan H, Liu Y, Fong W, Liu M (1995). [Chemical components of decoction of radix Paeoniae and radix Glycyrrhizae]. *Zhongguo Zhong yao za zhi* **20**(9):550-551, 576.

Tanaka R, Ariyama H, Qin B, Shibata Y, Takii Y, Kusaba H, Baba E, Mitsugi K, Harada M, Nakano S (2005). Synergistic interaction between oxaliplatin and SN-38 in human gastric cancer cell lines in vitro. *Oncology Reports* **14**(3):683-688.

Tang W, Eisenbrand G. (1992). *Chinese drugs of plant origin: Chemistry, pharmacology and use in traditional and modern medicine*. Berlin: Springer-Verlag.

Tang W, Gao Y, Chen G, Gao H, Dai X, Ye J, Chan E, Huang M, Zhou S (2005). A randomized, double-blind and placebo-controlled study of a Ganoderma lucidum polysaccharide extract in neurasthenia. *Journal of medicinal food* **8**(1):53-58.

Tausk FA (1998). Alternative medicine. Is it all in your mind? *Archives of Dermatology* **134**(11):1422-1425.

Teklu S, Gundersen LL, Larsen T, Malterud KE, Rise F (2005). Indolizine 1-sulfonates as potent inhibitors of 15-lipoxygenase from soybeans. *Bioorganic and Medicinal Chemistry* **13**(9):3127-3139.

Tham SM, Angus JA, Tudor EM, Wright CE (2005). Synergistic and additive interactions of the cannabinoid agonist CP55,940 with mu opioid receptor and alpha2-adrenoceptor agonists in acute pain models in mice. *British Journal of Pharmacology* **144**(6):875-884.

Thanou M, Verhoef JC, Junginger HE (2001). Oral drug absorption enhancement by chitosan and its derivatives. *Advanced drug delivery reviews* **52**(2):117-126.

Tildesley NT, Kennedy DO, Perry EK, Ballard CG, Wesnes KA, Scholey AB (2005). Positive modulation of mood and cognitive performance following administration of acute doses of Salvia lavandulaefolia essential oil to healthy young volunteers. *Physiology and Behavior* **83**(5):699-709.

Tomita M, Hayashi M, Awazu S (1995). Absorption-enhancing mechanism of sodium caprate and decanoylcarnitine in Caco-2 cells. *Journal of Pharmacology and Experimental Therapeutics* **272**(2):739-743.

Touma SE, Goldberg JS, Moench P, Guo X, Tickoo SK, Gudas LJ, Nanus DM (2005). Retinoic acid and the histone deacetylase inhibitor trichostatin a inhibit the proliferation of human renal cell carcinoma in a xenograft tumor model. *Clinical Cancer Research* **11**(9):3558-3566.

Tsai JC, Guy RH, Thornfeldt CR, Gao WN, Feingold KR, Elias PM (1996). Metabolic approaches to enhance transdermal drug delivery. 1. Effect of lipid synthesis inhibitors. *Journal of Pharmaceutical Sciences* **85**(6):643-648.

Tsen LC, Segal S, Pothier M, Bader AM (2000). Alternative medicine use in presurgical patients. *Anesthesiology* **93**(1):148-151.

Ung CY, Li H, Cao ZW, Li YX, Chen YZ (2007). Are Herb-Pairs of Traditional Chinese Medicine Distinguishable from Others? Pattern Analysis and Artificial Intelligence Classification Study of Traditionally-Defined Herbal Properties. *Journal of Ethnopharmacology*:accepted.

Ung CY, Li H, Kong CY, Wang JF, Chen YZ (2006). Usefulness of Traditionally-Defined Herbal Properties for Distinguishing Prescriptions of Traditional Chinese Medicine from Non-Prescription Recipes. *Journal of Ethnopharmacology*(In press):doi:10.1016/j.jep.2006.1006.1007.

Ursing C, Wikner J, Brismar K, Rojdmark S (2003). Caffeine raises the serum melatonin level in healthy subjects: an indication of melatonin metabolism by cytochrome P450(CYP)1A2. *Journal of Endocrinological Investigation* **26**(5):403-406.

Ushiroyama T, Ikeda A, Sakuma K, Ueki M (2005). Chai-hu-gui-zhi-gan-jiang-tang regulates plasma interleukin-6 and soluble interleukin-6 receptor concentrations and improves depressed mood in climacteric women with insomnia. *American Journal of Chinese Medicine* **33**(5):703-711.

van Waardenburg RC, de Jong LA, van Eijndhoven MA, Verseyden C, Pluim D, Jansen LE, Bjornsti MA, Schellens JH (2004). Platinated DNA adducts enhance poisoning of DNA topoisomerase I by camptothecin. *Journal of Biological Chemistry* **279**(52):54502-54509.

Verhaeghe R (1998). The use of low-molecular-weight heparins in cardiovascular disease. *Acta Cardiologica* **53**(1):15-21.

Viljoen A, van Vuuren S, Ernst E, Klepser M, Demirci B, Baser H, van Wyk BE (2003). Osmitopsis asteriscoides (Asteraceae)-the antimicrobial activity and essential oil composition of a Cape-Dutch remedy. *J Ethnopharmacol* **88**(2-3):137-143.

Wakabayashi I (1999). Inhibitory effects of baicalein and wogonin on lipopolysaccharide-induced nitric oxide production in macrophages. *Pharmacology and Toxicology* **84**(6):288-291.

Wang J, Cai C, Kong C, Cao Z, Chen Y (2005a). A computer method for validating traditional Chinese medicine herbal prescriptions. *American Journal of Chinese Medicine* **33**(2):281-297.

Wang J, Yang FZ, Zhao M, Zhang YH, Zhang YX, Liu Y, Liu WM, Wang FS, Xu SL, Yu ZM and others (2006). Randomized double-blinded and controlled clinical trial on treatment of HIV/AIDS by Zhongyan-4. *Chinese journal of integrative medicine* **12**(1):6-11.

Wang JF, Cai CZ, Kong CY, Cao ZW, Chen YZ (2005b). A computer method for validating traditional Chinese medicine herbal prescriptions. *American Journal of Chinese Medicine* **33**(2):281-297.

Wang JF, Cai CZ, Kong CY, Cao ZW, Chen YZ (2005c). A computer method for validating traditional Chinese medicine herbal prescriptions. *American Journal of Chinese Medicine* **33**(2):281-297.

Wang JF, Zhou H, Han LY, Chen X, Chen YZ, Cao ZW (2005d). Traditional Chinese medicine information database. *Clinical Pharmacology and Therapeutics* **78**(1):92-93.

Wang L, Higashiura K, Ura N, Miura T, Shimamoto K (2003). Chinese medicine, Jiang-Tang-Ke-Li, improves insulin resistance by modulating muscle fiber composition and muscle tumor necrosis factor-alpha in fructose-fed rats. *Hypertension Research* **26**(7):527-532.

Wang LQ. (2004). *Zhong Yi Lin Chuang Chang Yong Yao Dui*. Beijing: Xue Yan Publisher.

Wang M, Lamers RJ, Korthout HA, van Nesselrooij JH, Witkamp RF, van der Heijden R, Voshol PJ, Havekes LM, Verpoorte R, van der Greef J (2005e). Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacology. *Phytotherapy Research* **19**(3):173-182.

Wang Q, Morris ME (2007). Flavonoids modulate Monocarboxylate transporter-1 mediated transport of {gamma}-hydroxybutyrate in vitro and in vivo. *Drug Metabolism and Disposition* **35**:201-208.

Wang Z, Cheng Z, Fang X (1999). [Antiviral action of combined use of rhizoma Polygoni cuspidati and radix Astragali on HSV-1 strain]. *Zhongguo Zhong yao za zhi* **24**(3):176-180; 192.

Wang Z, Du Q, Wang F, Liu Z, Li B, Wang A, Wang Y (2004). Microarray analysis of gene expression on herbal glycoside recipes improving deficient ability of spatial learning memory in ischemic mice. *Journal of Neurochemistry* **88**(6):1406-1415.

Watano T, Nakazawa K, Obama T, Mori M, Inoue K, Fujimori K, Takanaka A (1993). Non-competitive antagonism by hirsuteine of nicotinic receptor-mediated dopamine release from rat pheochromocytoma cells. *Japanese Journal of Pharmacology* **61**(4):351-356.

Wcw W, A L, At L, Kt L, Cym L, Pc L, Ely W, Jl T (2006). Effectiveness of a Chinese herbal medicine preparation in the treatment of cough in uncomplicated upper respiratory tract infection: a randomised double-blinded placebo-control trial. *Cough* **2**:5.

Wen MC, Wei CH, Hu ZQ, Srivastava K, Ko J, Xi ST, Mu DZ, Du JB, Li GH, Wallenstein S and others (2005). Efficacy and tolerability of anti-asthma herbal medicine intervention in adult patients with moderate-severe allergic asthma. *Journal of Allergy and Clinical Immunology* **116**(3):517-524.

West DP, Zhu YF (2003). Evaluation of aloe vera gel gloves in the treatment of dry skin associated with occupational exposure. *American Journal of Infection Control* **31**(1):40-42.

Wheeler DL, Church DM, Edgar R, Federhen S, Helmberg W, Madden TL, Pontius JU, Schuler GD, Schriml LM, Sequeira E and others (2004). Database resources of

the National Center for Biotechnology Information: update. *Nucleic Acids Res* **32**(Database issue):D35-40.

Williamson EM (2001). Synergy and other interactions in phytomedicines. *Phytomedicine* **8**(5):401-409.

Winau F, Westphal O, Winau R (2004). Paul Ehrlich--in search of the magic bullet. *Microbes Infect* **6**(8):786-789.

Winther K, Apel K, Thamsborg G (2005). A powder made from seeds and shells of a rose-hip subspecies (Rosa canina) reduces symptoms of knee and hip osteoarthritis: a randomized, double-blind, placebo-controlled clinical trial. *Scandinavian Journal of Rheumatology* **34**(4):302-308.

Wozniak D, Lamer-Zarawska E, Matkowski A (2004). Antimutagenic and antiradical properties of flavones from the roots of Scutellaria baicalensis georgi. *Nahrung* **48**(1):9-12.

Wu HQ, Xie L, Jin XN, Ge Q, Jin H, Liu GQ (1989). [The effect of vanillin on the fully amygdala-kindled seizures in the rat]. *Yao Xue Xue Bao* **24**(7):482-486.

Xiao B, Guo J, Liu D, Zhang S (2007). Aloe-emodin induces in vitro G2/M arrest and alkaline phosphatase activation in human oral cancer KB cells. *Oral Oncology*.

Xinquan C, Lixia D. (2002). *Traditional Chinese Medicinal Herbal Active ingredients Analytical Manual*. Xinquan C, editor. Beijing: Xueyuan.

Xue Y, Yap CW, Sun LZ, Cao ZW, Wang JF, Chen YZ (2004). Prediction of P-glycoprotein substrates by a support vector machine approach. *Journal of Chemical Information and Computer Sciences* **44**(4):1497-1505.

Yagi A, Kabash A, Okamura N, Haraguchi H, Moustafa SM, Khalifa TI (2002). Antioxidant, free radical scavenging and anti-inflammatory effects of aloesin derivatives in Aloe vera. *Planta Medica* **68**(11):957-960.

Yamahara J, Mochizuki M, Rong HQ, Matsuda H, Fujimura H (1988). The anti-ulcer effect in rats of ginger constituents. *Journal of Ethnopharmacology* **23**(2-3):299-304.

Yang YZ (2001). *Shang Han Zha Bing Lun Jie Xi*. Wang Wen Publisher.

Yesilada E, Kupeli E (2002). Berberis crataegina DC. root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. *J Ethnopharmacol* **79**(2):237-248.

Yilmaz Y, Toledo RT (2004). Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. *J Agric Food Chem* **52**(2):255-260.

Yim TK, Ko KM (2002). Antioxidant and Immunomodulatory Activities of Chinese Tonifying Herbs. *Pharmaceutical Biology* **40**(5):329-335.

Yokozawa T, Ishida A, Kashiwada Y, Cho EJ, Kim HY, Ikeshiro Y (2004). Coptidis Rhizoma: protective effects against peroxynitrite-induced oxidative damage and elucidation of its active components. *Journal of Pharmacy and Pharmacology* **56**(4):547-556.

Yokozawa T, Satoh A, Cho EJ, Kashiwada Y, Ikeshiro Y (2005). Protective role of Coptidis Rhizoma alkaloids against peroxynitrite-induced damage to renal tubular epithelial cells. *J Pharm Pharmacol* **57**(3):367-374.

You S, Zhou M, Xue B, Fang T, Jiang W, Li C, Xu H, Jiang J, Wang Y, Xu S (1998). A clinical study on bing gan ling oral liquid for treatment of hepatitis C. *Journal of Traditional Chinese Medicine* **18**(3):209-214.

Yu FL, Bender W, Fang Q, Ludeke A, Welch B (2003). Prevention of chemical carcinogen DNA binding and inhibition of nuclear RNA polymerase activity by organosulfur compounds as the possible mechanisms for their anticancer initiation and proliferation effects. *Cancer Detection and Prevention* **27**(5):370-379.

Yuan R, Lin Y (2000a). Traditional Chinese medicine: an approach to scientific proof and clinical validation. *Pharmacology & Therapeutics* **86**(2):191-198.

Yuan R, Lin Y (2000b). Traditional Chinese medicine: an approach to scientific proof and clinical validation. *Pharmacology & Therapeutics* **86**:191-198.

Zhang E. (1998a). *Highly efficacious Chinese patent medicines.* Shanghai: Shanghai Univ. Tradi. Chin. Med. Pub. Co.

Zhang E. (1998b). *Prescriptions of traditional Chinese medicine.* Shanghai: Shanghai Univ. Tradi. Chin. Med. Pub. Co.

Zhang G, Miura Y, Yagasaki K (2000). Induction of apoptosis and cell cycle arrest in cancer cells by in vivo metabolites of teas. *Nutrition and Cancer* **38**(2):265-273.

Zhang JY (2005a). *Lei Jing*. Xue Yan Publisher.

Zhang L, Chang CJ, Bacus SS, Hung MC (1995). Suppressed transformation and induced differentiation of HER-2/neu-overexpressing breast cancer cells by emodin. *Cancer Research* **55**(17):3890-3896.

Zhang L, Lau YK, Xia W, Hortobagyi GN, Hung MC (1999). Tyrosine kinase inhibitor emodin suppresses growth of HER-2/neu-overexpressing breast cancer cells in athymic mice and sensitizes these cells to the inhibitory effect of paclitaxel. *Clinical Cancer Research* **5**(2):343-353.

Zhang S, Yang X, Morris ME (2004a). Flavonoids are inhibitors of breast cancer resistance protein (ABCG2)-mediated transport. *Molecular Pharmacology* **65**(5):1208-1216.

Zhang WB, Chen CX, Sim SM, Kwan CY (2004b). In vitro vasodilator mechanisms of the indole alkaloids rhynchophylline and isorhynchophylline, isolated from the hook of Uncaria rhynchophylla (Miquel). *Naunyn Schmiedebergs Arch Pharmacol* **369**(2):232-238.

Zhang Y, Luo Y, Zhai Q, Ma L, Dorf ME (2003). Negative role of cAMP-dependent protein kinase A in RANTES-mediated transcription of proinflammatory mediators through Raf. *FASEB Journal* **17**(6):734-736.

Zhang YM, Rock CO (2004c). Evaluation of epigallocatechin gallate and related plant polyphenols as inhibitors of the FabG and FabI reductases of bacterial type II fatty-acid synthase. *J Biol Chem* **279**(30):30994-31001.

Zhang ZJ (2005b). *Jing Gui Yu Han Jing*. Xue Yan Publisher.

Zhao NC, Jin WB, Zhang XH, Guan FL, Sun YB, Adachi H, Okuyama T (1999). Relaxant effects of pyranocoumarin compounds isolated from a Chinese medical plant, Bai-Hua Qian-Hu, on isolated rabbit tracheas and pulmonary arteries. *Biological and Pharmaceutical Bulletin* **22**(9):984-987.

Zhao WH, Hu ZQ, Okubo S, Hara Y, Shimamura T (2001). Mechanism of synergy between epigallocatechin gallate and beta-lactams against methicillin-resistant

Staphylococcus aureus. *Antimicrobial Agents and Chemotherapy* **45**(6):1737-1742.

Zhong SQ, Sun LJ, Yan YZ, Sun YQ, Zhong YY (2005). Effect of Xuesaitong soft capsule on hemorrheology and in auxiliarily treating patients with acute cerebral infarction. *Chinese journal of integrative medicine* **11**(2):128-131.

Zhou JR, Yu L, Mai Z, Blackburn GL (2004). Combined inhibition of estrogen-dependent human breast carcinoma by soy and tea bioactive components in mice. *International Journal of Cancer* **108**(1):8-14.

Zhu DY, Bai DL, Tang XC (1996). Recent studies on traditional Chinese medicinal plants. *Drug Development Research* **39**(2):147-157.

Zhu M, Li RC (1999). Receptor binding activities of Schefflera triterpenoids and oligosaccharides. *Planta Medica* **65**(2):99-103.

Zhu WF, Yan JF, Huang BQ (2006). [Application of Bayesian network in syndrome differentiation system of traditional Chinese medicine]. *Zhong Xi Yi Jie He Xue Bao* **4**(6):567-571.

Zicca A, Cafaggi S, Mariggio MA, Vannozzi MO, Ottone M, Bocchini V, Caviglioli G, Viale M (2002). Reduction of cisplatin hepatotoxicity by procainamide hydrochloride in rats. *European Journal of Pharmacology* **442**(3):265-272.