

# Plant substances as alternatives for animal products in traditional medicines

Report submitted to the Department  
for Environment Food and Rural Affairs

September 2006

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# Executive summary

An investigation of plant species as alternatives to the use of products obtained from endangered animal species (bear bile, rhino horn and tiger bone) was undertaken with financial support from the Department for Environment Food and Rural Affairs (DEFRA) and the International Fund for Animal Welfare (IFAW) Charitable Trust. The research was carried out by Middlesex University (UK) in collaboration with the Jodrell Laboratory, Royal Botanic Gardens, Kew (UK).

Products from several endangered species are used in Traditional Chinese Medicine for a variety of purposes. Bear bile (Xiong Dan) and rhino horn (Xi Jiao) are primarily classified as anti-inflammatory and fever-reducing remedies and tiger bone (Hu Gu) has been used as an anti-rheumatic/anti-arthritic remedy; the pathology of arthritis also involves inflammatory mechanisms. With the popularity of Traditional Medicine increasing, a continued demand for these products poses an ongoing and major threat to the survival of these species, all of which are listed under Appendix 1 of CITES. This study was undertaken in response to a recognised need for more research into possible herbal substitutes.

Based on both traditional use and scientific evidence for pharmacological actions, single herbs and TCM 'prescriptions' (combinations of herbs) were selected for investigation as alternatives to the use of bear bile, rhino horn and tiger bone in Traditional Chinese Medicine. A selection of 7 single herbs and 2 prescriptions were chosen for investigation as potential alternatives to bear bile; 9 single herbs and 6 prescriptions as potential alternatives to rhino horn; and 19 single herbs and two prescriptions as potential alternatives to tiger bone. As all three animal products are traditionally used to treat conditions associated with inflammatory processes, this area was chosen for investigation. The inflammatory response is a complex cascade of events, often triggered by infection (commonly by bacteria) and is one of the body's defence mechanisms in fighting disease. The inflammatory response forms one of the underlying pathologies of arthritis, fever, liver diseases, cancer and cardiovascular diseases. Therefore, preliminary studies were conducted to assess the effects of crude extracts, fractions and isolated compounds on bacterial growth and an anti-inflammatory mediator, nuclear factor-kappaB (NF- $\kappa$ B) *in vitro*. Cytochrome P450 3A4 inhibition tests were conducted to determine the effect of herbal extracts on this drug metabolising enzyme *in vitro*.

When recommending potential herbal alternatives it is essential to ensure that the correct plant species is being proposed. Verification of the plant material was carried out by comparing the chemical profiles of the samples obtained for research from commercial sources with chemical profiles of authentic and reference material from the Chinese Medicinal plant Authentication Centre, Royal Botanical Gardens, Kew. In addition, pesticide residues and metal concentrations were determined to confirm the quality of the product.

## Executive summary

Several of the herbs chosen for investigation as possible alternatives to bear bile were found to possess anti-bacterial activity (*Anemarrhena asphodeloides* Bge, *Gardenia jasminoides* Ellis, *Scutellaria baicalensis* Georgi, *Phellodendron amurense* Rupr., *Coptis chinensis* Franch. and *Rheum palmatum* L). Extracts of three herbs were also shown to have anti-inflammatory properties through the inhibition NF- $\kappa$ B activity (*Scutellaria baicalensis*, *Rheum palmatum* and *Coptis chinensis*). Preliminary results from the cytochrome P450 3A4 inhibition studies suggest that possible herb-herb interactions may occur in preparations containing both *Coptis chinensis* and *Scutellaria baicalensis* (such as Dia-Orengedokuto and Orengedokuto). Also, drug-herb interactions may occur when herbal preparations containing *Coptis chinensis* and/or *Scutellaria baicalensis* are co-administered with some pharmaceutical drugs metabolised by this enzyme. Further work is required to investigate the extent of these effects.

Water extracts of rhino horn did not demonstrate anti-bacterial nor anti-inflammatory properties, nor did they have any effect on the drug metabolising enzyme, cytochrome P450 3A4. However, certain Traditional Chinese Medicine prescriptions, both with and without rhino horn, did show anti-bacterial and anti-inflammatory properties in the assays used in this study. Further work using other bioassays is required to ascertain the contribution of the horn extracts to any activity shown by the prescriptions. The majority of herbs chosen as possible alternatives to rhino horn showed some anti-bacterial activity (17 out of 20). Extracts of several single herbs were also shown to have anti-inflammatory properties through the inhibition NF- $\kappa$ B activity (*Paeonia suffruticosa* Andr., *Trichosanthes kirilowii* Maxim., *Lophatherum gracile* Brongn., *Acorus calamus*, *Paeonia veitchii* Lynch, *Isatis indigotica* Fort., *Glycine max* L. and *Rehmannia glutinosa* Steud) as well as extracts of two prescriptions (Xi Jiao Dihuang Tang and Qing Ying Tang). To date, no scientific literature has been found suggesting that *Lophatherum gracile* has an anti-inflammatory effect and further studies are required to confirm these findings. *Salvia miltiorrhiza*, *Rehmannia glutinosa*, as well as *Scutellaria baicalensis* and *Coptis chinensis* showed inhibition of cytochrome P450 3A4. Since they are commonly used TCM herbs, further work may be required to determine potential adverse interactions with other remedies or orthodox medicines.

Preliminary results suggest that tiger bone may possess some anti-inflammatory properties through the inhibition of NF- $\kappa$ B activity. Three herbs included in existing Traditional Chinese Medicine prescriptions containing tiger bone also showed anti-inflammatory activity in the same assay *Angelica dahurica* Maxim., *Taxillus chinensis* (DC.) Danser and *Angelica sinensis* (Oliv.). None of the herbs investigated as alternatives to tiger bone were found to affect cytochrome P450 3A4 activity.

Supported by evidence of efficacy as anti-inflammatory and anti-bacterial agents as measured in this study, by information obtained from the available scientific literature, and by Traditional Chinese Medicine theory, a number of prescriptions and single herbs have been selected as suitable alternatives to the use of bear bile, rhino horn or tiger bone in Traditional Chinese Medicine. Most of the suggested herbal 'alternatives' to the animal products were found to already form part of one or more traditional prescriptions containing the animal products. This finding confirmed the practice in Traditional Chinese Medicine of combining remedies with similar functions for their additive and synergistic effects.

The inflammatory response is a complex cascade of events and nuclear factor-kappaB is only one anti-inflammatory mediator amongst many. Further studies are warranted to assess other pharmacological mechanisms through which the plants might mediate anti-inflammatory effects. Further work should also be carried out to investigate further the effect of herbal extracts on drug metabolising enzymes such as cytochrome P450 3A4.

If the findings of this study are to impact upon the use of products from endangered animal species in Traditional Chinese Medicine, the suggestions made for herbal alternatives will need to be acceptable to practitioners in terms of philosophy as well as potential efficacy. It will be necessary to discuss the findings of this study with TCM practitioners to determine whether the selected plant species would be considered suitable for use in Traditional Chinese Medicine as substitutes to bear bile, rhino horn and tiger bone. The suggested herbs and the evidence to support these suggestions will then need to be disseminated to practitioners and the public, both via scientific publications and through the more popular Traditional Chinese Medicine literature.



## Introduction and overview

### 1.1. Introduction

#### 1.1.1. The popularity of traditional medicine

The use of traditional medicine (TM) containing ingredients obtained from animals and plants has maintained its popularity in all regions of the developing world and is gaining in popularity in the industrialised countries. Countries in Africa, Asia and Latin America use TM to help meet some of their primary health care needs while in industrialised countries, traditional medicine use is seen as “Complementary” or “Alternative” (CAM) to orthodox or allopathic medicine. Thus, in Africa, up to 80% of the population uses traditional medicine for primary health care and in China traditional herbal preparations account for 30%–50% of the total medicinal consumption. In Europe, North America and other industrialised regions, over 50% of the population have used complementary or alternative medicine at least once. The popularity of TM has created a global market for herbal medicines that currently stands at over US \$ 60 billion annually and is growing steadily (WHO, 2004).

#### 1.1.2. The threat to endangered species from their use in traditional medicine

The growing market in TM poses a major threat to the survival of many endangered species, notably tigers, bears and rhinoceroses. Most TM “consumer” countries, including China, Japan, the UK and the USA, are Parties to the Convention on International Trade in Endangered Species (CITES), which bans international trade in these species between CITES member states. However, demand for medicines containing them continues and with it illegal trade in their parts and derivatives for the TM market. Tigers, rhinoceroses, and three species of bear, are listed under CITES Appendix 1 (2004); species that are the most endangered among CITES-listed animals and plants and are threatened with extinction.

Despite this protection, the number of tigers, bears and rhinoceroses in the wild continues to fall. Tiger numbers have dropped from more than 100,000 to between 4,800 and 7,300 individuals over the last century, three tiger species have become extinct, and, as tigers become increasingly difficult to find, other big cat species have begun to be hunted as an alternative. Whilst several parts of the tiger are used in TM, tiger bone is the most commonly used. It is believed to have an anti-inflammatory effect, particularly in cases of arthritis.

## Section 1

Bear populations are also declining around the world to the extent that the immediate survival of bears in key regions is threatened. It is recognised that bears are poached and illegally traded for use in TM, but the extent of illegal poaching is difficult to assess. Various bear parts are used in Asian TM, including the meat, gall bladder, brain, blood, bone and paw. Bear bile, extracted from the gall bladder, is most commonly used in TM, being prescribed for febrile diseases with high temperature and convulsions, inflammation of the liver, laryngitis, conjunctivitis and to reduce swelling and pain (for trauma, sprains, fractures and haemorrhoids).

Rhinoceros populations have also suffered severely in recent years. Three of the five species of rhinoceros are now critically endangered and threatened with extinction, with more than half of the world's remaining rhinoceroses lost during the 1970s (WWF, 2002). The use of rhinoceros horn in TM has largely been blamed for the decline in the population in Asia. Rhinoceros horn is prescribed in Asian TM as a detoxifying, anti-inflammatory and anti-convulsant agent and is used in the treatment of advanced stages of fever.

### 1.1.3. The need for research into the use of substitutes for specimens of endangered species

Parties to CITES have expressed concern over the continued and uncontrolled use of several endangered species in traditional medicine in view of the potential threat to the long-term survival of these species and the need to ensure the continued use and development of traditional medicines on a sustainable basis (CITES, 1997; Conf. 10.19). It has been recognised that problems of overexploitation must be addressed within the context of an improved understanding about the significance of traditional medicines in the world's health care systems. A resolution was therefore agreed at the 10th meeting of the Conference of Parties 1997 (CITES, 1997) calling for more research into the use of substitutes for specimens of endangered species in TM. The UK, represented by the Global Wildlife Division of the Department for Environment, Food and Rural Affairs (DEFRA), the UK CITES Management Authority, was instrumental in CITES in gaining agreement to this Resolution.

IFAW first began to address the issue of endangered species used in traditional medicine products in 1983 and have also emphasised the importance of research into herbal alternatives.

### 1.1.4. Middlesex University and traditional medicine

Against this background, the increasing popularity of TM in the industrialised countries, including the UK, led to the introduction of degree level education in several TM disciplines. Middlesex University was at the forefront of these developments, validating a degree in Herbal Medicine in 1994 and in Traditional Chinese Medicine (TCM) in 1996. Both programmes aim to provide an education and training to produce graduates who will be competent, reliable and caring practitioners. The TCM programme was developed and is delivered in collaboration with Beijing University of Chinese Medicine. The need to address issues surrounding the use of endangered species in TCM practice and to bring endangered species education into the curriculum is recognised by the academic group at Middlesex, as is the need for appropriate research in this area.

### 1.2. The current research project

In 2001 DEFRA and IFAW jointly commissioned Middlesex University to undertake research to investigate "Plant Substances as Alternatives for Animal Products Used in Traditional Chinese Medicine". The research was carried out at Middlesex and at the Jodrell Laboratories, Royal Botanic Gardens, Kew. The Royal Botanic Gardens at Kew are the UK appointed CITES Scientific Authority for Plants and carry out CITES projects and research into the trade in certain CITES-listed plants. In addition, Dr Bing Chan acted as external advisor on matters of TCM philosophy and practice.

The project was designed to be carried out in two parts. Project 1 was to investigate anti-inflammatory and anti-pyretic herbs as alternatives for bear bile and rhino horn. Project 2 was to investigate anti-inflammatory and anti-rheumatic herbs as alternatives for tiger bone. As the project progressed, further collaborations were established in order to achieve the aims of the project. These collaborations are listed in the acknowledgements. All collaborators have been vital to the success of the project and their input greatly valued.

#### 1.2.1. Aims of the project

The overall objective of the project is to provide original scientific data to support the promotion of alternatives to the use of animal products in TM in order to help prevent the further depletion of threatened and endangered wildlife.

The original aims for phase 1 and 2 of this research proposal were as follows:

- i) To identify the active chemical components of products derived from bear (bile), tiger (bone) and rhino (horn).
- ii) Once identified, to find plant substitutes.

The following report summarises the work that has been carried out over the past three years. From the report it can be seen that the initial aims of the project have been met. Having conducted an extensive literature review and consultations with Chinese medicine practitioners, the decision was taken to place the emphasis on the choice of herbs to investigate as possible alternatives to animal products based on TCM theory and philosophy rather than on similarities in chemical structure or in activity in Western pharmacological terms. The latter was taken into account and investigated once the choice of herbs had been made. This decision was taken as the team consider that the effective promotion of the use of alternatives to practitioners and patients of TCM will depend on their acknowledgment that the herbs possess the same or similar properties to the animal products as described by TCM philosophy. Also taken into consideration was the common usage in TCM of prescriptions consisting of a combination of herbs with a 'principal' ingredient. Several prescriptions, which include animal products as an ingredient, were therefore also investigated.

## Section 1

All three animal products under investigation (bear bile, rhino horn and tiger bone) are used in TM medicines for a variety of reasons, but all share a common use as an anti-inflammatory. Both bear bile (Xiong Dan) and rhino horn (Xi Jiao) are also classified in TCM as anti-pyretic remedies and tiger bone (Hu Gu) as an anti-rheumatic/anti-arthritic remedy. Herbs chosen for investigation based on TM theory were therefore investigated *in vitro* for their efficacy as anti-inflammatory agents. Since fever is often initiated by infection (commonly bacteria) anti-bacterial tests were also conducted as part of the bear bile and rhino horn projects. There has been concern expressed over possible herb-herb and drug-herb interactions and over the contamination of herbal medicines with metals and pesticide residues. This was investigated using cytochrome P450 3A4 assays, and quantitatively evaluating the chemical contaminants (metals and pesticide residues) in some selected herbs. In addition, the importance of authentication of herb samples is recognised and the plant species of the herbs in this study was investigated using chemical analysis.

An additional suggestion, though not included in the initial proposal for funding, was the possibility of extending the research to include animal trials, followed by human trials using volunteers to assess the effectiveness of herbal alternatives (Phase 4). It has not been felt that there is justification at this point to extend the project in this way and that this would, in any case, conflict with the mission and vision of both the University and the funding bodies.

### 1.3. Structure of the report

The report is divided into 7 sections. Following this overview, Sections 2, 3, and 4 present the rationale for the selection of herbs to investigate as potential alternatives to bear bile, rhinoceros horn and tiger bone respectively, identifying both herbs and combinations of herbs (prescriptions) chosen and briefly describing their properties as identified in the literature. Section 5 describes the methods used to prepare, authenticate and assess the biological activity of the chosen herbs. The assays allowed investigation of anti-bacterial activity, anti-inflammatory activity (NF- $\kappa$ B inhibition) and cytochrome P450 3A4 activity. Section 6 presents the main findings of the investigation into biological activity. The results of assays to measure contaminant levels (pesticide residues and metals) are shown in Appendices 2, 3A and 3B. A summary of the main findings and suggested future work is outlined in Section 7.

# Rationale for selection of herbs as potential alternatives to bear bile

## 2.1. Introduction

In order to identify which plants might be suitable to replace bear bile use in traditional medicine (TM), in particular, traditional Chinese medicine (TCM), it is important to understand the beliefs and evidence supporting its continued use. In this part of the report, sources of bear bile, its uses in TM and the identification of active constituents used in 'Western' medicine are discussed. Based on the findings of this research, the criteria for choosing herbs to investigate as potential alternatives were established, based on both the traditional uses and knowledge about medicinal properties of the different species of plants. Whilst single herbs have been selected for study, it is recognised that a mixture of different plant species is commonly prescribed in TCM, with the prescription relying on purported synergism of a combination of herbs, sometimes with animal parts and minerals. Therefore, prescriptions or combinations of herbs were also investigated as potential alternatives to the use of bear bile in TCM.

A summary of some of the known constituents and available research on the herbs and prescriptions chosen for study as potential alternatives to the use of bear bile in TCM is given at the end of this section.

## 2.2. Sources of bear bile

In TCM, bear bile was originally obtained from two members of the Ursidae family, namely, *Selenarctos thibetanus* (Asiatic black bear) and *Ursus arctos* (brown bear) (Bensky and Gamble, 1993; Chang and But, 1987). There is evidence to suggest that other species of bears such as *Ursus americanus* (American black bear) and *Helarctos malayanus* (sun bear) have also been exploited (Lin *et al.*, 1997). *Selenarctos thibetanus* (*Ursus thibetanus*), *Helarctos malayanus* and *Melursus ursinus* (sloth bear) are among bear species listed under CITES Appendix 1 (2004), banning international trade in their parts in CITES member states. However, due to demand for bear products in TCM, regulated bear farming is in operation in China and the Republic of Korea, where bile is artificially drained from bear gall bladders (Li, 2004). As a substitute to bear bile, bile derived from pig, water buffalo, goat, cattle and chicken have also been used in TCM and several of these have been sold as 'bear bile' (Lin *et al.*, 1997).

### 2.3. Bear bile and its constituents in TCM and Western medicine

Bear bile is composed of deconjugated tauroursodeoxycholic acid (TUDC), taurochenodeoxycholic acid (TCDC) and taurocholic acid (TC), of which the primary bile acids are known as ursodeoxycholic acid (UDCA), chenodeoxycholic acid (CDCA) and cholic acid, respectively (Espinoza *et al.*, 1993). In the UK, pharmaceutical products containing UDCA (e.g. Destolit®, Urdox®, Ursofalk®, Ursogal®), which may be obtained from other animal sources (e.g. ox), are indicated for the dissolution of gallstones (British Pharmacopoeia, 2000). UDCA has also been used in the treatment of some chronic inflammatory disorders such as liver fibrosis and chronic active hepatitis (Kowdley, 2000; Rolo *et al.*, 2000; Van Den Bogaert, 2003).

### 2.4. Bear bile in TCM

Bear bile (Xiong Dan; Fel Ursi) is described in TCM as having a 'bitter' taste and being 'cold' in nature. There are several ailments associated with the use of bear bile (Bensky and Gamble, 1993; Chang and But, 1987). These include gallstones, cholestatic hepatic diseases, febrile diseases with high fever and convulsions, pharyngolaryngitis, conjunctivitis, traumatic injuries, swelling, pain, sprains and fractures, haemorrhoids and cardiovascular diseases. The Association of Chinese Medicine and Philosophy and Earthcare Society (Hong Kong) in their report on 'Herbal alternatives to bear bile in Chinese medicine' included syphilis and several cancers as being treated by bear bile (IFAW report, 1994). Bile obtained from other species of animals has also been investigated; one study showed bear bile and pig bile to demonstrate comparable anti-inflammatory, analgesic and anti-convulsant properties and therefore, pig bile has been advocated as a suitable animal alternative to bear bile (Li *et al.*, 1995).

### 2.5. Selection of herbs and herbal prescriptions for investigation

#### 2.5.1. Criteria used for selection of single herbs

There is some scientific evidence to support the traditional use of bear bile in the treatment of inflammatory conditions (Li *et al.*, 1995), and its chief active constituent, UDCA, in the treatment of cholesterol gallstones and chronic liver inflammation (Van Den Bogaert, 2003), some cardiovascular diseases (Lee, 1999) and cancer (Im and Martinez, 2004). At the beginning of this study an extensive literature survey was conducted to select herbs to be investigated as possible alternatives to bear bile. One hundred and three plant species used as 'heat-clearing' herbs in TCM were identified. In order to select suitable herbs, criteria were then used which included herbs with constituents similar in structure and function to UDCA, a tetracyclic compound. For example, pentacyclic triterpenoids such as ursolic and oleanolic acid are attributed with anti-inflammatory, hepatoprotective and anti-neoplastic activities (Saraswat *et al.*, 2000; Syrovets *et al.*, 2000). However, these compounds are found in several plant species. In consultation with TCM practitioners, the criteria were further refined (Table 2.1.) in order to select herbs with both TCM and pharmacological properties similar to bear bile.

## Rationale for selection of herbs as potential alternatives to bear bile

**Table 2.1. Properties / functions of bear bile and UDCA used as criteria for herb selection**

Criteria	Properties and functions of bear bile and UDCA
A	'Cold' nature
B	'Bitter' taste
C	'Heat clearing'
D	'Fire-purging'
E	Anti-inflammatory properties
F	Hepatoprotective properties
G	Anti-neoplastic properties
H	Cardiovascular protective properties

Criteria A – D: TCM properties of bear bile (Bensky and Gamble, 1993; Chang and But, 1987).

Criteria E – H: Pharmacological / clinical effects of bear bile and UDCA reported in the literature (Li *et al.*, 1995; Lee, 1999; Van Den Bogaert, 2003; Im and Martinez, 2004).

**Table 2.2. TCM plants selected after consultation with TCM practitioners and from evaluation of TCM literature and pharmacological and clinical data**

Plant species with some similar properties to bear bile	A	B	C	D	E	F	G	H	TCM references
1. <i>Gardenia jasminoides</i> Ellis (syn.: <i>G. augusta</i> Merr.) (Rubiaceae) fruit = Zhi Zi	*	*	*	*	*	*	*		1, 2, 4
2. <i>Anemarrhena asphodeloides</i> Bge. (Anthericaceae) rhizome = Zhi Mu	*	*	*	*			*		1, 2, 4
3. <i>Scutellaria baicalensis</i> Georgi (Lamiaceae) root = Huang Qin	*	*	*		*	*	*	*	1, 2, 4
4. <i>Coptis chinensis</i> Franch. (Ranunculaceae) rhizome = Huang Lian	*	*	*		*	*	*	*	1, 2, 4
5. <i>Phellodendron amurense</i> Rupr (Rutaceae) bark = Huang Bai	*	*	*		*	*		*	4
6. <i>Andrographis paniculata</i> Nees (Acanthaceae) aerial parts = Chuan Xin Lian	*	*	*		*	*		*	1, 2, 4
7. <i>Rheum palmatum</i> L. (Polygonaceae) root and rhizome = Da Huang	*	*	*		*	*			1, 3

Criteria A – D (refer to Table 2.1.): based on TCM literature (Chang and But, 1987<sup>1</sup>; Bensky and Gamble, 1993<sup>2</sup>; Chang and But, 2001<sup>3</sup>; Chinese Pharmacopoeia, 2005<sup>4</sup>).

Criteria E – H (refer to Table 2.1.): based on pharmacological and clinical data (Section 2.4).

## Section 2

Out of the eight criteria listed (Table 2.1.) priority was given to plant species that complied with the properties of bear bile as described in TCM [Table 2.1; criteria A – D]. Therefore, all the herbs selected for study are traditionally used in the practice of Chinese medicine. Additional criteria for choice were based on evidence from published scientific studies [Table 2.1; E – H]. When assessing the suitability of the selected herbs as alternatives to bear bile, the known relevant biological activities and their constituents were also considered. The potential for using these species as alternatives to bear bile was then discussed with TCM practitioners. These discussions resulted in a reduction in the number of plant species from 103 species to 7 species (Table 2.2.).

### 2.5.2. Criteria used for selection of herbal prescriptions for study

In addition to the 7 selected herbs chosen for study, the use of prescriptions in TCM was also considered. In TCM, a mixture of different plant species is commonly prescribed, relying on the synergy between herbs, sometimes with animal parts and/or minerals. A literature survey was therefore conducted on TCM prescriptions containing bear bile with the aim of selecting prescriptions for investigation, to determine whether they demonstrate biological activity without bear bile. A major criterion for choosing TCM prescriptions for this study was that they contained not more than one animal product (i.e. bear bile). It was also important to choose prescriptions that did not contain endangered plant species (those restricted by CITES). A survey identified a TCM prescription (prescription X), which complied with the criteria. Prescription X is used in the treatment of laryngitis and contains bear bile and six herbs (Zhu, 1989). The herbal composition of prescription X is described in Table 2.3. In addition, two Chinese-Japanese (Kampo) patent medicines, Orenge dokuto and Diao-Orenge dokuto are also proposed as possible replacements for prescriptions containing bear bile. These two prescriptions were selected on the basis of being composed of herbs proposed from this investigation and also possessing some similar biological and TCM functions as bear bile.

**Table 2.3. Herbal composition of prescription X**

Herbs listed as being in prescription
Zhi Zi, fruit of <i>Gardenia jasminoides</i> (Rubiaceae)
Huang Lian, rhizome of <i>Coptis chinensis</i> (Ranunculaceae)
Ban Lan Gen, root of <i>Isatis indigotica</i> (Brassicaceae)
Jin Yin Hua, flower bud of <i>Lonicera japonica</i> (Caprifoliaceae)
Lian Qiao, fruit of <i>Forsythia suspensa</i> (Oleaceae)
Hu Po, fossil resin of <i>Pinus succinifer</i> (Pinaceae)

## 2.6. Single herbs chosen as potential alternatives to bear bile in TCM: summary of reputed and pharmacological effects

### 2.6.1. Huang Qin (Radix Scutellariae)

Huang Qin (skullcap) is prepared from the root of *Scutellaria baicalensis* Georgi (Lamiaceae). It functions to 'remove damp', 'quench fire' and counteract toxins. It is used as an anti-inflammatory agent and in the treatment of fever, hepatitis and acute conjunctivitis (Bensky and Gamble, 1993; Chang and But, 1987). The anti-pyretic, analgesic (Huang *et al.*, 1990), anti-hypercholesterolemic (Yotsumoto *et al.*, 1997), cardiovascular protective (Wang *et al.*, 2004) and anti-neoplastic (Fukutake *et al.*, 1998; Wozniak *et al.*, 2004) effects of *S. baicalensis* have been reported. In addition, the anti-inflammatory effect of *S. baicalensis* has been demonstrated *in vitro* and *in vivo* (van Loon, 1997; Cuellar *et al.*, 2001). Flavonoid constituents (baicalein, baicalin, wogonin and oroxylin A) of *S. baicalensis* have been researched for the mechanism of action of their anti-inflammatory effects (Chen *et al.*, 2000; Chen *et al.*, 2001; Kang *et al.*, 2003a; Huang *et al.*, 2004).

### 2.6.2. Huang Lian (Rhizoma Coptidis)

Huang Lian (goldthread rhizome) is prepared from the dried rhizome of *Coptis chinensis* Franch, *C. deltoidea* C.Y.Cheng.&Hsiao, or *C. teeta* Wallich (Ranunculaceae). It is used traditionally in the treatment of some inflammatory diseases, fever, conjunctivitis and some tumours (Hsu *et al.*, 1986; Chang and But, 1987). Some studies have associated Rhizoma Coptidis with potential anti-inflammatory, anti-oxidant, anti-hypercholesterolemic, and anti-neoplastic effects. *C. chinensis* has shown anti-inflammatory activity *in vivo* (Cuellar *et al.*, 2001), which may be mediated through the inhibition of interleukin-8 (IL-8) induction (Lee *et al.*, 1995). Berberine, a constituent of *C. chinensis*, is reported to inhibit cyclooxygenase-2 (COX-2) activity (Fukuda *et al.*, 1999). *C. chinensis* is reported to be anti-oxidant *in vitro* and *in vivo* (Liu and Ng, 2000; Schinella *et al.*, 2002) and has shown cholesterol lowering effects (Yotsumoto *et al.*, 1997; Yokozawa *et al.*, 2003). Rhizoma Coptidis has also shown potential anti-neoplastic effects *in vitro* (Fukutake *et al.*, 1998; Iizuka *et al.*, 2000). Anti-bacterial activity of Rhizoma Coptidis is cited in Chang and But (1987) and Tang and Eisenbrand (1992). Adverse effects associated with Rhizoma Coptidis include vomiting, dyspnoea and convulsions (Huang, 1999).

### 2.6.3. Huang Bai (Cortex Phellodendri)

Huang Bai (Amur corktree) is prepared from the dried stem bark of *Phellodendron amurense* Rupr. (Rutaceae). It functions as a 'heat-clearing', anti-inflammatory and anti-bacterial agent in TCM (Chang and But, 1987; Tang and Eisenbrand, 1992). Some studies indicate that *P. amurense* has anti-inflammatory (Cuellar *et al.*, 2001), hepatoprotective (Yotsumoto *et al.*, 1997), anti-oxidant (Kong *et al.*, 2001) and anti-bacterial (Chang and But, 1987) activities. In one study, the hepatoprotective effects of Cortex Phellodendri were reported to be less effective than Rhizoma Coptidis and Radix Scutellariae (Yotsumoto *et al.*, 1997).

### 2.6.4. Zhi Zi (Fructus Gardeniae)

Zhi Zi (cape jasmine fruit) is prepared from the fruit of *Gardenia jasminoides* Ellis (syn.: *G. augusta* Merr.) (Rubiaceae). It is reputed to 'reduce heat', 'remove heat from the blood', counteract toxicity and ease the mind (Pharmacopoeia of PRC, 2000). It is a 'fire-purging' febrifuge and is indicated in febrile diseases with restlessness. It is also used traditionally in the treatment of conjunctivitis, some tumours and externally for sprains and bruises (Chang and But, 1987; Bensky and Gamble, 1993). The anti-inflammatory, anti-neoplastic (Fukutake *et al.*, 2000) and hepatoprotective (Chiu *et al.*, 1989) activities of Fructus Gardeniae have been reported. Genipin and geniposide, constituents of the herb, have been reported to be analgesic (Harada *et al.*, 1974) and geniposide is reported to be anti-inflammatory *in vivo* (Yao *et al.*, 1991). Genipin, as well as crocetin have been reported to possess anti-neoplastic properties (Chang *et al.*, 1996; Kuo *et al.*, 2004). Fructus Gardeniae and its constituents, genipin and crocin, are reported to be choleric (Harada *et al.*, 1974; Tang and Eisenbrand, 1992). Crocin, a carotenoid constituent of *Gardenia jasminoides* possess anti-oxidant properties (Pham *et al.*, 2000). However, reversible acute hepatic damage has been observed with crocin (Tang and Eisenbrand, 1992) and diarrhoea was observed as a side effect of geniposide in mice (Bensky and Gamble, 1993). Gardenic acid and gardenodic acid A (constituents of *Gardenia jasminoides*) can cause abortion in early pregnancy (Pei-Gen and Nai-Gong, 1991).

### 2.6.5. Chuan Xin Lian (Herba Andrographis)

Chuan Xin Lian (green chiretta) is prepared from the dried aerial parts of *Andrographis paniculata* Nees (Acanthaceae). It is used in TCM to 'remove heat', 'counteract toxicity' and reduce swelling (Pharmacopoeia of PRC, 2000). It is used traditionally in the treatment of inflammatory diseases, fever, hepatitis and pharyngolaryngitis (Chang and But, 1987; Bensky and Gamble, 1993; Hocking 1997). There is some scientific evidence to support the traditional use of *A. paniculata* as an anti-pyretic, anti-inflammatory, hepatoprotective and cardiovascular protective agent. Clinical trials indicate that *A. paniculata* has some efficacy in treating fever, sore throat and cold symptoms, but was not as effective as paracetamol (Thamlikitkul *et al.*, 1991; Caceres *et al.*, 1999). Diterpene lactones (andrographolide, neoandrographolide, 14-deoxy-11, 12-didehydroandrographolide and 14-deoxyandrographolide) from *A. paniculata* have been reported to exert anti-inflammatory effects through the inhibition of nitric oxide (Zhang and Tan, 1999; Chiou *et al.*, 2000; Batkhuu *et al.*, 2002), and are also reputedly anti-pyretic (Chang and But, 1987). The anti-inflammatory action of andrographolide has been extensively researched (Habtemariam, 1999; Amroyan *et al.*, 1999; Chiou *et al.*, 2000; Shen *et al.*, 2002) and it is also reported to possess anti-neoplastic properties (Rajagopal *et al.*, 2003). The hepatoprotective activities of *A. paniculata* have been reported (Ram 2001; Trivedi *et al.*, 2001), and several hepatoprotective compounds have been isolated from *A. paniculata* (Jain *et al.*, 2000). Extracts of *A. paniculata* and 14-deoxy-11,12-didehydroandrographolide have demonstrated cardiovascular activity *in vivo* (Guo *et al.*, 1996; Zhang *et al.*, 1998). Overdose of the herb may cause dizziness, palpitations, gastric discomfort and loss of appetite (Chang and But, 1987; Huang, 1999). The herb extract has shown contraceptive effects *in vivo* (Zoha *et al.*, 1989).

### 2.6.6. Zhi Mu (Rhizoma Anemarrhena)

Zhi Mu is prepared from the dried rhizome of *Anemarrhena asphodeloides* Bge (Anthericaceae). It is classified as a 'fire-purging' anti-pyretic (Pharmacopoeia of PRC, 2000). *A. asphodeloides* and its constituent, sarsapogenin, are reputed to reduce fever *in vivo* (Chang and But, 1987; Huang, 1999). A xanthone-C-glucoside isolated from *A. asphodeloides*, mangiferin, has shown anti-oxidant effects (Sanchez *et al.*, 2000; Ma *et al.*, 2001). Mangiferin and other constituents of *A. asphodeloides*, *cis*-hinokiresinol, tigogenin and hecogenin have demonstrated anti-neoplastic properties (Yoshimi *et al.*, 2001; Corbiere *et al.*, 2003; Jeong *et al.*, 2003). *A. asphodeloides* is reputed to have an inhibitory effect on several bacteria (Chang and But, 1987).

### 2.6.7. Da Huang (Radix et Rhizoma Rhei)

Da Huang (rhubarb) is prepared from the dried root and rhizome of *Rheum palmatum* L., *R. tanguticum* Maxim. and *R. officinale* Baill. (Polygonaceae). It is used traditionally mainly as a laxative and also in the treatment of haemorrhoids, conjunctivitis and as a choleric agent (Hsu *et al.*, 1986; Tang and Eisenbrand, 1992; Huang, 1999). Radix et Rhizoma Rhei has been shown to have cholesterol reducing effects through the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity (Kim *et al.*, 2002). The rhizome of *R. undulatum* and constituent stillbenes (rhapontigenin, piceatannol, resveratrol, chrysophanol 8-O- $\beta$ -D-(6'-galloyl)-glucopyranoside and aloe-emodin 1-O- $\beta$ -D-(glucopyranoside) have been shown to exert anti-inflammatory effects through the inhibition of nitric oxide production *in vitro* (Matsuda *et al.*, 2000; Kageura *et al.*, 2001). Species of *Rheum* used in Da Huang listed above have shown anti-oxidant effects *in vitro* (Matsuda *et al.*, 2001b).

## 2.7. Prescriptions chosen for investigation as potential alternatives to bear bile in TCM

### 2.7.1. Orengedokuto

Orengedokuto (Huanglian-Jie-Du-Tang, TJ-15) is a traditional kampo patent medicine which is approved as an ethical medicine by the Ministry of Health and Welfare of Japan, and is listed in the Pharmacopoeia of Japan for the treatment of cerebrovascular disease, hypertension, gastritis and liver diseases (Ohta *et al.*, 1998; Maclean and Taylor, 2000). Orengedokuto is composed of four herbs (Table 2.4.).

In this current study, the four herbs were individually studied and proposed as herbal alternatives to bear bile. There has been extensive scientific research, by other workers, into the pharmacological properties of Orengedokuto. Similar to bear bile, it has been associated with anti-inflammatory (Dai *et al.*, 2000; Fukutake *et al.*, 2000), hepatoprotective (Ohta *et al.*, 1998; Seikiya *et al.*, 2002) and anti-neoplastic (Fukutake *et al.*, 2000) effects.

## Section 2

**Table 2.4. Composition of herbs in Orengedokuto (Seikiya *et al.*, 2002)**

Herbs	Ratio
Huang Qin (Radix Scutellariae)	3.0
Huang Lian (Rhizoma Coptidis)	2.0
Huang Bai (Cortex Phellodendri)	1.5
Zhi Zi (Fructus Gardeniae)	2.0

Water extracts of Rhizoma Coptidis, Radix Scutellariae and Fructus Gardeniae (three of the components of orengedokuto) also showed some potential anti-neoplastic activity, but with lower efficacy than the formula (Fukutake *et al.*, 1998; Fukutake *et al.*, 2000). Fructus Gardeniae has also been associated with potential anti-inflammatory activity (which may be mediated via inhibition of COX-2 activity) (Fukutake *et al.*, 2000). Orengedokuto has also been reported to inhibit hepatic cholesterol ester formation by inhibiting the activity of acyl-coenzymeA:cholesterol acyltransferase (ACAT) *in vitro* (Yotsumoto *et al.*, 1997). In addition, extracts of Radix Scutellariae, Rhizoma Coptidis and Cortex Phellodendri decreased ACAT activity, whereas Fructus Gardeniae had no significant effect (Yotsumoto *et al.*, 1997). However, oral administration of Orengedokuto was not able to significantly reduce fever caused by a bacterial pyrogen *in vivo* (Itami *et al.*, 1992).

### 2.7.2. Dia-Orengedokuto

Dia-Orengedokuto contains the same herbs as Orengedokuto with an additional herb, Radix et Rhizoma Rhei and is used in the treatment of atherosclerosis (Kim *et al.*, 2002). Water and ethanol extracts of Dia-Orengedokuto inhibited the activity of HMG-CoA reductase more potently than extracts of Orengedokuto (Kim *et al.*, 2002). Of the herbal constituents of Dia-Orengedokuto, Rhizoma Coptidis was more potent at reducing HMG-CoA reductase activity, followed by Radix et Rhizoma Rhei (Kim *et al.*, 2002). Constituents of bear bile (CDCA and cholic acid) are also inhibitors of HMG-CoA reductase, which leads to reduction in hepatic cholesterol levels (Björkhem *et al.*, 1993).

## 2.8. Alternatives to bear bile: summary

Based on both traditional uses and knowledge of the medicinal properties of the different species of plants, a selection of 7 single herbs and 2 prescriptions consisting of a combination of herbs, were chosen as alternatives to bear bile in TCM. In addition, a TCM prescription containing bear bile was considered for study (prescription X). From Table 2.2., it is apparent that none of the 7 herbs listed as having similar properties to bear bile fulfil all the functions reputedly associated with bear bile. In addition, some TCM prescriptions containing bear bile also contain some of the suggested herbal replacements. It may be that, in some circumstances combinations of herbs, based on TCM principles, may be more suitable as replacements for bear bile in prescriptions containing the animal product.

The Association of Chinese Medicine and Philosophy and Earthcare Society (Hong Kong) has published a report on 'The Herbal Alternatives to Bear Bile in Chinese Medicine' based on TCM philosophy (IFAW report, 1994). They suggest 54 herbs as alternatives to bear bile (Appendix 1). Five of the seven herbs listed in Table 2.2 were also proposed in the IFAW report as alternatives to bear bile, Huang Qin (*Radix Scutellariae*), Huang Bai (*Cortex Phellodendri*), Zhi Zi (*Fructus Gardeniae*), Chuan Xin Lian (*Herba Andrographitis*) and Da Huang (*Radix et Rhizoma Rhei*). The underlying pathology of several of the ailments treated with bear bile is inflammation. In humans, inflammation is often initiated by infection, frequently caused by different species of bacteria (Moltz, 1993). Therefore, in this study, the herbs and prescription selected for investigation as possible alternatives to bear bile were tested in anti-bacterial and anti-inflammatory assays. The methods used and the results obtained in the present study are presented in Sections 5 and 6.

# Rationale for selection of herbs as potential alternatives to rhino horn

### 3.1. Introduction

This section addresses the selection of potential herbal alternatives to rhino horn in TCM. As with the choice of herbs to investigate as potential alternatives for the use of bear bile, selection has been based on investigation of both traditional use and the medicinal properties of the different species of plants. In this part of the report, sources of rhino horn, its uses in TCM and its reported pharmacological activities are discussed. In addition, TCM prescriptions traditionally containing rhino horn were investigated to determine their pharmacological potential in the presence and absence of the animal product. A summary of some of the known constituents and available research on the herbs and prescriptions selected for further investigation is given.

### 3.2. Sources of rhino horn

The black rhino (*Deceros bicornis*, Rhinocerotidae) population decreased by 95% between 1970 and 1993 (WWF, 2002). Due to a decline in their population, all five species (*Deceros bicornis*, *Cerathotherium sinum*, *Dicerorhinus sumatrensis*, *Rhinoceros sondaicus*, *Rhinoceroos unicornis*) of Rhinocerotidae are listed under CITES Appendix 1 (2004), therefore banning international commercial trade in their parts. Water buffalo horn has been used as an animal substitute for rhino horn, but generally at higher doses (Bensky and Barolet, 1990). Other alternatives to rhino horn include horns from cattle and the Saiga antelope (But *et al.*, 1990).

### 3.3. Rhino horn and its constituents

The primary constituent of rhino horn is keratin; constituents also include other proteins, amino acids, peptides, sterols, amines and calcium (Ingaki and Oida, 1970; Lee and Kim, 1974; Chang and But, 1987). Aqueous extracts of horns from rhino, Saiga antelope (*Saiga tatarica*), water buffalo (*Bubalus bubalis*) and cattle (*Bos taurus domesticus*) are reported to be anti-pyretic (But *et al.*, 1990). But and Tam (1991) investigated the anti-pyretic properties of herbal prescriptions containing either rhino horn or buffalo horn; separate rhino and buffalo horn extracts were found to be anti-pyretic and the combined horn-herb extracts were also anti-pyretic. In another study, rhino horn did not show anti-pyretic activity *in vivo* (Laburn and Mitchel, 1997). But *et al.* (1990) cited other studies conducted in Asia on the anti-pyretic properties of rhino horn with contradictory conclusions, but mainly with negative results. Scientific research into the anti-pyretic properties of rhino horn has shown that it is effective at reducing temperature in febrile animals only at high concentrations.

### 3.4. Rhino horn in TCM

In TCM, rhino horn is considered as having a strong action of ‘clearing heat’, ‘removing heat from the blood’, as well as arresting convulsions (Xu, 1994). The low efficacy of the rhino horn extracts to reduce temperature in febrile animals could in part be explained by the differences in concepts of the pathology of fever between Western medicine and TCM. The major difference is that in TCM febrile diseases can manifest without an increase in body temperature (Hsu *et al.*, 1986; Xu, 1994). In contrast, febrile diseases are associated with an increase in body temperature in Western medicine (Moltz, 1993).

Rhino horn (Xi Jiao; Cornu Rhinocerotis) is used as a detoxifying, anti-convulsant and anti-inflammatory agent. A major use of rhino horn is in the treatment of advanced stages of fever in the ying and blood conformation, complicated by delirium or coma. It is often used in combination with other TCM remedies and the horn is reputed to be a potent anti-convulsant in these remedies (Chang and But, 1987). Haemorrhagic conditions (e.g. erythema, haematemesis and epistaxis) sometimes manifest symptoms associated with conditions treated by rhino horn. Rhino horn has also been associated with the treatment of cardiovascular diseases (Chang and But, 1987).

### 3.5. Selection of herbs and prescriptions for investigation

#### 3.5.1. Criteria used for selection of single herbs

After consulting TCM practitioners a set of criteria was developed (Table 3.1.) and used to conduct a survey of TCM literature, to identify herbs to be investigated as possible alternatives to rhino horn (Table 3.2.). This was used in consultation with TCM practitioners to select nine plant species (Table 3.2.) for further investigation.

**Table 3.1. Properties / functions of rhino horn used as criteria for herb selection**

Criteria	Properties and functions of rhino horn
A	‘Cold’ nature
B	‘Bitter’ taste
C	‘Salty’ taste
D	‘Blood cooling’
E	‘Heat clearing’
F	Anti-convulsant
G	Anti-inflammatory properties
H	Anti-pyretic properties
I	Reduce haemorrhage

## Section 3

**Table 3.2. TCM herbs selected after consultation with TCM practitioners and from evaluation of TCM literature and pharmacological and clinical data**

Herbs with some similar properties to rhino horn	A	B	C	D	E	F	G	H	I	TCM references
1. <i>Scrophularia ningpoensis</i> Hemsl. (Scrophulariaceae) root = Xuan Shen	*	*	*	*	*		*	*		1, 2
2. <i>Rehmannia glutinosa</i> Steud (Scrophulariaceae) root = Sheng Di Huang	*	*		*	*		*			1, 2
3. <i>Paeonia suffruticosa</i> Andr. (Paeoniaceae) root = Mu Dan Pi	*	*		*	*	*	*			1, 2
4. <i>Paeonia veitchii</i> Lynch or <i>P. lactiflora</i> Pall. (Paeoniaceae) root = Chi Shao	*	*		*	*	*	*		*	2
5. <i>Arnebia euchroma</i> I.M.Johnst. (Boraginaceae) root = Zi Cao	*		*	*	*		*			1
6. <i>Isatis indigotica</i> (Brassicaceae) root = Ban Lan Gen	*	*		*	*		*	*		1, 2
7. <i>Lonicera japonica</i> Thunb. (Caprifoliaceae) flower bud = Jin Yin Hua		*		*	*	*	*			1, 2
8. <i>Forsythia suspensa</i> Vahl (Oleaceae) fruit = Lian Qiao	*	*			*		*	*		1, 2
9. <i>Salvia miltiorrhiza</i> Bge (Lamiaceae) root = Dan Shen *	*			*		*				1, 2

Criteria A – I (refer to Table 3.1.): based on TCM literature (Bensky and Gamble, 1993<sup>1</sup>; Chinese Pharmacopoeia, 2005<sup>2</sup>).

### 3.5.2. Criteria used for selection of herbal prescriptions

When combined with other TCM remedies to form prescriptions, rhino horn is reputed to play an important role. Therefore, another literature survey was conducted to ascertain TCM prescriptions containing rhino horn, which could be studied in biological assays with and without rhino horn. Five TCM prescriptions were selected for study on the basis of containing rhino horn as the only animal component. The prescriptions selected were Qing Ying Tang, Qingwen Baidu Yin, Xi Jiao Dihuang Tang, Sheng Xi Dan and Qing Gong Tang. Also selected was a TCM prescription composed only of herbs, Zhi Zi Jin Hua, which was used as a TCM 'control'. All six prescriptions are used to treat epidemic febrile diseases and their compositions are described in Table 3.3.

## Rationale for selection of herbs as potential alternatives to rhino horn

**Table 3.3. The distribution of 23 herbs and one mineral in six TCM prescriptions.**

	TCM prescriptions traditionally containing rhino horn					
	TCM control	Qingwen Baidu Yin	Xi Jiao Dihuang Tang	Qing Ying Tang	Sheng Xi Dan	Qing Gong Tang
1. Xuan Shen, root of <i>Scrophularia ningpoensis</i> (Scrophulariaceae)	Zhi Zi Jin Hua	*		*	*	*
2. Sheng Di Huang, root of <i>Rehmannia glutinosa</i> (Scrophulariaceae)		*	*	*	*	
3. Mu Dan Pi, root of <i>Paeonia suffruticosa</i> Andr. (Paeoniaceae)		*	*			
4. Chi Shao, root of <i>Paeonia lactiflora</i> , <i>P. veitchii</i> (Paeoniaceae)		*	*			
5. Zi Cao, root of <i>Arnebia euchroma</i> (Boraginaceae)					*	
6. Ban Lan Gen, root of <i>Isatis indigotica</i> (Brassicaceae)					*	
7. Jin Yin Hua, flower bud of <i>Lonicera japonica</i> (Caprifoliaceae)	*			*	*	
8. Lian Qiao, fruit of <i>Forsythia suspensa</i> (Oleaceae)		*		*	*	*
9. Dan Shen, root of <i>Salvia miltiorrhiza</i> (Lamiaceae)				*		
10. Zhi Mu, rhizome of <i>Anemarrhena asphodeloides</i> (Anthericaceae)	*	*				
11. Zhi Zi, fruit of <i>Gardenia jasminoides</i> (Rubiaceae)	*	*				
12. Huang Qin, root of <i>Scutellaria baicalensis</i> (Lamiaceae)	*	*			*	
13. Huang Lian, rhizome of <i>Coptis chinensis</i> (Ranunculaceae)	*	*		*		
14. Huang Bai, cortex of <i>Phellodendron amurense</i> (Rutaceae)	*					
15. Da Huang, root and rhizome of <i>Rheum palmatum</i> (Polygonaceae)	*					
16. Tian Hua Fen, root of <i>Trichosanthes kirilowii</i> (Cucurbitaceae)	*				*	
17. Lian Zi Xin, seed of <i>Nelumbo nucifera</i> (Nelumbonaceae)						*
18. Mai Men Dong, root of <i>Ophiopogon japonicus</i> (Convallariaceae)				*		*
19. Dan Zhu Ye, aerial part of <i>Lophatherum gracile</i> (Poaceae)		*		*		*
20. Jie Geng, root of <i>Platycodon grandiflorum</i> (Campanulaceae)		*				
21. Gan Cao, root of <i>Glycyrrhiza uralensis</i> or <i>G. glabra</i> (Leguminosae)		*				
22. Chang Pu, rhizome of <i>Acorus calamus</i> , <i>A. tatarinowii</i> (Acoraceae)					*	
23. Dan Dou Chi, seed of <i>Glycine max</i> (Leguminosae)					*	
24. Shi Gao, calcium sulphate		*				

Zhi Zi Jin Hua was used as a TCM control prescription in biological tests. Herbs numbered 1 to 7 are also listed in Table 2.6 as possible alternatives to rhino horn.

The six prescriptions were made up of a total of 23 herbs and one mineral (Table 3.3.). It became apparent from Table 3.3., that all nine possible herbal 'alternatives' to rhino horn already existed in one or more prescriptions used in this study. This finding confirmed the practice in TCM of combining remedies with similar functions for their additive and synergistic effects. However, it challenged the logic of replacing one plant species with a specific range of functions with another species that has different properties, and that might already exist in a prescription. Therefore, the principles governing the composition of TCM prescriptions were considered in determining the suitability of the selected herbs as alternatives to rhino horn.

### 3.6. Single herbs as potential alternatives to rhino horn: reputed and pharmacological effects

#### 3.6.1. Ban Lan Gen (*Radix Isatidis*)

Ban Lan Gen (Dyer's woad) is prepared from the dried root of *Isatis indigotica* Fort. (Brassicaceae). Similar to rhino horn, it is a 'blood cooling' febrifuge with the TCM properties 'cold' and 'bitter' (Hsu *et al.*, 1986). Ban Lan Gen is used traditionally as an anti-inflammatory, anti-bacterial and anti-viral remedy, and is often used in the treatment of seasonal febrile diseases (Hsu *et al.*, 1986; Ho and Chang, 2002). Ho and Chang (2002) reported that the methanolic extract of the dried roots of *I. indigotica* was anti-pyretic, anti-inflammatory and analgesic. Ethanol extracts of the roots of *I. indigotica* and organic acids isolated from *I. indigotica* (2-aminobenzoic acid, benzoic acid, salicylic acid, syringic acid and 3-(2'-carboxyphenyl)-4(3H)-quinazoline) showed anti-endotoxin activity (Wu *et al.*, 1997). The alkaloid tryptanthrin, isolated from *I. indigotica*, inhibited nitric oxide, PGE<sub>2</sub> (Ishihara *et al.*, 2000) as well as 5-lipoxygenase (5-LOX) and COX-2 activities (Danz *et al.*, 2002), indicating anti-inflammatory properties. Isaindigotone, also an alkaloid from *I. indigotica*, inhibited 5-LOX activity, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) generation and nitric oxide (Molina *et al.*, 2001).

#### 3.6.2. Chi Shao (*Radix Paeoniae Rubra*)

Chi Shao is prepared from the dried roots of *Paeonia lactiflora* Pall. (white peony) or *P. veitchii* Lynch (red peony) (Paeoniaceae). Similar to rhino horn, it is classified as a 'blood cooling' febrifuge in TCM (Hsu *et al.*, 1986; Wiseman and Ye, 1998). It has been reputed to possess analgesic and anti-convulsive effects (Hsu *et al.*, 1986; Ding *et al.*, 2000). *P. lactiflora* and its constituent paeonol, are reported to be anti-oxidant (Goto *et al.*, 1999; Ohsugi *et al.*, 1999). *P. lactiflora* demonstrated anti-inflammatory action *in vitro* (Huang *et al.*, 1990) and reduced liver damage *in vivo* (Qi, 1991). Radix Paeoniae Rubra showed anti-neoplastic properties *in vitro* (Lee *et al.*, 2002). Paeoniflorin isolated from *P. lactiflora* has been shown to be an anti-hyperlipidemic agent *in vivo* (Yang *et al.*, 2004), reduce haemorrhage due to bacterial infection and possess anti-inflammatory properties *in vitro* (Ding *et al.*, 2000). Another compound, 1,2,3,4,6-penta-O-galloyl- $\beta$ -D-glucose isolated from the root of *Paeonia lactiflora* has been shown to possess anti-oxidant, anti-neoplastic and anti-inflammatory effects (Lee *et al.*, 2003). Resveratrol, also isolated from the root of *Paeonia lactiflora*, has been reported to have anti-oxidant and anti-neoplastic activities, *in vitro* (Kang *et al.*, 2003b).

### 3.6.3. Mu Dan Pi (Cortex Moutan)

Mu Dan Pi is prepared from the dried root bark of *Paeonia suffruticosa* Andr. (Paeoniaceae). Similar to rhino horn, it is used as a 'blood-cooling' febrifuge in TCM (Hsu *et al.*, 1986; Wiseman and Ye, 1998). The TCM herbs Mu Dan Pi and Chi Shao have been shown to have some similar pharmacological and phytochemical properties (Lin *et al.*, 1999; Ding *et al.*, 2000). Both herbs contain the compounds paeoniflorin, resveratrol and 1,2,3,4,6-penta-*O*-galloyl- $\beta$ -D-glucose (pharmacological properties are listed under Chi Shao). Recently, 1,2,3,4,6-penta-*O*-galloyl- $\beta$ -D-glucose has been demonstrated to exert anti-inflammatory activity through the inhibition of IL-8 via NF- $\kappa$ B binding inhibition (Oh *et al.*, 2004) and inhibition of iNOS and COX-2 (Lee *et al.*, 2003). The major lipophilic compound from *P. suffruticosa*, paeonol and minor constituents, 2,5-dihydroxy-4-methoxyacetophenone and 2,5-dihydroxy-4-methylacetophenone have also demonstrated anti-inflammatory effects (Lin *et al.*, 1999). Methanolic extracts of *P. suffruticosa* inhibited IL-8 production (Oh *et al.*, 2003); water extracts showed anti-oxidant effects (Liu and Ng, 2000), *in vitro*. Some compounds from *P. suffruticosa* namely suffruticosides A, B, C, and D, galloyl-oxypaeoniflorin, and galloyl-paeoniflorin has been reported to exhibit more potent anti-oxidant effects than  $\alpha$ -tocopherol (Matsuda *et al.*, 2001a).

### 3.6.4. Dan Shen (Radix Salvia Miltiorrhizae)

Dan Shen (red sage root) is prepared from the root of *Salvia miltiorrhiza* Bge (Lamiaceae). Tanshinones from *S. miltiorrhiza* are reported to be anti-inflammatory in rats with infective arthritis (Duke and Ayensu, 1985) and in mice with croton oil induced inflammation of the ear (Tang and Eisenbrand, 1992), however the mechanism of action was not established in these studies. A diterpene, tanshinone IIA, isolated from the root of *S. miltiorrhiza* demonstrated anti-inflammatory effects through the inhibition of iNOS expression and production of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 (Jang *et al.*, 2003). Tanshinones from *S. miltiorrhiza* root have also demonstrated anti-inflammatory activity in mice and were active against 5-LOX in porcine leukocytes, but were not as active as the crude extracts (Chang and But, 1986; Paulus and Bauer, 2000).

### 3.6.5. Jin Yin Hua (Flos Lonicerae)

Jin Yin Hua (honeysuckle) is prepared from the dried flower buds of *Lonicera japonica* Thunb. (Caprifoliaceae). It is used traditionally as an anti-bacterial, anti-inflammatory and anti-pyretic remedy (Chang and But, 1987; Tang and Eisenbrand, 1992). A water extract of *L. japonica* demonstrated anti-inflammatory properties by inhibiting NF- $\kappa$ B activity, inducible nitric oxide and TNF- $\alpha$  *in vitro* (Lee *et al.*, 2001); anti-inflammatory effects of aqueous extracts of *L. japonica* have also been shown *in vivo* (Tae *et al.*, 2003). Oral administration of a butanol extract of *L. japonica* had mild anti-inflammatory activity against acute granulomatous and chronic inflammatory models *in vivo* (Lee *et al.*, 1998). Other studies provide some information regarding the compounds that might be responsible for the anti-inflammatory effects of the crude extracts. Lonicerosides A and C, saponins from the aerial parts of *L. japonica*, caused inhibition of ear oedema *in vivo* (Kwak *et al.*, 2003). Some compounds (e.g. methyl caffeate, 3,4-di-*O*-caffeoylquinic acid, methyl 3,4-di-*O*-caffeoylquinic acid) isolated from *L. japonica*, inhibited platelet aggregation (Chang and Hsu, 1992); methyl caffeate and methyl 3,4-di-*O*-caffeoylquinic acid also potently inhibited thromboxane formation from endogenous arachidonic acid (Chang and Hsu, 1992) and ochnaflavone, also isolated from *L. japonica*, strongly inhibited rat platelet phospholipase A<sub>2</sub> (Chang *et al.*, 1994).

### 3.6.6. Lian Qiao (Fructus Forsythia)

Lian Qiao (rengyo) is prepared from the dried fruit of *Forsythia suspensa* Vahl. (Oleaceae). It is used traditionally as an anti-pyretic and an anti-inflammatory agent in the treatment of bacterial infections (Chang and But, 1986; Tang and Eisenbrand, 1992). Water extracts of Fructus Forsythia have been reputed to reduce inflammation and fever *in vivo* (Chang and But, 1987). Methanol and *n*-hexane fractions of aqueous extracts of *F. suspensa* have been shown to have anti-inflammatory effects *in vivo* (Ozaki *et al.*, 1997). One of the anti-inflammatory constituents of the *n*-hexane extract was found to be 3 $\beta$ -aceto-20,25-epoxydammarane-24-ol (Ozaki *et al.*, 2000).

### 3.6.7. Sheng Di Huang (Radix Rehmanniae)

Sheng Di Huang (Chinese foxglove root) is prepared from the root of *Rehmannia glutinosa* Steud (Scrophulariaceae). Similar to rhino horn, it is classified in TCM as an anti-inflammatory and a 'blood-cooling' febrifuge (Hsu *et al.*, 1986). Due to its similarity in TCM functions as rhino horn, when combined with rhino horn in TCM prescriptions, the two remedies are often considered as the most important ingredients (Xu, 1994). However, *R. glutinosa* is not attributed with the anti-convulsant properties of rhino horn (Xu, 1994). *Rehmannia glutinosa* has shown potential anti-inflammatory effects through the inhibition of COX (Prieto *et al.*, 2003), TNF- $\alpha$  and IL-1 (Kim *et al.*, 1999) secretion, *in vitro*.

### 3.6.8. Xuan Shen (*Radix Scrophulariae*)

Xuan Shen (figwort root), prepared from the dried roots of *Scrophularia ningpoensis* Hemsl possesses anti-inflammatory properties and is a 'blood-cooling' febrifuge (Hsu *et al.*, 1986; Wiseman and Ye, 1998). Phenylpropanoid glycosides (angoroside C and acteoside) isolated from the root of *S. ningpoensis* have demonstrated anti-oxidant effects *in vitro* (Li *et al.*, 2000). Constituent iridoids glycosides (aucubin, verbenalin, and loganin) are reported to show anti-inflammatory effects *in vivo* (Recio *et al.*, 1994); aucubin has been shown to exert anti-inflammatory activity through the inhibition of the leukotriene, LTC<sub>4</sub>, *in vitro* (Bermejo *et al.*, 2000).

### 3.6.9. Zi Cao (*Radix Arnebiae*)

Zi Cao (purple gromwell root) is prepared from the root of *Arnebia euchroma* I.M.Johns+ (Boraginaceae). Like rhino horn, it is classified as a 'blood cooling' febrifuge in TCM (Hsu *et al.*, 1986). *Arnebia euchroma* demonstrated anti-inflammatory activity, *in vivo* (Kaith *et al.*, 1996) and *in vitro* through the inhibition of COX-2 activity (Subbaramaiah *et al.*, 2001). Shikonin, a compound isolated from the root of *Arnebia euchroma* has also been reported to demonstrate anti-inflammatory activity, *in vivo* and *in vitro* (Wang *et al.*, 1994; Ko *et al.*, 1995).

## 3.7. Prescriptions traditionally containing rhino horn: reputed and pharmacological effects

### 3.7.1. Qing Ying Tang

Qing Ying Tang is a decoction for 'clearing heat' in the 'ying' system and contains eight herbs in addition to rhino horn (Zhu, 1989; Zou, 1989; Xu, 1994). Rhino horn (*Rhinoceros Cornu*) and Sheng Di Huang (*Radix Rehmanniae*) are the two principal (important) remedies and they function by 'clearing heat' from the 'ying' and blood systems. Other prescription components are described in Table 3.3. The combined horn-herb extracts, and prescription absent from animal product, were anti-pyretic *in vivo* (But and Tam, 1991).

### 3.7.2. Qingwen Baidu Yin

The prescription Qingwen Baidu Yin is an anti-pyretic and anti-toxic decoction used to 'clear away' heat from 'qi' and blood systems (Xu, 1994). The prescription contains 12 herbs, one mineral and rhino horn. Five of the herbs (Sheng Di Huang, Xuan Shen, Lian Qiao, Huang Lian and Dan Zhu Ye) are included in the Qing Ying Tang prescription. The two important components are regarded as rhino horn and Sheng Di Huang. Other prescription components are described in Table 3.3. A modified version of Qingwen Baidu Yin, with buffalo horn substituted for rhino horn, was found to be effective in reducing fever *in vivo*, but the prescription without added animal product was not tested (Xie, 1993).

### 3.7.3. Xi Jiao Dihuang Tang

Xi Jiao Dihuang Tang is a prescription used to 'clear away' heat from 'qi' and blood systems (Zhu, 1989; Bensky and Barolet, 1990; Xu, 1994) It contains rhino horn (the principal component) and three herbs, as described in Table 3.3. All four components of Xi Jiao Dihuang Tang are also included in Qingwen Baidu Yin.

### 3.7.4. Sheng Xi Dan

Sheng Xi Dan is also known as 'magical rhinoceros special pill' (Bensky and Barolet, 1990). It is composed of rhino horn and 10 herbs (Table 3.3.).

### 3.7.5. Qing Gong Tang

Qing Gong Tang is used in the treatment of epidemic febrile diseases. It is composed of rhino horn and five herbs (Table 3.3.).

### 3.7.6. Zhi Zi Jin Hua

Zhi Zi Jin Hua is listed in the Pharmacopoeia of the People's Republic of China (1997) as an anti-pyretic agent and it is composed of eight herbs (Table 3.3.).

## 3.8. Alternatives to rhino horn: summary

Based on both traditional use and knowledge of the medicinal properties of the different species of plants, a selection of 9 single herbs were chosen for investigation as alternatives to rhino horn in TCM. Six TCM prescriptions were also investigated; five contained rhino horn as the only animal component and one TCM prescription was composed only of herbs. All six prescriptions are used in TCM to treat epidemic febrile diseases. The nine single herbs chosen as possible herbal 'alternatives' to rhino horn were found to be included in one or more prescriptions used in this study. This finding confirmed the practice in TCM of combining remedies with similar functions for their additive and synergistic effects. As with the bear bile research, it may be that combinations of the herbs, based on TCM principles, may be more suitable as replacements for animal product use.

Rhino horn is used in TCM as a detoxifying, anti-convulsant and anti-inflammatory agent. A major use of rhino horn is in the treatment of advanced stages of fever in the 'ying' and blood conformation, complicated by delirium or coma. The herbs and prescriptions selected for investigation were tested in anti-bacterial and anti-inflammatory assays. The methods used and results obtained are reported in Sections 5 and 6 of this report.

# Rationale for selection of herbs as potential alternatives to tiger bone

## 4.1. Introduction

This section addresses the selection of potential alternatives to tiger bone in TCM. In terms of finding herbal alternatives to animal products in traditional practices of medicine, the need is perhaps most urgent with that of tiger bone, where hunting has driven the species to the brink of extinction. As with the herbs selected to investigate as potential alternatives for bear bile and rhino horn, selection has been based on investigation of both traditional use and the medicinal properties of the different species of plants and fungi. Sources of tiger bone and the devastating effect of hunting on the tiger population, its uses in TCM and its reported pharmacological activities are discussed. As with the research seeking alternatives to bear bile and rhino horn, both single herbs and prescriptions were selected for investigation. A summary of some of the known constituents and available pharmacological and clinical research on the selected herbs / fungi, and the prescriptions, is given.

## 4.2. Sources of tiger bone

One of the world's most endangered animal species is the tiger, *Panthera tigris* (Felidae). There are only five out of eight remaining subspecies (*P. t. amoyensis*, *P. t. sumatrae*, *P. t. altaica*, *P. t. corbetti* and *P. t. tigris*) surviving today, with an estimated population of 5,000 – 7,500 (WWF, 2000). Consequently, *P. tigris* is included in Appendix 1 of CITES (2003).

In view of the concerns relating to the declining numbers of tigers, investigations were conducted to identify plants or fungi that may be suitable alternatives to tiger bone in TCM. Both TCM principles and pharmacological activities were considered to assist with the identification of suitable species in this study.

## 4.3. Tiger bone and its constituents

Tiger bone is reported to contain collagen, fats, calcium phosphate, calcium carbonate and magnesium phosphate; the gelatin is reputed to be composed of 17 amino acids (Chang and But, 1987). Generally, bone is primarily composed of inorganic calcium salts (65–70%); smaller amounts of chondroitin sulphate, keratin sulphate and phospholipids are also reported to be present (Brody, 1994). In the UK, chondroitin sulphate is included in some over-the-counter remedies that are used to relieve symptoms of arthritis.

## Section 4

There is a general lack of published literature on tiger bone and there is limited evidence to support the pharmacotherapeutic potential of tiger bone for alleviating symptoms of arthritis (Chang and But, 1987). Suspensions of both tiger bone and dog bone are reported to be anti-inflammatory *in vivo* (Chang and But, 1987); however, the doses used in this study appeared to be much higher than would usually be administered therapeutically. Tiger bone powder is reported to reduce total neutrophil concentration and to inhibit leukocyte and lymphocyte proliferation *in vivo* (Chang and But, 1987). Both tiger and dog gelatin are reported to be analgesic *in vivo* (Chang and But, 1987), and tiger and dog bones are reported to be sedative *in vivo* (Chang and But, 1987).

Chang and But (1987) also cite research conducted on a TCM prescription, 'compound union pill', containing tiger bone which promoted healing of fractures in rabbits. Analysis of each component of this prescription showed that tiger bone was one of the most effective ingredients to replenish bone density and promote healing (Chang and But, 1987).

### 4.4. Tiger bone in TCM

Tiger bone (Hu Gu) is described in TCM as having a 'pungent' taste and 'warm' nature (Chang and But, 1987; Bensky and Gamble, 1993). It is believed to 'dispel wind-dampness', 'disperse wind cold' and 'strengthen the sinews and bones' (Bensky and Gamble, 1993). Tiger bone is used in TCM to treat symptoms such as bone and muscle pain, limb spasms, lower back pain and chills. It is used to treat pathologic states classified under 'painful obstruction disorders' in TCM. This group of disorders best fits the Western term 'arthritic disorders' which includes various rheumatic diseases and osteoarthritis (Guillaume and Chieu, 1996).

### 4.5. Selection of herbs

#### 4.5.1. Criteria used for selection of single herbs

There is some limited scientific evidence to support the use of tiger bone as an anti-inflammatory and an analgesic agent in traditional medicine. In order to identify species, which possessed the appropriate TCM properties and functions, an extensive literature survey was initially conducted using the criteria described in Table 4.1. Forty-six species (Table 4.2.) were identified based on the criteria. Although, all the species listed in Table 4.2 may be used in combination with other TCM remedies in the treatment of arthritis and rheumatism, several of them are not categorised as anti-rheumatics in TCM *Materia Medica*, and may have other functions (e.g. as a tonic).

## Rationale for selection of herbs as potential alternatives to tiger bone

**Table 4.1. Properties / functions of tiger bone used as criteria for species selection**

Criteria	Properties and functions of tiger bone
A	For arthritic and rheumatic conditions
B	Analgesic
C	'Warm' nature
D	'Pungent' or 'acid'
E	'Sweet' taste
F	Heals wounds and fractures
G	Alleviates pain in lower back and knees
H	Expels wind dampness or cold

### 4.5.2. Criteria used for the selection of prescriptions

In addition to the literature survey conducted to identify appropriate species, another survey was conducted to identify TCM prescriptions that included tiger bone as a component. Those prescriptions identified were generally indicated for arthritis or related disorders. In consultation with TCM practitioners, two TCM prescriptions that contained tiger bone as the only animal component and non-endangered plant / fungal species (those not restricted by CITES) were selected for further investigation (prescription compositions are described in Table 4.3.). The two TCM prescriptions were composed of a combined total of 19 different TCM herbs and one fungus; eight of these species were identified in the initial literature survey of the herbs (Table 4.2.).

Since TCM often uses remedies with similar functions to form prescriptions, preliminary pharmacological investigations were conducted to assess the potential anti-inflammatory effects of the two TCM prescriptions; 19 species were also subjected to analysis in the bioassays (Table 4.3. and 4.4.). This approach was designed to identify species used in the prescriptions, which may have anti-inflammatory properties via the biological pathway tested. In addition to the preliminary pharmacological investigations, which were conducted in this study to identify any scientific basis for the reputed activities of the 19 selected remedies, a literature search was also conducted. This aim of this investigation was to identify any pharmacological or clinical studies relating to the potential anti-inflammatory / anti-rheumatic / analgesic effects of the 19 selected remedies (Tables 4.3. and 4.4.). This exercise was to provide information previously reported that might also assist in the identification of possible alternatives to tiger bone. The results of this study are summarised in section 4.6.

Table 4.2. TCM species identified from evaluation of TCM literature using criteria based on the TCM functions and properties of tiger bone

TCM herbs	A	B	C	D	E	F	G	H
	Arthritis Rheumatic	Analgesic	Warm	Pungent (acid)	Sweet	Wounds & fractures	Lower back & knee pain	Expels wind dampness / cold
1. Fang Feng, root of <i>Saposhnikovia divaricata</i> (Turcz.) Schischk. (Apiaceae)	*	*	*	*	*			*
2. Wei Ling Xian, root and rhizome of <i>Clematis chinensis</i> Osb. (Ranunculaceae)	*	*	*	*		*		*
3. Nao Yang Hua, aerial part of <i>Rhododendron molle</i> Siebold & Zucc. (Ericaceae)	*	*	*	*		*	*	
4. Du Huo, root of <i>Angelica pubescens</i> Maxim. (Apiaceae)	*	*	*	*				*
5. Fu Zi, prepared daughter root tuber of <i>Aconitum carmichaelii</i> Debx. (Ranunculaceae)	*	*	*	*				
6. Cang Er Zi, fruit of <i>Xanthium sibiricum</i> Patrini ex Widd. (Asteraceae)	*	*	*	*				*
7. Xi Xin, whole plant of <i>Asarum heterotropoides</i> forma <i>manshuricum</i> (Maxim.) Kitag. (Aristolochiaceae)*	*	*	*	*				
8. Zu Shi Ma, bark or root bark of <i>Daphne giraldi</i> Nitsche (Thymelaeaceae)	*	*	*	*				
9. Yang Jin Hua, corolla of <i>Datura metel</i> L. (Solanaceae)	*	*	*	*				
10. Chuan Shan Long, rhizome of <i>Dioscorea nipponica</i> Makino (Dioscoreaceae)	*	*	*		*	*		*
11. Ba Jiao Feng, leaves, stems and fibrous roots of <i>Alangium chinense</i> (Lour.) Harms (Alangiaceae)	*	*	*			*		
12. Qi Ye Lian, roots, stems and leaves of <i>Schefflera arboricola</i> Hayata (Araliaceae)	*	*	*			*		

Continued

## Rationale for selection of herbs as potential alternatives to tiger bone

Table 4.2. TCM species identified from evaluation of TCM literature using criteria based on the TCM functions and properties of tiger bone

TCM herbs	A	B	C	D	E	F	G	H
	Arthritis Rheumatic	Analgesic	Warm	Pungent (acid)	Sweet	Wounds & fractures	Lower back & knee pain	Expels wind dampness / cold
13. Chuan Xiong, rhizome of <i>Ligusticum chuanxiong</i> Hort. (Apiaceae)	*	*	*					*
14. Qing Feng Teng, stem of <i>Sinomenium acutum</i> Rehd. (Menispermaceae)	*	*	*					*
15. Xue Shang Yi Zhi Hao, root of <i>Aconitum brachypodum</i> Diels (Ranunculaceae)	*	*	*					
16. Qin Jiao, root of <i>Gentiana macrophylla</i> Pall. (Gentianaceae)	*	*		*				*
17. Fang Ji, root of <i>Stephania tetrandra</i> S.Moore (Menispermaceae)	*	*		*				
18. Lei Gong Teng, root of <i>Tripterygium wilfordii</i> Hook.f. (Celastraceae)	*	*		*				
19. Chou Wu Tong, twigs and leaves of <i>Clerodendron trichotonum</i> Thunb. (Verbenaceae)	*	*			*			
20. Dang Gui, root of <i>Angelica sinensis</i> (Oliv.) Diels (Apiaceae)	*		*	*	*			
21. Ji Xue Teng, root and stem of <i>Spatholobus suberectus</i> Dunn. (Fabaceae)	*		*	*	*		*	
22. Yin Yang Huo, aerial parts of <i>Epimedium sagittatum</i> Maxim. (Berberidaceae)	*		*	*			*	
23. Cang Zhu, rhizome of <i>Atractylodes lancea</i> (Thunb.) DC. or <i>A. chinensis</i> (D.C.) Koidz. (Asteraceae)	*		*	*				*
24. Ding Gong Teng, root and stem of <i>Erycibe obtusifolia</i> Benth. (Convolvulaceae)	*		*	*				*

Continued

Table 4.2. TCM species identified from evaluation of TCM literature using criteria based on the TCM functions and properties of tiger bone

TCM herbs	A	B	C	D	E	F	G	H
	Arthritis Rheumatic	Analgesic	Warm	Pungent (acid)	Sweet	Wounds & fractures	Lower back & knee pain	Expels wind dampness / cold
25. Ci Wu Jia, root and rhizome of <i>Eleutherococcus senticosus</i> (Rupr. & Maxim.) Rupr. (Araliaceae)	*		*	*				
26. Yun Xiang Cao, whole plant of <i>Cymbopogon distans</i> (Nees ex Steudel) Will. (Poaceae)	*		*	*				
27. Rou Gui, bark of <i>Cinnamomum cassia</i> D. Don (Lauraceae)	*			*	*			
28. Tou Gu Cao, whole plant of <i>Impatiens balsamina</i> L. (Balsaminaceae)	*	*			*			*
29. Xu Chang Qing, root and rhizome of <i>Cynanchum paniculatum</i> Bunge (Asclepiadaceae)	*	*						
30. Xue Lian, root of <i>Saussurea laniceps</i> Hand.-Mazz (Asteraceae)	*		*		*			
31. Xiang Jia Pi, root bark of <i>Periploca sepium</i> Bunge (Asclepiadaceae)	*		*		*			
32. Lu Xian Cao, whole plant, <i>Pyrola calliantha</i> Andres and <i>P. decorata</i> Andres (Pyrolaceae)	*		*				*	
33. Yi Ye Qiu, shoot and root of <i>Securinega suffruticosa</i> (Pall.) Rehd. (Phyllanthaceae)	*		*				*	
34. Huang Jing Zi, fruit of <i>Vitex negundo</i> L. (Lamiaceae)	*		*					
35. Shi Diao Lan, whole plant of <i>Lysionotus pauciflorus</i> Maxim. (Gesneriaceae)	*		*					
36. Kun Ming Shaun Hai Tang, root of <i>Tripterygium hypoglaucom</i> Hutchinson (Celastraceae)	*		*					
37. Mu Gua, fruit of <i>Chaenomeles speciosa</i> (Sweet) Nakai (Rosaceae)	*		*					

## Rationale for selection of herbs as potential alternatives to tiger bone

**Table 4.2. TCM species identified from evaluation of TCM literature using criteria based on the TCM functions and properties of tiger bone**

TCM herbs	A	B	C	D	E	F	G	H
	Arthritis Rheumatic	Analgesic	Warm	Pungent (acid)	Sweet	Wounds & fractures	Lower back & knee pain	Expels wind dampness / cold
38. Sang Zhi, young branches of <i>Morus alba</i> L. (Moraceae)	*			*				*
39. Fu Zi, prepared daughter root tuber of <i>Aconitum Carmichaelii</i> Debx. (Ranunculaceae)	*			*				
40. Nu Zhen Zi, fruit of <i>Ligustrum lucidum</i> Ait. (Oleaceae)	*				*		*	
41. Sang Ji Sheng is the stem and branch of <i>Taxillus chinensis</i> (DC.) Danser (Loranthaceae)	*				*		*	
42. Jin Gi Er, seed of <i>Caragana microphylla</i> Lam. (Leguminosae)	*				*			
43. Man Shan Xiang, <i>Lysimachia capillipes</i> Hemsl. (Primulaceae)	*				*			
44. Qian Cao, root and stem of <i>Rubia cordifolia</i> L. (Rubiaceae)	*					*		
45. Jiu Jie Feng, root, aerial part or whole plant of <i>Sarcandra glabra</i> (Thunberg) Nakai (Chloranthaceae)	*					*		
46. Ma Qian Zi, seed of <i>Strychnos nux-vomica</i> L. (F of China) (Loganiaceae)	*					*		

References: Chinese Pharmacopoeia, 2005; Chang and But, 1987; Bensky and Gamble, 1993 and 2004; Chang and But, 2001.

## Section 4

**Table 4.3. Composition of herbs as described TCM prescriptions, traditionally containing tiger bone**

Herbs	Yang Xue Gu Feng Tang	Du Huo Ji Sheng Tang
Bai Shao, root of <i>Paeonia lactiflora</i> (Ranunculaceae) $\phi$	*	*
Dang Gui, root of <i>Angelica sinensis</i> (Apiaceae) $\phi$	*	*
Du Huo, root of <i>Angelica pubescens</i> (Apiaceae) $\phi$	*	*
Fu Ling, sclerotium of <i>Poria cocos</i> (Polyporaceae) fungus	*	*
Niu Xi, root of <i>Achyranthes bidentata</i> (Amaranthaceae) $\phi$	*	*
Qin Jiao, root of <i>Gentiana macrophylla</i> (Gentianaceae) $\phi$	*	*
Di Huang, root of <i>Rehmannia glutinosa</i> (Scrophulariaceae)	*	*
Bai Zhu, rhizome of <i>Atractylodes macrocephala</i> (Asteraceae) $\phi$	*	
Gui Zhi, twigs of <i>Cinnamomum cassia</i> (Lauraceae) $\phi$	*	
Mu Xiang, root of <i>Saussurea costus</i> (Falc.) Lipsch. (Asteraceae) $\phi$	*	
Sang Chi (Sang zhi), twig of <i>Morus alba</i> (Moraceae)	*	
Xu Duan, rhizome of <i>Dipsacus asper</i> (Dipsacaceae)	*	
Chuan Xiong, rhizome of <i>Ligusticum chuanxiong</i> (Apiaceae) $\phi$		*
Du Zhong, bark of <i>Eucommia ulmoides</i> (Eucommiaceae) $\phi$		*
Fang Feng, root of <i>Saposhnikovia divaricata</i> (Apiaceae) $\phi$		*
Gan Cao, rhizome of <i>Glycyrrhiza uralensis</i> (Leguminosae) $\phi$		*
Ren Shen, root of <i>Panax ginseng</i> (Araliaceae) $\phi$		*
Rou Gui, bark of <i>Cinnamomum cassia</i> (Lauraceae)		*
Sang Ji Sheng, stem and branch of <i>Taxillus chinensis</i> (Loranthaceae) $\phi$		*

$\phi$  Species also tested individually in biological activity tests

**Table 4.4. Other species selected for biological activity tests (not listed in Table 4.3.)**

Herbs
Cang Zhu, rhizome of <i>Atractylodes lancea</i> + (Asteraceae)
Mu Gua, fruit of <i>Chaenomeles speciosa</i> (Rosaceae)
Wei Lin Xian, root and rhizome of <i>Clematis chinensis</i> (Ranunculaceae)
Yin Yang Huo, aerial parts of <i>Epimedium sagittatum</i> (Berberidaceae)
San Qi, root of <i>Panax pseudoginseng</i> (Araliaceae)

+ Other plant species may be used for the Chinese name specified (not listed in Table 4.4.)

## 4.6. Single herbs and other TCM remedies as potential alternatives to tiger bone: reputed and pharmacological effects

### 4.6.1. Bai Shao (*Radix Paeoniae Alba*)

Bai Shao is the dried root of *Paeonia lactiflora* Pall (Ranunculaceae) and is used to treat a variety of disorders in TCM including spasmodic pain of the limbs; *Paeonia* root may also be used for analgesic effects (Pharmacopoeia of PRC, 2000; Tang and Eisenbrand, 1992). Few studies have been conducted to investigate the anti-inflammatory potential of *P. lactiflora*, however, it has been associated with activity against COX (Prieto *et al.*, 2003). Paeoniflorin, isolated from the root, is reported to be anti-inflammatory (Tang and Eisenbrand, 1992).

### 4.6.2. Bai Zhu (*Rhizoma Atractylodis Macrocephalae*)

Bai Zhu is the dried rhizome of *Atractylodes macrocephala* Koidz. (Asteraceae) and it is indicated in TCM for a number of disorders, which include oedema (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). Limited research has been conducted to investigate the anti-inflammatory potential of *A. macrocephala*, but it has been associated with activity against COX (Prieto *et al.*, 2003).

### 4.6.3. Cang Zhu (*Rhizoma Atractylodis*)

Cang Zhu is the dried rhizome of *Atractylodes lancea* DC. or *A. chinensis* Koidz (Asteraceae) and it has been used in TCM for the treatment of rheumatic arthralgia (Pharmacopoeia of PRC, 2000). Some compounds (including phenols, polyacetylenes, atractylon (sesquiterpene) and osthole (coumarin)) from *A. lancea* rhizomes and lipophilic extracts have been associated with inhibition of COX-1 and 5-LOX (Resch *et al.*, 1998; Resch *et al.*, 2001). A Japanese prescription used traditionally for arthritis treatment and composed of seven crude drugs including *A. lancea* was anti-inflammatory *in vivo* (Kimura *et al.*, 1991). Pharmacological studies regarding *A. chinensis* are lacking.

### 4.6.4. Chuan Xiong (*Rhizoma Chuanxiong*)

Chuan Xiong is the dried rhizome of *Ligusticum chuanxiong* Hort. (Apiaceae), which is used in TCM for various conditions such as rheumatic arthralgia (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). There is a relative lack of research regarding the pharmacological basis of the reputed anti-inflammatory / anti-rheumatic activity of *L. chuanxiong*. An alcohol extract is reported as anti-inflammatory and analgesic (Chang and But, 2001). Tetramethylpyrazine, an alkaloid from *L. chuanxiong*, has been associated with anti-inflammatory activity in both the early and late stages of inflammation (Ozaki, 1992).

### 4.6.5. Dang Gui (Radix Angelicae Sinensis)

Dang Gui is prepared from the dried root of *Angelica sinensis* (Oliv.) Diels (Apiaceae) and it is indicated in TCM for a number of disorders including rheumatic arthralgia and traumatic injuries (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). There is a relative lack of research regarding the pharmacological basis of the reputed anti-inflammatory / anti-rheumatic activity of *A. sinensis*. Ferulic acid, reported to occur in *A. sinensis*, has been associated with anti-inflammatory activity in both the early and late stages of inflammation (Ozaki, 1992).

### 4.6.6. Di Huang (Radix Rehmanniae)

Di Huang is the fresh or dried root tuber of *Rehmannia glutinosa* Steud. (Scrophulariaceae) and is used in TCM to treat various disorders and may be used as a tonic (processed roots) or haemostatic (fresh and dried roots) (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). An extract (100% methanol) of *R. glutinosa* root showed inhibition of COX-2 and iNOS activity (Hong *et al.*, 2002). An aqueous extract of *R. glutinosa* root has been suggested to inhibit TNF- $\alpha$  secretion by inhibiting IL-1 secretion and *R. glutinosa* root extract may have anti-inflammatory activity in the CNS (Kim *et al.*, 1999). However, *in vivo*, an ethanolic extract of *R. glutinosa* root was ineffective on the development of oedema in arthritic rats and on chronic and acute inflammation (Kubo *et al.*, 1994). It is also reported that it is only the decoction which has an anti-inflammatory effect and not an alcohol extract (Chang and But, 2001).

### 4.6.7. Du Huo (Radix Angelicae Pubescentis)

Du Huo is the dried root of *Angelica pubescens* Maxim. (Umbelliferae), which has been used in TCM as an analgesic and anti-rheumatic agent (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). Anti-inflammatory and analgesic activities of *A. pubescens* root are well documented (Chen *et al.*, 1995; Kosuge *et al.*, 1985; Liu *et al.*, 1998a; Liu *et al.*, 1998b; Prieto *et al.*, 2003). Methanol, chloroform and ethyl acetate extracts are reported to reduce pain and oedema *in vivo*; columbianadin, columbianetin acetate, bergapten, umbelliferone and caffeic acid were anti-inflammatory and analgesic *in vivo*; osthole and xanthotoxin were anti-inflammatory *in vivo* (Chen *et al.*, 1995). *A. pubescens*, *A. pubescens f. biserrata*, linoleic acid, osthole, osthenol and some polyacetylenes (e.g. falcarindiol) have also been associated with inhibition of COX and 5-LOX (Liu *et al.*, 1998a; Liu *et al.*, 1998b). *A. pubescens* is also reported to be effective in attenuating persistent hindpaw inflammation and hyperalgesia in rats (Wei *et al.*, 1999).

### 4.6.8. Du Zhong (Cortex Eucommiae)

Du Zhong is the dried stem bark of *Eucommia ulmoides* Oliver (Eucommiaceae) and is reputed in TCM to strengthen the tendons and bones (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). The chemical composition of *E. ulmoides* has been subjected to some investigation and pharmacological activities have been associated with some constituents. However, there is a comparative lack of research regarding pharmacological studies associated with anti-inflammatory or anti-rheumatic activities. A decoction is reported to be analgesic and anti-inflammatory (which may be related to enhancement of adrenocortical function) *in vivo* (Chang and But, 2001).

### 4.6.9. Fang Feng (Radix Saposhnikoviae)

Fang Feng is the dried root of *Saposhnikovia divaricata* (Turcz.) Schischk. (Apiaceae) and it is used in TCM to treat rheumatic arthralgia (Pharmacopoeia of PRC, 2000). The ethanol extract of Fang Feng is reported to be analgesic and anti-inflammatory (Chang and But, 2001). Analgesic components of *S. divaricata* are reported to be chromones, coumarins, polyacetylenes and 1-acylglycerols; the most potent analgesia was associated with chromones such as divaricatol, ledebouriellol and hamaudol (Okuyama *et al.*, 2001). Imperatorin and deltoin, isolated from *S. divaricata* root, inhibited the expression of the iNOS protein (Wang *et al.*, 1999).

### 4.6.10. Fu Ling (Poria)

Fu Ling is the dried sclerotium of the fungus *Poria cocos* (Polyporaceae), which is indicated in TCM for a number of disorders and is reputed to cause diuresis and to calm the mind (Pharmacopoeia of PRC, 2000). *P. cocos* is reported to inhibit 5-LOX and phospholipase A<sub>2</sub> activities and dehydrotumulosic and pachymic acids, which have been isolated from *P. cocos*, are reported to inhibit leukotriene B<sub>4</sub> (LTB<sub>4</sub>) release and to inhibit phospholipase A<sub>2</sub> activity (Cuellar *et al.*, 1996; Giner *et al.*, 2000; Giner-Larza *et al.*, 2000; Prieto *et al.*, 2003). A triterpene derivative (3 $\beta$ -*p*-hydroxybenzoyldehydrotumulosic acid) isolated from *P. cocos* showed anti-inflammatory activity *in vivo* (Yasukawa *et al.*, 1998).

### 4.6.11. Gui Zhi (Ramulus Cinnamomi)

Gui Zhi is the dried young branches of *Cinnamomum cassia* (Lauraceae), which has been used in TCM for the treatment of arthralgia and oedema (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). An extract (100% methanol) of *C. cassia* twigs showed inhibition of COX-2 and iNOS activity (Hong *et al.*, 2002), which may explain some of the reputed effects. The active constituents responsible for activities observed in pharmacological studies require further investigation.

### 4.6.12. Ji Xue Teng (Caulis Spatholobi)

Ji Xue Teng is the root and stem of *Spatholobus suberectus* Dunn (Fabaceae) and it has been used in TCM for various conditions, which include knee pain or generalised joint soreness (Bensky and Gamble, 1993).

Ji Xue Teng is reported to promote beneficial effects on artificially-induced arthritis *in vivo* (Bensky and Gamble, 1993). In addition, the alternative plant species used to prepare Ji Xue Teng, *S. suberectus* stem, was active against COX-1, phospholipase A<sub>2</sub>, 5-LOX and 12-LOX activities, but did not inhibit COX-2 activity (Li *et al.*, 2003).

### 4.6.13. Lu Lu Tong (Fructus Liquidambaris)

Lu Lu Tong is the dried ripe fruit of *Liquidambar formosana* Hance (Hamamelidaceae) and it is indicated for arthralgia with numbness and muscular contracture (Pharmacopoeia of PRC, 2000). Some studies have investigated the chemistry of *Liquidambar* species, however, pharmacological studies are limited; anti-inflammatory / anti-rheumatic effects have not been substantially investigated.

### 4.6.14. Mu Gua (Fructus Chaenomelis)

Mu Gua is the dried nearly ripe fruit of *Chaenomeles speciosa* Nakai (Rosaceae) and it is indicated in TCM for arthritis with ankylosis (Pharmacopoeia of PRC, 2000). Glucosides from *C. speciosa* were anti-inflammatory (effects included inhibition of TNF- $\alpha$  and PGE<sub>2</sub>) *in vivo* (Chen and Wei, 2003; Dai *et al.*, 2003).

### 4.6.15. Mu Xiang (Radix Aucklandiae)

Mu Xiang is the dried root of *Saussurea costus* (Falc.) Lipsch (Syn.: *Saussurea lappa*) (Asteraceae) and it is used in TCM for treating some types of pain (Pharmacopoeia of PRC, 2000). An ethanolic extract of *S. lappa* is reported to show anti-inflammatory and anti-arthritic activity (Gokhale *et al.*, 2002). Sesquiterpene lactones (e.g. costunolide, cynaropicrin) from *Saussurea lappa* have been associated with anti-inflammatory activity (Cho *et al.*, 2000; Gokhale *et al.*, 2003; Matsuda *et al.*, 2003; Cho *et al.*, 2004). The anti-inflammatory activity of the sesquiterpene lactone fraction of *S. lappa* has been suggested to be due to stabilisation of lysosomal membranes and an anti-proliferative effect (Gokhale *et al.*, 2003). Cynaropicrin inhibited TNF- $\alpha$  release, attenuated nitric oxide accumulation and dose-dependently suppressed the proliferation of lymphocytes (Cho *et al.*, 2000); costunolide inhibited IL-1 $\beta$  gene expression (Kang *et al.*, 2004). Some amino acid-sesquiterpene conjugates (saussureamines A and B) from a methanolic extract of *S. lappa* roots inhibited activation of NF- $\kappa$ B (Matsuda *et al.*, 2003).

### 4.6.16. Niu Xi (*Radix Achyranthis Bidentatae*)

Niu Xi is prepared from the dried root of *Achyranthes bidentata* Blume (Amaranthaceae) and is used in TCM as a tonic and for soreness of the lumbar and knee joints with weakness in the legs (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). There is a general lack of research regarding the study of potential anti-inflammatory / anti-rheumatic effects of *A. bidentata*. An oligosaccharide (AbPS) isolated from *A. bidentata* significantly enhanced the humoral immune response and antagonised the immunosuppressive effects of cyclosporin A; AbPS increased the production of TNF and the activity of natural killer cells; in tumour patients treated by chemotherapy or radiotherapy, AbPS maintained their peripheral white blood cell count and improved the quality of life (Li, 2000). Root polysaccharides induced IL-1 and TNF- $\alpha$  synthesis and secretion from mouse peritoneal macrophages *in vitro*, indicating immunopotentiating activity (Xiang and Li, 1993). Leflunomide (disease modifying anti-rheumatic drug in clinical use) and its active metabolite are associated with inhibition of IL-1 $\beta$ , TNF- $\alpha$  and NF- $\kappa$ B (Breedveld and Dayer, 2000; Elkayam *et al.*, 2003), thus *A. bidentata* saccharides may be of no therapeutic benefit in rheumatoid arthritis (RA) via these mechanisms (in view of the reported association with immunopotentiating effects).

### 4.6.17. Qin Jiao (*Radix Gentianae Macrophyllae*)

Qin Jiao is the dried root of *Gentiana macrophylla* Pall. It has been used in TCM mainly for the treatment of rheumatic or rheumatoid arthritis with muscular contracture and severe joint pain (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). Compounds identified in *Gentiana macrophylla* include various secoiridoids. The secoiridoid glucoside, gentiopicroside, is reported to show anti-inflammatory activity *in vivo* (Tang and Eisenbrand, 1992).

### 4.6.18. Ren Shen (*Radix Ginseng*)

Ren Shen is the dried root of *Panax ginseng* C.A.Mey (Araliaceae) and it is indicated for various conditions, including general weakness with irritability and insomnia in chronic diseases, and may be included in prescriptions as a tonic (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). *P. ginseng* has been extensively researched regarding its chemistry and its pharmacological and clinical effects. Ginsenoside Rg<sub>3</sub> inhibited COX-2 expression and NF- $\kappa$ B activation (Keum *et al.*, 2003).

### 4.6.19. Rou Gui (*Cortex Cinnamomi*)

Rou Gui is the dried stem bark of *Cinnamomum cassia* D.Don (Lauraceae), which was used traditionally for cold and pain in the knees and for some inflammatory disorders (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). An extract (70% methanol) of *C. cassia* cortex showed an inhibitory effect on acute inflammation *in vivo* (Kubo *et al.*, 1996). Further investigation is required to identify the active constituents responsible for these effects.

### 4.6.20. San Qi (Radix Notoginseng)

San Qi is the dried root of *Panax notoginseng* (Burkill) Chen (Araliaceae), which is used in TCM to alleviate traumatic swelling and pain, amongst other conditions (Pharmacopoeia of PRC, 2000; Tang and Eisenbrand, 1992). Saponins from *Panax notoginseng* are reported to be anti-inflammatory, which may be associated with inhibition of phospholipase A<sub>2</sub> activity (Hao and Yang, 1986; Tang and Eisenbrand, 1992; Li and Chu, 1999). Another study showed that *P. notoginseng* did not produce any significant effect on inflammation and hyperalgesia *in vivo* (Wei *et al.*, 1999).

### 4.6.21. Sang Zhi (Ramulus Mori)

Sang Zhi is the dried young branches of *Morus alba* L. (Moraceae) and is used for the treatment of arthritis and rheumatism (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). An extract (100% methanol) of *M. alba* twigs showed inhibition of iNOS activity (Hong *et al.*, 2002). Mulberroside A and oxyresveratrol, obtained from *M. alba* cortex, have been investigated for their anti-inflammatory activity and were shown to significantly reduce paw oedema (Chung *et al.*, 2003). The anti-inflammatory properties of oxyresveratrol were associated with inhibition of NOS expression through down-regulation of NF- $\kappa$ B binding and inhibition of COX-2 activity (Chung *et al.*, 2003).

### 4.6.22. Wei Ling Xian (Radix Clematidis)

Wei Ling Xian is the dried root and rhizome of *Clematis chinensis* Osb. (Ranunculaceae), and it is indicated in TCM for rheumatic or rheumatoid arthralgia with numbness of the limbs (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). Stems of *C. chinensis* have also been used in TCM to treat rheumatic arthritis and other inflammatory conditions (Xu *et al.*, 1996). In one study, an ethanol extract of *C. chinensis* stems only inhibited COX-1 activity *in vitro* at high concentrations (Li *et al.*, 2003). Few pharmacological studies have been conducted to investigate any scientific basis for the reputed anti-inflammatory / anti-rheumatic effects of *C. chinensis*.

### 4.6.23. Xu Duan (Dipsaci Radix)

Xu Duan is the dried root of *Dipsacus asperoides* C.Y.Cheng & T.M.Ai (Dipsacaceae), which is indicated in TCM for aching and weakness of the loins and knees, rheumatic arthralgia and traumatic injuries (Pharmacopoeia of PRC, 2000). *D. asperoides* has not been subjected to substantial investigation regarding its chemistry and pharmacological activities.

#### 4.6.24. Yin Yang Huo (Herba Epimedii)

Yin Yang Huo is prepared from the dried aerial parts of *Epimedium sagittatum* (S. et Z) Maxim (Berberidaceae); it is indicated in TCM for weakness of the limbs and rheumatic or rheumatoid arthralgia with numbness or muscle contracture, and has also been included in prescriptions as a tonic (Tang and Eisenbrand, 1992; Pharmacopoeia of PRC, 2000). A flavonoid extract from *E. sagittatum* was effective in preventing osteoporosis *in vivo*, which may be mediated by enhancement of osteoblast development (Chen *et al.*, 2004). Other studies, relating to the potential anti-inflammatory / anti-rheumatic effects of the species used to prepare Yin Yang Huo are limited, and further investigations are necessary to establish the basis for their clinical use.

### 4.7. Alternatives to tiger bone: summary

Based on both traditional uses and evidence from scientific research, a selection of 19 single herbs and 2 prescriptions (Tables 4.3. and 4.4.) were selected. Five other species present in some TCM prescriptions also traditionally containing tiger bone, were chosen for further study (Table 4.4.). In TCM, tiger bone is traditionally used to treat conditions involving inflammation and this property is supported by research suggesting that suspensions of both tiger bone and dog bone are anti-inflammatory *in vivo*. Preliminary pharmacological investigations were therefore conducted to assess the potential anti-inflammatory effects of the selected herbs and prescriptions. The methods used are reported in Section 5 of this report and the findings are reported in Section 6. This approach was designed to identify species used in the prescriptions, which may have anti-inflammatory properties via the biological pathway tested.

# Biological and chemical methods used to study plant and fungal material

## 5.1. Introduction

Both rhino horn and bear bile are primarily classified in TCM as anti-inflammatory and fever-reducing remedies and tiger bone has been used as an anti-arthritic / anti-rheumatic remedy (Hsu *et al.*, 1986); the pathology of arthritis also involves inflammatory mechanisms. Thus, assays were selected to evaluate the anti-inflammatory potential of the TCM material.

The inflammatory response is a complex cascade of events, often triggered by infection (commonly by bacteria) and is one of the body's defence mechanisms in fighting disease. The inflammatory response forms one of the underlying pathologies of arthritis (McEvoy, 2004); fever (Ivanov and Romanovsky 2004); liver diseases (Tanasescu, 2004), cancer (Ross *et al.*, 2004) and cardiovascular diseases (Brown and Jones, 2004). Therefore, preliminary studies were conducted to assess the effects of crude extracts, fractions and isolated compounds on bacterial growth and an anti-inflammatory mediator, nuclear factor-kappaB (NF- $\kappa$ B), *in vitro*. NF- $\kappa$ B is a transcriptional factor which regulates the genes of several pro-inflammatory chemicals, such as cytokines (IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ ), enzymes (COX-2, iNOS), adhesion molecules and in a self-regulatory way its inhibitory protein, I- $\kappa$ B (Bremner and Heinrich, 2002; Ross *et al.*, 2004). CYP3A4 inhibition tests, using testosterone 6 $\beta$ -hydroxylation as a probe for enzyme activity, were conducted in human liver microsomes, to determine the effect of herbal extracts on drug metabolising enzyme *in vitro*.

In this study, TCM material was extracted using solvents of varying polarity and the resulting extracts were tested in a range of bioassays. Preliminary studies were conducted using aqueous extracts since this extraction procedure reflects the preparation of decoctions in TCM. Prior to testing in the bioassays, the chemical profiles of the plant material, obtained from commercial companies, were compared with the chemical profiles of authentic and other reference material, obtained from the Chinese Medicinal Plant Authentication Centre (CMPAC), Royal Botanic Gardens, Kew.

Verification of the TCM material is essential to ensure that, when interpreting data regarding the chemistry and pharmacological activities of each species, the results refer to the correct species. This is of particular concern, as TCM material may be adulterated or deliberately substituted with other plant species. A TCM name for a herb may refer to more than one plant species, for example Chi Shao is prepared from the dried root of *Paeonia lactiflora* or *Paeonia veitchii*. Thus, when recommending potential alternatives to the animal material (bear bile, rhino horn, tiger bone), it is essential to ensure the correct species is being proposed as an alternative. This process involved comparison of the chemical profiles of the commercial material with verified TCM material (available from the collections held at CMPAC, Royal Botanic Gardens, Kew) using various chromatographic techniques. Some other concerns associated with the use of traditional herbal medicines (in particular TCM) are the safety of the remedies (Koh and Woh, 2000). Therefore, in addition, it was important to determine if the herbs contained high levels of pesticide residues (Appendix 2) and heavy metals (Appendix 3).

## 5.2. Materials

Many of the samples of the TCM material were kindly donated by Jo Liu of Mayway (UK) Ltd. (Hanwell, UK) and Paul Skipworth of Kingham Herbs and Tinctures (UK). Rhino horn was kindly donated by the CITES team (Special Operations District, Heathrow airport) and tiger bone (obtained from the rib of a male hybrid Amur tiger), was donated by the National Museums of Scotland. Ursodeoxycholic acid (UDCA) was purchased from Sigma chemicals (UK). Authentic and other reference (market) samples of TCM material, used to assist with authentication of the trade TCM material, were obtained from CMPAC, Royal Botanic Gardens, Kew.

## 5.3. Authentication techniques for trade TCM material

TCM material (trade samples and reference material) was ground using a pestle and mortar or grinding equipment, to provide sufficient material for authentication procedures and bioassays. Ground material was then extracted using aqueous 80% methanol, and as different types of compounds were targeted to assist with the authentication process, the ground material of some species was also extracted using other solvents (water, ethanol, dichloromethane or hexane). Following extraction, extracts were filtered, evaporated to dryness (aqueous extracts were freeze-dried) and reconstituted in the appropriate solvent, prior to HPLC and LC-MS analysis.

### 5.3.1. HPLC (UV-DAD) method

Analytical HPLC was carried out using a Waters LC600 pump and a 996 photodiode array detector. A Merck LiChrospher 100RP-18 (250 x 4.0 mm i.d. 5 µm particle size) column (maintained at 30°C) was used for analysis with a flow rate of 1 ml/min. The mobile phase consisted of 2% aqueous acetic acid (A) and methanol : acetic acid : water (18:1:1). Initial conditions were 75% A and 25% B; the proportion of B increased with a linear gradient, reaching 100% at t = 20 min. This was followed by an isocratic elution of 100% B until t = 25 min. Injections (30 µl) were made by an autosampler.

### 5.3.2. LC-MS method

LC-MS analysis was conducted at the Royal Botanic Gardens, Kew, by Dr G. Kite. Aqueous 80% methanol extracts were analysed using a Thermo-Finnigan LC/MS/MS system consisting of a 'Surveyor' autosampling LC system, interfaced to a 'LCQ Classic' quadrupole ion trap mass spectrometer.

Chromatographic separation of compounds was performed on a 250 mm x 4.6 mm i.d., 5 µm Supelco Discovery-C18 column using a 1 ml/min mobile phase gradient programmed from water (A), methanol (B) and methanol containing 5% acetic acid (C). The gradient programme (A:B:C) was 80:0:20 (t = 0 min), 0:80:20 (t = 20 min), 0:80:20 (t = 25 min), 80:0:20 (t = 27 min), 80:0:20 (t = 37 min). Data analysis was performed using Xcalibur 1.2 software (Thermo-Finnigan).

### 5.3.3. TD-GC-MS method

The TD-GC-MS system used consisted of a Perkin-Elmer ATD400 thermal desorption unit, a Perkin-Elmer AutoSystem XL GC and a Perkin-Elmer TurboMass MS (quadrupole). Chromatography was performed on a 30 m x 0.25 mm i.d. x 0.25 µm DB-5MS column (J. & W. Scientific, USA) using an oven program of 60–300°C at 6°C/min. The carrier gas was helium at a flow rate of 1 ml/min. The TCM material was analysed using desorption; the inlet split flow was 0 ml/min and the outlet split flow was 18.75 ml/min, the desorption flow was 60 ml/min, the desorption temperature was 150°C, the trap temperature was 4°C and the pressure was 14.6 psi. Detection was by mass-spectrometry; the MS was fitted with an EI source operated at 70eV with a source temperature of 180°C, and mass spectra were recorded in the range m/z 38–300. The software was Turbomass, version 4.1.1. Approximately 2 mg of dried TCM material was desorbed for each analysis. Compounds were identified by comparing mass spectra with published data (Ausloss *et al.*, 1992; Adams, 2001).

## 5.4. Methods for fractionation and isolation of compounds

### 5.4.1. Fractionations of *Scutellaria baicalensis* and isolation of compounds

Dried powdered root of *S. baicalensis* (35 g) was extracted in MeOH (800 ml) using a Soxhlet apparatus for 19 h (64°C). The extract was concentrated to about 300 ml and partitioned using hexane (100 ml). Column chromatography using normal phase silica gel (60A S-230/70 mesh, SL06SA4, YMC Co. Ltd) was conducted on the dried methanol layer (12 g). The mobile phase used was a step gradient elution of 10 combinations (each 500 ml) of CHCl<sub>3</sub>, Me<sub>2</sub>CO, MeOH and H<sub>2</sub>O in increasing polarity, starting with 100% CHCl<sub>3</sub> and ending with 95% MeOH in H<sub>2</sub>O. A total of 20 fractions were collected and concentrated separately to dryness. HPLC-(UV-DAD) analysis, anti-bacterial and anti-inflammatory (NF-κB) tests were conducted on the crude extracts of *S. baicalensis* and each of the 20 fractions. Fraction 4 (SB4) was selected for further fractionation based on the results obtained from NF-κB tests. Preparative TLC was adopted as a method to fractionate SB4 using a mobile phase of hexane: CHCl<sub>3</sub>: EtOAc (1:1:1). Nine fractions were obtained and tested in anti-bacterial and anti-inflammatory (NF-κB) assays. The fifth fraction of SB4 (SB4v) demonstrated potent NF-κB inhibition. Therefore SB4v was fractionated using preparative HPLC-UV-DAD and NMR spectroscopy was used to identify the compounds isolated. NMR data were acquired and interpreted at the Royal Botanic Gardens, Kew, by Dr N. Veitch.

### 5.4.2. Fractionation of *Salvia miltiorrhiza* and Qing Ying Tang

Dried powdered root of *S. miltiorrhiza* (72 g) was extracted in MeOH (800 ml) by using a Soxhlet apparatus for 17 h (64°C). The extract was concentrated to dryness and 11 g was subjected to silica column chromatography. A gradient elution of 8 combinations of CHCl<sub>3</sub>, MeOH and H<sub>2</sub>O in increasing polarity, starting with 100% CHCl<sub>3</sub> and ending with 85% MeOH in H<sub>2</sub>O, was employed. A total of 17 fractions were collected.

Lypophilised crude hot water extract of the TCM prescription, Qing Ying Tang (2.4 g) was fractionated using flash chromatography (reversed-phase). A stepwise gradient elution system was used starting with 5% MeOH in water and ending with 100% MeOH in volumes of 600 ml each. Twenty-four fractions were collected. Anti-bacterial tests were performed on the fractions obtained from which *S. miltiorrhiza* and Qing Ying Tang. The fractions which showed some anti-bacterial activity were tested in an anti-inflammatory (NF-κB) assay.

### 5.5. Method for anti-bacterial tests

Assays were conducted at the Royal Botanic Gardens, Kew with Dr T. Kokubun. The method for assessing anti-bacterial activity was a modification of that described by Rehalison *et al.* (1991). For this study, 20 µl aliquots of each herb extract (5 mg/ml) or fraction (1 mg/ml) were applied to three replicate TLC plates (20 x 10 cm<sup>2</sup>, pre-coated aluminium-backed silica gel, 60F<sub>254</sub> sheets, Merck, Germany). The TLC plates were developed in a tank containing one of the following solvent systems: chloroform : acetone (4:1) or (17:3), or chloroform : acetone : water (7:3:1). Developed plates were observed under UV light (254 nm and 355 nm). One TLC plate was subjected to chemical analysis and two were used to conduct anti-bacterial tests. The TLC plate for chemical analysis was sprayed evenly with *p*-anisaldehyde (0.5 ml in 50 ml HOAc and 1 ml conc. H<sub>2</sub>SO<sub>4</sub>); the chemical profile of the sprayed plate was examined under UV light (355 nm). After heating, plates were re-examined under UV light (355 nm).

Two of the developed TLC plates were fixed onto separate culture dishes and chloramphenicol (3 µg/ml; positive control) was applied to a solvent-free area on each TLC plate. A small colony of previously cultured bacteria (*Pseudomonas syringae*, ID No. IMI347448, CABI Bioscience, UK or *Bacillus subtilis*, ID No. IMI347329, CABI Bioscience, UK) was suspended in water and added to 50 ml nutrient agar solution to form a seeded medium. This medium was used as an overlay on the TLC plate to form bioautograms with a layer of approx 1 mm thickness of medium. The bioautographs were sealed and incubated overnight (37°C, 100 % relative humidity).

After incubation the bioautographs were stained with *p*-iodonitrotetradium violet (2-[4-indophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride, Sigma, USA) diluted in ethanol (0.5 mg/ml, diluted 10-fold immediately prior to use). The dishes were incubated (at 37°C) for a further two hours and then visually examined. Areas on the plate with inhibited or reduced bacterial growth appeared as white spots against a pink background. Areas of inhibition were compared to corresponding areas in the chemically treated plates to ascertain groups of compounds responsible for the inhibition of bacterial growth.

## 5.6. NF- $\kappa$ B studies using the IL-6 promoter assay method

Dr P. Bremner conducted the assays as part of the collaboration with Professor M. Heinrich, School of Pharmacy, London. The assay used was as described by Bork *et al.* (1999). HeLa S3 cells were stably transfected with IL-6 promoter fused with a Luciferase reporter gene for 24 hours. Extracts/fractions (final incubation concentration of 100  $\mu$ g/ml in DMSO) and compounds (prepared in acetone) were placed in 96-well plates and incubated with the transfected cells (at 37°C; 95% humidity) for 1 hour. The cells were then stimulated with 50 ng/ml (final concentration) of either phorbol myristate acetate (PMA, Sigma, UK) or TNF- $\alpha$  (Sigma, UK) and incubated for a minimum of 7 hours (maximum 14 hours) before harvesting. To each well, 100  $\mu$ l of lysis buffer (25 mM Tris-phosphate pH 7.8, 8 mM MgCl<sub>2</sub>, 1 mM DTT, 1% Triton X-100 and 7% glycerol) was added and left for 15 minutes. After harvesting the cells, beetle Luciferin (50  $\mu$ l, Promega, USA) was added to the lysed cells (15  $\mu$ l) in a 96-well plate by an automated Luminoter/photometer (Anthos Lucy 1, Rosys Anthos, Switzerland) and the light emission was measured following a reaction time of 10 seconds. The light emission of the lysis buffer was obtained as a background reading and subtracted from each experimental value. Positive controls consisted of cells stimulated with either PMA or TNF- $\alpha$  only and negative controls involved cells subjected to no stimulation.

## 5.7. Method for cytochrome P450 3A4 inhibition studies

A Tecan Genesis 150 RSP (Tecan UK Ltd, Reading, UK) was used to incubate the test solutions in 96-well plates using an automated set-up and timed procedures. The following sequence was performed, in duplicate, by the RSP. Ketoconazole (0.1, 0.3, 1, 3 and 10  $\mu$ M), herbal extracts and fractions (1000  $\mu$ g/ml in water), compounds (200  $\mu$ g/ml in 2.5% acetone) and negative controls (water or 2.5% acetone) were placed in 96-well plates.

Phosphate buffer (0.1 M; 245  $\mu$ l) was added to human liver microsomes containing testosterone (100 nM; 50  $\mu$ l). A test solution (50  $\mu$ l) was then added. The plates were pre-incubated for 2 minutes. NADPH regenerating mixture (155  $\mu$ l) was added to each tube (to give a final volume of 0.5 ml) and incubated at 37  $\pm$  2°C for 20 minutes. Phosphoric acid (0.15 M; 0.1 ml) was added to terminate the reactions.

Instrumental quality control samples were performed (for each 96-well plate) by preparing tubes containing the human liver microsomes (50  $\mu$ l) (with no added testosterone) and phosphate buffer (0.1 M; 245  $\mu$ l). After the 2 minutes pre-incubation, NADPH mixture (155  $\mu$ l) was added to each tube (to a final volume of 0.45 ml) and incubated as described above. On the termination of the reactions, by the addition of phosphoric acid, 6 $\beta$ -hydroxytestosterone (50  $\mu$ l; 10 and 100  $\mu$ M) was used to generate duplicate quality samples at two concentrations (1  $\mu$ M and 10  $\mu$ M). All the incubation tubes were centrifuged at 3000 rpm for 10 minutes at ambient temperature. Aliquots of each sample were placed in HPLC vials using the RSP.

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The incubation procedure described above resulted in a 10 times dilution of the test solutions. Therefore, the herbal extracts and compounds were tested at final concentrations of 100 µg/ml and 20 µg/ml, respectively. Baicalin (45 µM), baicalein (74 µM), scutellarein (69 µM) and chrysin (79 µM) were tested. The final concentrations for ketoconazole were 0.01, 0.03, 0.1, 0.3 and 1 µM.

### 5.7.1. CYP3A4 LC-MS method

Liquid chromatographic-mass spectrometric (LC-MS) analysis was conducted using a 1100 HPLC system (Agilent, Berks., UK) interfaced with mass spectrometer. The chromatographic determination of 6β-hydroxytestosterone was performed on a 150 mm x 4.6 mm i.d., 5 µm Luna Phenyl Hexyl column (Phenomenex, Cheshire, UK) with a Sentry 20 mm x 2.9 mm Symmetry C<sub>8</sub> guard cartridge (Waters, Mass., USA) at 25°C. A flow rate of 1.5 ml/min was used with mobile phase gradient programmed between ammonium acetate buffer (A) and acetonitrile (B). The gradient programme (A:B) was 81:19 (t = 0); 73:27 (t = 3 minutes); 10:90 (t = 10 min) and then back to the original mobile phase ratio of 81:19 at t = 11 minutes till t = 11.5 minutes. The injection volume was 50 µl. The MS detection was made in the positive ion mode, with Turbolonspray, 500°C, flow split 1:5. The nebuliser gas was set at 8 (arbitrary scale), auxiliary gas at 6 L/min, and the curtain gas was set at 10 L/min. The ionspray voltage was 4800 mV, orifice voltage (declustering potential) was 31 mV, ring voltage (focussing potential) was 180 mV and the Q0 voltage (entrance potential) was -5 mV. The ion was monitored at m/z = 305.6 with a scan time of 500 ms. Data were acquired for 10 minutes per sample. The approximate retention time for 6β-hydroxytestosterone was 8.5 minutes.

## 5.8. Statistical analysis

The values obtained for the NF-κB and CYP450 assays were expressed as mean values ± SD. The Student's 1-sample *t*-test was used to determine statistical differences between test and control groups. The difference was considered statistically significant when  $p < 0.05$ .

## Section 6

# Results and discussion

## 6.1. Bear bile: bioassay results and discussion

Results of the authentication study indicate that at least five of the seven TCM herbs (Table 6.1.) showed similar chemical profiles to the reference and authentic material, thus indicating they were the correct plant species, as described in TCM.

**Table 6.1. TCM herbs for which the plant species were identified**

Chuan Xin Lian, aerial part of <i>Andrographis paniculata</i> (CXL)
Zhi Mu, rhizome of <i>Anemarrhena asphodeloides</i> (ZM)
Zhi Zi, fruit of <i>Gardenia jasminoides</i> (ZZ)
Huang Qin, root of <i>Scutellaria baicalensis</i> (SB)
Huang Bai, cortex of <i>Phellodendron amurense</i> (HB)
TCM herbs for which the plant species were not verified
Huang Lian, Rhizoma <i>Coptidis</i> (HL)*
Da Huang, Radix et Rhizoma <i>Rhei</i> (DH)*

### 6.1.1. Anti-bacterial tests

The seven herbs described in Table 6.1., as well as prescription X were tested for their effect on Gram-positive and Gram-negative bacteria. Some components from ethyl acetate extracts (100 µg) of the following six herbs, separated on TLC plates, showed inhibitory action against the growth of *Bacillus subtilis* (Gram-positive bacteria): *Anemarrhena asphodeloides*, *Gardenia jasminoides*, *Scutellaria baicalensis*, *Phellodendron amurense*, *Coptis chinensis* and *Rheum palmatum*. In addition, some fractions from the ethyl acetate extract (100 µg) of *Rheum palmatum* and fractions from the methanol extract (20 µg) of *Scutellaria baicalensis* also inhibited the growth of *Pseudomonas syringae* (Gram-positive bacteria). Out of the seven herbs tested only *Andrographis paniculata* showed no inhibitory effect against either *Bacillus subtilis* or *Pseudomonas syringae* at 100 µg. However, some TCM literature cites studies conducted in China, which have shown that *Andrographis paniculata* inhibits several Gram-negative and Gram-positive bacteria in vitro, but the concentrations tested were not stated (Hsu et al., 1986; Chang and But, 1987; Huang, 1999). Prescription X (100 µg) did not inhibit either *Bacillus subtilis* or *Pseudomonas syringae* at the concentration tested.

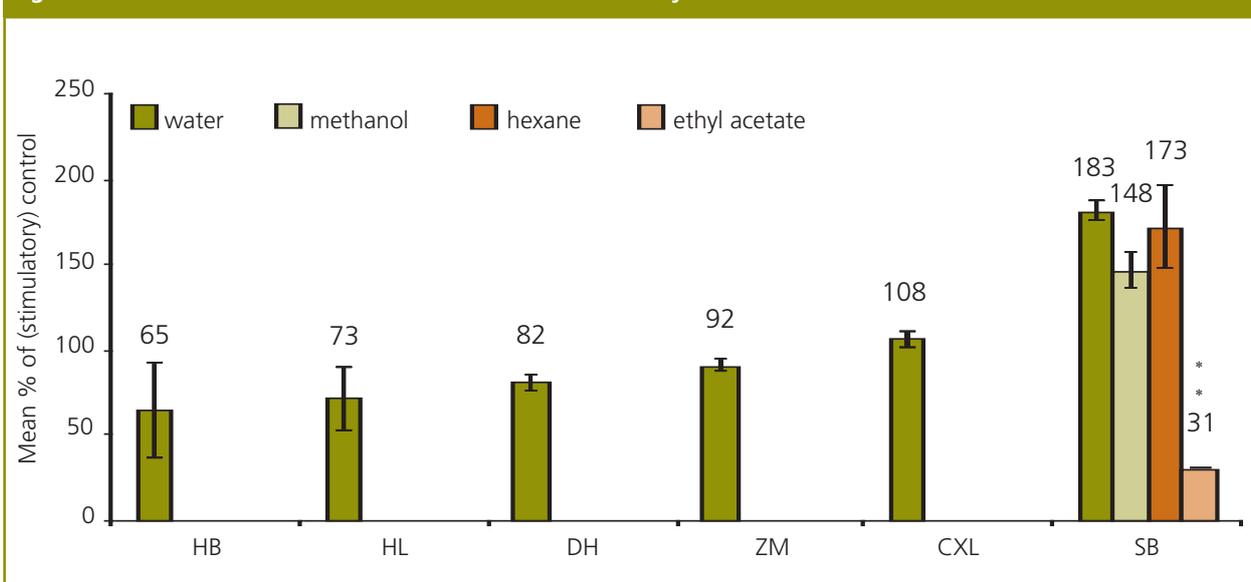
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### 6.1.2. Anti-inflammatory (NF- $\kappa$ B) tests

Herbal extracts (100  $\mu$ g/ml) were tested in either TNF- $\alpha$  or PMA-stimulated HeLa cells using an IL-6 promoter method to determine their effect on NF- $\kappa$ B activity; results are shown in Fig. 6.1. Water extracts of *Rheum palmatum* (DH) showed significant ( $p < 0.05$ ) inhibition of NF- $\kappa$ B activity. Water extracts of *Coptis chinensis* (HL), *Phellodendron amurense* (HB) and *Anemarrhena asphodeloides* (ZM) reduced NF- $\kappa$ B activity; however, these reductions were not statistically significant. A water extract of *Andrographis paniculata* (CXL) did not affect NF- $\kappa$ B production. Also, prescription X reduced Luciferase values by 16%, but this was not statistically significant (data not shown).

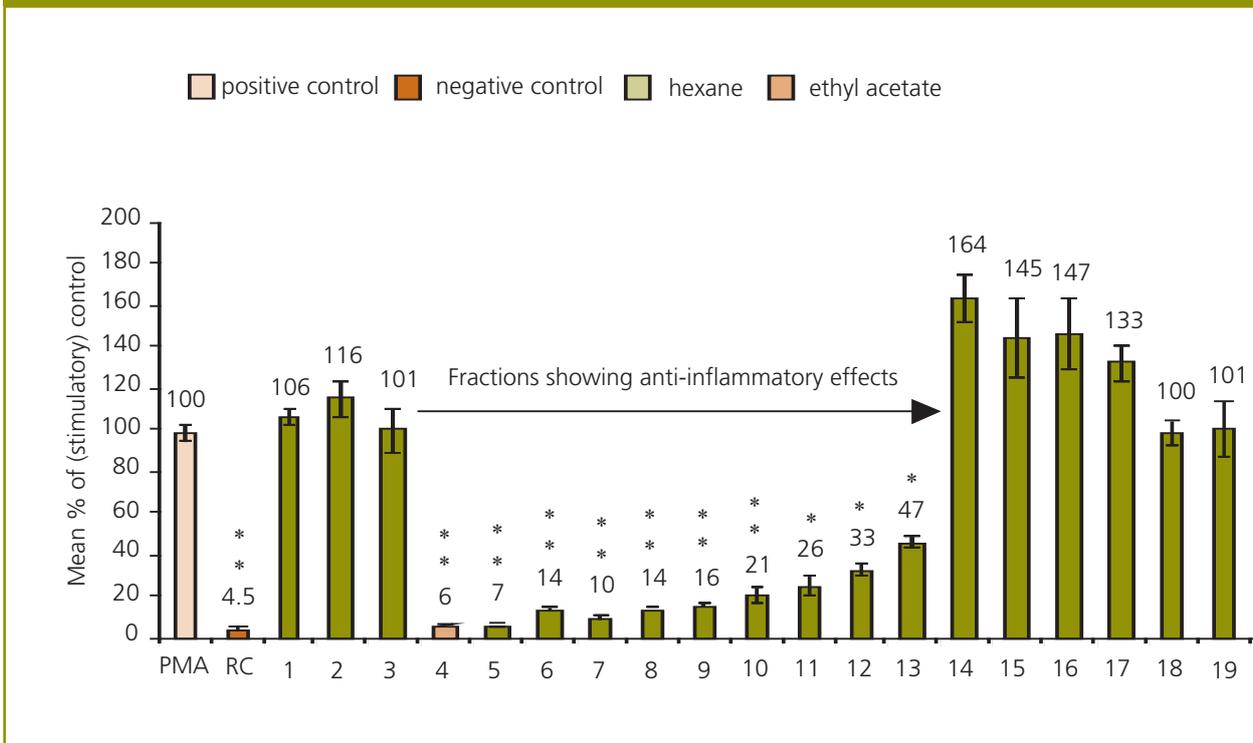
Although potent inhibition of NF- $\kappa$ B activity was measured for the ethyl acetate extract of *Scutellaria baicalensis* (69%,  $p < 0.001$ ), stimulation was obtained for the corresponding water, methanol (SBM) and hexane (SBH) extracts (Fig. 6.1.). The effects of fractions (SB1 – SB20; obtained from methanol extract) of *S. baicalensis* on NF- $\kappa$ B activity are shown in Fig 6.2.

Fig. 6.1. The effects of six herbal extracts on NF- $\kappa$ B activity



Cells for water extracts (100  $\mu$ g/ml) were stimulated with phorbol myristate acetate (PMA; 50 ng/ml) and the ethyl acetate extract (100  $\mu$ g/ml) with TNF- $\alpha$  (50  $\mu$ g/ml). Induced IL-6 promoter activity was measured as light emission (Luciferase values) expressed as a percentage relative to cells stimulated by PMA or TNF- $\alpha$  only. The data represent mean ( $n = 3$ )  $\pm$  SD. \* $p < 0.05$  and \*\* $p < 0.001$  indicate statistically significant differences from cells treated with PMA or TNF- $\alpha$  only.

Codes for herbs presented in Fig. 6.1: HB: Huang bai, cortex of *Phellodendron amurense*; HL: Huang lian, Rhizoma Coptidis; DH: Da Huang, Radix et Rhizoma Rhei; ZM: Zhi Mu, rhizome of *Anemarrhena asphodeloides*; CXL: Chuan Xin Lian, aerial part of *Andrographis paniculata*; SB: Huang Qin, root of *Scutellaria baicalensis*.

Fig. 6.2. The effects of fractions obtained from methanol extract of *Scutellaria baicalensis* on NF- $\kappa$ B activity

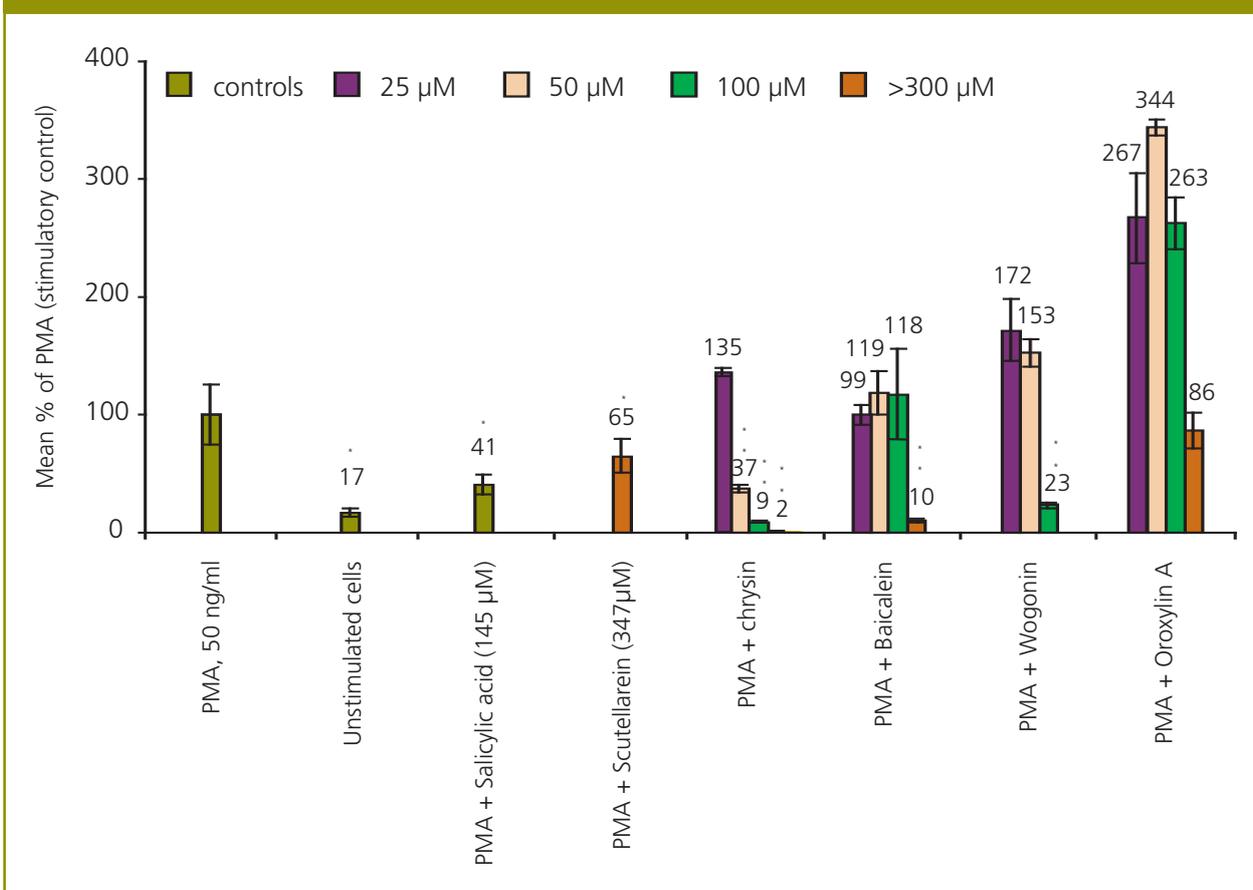
Extracts (100  $\mu$ g/ml) were tested in PMA-stimulated cells. IL-6 gene promoter activity was measured as outlined in the legend for Fig. 6.1. Negative controls: resting cells (RC; unstimulated); positive controls: PMA stimulated cells. The data represent mean ( $n = 9$  for controls and  $n = 3$  for test samples)  $\pm$  SD. \* $p = 0.001$ , \*\* $p < 0.001$  indicate statistically significant differences from PMA-stimulated cells. Colour code: yellow = positive control (stimulatory); red = resting cells (inhibitory); purple = other fractions obtained from fractionating the crude extract; orange = fraction further fractionated. Fraction 4v (obtained from fractionating fraction 4) = 3%,  $p < 0.001$ .

Fractions SB4-SB13 demonstrated significant inhibition of NF- $\kappa$ B activity ( $p < 0.001$ ), in contrast to the stimulatory effects of some fractions (SB14-SB17) and the crude methanolic extract (SBM). Three compounds, chrysin, wogonin and oroxylin A, were isolated and identified from SB4 using TLC, HPLC (UV-DAD) and NMR. These three flavonoids were also identified from their characteristic UV profiles, as present in active fractions SB4 to SB12 from HPLC-(UV-DAD) analysis. When the constituents of *S. baicalensis* (chrysin, wogonin, oroxylin A, baicalein, baicalin and scutellarein) were tested, the NF- $\kappa$ B inhibitory and stimulatory activities of some of these compounds were found to be dependent on their concentrations (Fig. 6.3). Salicylic acid, an anti-inflammatory compound, which inhibits COX activity, was used as a further control in this assay. Chrysin inhibited NF- $\kappa$ B activity in a dose-dependant manner (at 50–393  $\mu$ M,  $p < 0.001$ ), showing more inhibitory activity than salicylic acid (145  $\mu$ M,  $p < 0.01$ ; Fig 6.3). Baicalein and wogonin demonstrated a significant reduction in NF- $\kappa$ B activity, only at 370 and 100  $\mu$ M, respectively. However, chrysin and baicalein had some associated cytotoxicity at 393  $\mu$ M and 370  $\mu$ M, respectively.

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At lower concentrations chrysin (25  $\mu\text{M}$ ) and wogonin (25 and 50  $\mu\text{M}$ ) demonstrated significant stimulation of NF- $\kappa\text{B}$  activity (Fig. 6.3.). Oroxylin A also stimulated NF- $\kappa\text{B}$  activity at 25–100  $\mu\text{M}$  ( $p < 0.05$ ) but showed no significant effect at 352  $\mu\text{M}$ . However, oroxylin A (70  $\mu\text{M}$ ) has been reported to inhibit LPS-induced NF- $\kappa\text{B}$  activity in RAW264.7 macrophages via the inhibition of NF- $\kappa\text{B}$  complex (Chen *et al.*, 2000). The anti-inflammatory pathway tested in the current study was via the inhibition of NF- $\kappa\text{B}$  activity (as assessed using IL-6 promoter assay). Recently, baicalein (24, 48 and 96  $\mu\text{M}$ ) has been reported to potently inhibit IL-12 production in LPS-activated macrophages via the inhibition of NF- $\kappa\text{B}$  binding activity (Kang *et al.*, 2003a).

**Fig. 6.3. The effects of flavonoids from *Scutellaria baicalensis*, and salicylic acid on NF- $\kappa\text{B}$  activity.**



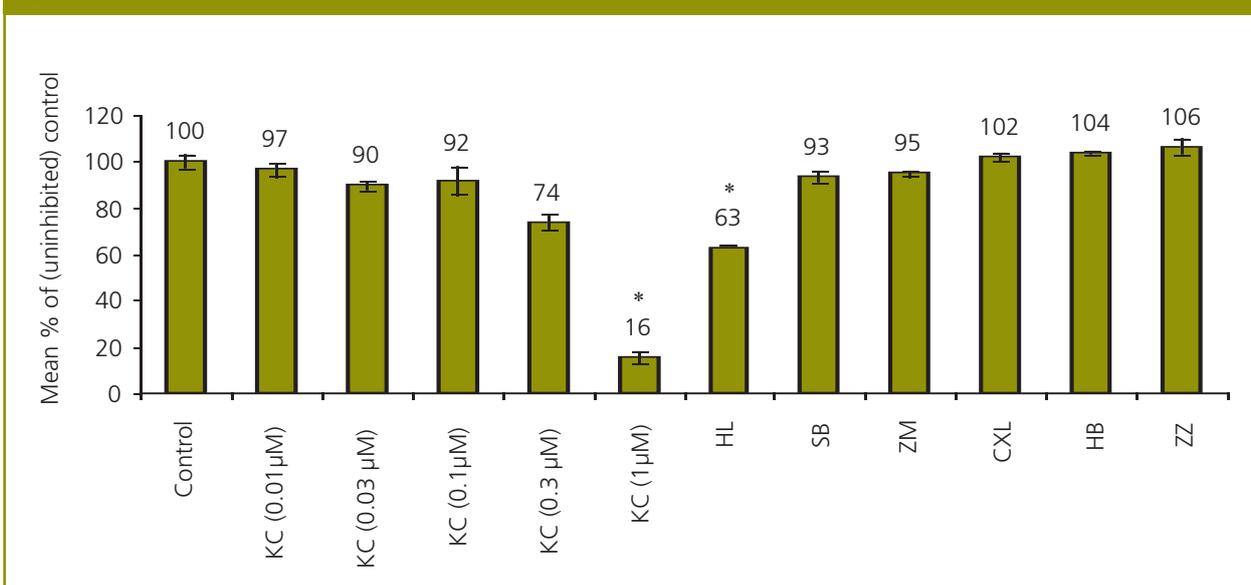
Compounds were tested in PMA-stimulated cells; IL-6 promoter activity was measured as outlined in the legend for Fig. 6.1. The data represent mean ( $n = 8$  for PMA,  $n = 9$  for resting cells and  $n = 3$  for test compounds)  $\pm$  SD.

\* $p < 0.01$ , \*\* $p < 0.001$  indicate statistically significant differences from PMA-stimulated cells. Scutellarein (5,6,7,4'-tetrahydroxyflavone); baicalin (5,6-dihydroxy-7-glucuronide); chrysin (5,7-dihydroxyflavone); baicalein (5,6,7-trihydroxyflavone); wogonin (5,7-dihydroxy-8-methoxyflavone); oroxylin A (5,7-dihydroxy-6-methoxyflavone).

### 6.1.3. Cytochrome P450 3A4 tests

Results for the CYP3A4 inhibition assay, using testosterone 6 $\beta$ -hydroxylation as a probe for enzyme activity in human liver microsomes, are presented in Figs 6.4 and 6.5. Crude hot water extracts of *Coptis chinensis* (100  $\mu$ g/ml) significantly reduced CYP3A4 activity by 37% ( $p < 0.01$ ) compared to that of the negative (uninhibited) control (Fig. 6.4). Water extracts of *Scutellaria baicalensis*, *Anemarrhena asphodeloides*, *Andrographis paniculata*, *Phellodendron amurense* and *Gardenia jasminoides* showed no significant effect CYP3A4 activity at 100  $\mu$ g/ml (Fig. 6.4).

Fig. 6.4. The effects of six TCM herbs and ketoconazole on CYP3A4 activity.

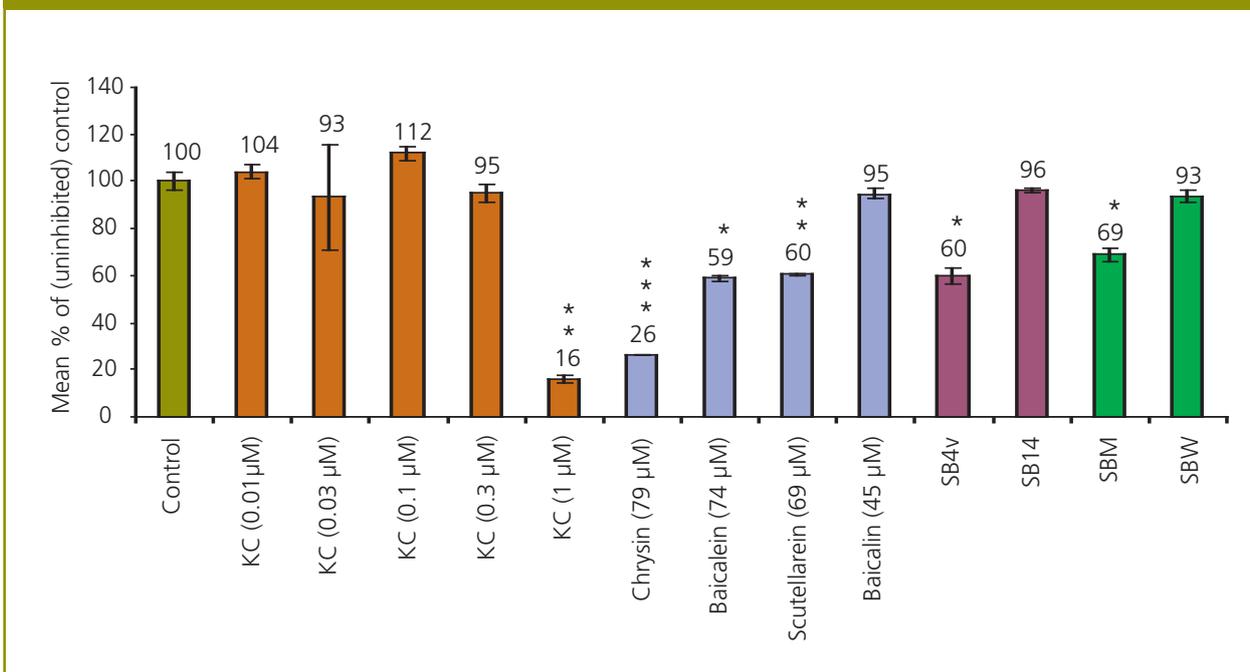


The assay was conducted using testosterone 6 $\beta$ -hydroxylation as a probe for enzyme activity in human liver microsomes. Results were calculated as mean % of uninhibited controls (water); ketoconazole (KC) was used as positive control. Data represent mean ( $n = 2$ )  $\pm$  SD. \* $p < 0.01$  indicate statistically significant differences from groups only treated with water.

Codes for herbs presented in Fig. 6.4: HL: Huang lian, *Coptis chinensis*; Huang qin, root of *Scutellaria baicalensis*; ZM: Zhi Mu, rhizome of *Anemarrhena asphodeloides*; CXL: Chuan Xin Lian, aerial part of *Andrographis paniculata*; SB: HB: Huang Bai, cortex of *Phellodendron amurense*; ZZ: Zhi Zi, fruit of *Gardenia jasminoides*.

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**Fig. 6.5.** The effects of fractions and flavonoid compounds of *Scutellaria baicalensis* (SB) and ketoconazole on CYP3A4 activity.



The assay was conducted using testosterone 6 $\beta$ -hydroxylation as a probe for enzyme activity in human liver microsomes. Results were calculated as mean % of uninhibited controls (water); ketoconazole (KC) was used as positive control. Data represent mean (n = 2) + SD. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 indicate statistically significant differences from groups only treated with water. Colour codes: red = unstimulated controls (water or 2.5% acetone); yellow = ketoconazole; blue = known compounds of *S. baicalensis*; plum = fractions obtained from fractionating *S. baicalensis*; green = crude extracts of *S. baicalensis*.

However, a methanolic extract of *Scutellaria baicalensis* (SBM), a fraction (SB4v) and two constituent flavonoids, baicalein (74  $\mu$ M) and scutellarein (69  $\mu$ M), from *Scutellaria baicalensis* all inhibited CYP3A4 activity by 30–40 % (p<0.05) compared to the negative (uninhibited) control (Fig. 6.5). Chrysin (79  $\mu$ M), showed greater inhibition by lowering CYP3A4 activity to 74% (p<0.001) compared to the negative (uninhibited) control (Fig. 6.5). The IC<sub>50</sub> value obtained for the positive control, ketoconazole in the CYP3A4 studies was 0.6  $\mu$ M, which was high, compared to reported values of 0.1  $\mu$ M (McKillop *et al.*, 1999) and 0.04 mM (Sai *et al.*, 2000). The high positive control value observed may indicate that moderate inhibition might not have been detected with some of the test extracts at the concentration tested.

#### 6.1.4. Conclusions

Results from this study have shown that *Anemarrhena asphodeloides*, *Gardenia jasminoides*, *Scutellaria baicalensis*, *Phellodendron amurense*, Rhizoma Coptidis and Radix et Rhizoma Rhei all possess some anti-bacterial activity. Also, results from this study have further confirmed the anti-inflammatory properties of Radix et Rhizoma Rhei and *Scutellaria baicalensis* through the inhibition NF- $\kappa$ B activity. The results from both the anti-bacterial and anti-inflammatory tests have highlighted the herbs which may be investigated further through bioactivity guided fractionations.

Preliminary results from the CYP3A4 studies suggest that possible herb-herb interactions may occur in preparations containing both Rhizoma Coptidis and *Scutellaria baicalensis* (such as Dia-Orengedokuto and Orengedokuto). Also, drug-herb interactions may occur when herbal preparations containing Rhizoma Coptidis and/or *Scutellaria baicalensis* are co-administered with some pharmaceutical drugs, which are metabolised by CYP3A4. However, further work is required to investigate the extent of these effects.

## 6.2. Rhino horn: bioassay results and discussion

Twenty-two herbs that could be potential substitutes for rhino horn were assayed for activity (Table 6.2). The extracts of the 22 herbs were chemically profiled along with authenticated TCM material. At least 13 of these herbs showed similar chemical profiles to the authenticated TCM samples, thus indicating they were the correct plant species, as described in TCM. Further work is necessary on 9 of the plant species to confirm their identification.

### 6.2.1. Anti-bacterial tests

Results for anti-bacterial activity of extracts (100  $\mu$ g) of rhino horn and TCM prescriptions are summarised in Table 6.3. In addition, 24 fractions were obtained from flash chromatography of water extracts of Qing Ying Tang; fractions QYT9, QYT15, QYT16 and QYT17 showed some inhibitory activity against *Bacillus subtilis* but did not inhibit *Pseudomonas syringae*. The anti-bacterial tests were qualitative, so although some prescriptions with and without rhino horn demonstrated anti-bacterial activity, the contribution of the horn extracts in the prescriptions could not be evaluated. However, rhino horn alone did not inhibit the growth of either *Bacillus subtilis* or *Pseudomonas syringae*.

Crude ethyl acetate extracts (100  $\mu$ g) of Radix et Rhizoma Rhei and fractions from methanolic extracts (20  $\mu$ g) of *Salvia miltiorrhiza*, *Scutellaria baicalensis* and *Lonicera japonica* showed anti-bacterial activity against both *Bacillus subtilis* and *Pseudomonas syringae*. Ethyl acetate extracts (100  $\mu$ g) of 17 herbs showed some inhibitory activity against *Bacillus subtilis* and are listed in Table 6.2 (samples 4–20).

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**Table 6.2. TCM herbs investigated in biological assays**

Abbreviation	TCM herbs
MDP	Mu Dan Pi, root of <i>Paeonia suffruticosa</i>
JG	Jie Geng, root of <i>Platycodon grandiflorum</i>
GC	Gan Cao, root of <i>Glycyrrhiza uralensis</i>
XS	Xuan Shen, root of <i>Scrophularia ningpoensis</i>
SDH	Sheng Di Huang, root of <i>Rehmannia glutinosa</i>
JYH	Jin Yin Hua, flower bud of <i>Lonicera japonica</i>
LQ	Lian Qiao, fruit of <i>Forsythia suspensa</i>
DS	Dan Shen, root of <i>Salvia miltiorrhiza</i>
DZY	Dan Zhu Ye, aerial part of <i>Lophatherum gracile</i>
ZM (B)	Zhi Mu, rhizome of <i>Anemarrhena asphodeloides</i>
ZZ (B)	Zhi Zi, fruit of <i>Gardenia jasminoides</i>
HQ (B)	Huang Qin, root of <i>Scutellaria baicalensis</i>
HB (B)	Huang Bai, cortex of <i>Phellodendron amurense</i>
Abbreviation	TCM herbs
HL (B)	Huang Lian, Rhizoma Coptidis
DH (B)	Da Huang, Radix et Rhizoma Rhei
ZC	Zi Cao, Radix Arnebiae
BLG	Ban Lan Gen, Radix Isatidis
MMD	Mai Men Dong, Ophiopogonis Radix
DDC	Dan Dou Chi, Semen Sojae Praeparatum
CP	Chang Pu, Rhizoma Acori Graminei
CSY	Chi Shao, Radix Paeoniae Rubra
THF	Tian Hua Fen, Radix Trichosanthis

(B) Results have also been presented in the bear bile project (section 6.1).

TCM pharmaceutical names are used in the text for herbs that require further investigation to assist with their authentication.

**Table 6.3. Anti-bacterial activity of rhino horn and TCM prescriptions**

Rhino horn and TCM prescriptions	Anti-bacterial tests	
	<i>B. subtilis</i>	<i>P. syringae</i>
Rhino horn	nd	nd
Qing Ying Tang plus rhino horn (QYT + RH)	Inhibition	nd
Qing Ying Tang without rhino horn (QYT)	Inhibition	nd
Sheng Xi Dan plus rhino horn (SXD+RH)	Inhibition	Inhibition
Sheng Xi Dan without rhino horn (SXD)	Inhibition	Inhibition
Qing Gong Tang plus rhino horn (QGT+RH)	Inhibition	nd
Qing Gong Tang without rhino horn (QGT)	Inhibition	Inhibition
Qingwen Baidu Yin plus rhino horn (QWBY + RH)	nd	nd
Qingwen Baidu Yin without rhino horn (QWBY)	nd	nd
Xi Jiao Dihuang Tang plus rhino horn (XJDHT + RH)	nd	nd
Xi Jiao Dihuang Tang without rhino horn (XJDHT)	nd	nd
Zhi Zi Jin Hua (ZZJH)	nd	nd

nd: no inhibition detected

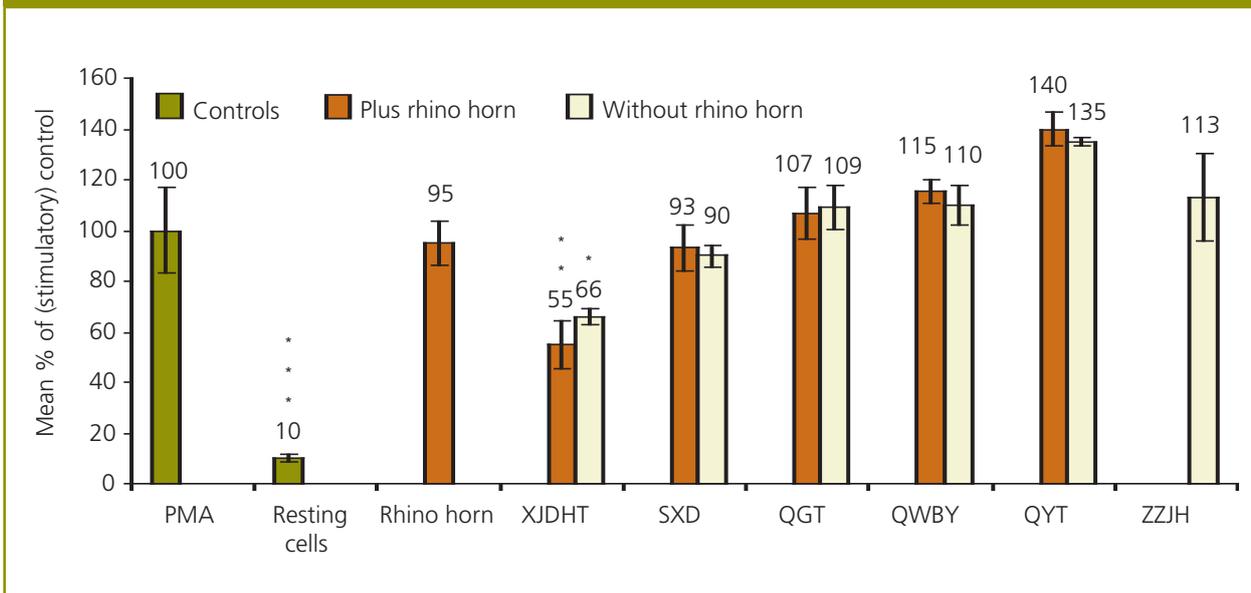
### 6.2.2. Anti-inflammatory (NF- $\kappa$ B) tests

Water extracts (100  $\mu$ g/ml) of five TCM prescriptions containing rhino horn and five without rhino horn, rhino horn alone, as well as Zhi Zi Jin Hua (composed only of herbs) were tested in PMA-stimulated HeLa cells using IL-6 promoter assay (Fig 6.6). Only the prescriptions Xi Jiao Dihuang Tang without rhino horn (XJDHT) and Xi Jiao Dihuang tang with rhino horn (XJDHT+RH) showed significant inhibitory effect on NF- $\kappa$ B activity (Fig 6.6.). XJDHT+RH and XJDHT reduced NF- $\kappa$ B activity by 45 % ( $p < 0.01$ ) and 34 % ( $p < 0.05$ ), respectively, compared to fully stimulated cells by PMA, indicating that rhino horn might contribute to the inhibitory effect. However, since rhino horn extract alone did not show any apparent effect on the NF- $\kappa$ B activity and results from the other prescriptions were not conclusive, further work is required to clarify the contribution of the horn extract and whether there is a synergistic effect.

The TCM prescription Qing Ying Tang (QYT) demonstrated stimulatory effect on NF- $\kappa$ B activity (Fig. 6.6.). However, when fractions obtained from QYT (QYT9, QYT15, QYT16 and QYT17), that showed anti-bacterial activity were tested in the NF- $\kappa$ B assay they demonstrated significant inhibition of NF- $\kappa$ B activity (Fig. 6.7(A)).

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Fig. 6.6. The effects of rhino horn extract, TCM prescriptions (with and without rhino horn) on NF- $\kappa$ B activity.

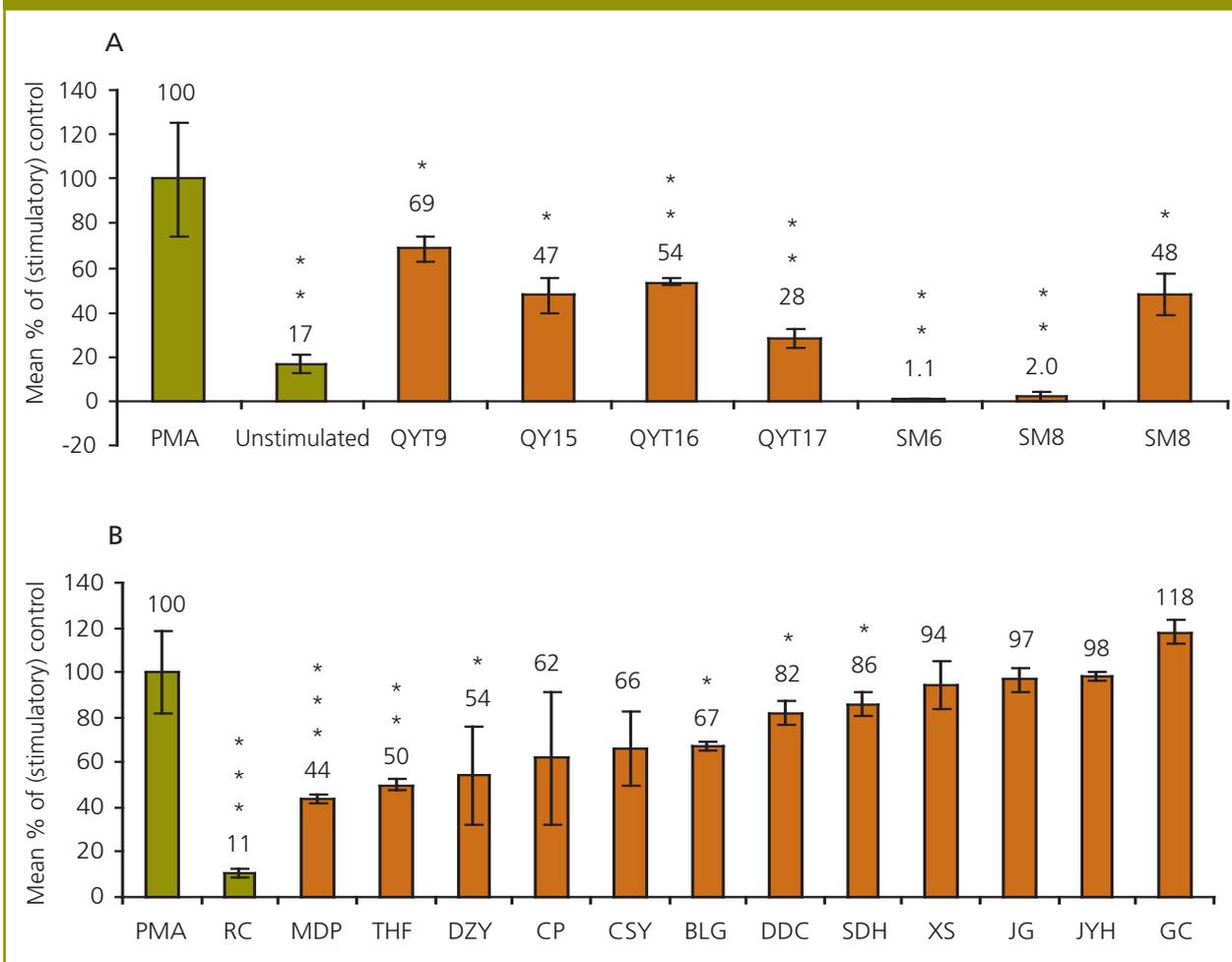


Water extracts (100  $\mu$ g/ml) were tested in PMA-stimulated cells as outlined in Fig. 6.1. Resting cells were used as negative controls; cells stimulated with PMA alone were used as positive controls.

The data represent mean ( $n = 5$  for controls and  $n = 3$  for test samples)  $\pm$  SD. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  indicate statistically significant differences from groups only treated with PMA. The abbreviations for the prescriptions are described in Table 6.3.

*Salvia miltiorrhiza* is one of eight herbs making up the QYT prescription. Fractions SM6 and SM7 from the methanolic extract of *Salvia miltiorrhiza* potentially inhibited NF- $\kappa$ B activity to below levels obtained for resting cells, and SM8 reduced NF- $\kappa$ B activity to 52% of that of PMA (fully stimulated cells) (Fig. 6.7.(A)).

Crude hot water extracts of some of the 16 herbs (found in some of the TCM prescriptions investigated in this study) were also tested in PMA-stimulated HeLa cells in NF- $\kappa$ B tests and the results are presented in Fig. 6.7.(B). *Paeonia suffruticosa* and *Radix Trichosanthis* significantly reduced NF- $\kappa$ B activity by about 50% of that obtained by cells fully stimulated with PMA. Other herbs showing statistically significant reduction in NF- $\kappa$ B activity were *Lophatherum gracile*, *Radix Isatidis*, *Rhizoma Coptidis*, *Semen Sojae Praeparatum* and *Rehmannia glutinosa* (Fig 6.7.(B)).

Fig. 6.7. The effects of TCM remedies on NF- $\kappa$ B activity.

(A) The effects of fractions (SM6, SM7 and SM8) of a methanol extract of *Salvia miltiorrhiza* (SM) and water extract of Qing Ying Tang (QYT).

(B) The effects of 12 TCM herbs (full names are described in Table 6.2).

Extracts (100  $\mu$ g/ml) were tested in PMA-stimulated cells as outlined in Fig. 6.1. Resting cells (RC) were used as negative controls; cells stimulated with PMA alone were used as positive controls. The induced IL-6 promoter activity was measured as light emission (Luciferase values) and expressed as a percentage relative to cells stimulated with PMA alone. The data represent mean ( $n = 5$  for controls and  $n = 3$  for test samples)  $\pm$  SD. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  indicate statistically significant differences from groups only treated with PMA.

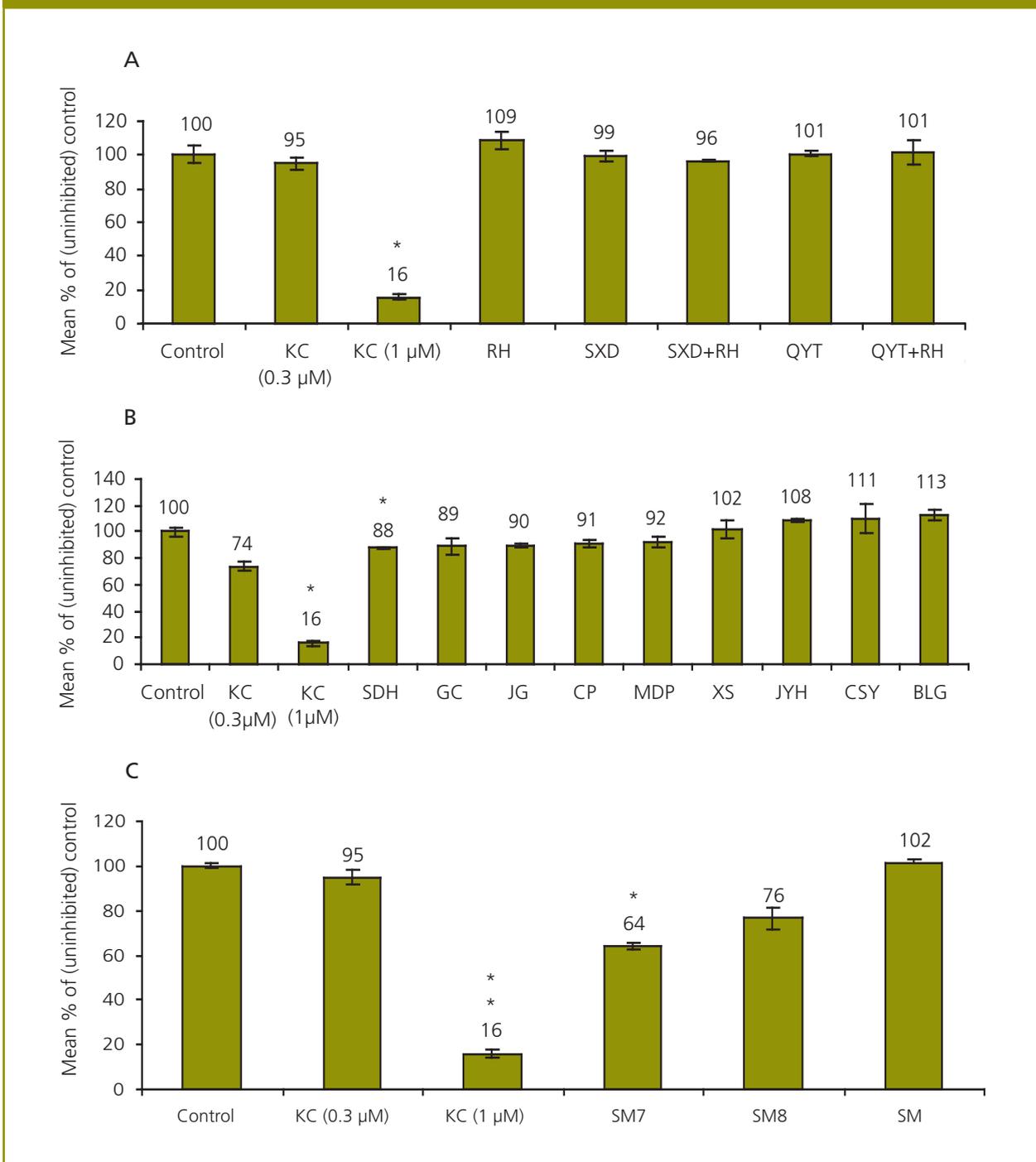
### 6.2.3. Cytochrome P450 3A4 tests

Water extracts (100  $\mu$ g/ml) of rhino horn and TCM prescriptions Sheng Xi Dan and Qing Ying Tang (with and without rhino horn) showed no apparent significant effect on  $6\beta$ -testosterone hydroxylation due to inhibition of CYP3A4 activity (Fig. 6.8. (A)).

The IC<sub>50</sub> value obtained for the positive control, ketoconazole, in these studies was 0.5  $\mu$ M. Water extracts (100  $\mu$ g/ml) of 14 herbs were also tested and only Rhizoma Coptidis and *Rehmannia glutinosa* showed any significant effect on CYP3A4 activity compared to the control (containing no inhibitor) (Fig. 6.8.(B)). Fraction SM7 obtained from a methanolic extract of *Salvia miltiorrhiza* also demonstrated inhibitory activity against CYP3A4 activity compared to the control (Fig. 6.8. (C)). The inhibitory effects of some *Scutellaria* constituents are discussed under the bear bile project (section 6.1.).

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Fig. 6.8. The effects of TCM remedies and ketoconazole on microsomal CYP3A4 activity.



(A) Rhino horn (RH) and Shen Xi Dan (SXD) and Qing Ying Tang (GYT) with and without RH.

(B) Nine TCM herbs (full names are described in Table 6.2.).

(C) Fractions (SM7 & SM8) of a methanol extract of *Salvia miltiorrhiza* (SM).

The CYP3A4 inhibition assay was conducted using testosterone 6 $\beta$ -hydroxylation as a probe for enzyme activity in human liver microsomes. Results were calculated as mean percent of uninhibited control (water); ketoconazole (KC) was used as positive control. The data represent mean (n = 2)  $\pm$  SD. \* $p$ <0.05, \*\* $p$ <0.01 indicate statistically significant differences from groups only treated with vehicle solution.

#### 6.2.4. Conclusions

Water extracts of rhino horn did not demonstrate anti-bacterial or anti-inflammatory properties, nor did they have any effect on the drug metabolising enzyme, CYP3A4. However, some TCM prescriptions with and without rhino horn showed some anti-bacterial and anti-inflammatory properties in the assays used in this study. However, further work using other bioassays is required to ascertain the contribution of the horn extracts to any activities of the TCM prescriptions.

Most of the herbs (17 out of 22) showed some anti-bacterial activity against *Bacillus subtilis*, indicating potential pharmacological effects. Also, results from this study indicate potential anti-inflammatory properties of four out of the nine herbs selected to study as possible alternatives to rhino horn: *Paeonia suffruticosa*, Radix Isatidis, *Rehmannia glutinosa* and *Salvia miltiorrhiza*. In addition Radix Trichosanthis, Semen Sojae Praeparatum, Rhizoma Coptidis and *Lophatherum gracile*, found in different prescriptions also showed anti-inflammatory properties. To date no scientific literature in the English language has been obtained for the anti-inflammatory effects of *Lophatherum gracile* and therefore further studies are required to verify the data obtained from this study. The TCM prescriptions Xi Jiao Dihuang Tang and Qing Ying Tang also showed a significant anti-inflammatory effect through the inhibition of NF- $\kappa$ B activity.

*Salvia miltiorrhiza*, *Rehmannia glutinosa*, as well as *Scutellaria baicalensis* and *Rhizoma Coptidis*, showed inhibitory effects on CYP3A4 during preliminary studies. Since they are commonly used TCM herbs, further work may be required to determine potential adverse interactions with other remedies.

### 6.3. Tiger bone: bioassay results and discussion

The extracts of the twenty-three herbs and one fungus were chemically profiled along with authenticated TCM material, using various analytical techniques (HPLC (UV-DAD), LC-MS and GC-MS). At least 10 of the species showed similar chemical profiles to the authenticated TCM samples, thus indicating they were the correct species, as described in TCM. Further analysis of the remaining TCM material is necessary to confirm their identification. Eighteen of the herbs were investigated in biological assays for the tiger bone project (Table 6.4.).

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**Table 6.4. TCM samples studied in biological assays**

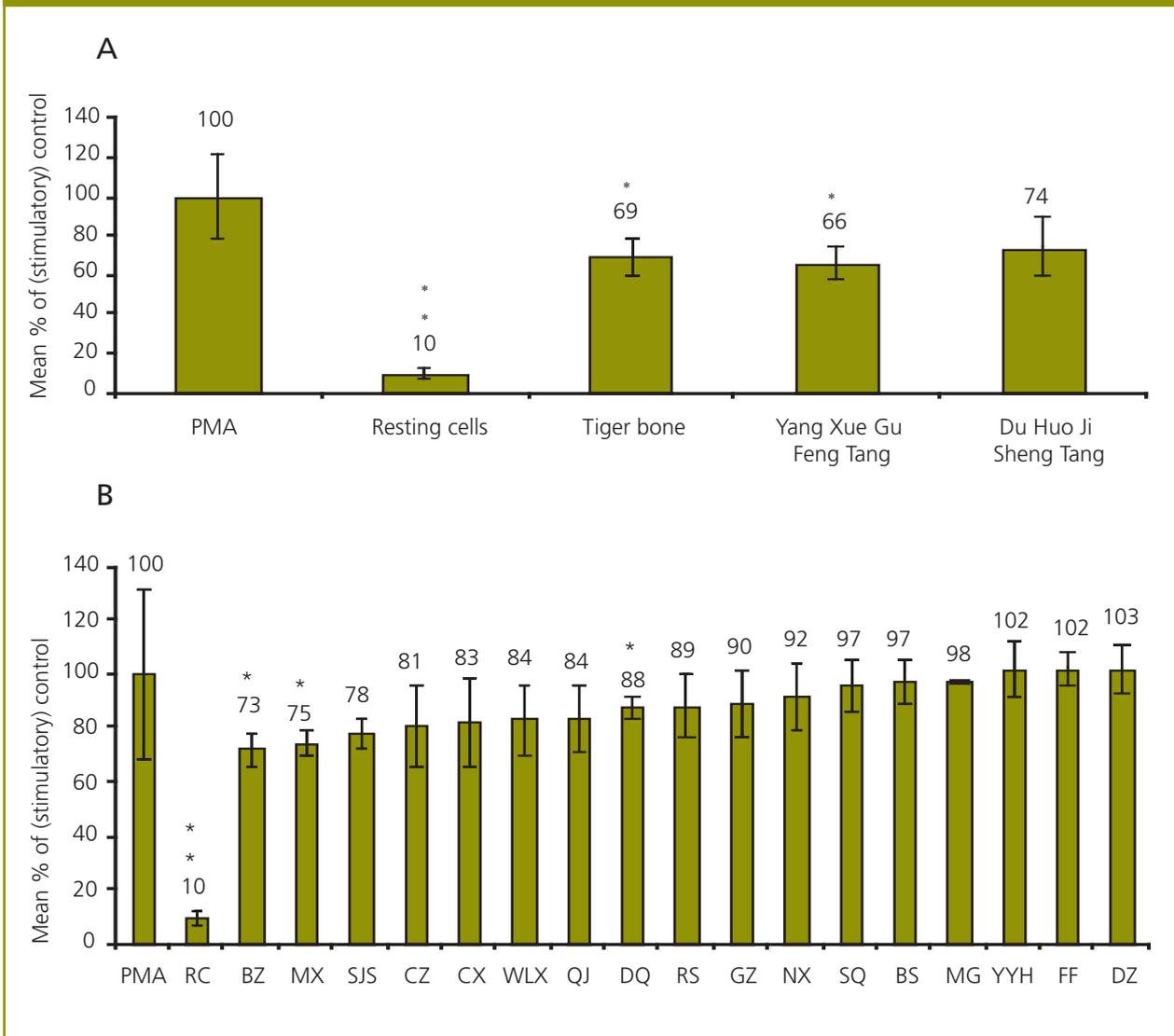
	TCM samples studied in biological assays
Tiger bone	<i>Panthera tigris</i>
DH(b)	Du Huo, root of <i>Angelica pubescens</i>
WLX (a)	Wei Lin Xian, root of <i>Clematis chinensis</i>
BS (a)	Bai Shao, root of <i>Paeonia lactiflora</i>
YYH (a)	Ying Yang Huo, aerial part of <i>Epimedium sagittatum</i>
FF (a)	Fang Feng, root of <i>Saposhnikovia divaricata</i>
DZ (a)	Du Zhong, bark of <i>Eucommia ulmoides</i>
MX	Mu Xiang, root of <i>Saussurea costus</i>
CZ	Cang Zhu, root of <i>Atractylodes lancea</i>
RS	Ren Shen, root of <i>Panax ginseng</i>
SQ	San Qi, root of <i>Panax pseudoginseng</i>
BZ	Bai Zhu, roots of <i>Atractylodes macrocephala</i>
SJS	Sang Ji Sheng, stem and branch of <i>Taxillus chinensis</i>
CX	Chuan Xiong, rhizome of <i>Ligusticum chuanxiong</i>
QJ	Qin Jiao, leaf of <i>Gentiana macrophylla</i>
DQ	Dang Gui, root of <i>Angelica sinensis</i>
GZ	Gui Zhi, twigs of <i>Cinnamomum cassia</i>
NX	Niu Xi, root of <i>Achyranthes bidentata</i>
MG	Mu Gua, fruit of <i>Chaenomeles speciosa</i>

(a) Herbs tested in NF- $\kappa$ B assay only; (b) Herbs tested CYP3A4 assay only

### 6.3.1. Anti-inflammatory (NF- $\kappa$ B) tests

Crude hot water extracts (100  $\mu$ g/ml) of tiger bone and the prescription Yang Xue Gu Feng Tang (without tiger bone) reduced NF- $\kappa$ B activity (via the inhibition of IL-6 promoter activity) by 31% and 34%, respectively ( $p < 0.05$ ), in PMA-stimulated HeLa cells (Fig. 6.9(A)). However, the reduction of NF- $\kappa$ B activity (26%) by the prescription, Du Huo Ji Sheng Tang (without tiger bone) was not statistically significant (Fig. 6.9(A)). Results for crude hot water extracts (100  $\mu$ g/ml) of 17 herbs are shown in Fig 6.9(B). Four of these herbs produced a statistically significant reduction in NF- $\kappa$ B activity: *Atractylodes macrocephala* (Bai Zhu), *Saussurea costus* (Mu Xiang), *Taxillus chinensis* (Sang Ji Sheng) and *Angelica sinensis* (Dang Qui).

**Fig. 6.9. The effects of TCM prescriptions, tiger bone and remedies traditionally found in tiger bone prescriptions, on NF-κB activity.**



(A) The effects of tiger bone and two TCM prescriptions on NF-κB activity.

(B) The effects of 17 herbs on NF-κB activity (full names are described in Table 6.4.).

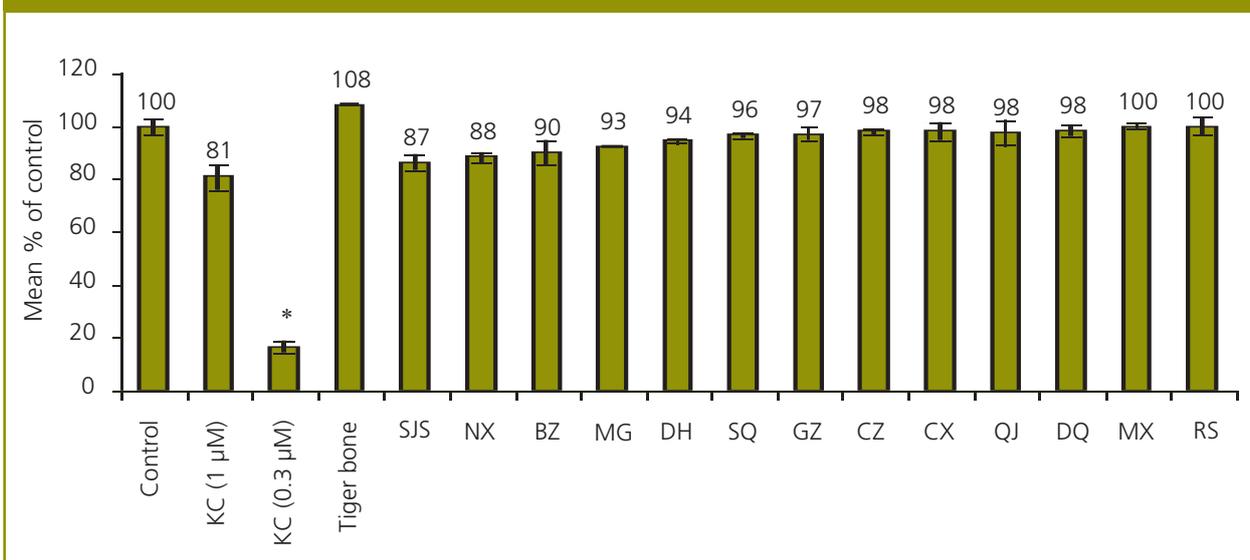
Extracts (100 µg/ml) were tested in PMA-stimulated cells as outlined in Fig. 6.1. The full names of the herbs are as described in Table 6.4. Resting cells (RC; unstimulated) were used as negative controls; cells stimulated with PMA were used as positive controls. The induced IL-6 promoter activity was measured as light emission (Luciferase values) and expressed as a percentage relative to cells stimulated by PMA only. The data represent mean (n = 7 for controls and n = 3 for test samples) + SD. \**p*<0.05 and \*\**p*<0.01 indicate statistically significant differences from groups only treated with PMA.

Although, the inhibitory effects of the herbs were not potent at the concentrations tested, the results have highlighted the herbs that could be investigated further, using other assays to assess potential pharmacological mechanisms that could be related to anti-inflammatory activity.

### 6.3.2. Cytochrome P450 3A4 tests

Crude hot water extracts (100 µg/ml) of tiger bone and thirteen individual herbs were tested for their inhibitory potential on the metabolising enzyme CYP3A4. Results for the CYP3A4 inhibition assay using testosterone 6β-hydroxylation as a probe for enzyme activity in human liver microsomes is presented in Fig. 6.10. None of the extracts tested showed any apparent significant effect on 6β-testosterone hydroxylation due to inhibition of CYP3A4 activity. These results suggest that tiger bone and the herbs tested may not be modulators of CYP3A4. However, there has been some reported evidence to suggest that some TCM herbs such as *Angelica dahurica* is an inhibitor of CYP3A4, which was demonstrated by the longer pre-incubation time of the microsomal assay (Guo *et al.*, 2001). Also, the IC<sub>50</sub> value obtained for the positive control, ketoconazole in this study was 0.5 µM, which was high compared to reported values of 0.1 µM (McKillop *et al.*, 1999) and 0.04 mM (Sai *et al.*, 2000); and therefore moderate inhibitors may not have been detected.

Fig. 6.10. The effects of water extracts of tiger bone and 13 TCM herbs on CYP3A4 activity.



The CYP3A4 inhibition assay was conducted using testosterone 6β-hydroxylation as a probe for enzyme activity in human liver microsomes. Results were calculated as mean % of uninhibited control (water); ketoconazole (KC) was used as positive control of inhibition. The data represent mean (n = 2) ± SD. \*p<0.01 indicate statistically significant differences from groups only treated with water. The full names of the herbs are described in Table 6.4.

### 6.3.3. Conclusions

Tiger bone may possess some anti-inflammatory properties, through the inhibition of IL-6 promoter activity. However, further tests are required to confirm this activity. The following 3 herbs (not restricted by CITES, 2004) contained in some existing TCM prescriptions that included tiger bone, showed some potential anti-inflammatory activity: *Atractylodes macrocephala* (Bai Zhu), *Taxillus chinensis* (Sang Ji Sheng), and *Angelica sinensis* (Dang Qui). Although the inhibitory effects of the herbs were not potent at the concentrations tested, the results have highlighted the herbs which could be investigated further. None of the herbs investigated had any effect on CYP3A4; since TCM remedies are sometimes co-administered with pharmaceutical drugs, there is a need for further investigation to determine if the herbs affect different CYP enzymes by different mechanisms.

## Conclusions

### 7.1. General conclusions

Following consultation with TCM practitioners to identify potential TCM prescriptions and herbs, consideration of the TCM uses of the plants and their reported pharmacological activities in the literature were used as criteria to select the herbs that could have potential as alternatives to bear bile, rhino horn and tiger bone. Most of the suggested herbal 'alternatives' to the animal products are listed as ingredients in one or more TCM prescriptions which traditionally contained the animal products. This finding confirmed the practice in TCM of combining remedies with similar functions for their additive and synergistic effects. Following authentication studies, many of the individual TCM herbs and prescriptions were tested for anti-inflammatory and anti-bacterial activity and for their effects on the enzyme CYP3A4. As a result of the bioassays and with consideration of the traditional uses of the herbs, a number have been selected as potential alternatives to the animal products.

### 7.2. Herbs selected as potential alternatives to animal products

#### 7.2.1. Herbs proposed as potential alternatives to bear bile used in TCM

The following seven species are proposed as potential alternatives to bear bile based on evidence from published TCM and other scientific literature, as well as results from anti-inflammatory and anti-bacterial tests conducted in this study. Of these seven species, five were also highlighted in the IFAW report (1994); Appendix 1 of this report. The five herbs are Zhi Zi, Huang Qin, Huang Bai, Da Huang and Chuan Xin Lian.

1. *Gardenia jasminoides* Ellis (syn.: *G. augusta* Merr.) (Rubiaceae) fruit = Zhi Zi
2. *Scutellaria baicalensis* Georgi (Lamiaceae) root = Huang Qin
3. *Coptis chinensis* Franch. (Ranunculaceae) rhizome = Huang Lian
4. *Phellodendron amurense* Rupr (Rutaceae) bark = Huang Bai
5. *Rheum palmatum* L. (Polygonaceae) root and rhizome = Da Huang
6. *Anemarrhena asphodeloides* Bge. (Anthericaceae) rhizome = Zhi Mu
7. *Andrographis paniculata* Nees (Acanthaceae) aerial parts = Chuan Xin Lian

### 7.2.2. Prescriptions proposed as potential alternatives to bear bile used in TCM

Two Kampo medicines are proposed as potential alternatives to bear bile based on published TCM and other scientific literature.

Orengedokuto is composed of herbs (1) – (4) listed in Section 7.2.1.

Dia-Orengedokuto is composed of herbs (1) – (5) in Section 7.2.1.

### 7.2.3. Herbs proposed as potential alternatives to rhino horn used in TCM

The following species are proposed as potential alternatives to rhino horn based on evidence from published TCM and other scientific literature, as well as results from anti-inflammatory and / or anti-bacterial tests conducted in this study.

1. *Scrophularia ningpoensis* Hemsl. (Scrophulariaceae) root = Xuan Shen
2. *Rehmannia glutinosa* Steud (Scrophulariaceae) root = Sheng Di Huang
3. *Paeonia suffruticosa* Andr. (Paeoniaceae) root = Mu Dan Pi
4. *Paeonia veitchii* Lynch or *P. lactiflora* Pall. (Paeoniaceae) root = Chi Shao
5. *Arnebia euchroma* I.M.Johnst. (Boraginaceae) root = Zi Cao
6. *Isatis indigotica* (Brassicaceae) root = Ban Lan Gen
7. *Lonicera japonica* Thunb. (Caprifoliaceae) flower bud = Jin Yin Hua
8. *Forsythia suspensa* Vahl (Oleaceae) fruit = Lian Qiao
9. *Salvia miltiorrhiza* Bge (Lamiaceae) root = Dan Shen

#### 7.2.4. Herbs proposed as potential alternatives to tiger bone used in TCM

The following species are proposed as potential alternatives to tiger bone. Herb selection was based on evidence from published TCM and other scientific literature. Further evidence for the potential anti-inflammatory effects of herbs (10) to (12) was obtained from the present study.

1. *Saposhnikovia divaricata* (Turcz.) Schischk. (Apiaceae) root = Fang Feng
2. *Clematis chinensis* Osb. (Ranunculaceae) root and rhizome = Wei Ling Xian
3. *Angelica pubescens* Maxim. (Apiaceae) root = Du Huo
4. *Ligusticum chuanxiong* Hort. (Apiaceae) rhizome = Chuan Xiong
5. *Gentiana macrophylla* Pall. (Gentianaceae) root = Qin Jiao
6. *Epimedium sagittatum* Maxim. (Berberidaceae) aerial parts = Yin Yang Huo
7. *Atractylodes lancea* (Thunb.) DC. or *A. chinensis* (D.C.) Koidz. (Asteraceae) rhizome = Cang Zhu
8. *Cinnamomum cassia* D.Don (Lauraceae) bark = Rou Gui
9. *Morus alba* L. (Moraceae) young branches = Sang Zhi
10. *Angelica sinensis* (Oliv.) Diels (Apiaceae) root = Dang Gui
11. *Taxillus chinensis* (DC.) Danser (Loranthaceae) stem and branch = Sang Ji Sheng
12. *Atractylodes macrocephala* (Asteraceae) rhizome = Bai Zhu
13. *Spatholobus suberectus* Dunn. (Fabaceae) root and stem = Ji Xue Teng
14. *Chaenomeles speciosa* (Sweet) Nakai (Rosaceae) fruit = Mu Gua
15. *Cinnamomum cassia* (Lauraceae) twigs = Gui Zhi

### **7.3. Future work**

Further research should be undertaken regarding the potential anti-inflammatory activity of the species described above (Section 7.1–7.3). Further studies are therefore warranted to assess other pharmacological mechanisms, using other bioassay systems, through which the plants may mediate their anti-inflammatory effects, in addition to further consultation with TCM practitioners.

The results from this study will be disseminated via scientific publications and in the more popular TCM literature.

The results from this study should also be discussed with TCM practitioners to determine whether the selected plant species would be considered for use in TCM as substitutes to bear bile, rhino horn and tiger bone.

# References

- Adams, R.P. (2001). *Identification of Essential Oil Components by Gas Chromatography / Quadrupole Mass Spectroscopy*. Allured Publishing Corporation, Illinois, USA.
- Amroyan, E., Gabrielian, E., Panossian, A., Wikman, G. & Wagner, H. (1999). Inhibitory effect of andrographolide from *Andrographis paniculata* on PAF-induced platelet aggregation. *Phytomedicine* 6(1): 27–31.
- Ausloos, P., Clifton, C., Lias, S.G., Shamim, A, & Stein, S. (1992). *NIST/EPA/NIH Mass Spectral Database (v. 4.0)*. US. Department of Commerce, Gaitherburg, USA.
- Batkhuu, J., Hattori, K., Takano, F., Fushiya, S., Oshiman, K. & Fujimiya, Y. (2002) Suppression of NO production in activated macrophages *in vitro* and *ex vivo* by neoandrographolide isolated from *Andrographis paniculata*. *Biol Pharm Bull.* 25(9): 1169–74.
- Bensky, D. & Barolet, R. (1990). *Chinese Herbal Medicine: Formulas and strategies*. Eastland Press.
- Bensky, D. & Gamble, A. (1993). *Chinese Herbal Medicine Materia Medica*. Eastland Press.
- Bermejo Benito, P., Diaz Lanza, A.M., Silvan Sen, A.M., De Santos Galindez, J., Fernandez Matellano, L., Sanz Gomez, A. & Abad Martinez, M.J. (2000). Effects of some iridoids from plant origin on arachidonic acid metabolism in cellular systems. *Planta Med.* 66(4): 324–8.
- Björkhem, I., Andersson, U., Sudjama-Sugiaman, E., Eggertsen, G. & Hylemon, P. (1993). Studies on the link between HMG-CoA reductase and cholesterol 7 alpha-hydroxylase in lymph-fistula rats: evidence for both transcriptional and post-transcriptional mechanisms for down-regulation of the two enzymes by bile acids. *J Lipid Res.* 34(9): 1497–503.
- Bork, P.M., Bacher, S., Schmitz, M.L., Kaspers, U. & Heinrich, M. (1999). Hypericin as a non-antioxidant inhibitor of NF-kappa B. *Planta Med.* 65(4): 297–300.
- Breedveld, F.C. & Dayer, J.M. (2000). Leflunomide: mode of action in the treatment of rheumatoid arthritis. *Ann. Rheum. Disease.* 59 (11): 841–849.
- Bremner, P. & Heinrich, M. (2002) Natural products as targeted modulators of the nuclear factor-kappaB pathway. *J Pharm Pharmacol.* 54(4): 453–72.
- British Pharmacopoeia (2000). The Stationery Office Books. ISBN 011322320X.
- Brody, T. (1994). *Nutritional Biochemistry*. United Kingdom: Academic Press Inc.
- Brown, M.A. & Jones, W.K. (2004). NF-kappaB action in sepsis: the innate immune system and the heart. *Front Biosci.* 9:1201–17.
- But, P.P.H., Lung, L.C. & Tam, Y.K. (1990) Antipyretic effects of rhinoceros horn and other animal horns. *J Ethnopharmacol.* 30: 157–168.

## References

- But, P.P.H. & Tam, Y.K. (1991). Ethnopharmacology of rhinoceros horn. II: Antipyretic effects of prescriptions containing rhinoceros horn or water buffalo horn. *J Ethnopharmacol.* 33: 45–50.
- Caceres, D.D., Hancke, J.L., Burgos, R.A., Sandberg, F. & Wikman, G.K. (1999). Use of visual analogue scale measurements (VAS) to assess the effectiveness of standardized *Andrographis paniculata* extract SHA-10 in reducing the symptoms of common cold. A randomised double blind-placebo study. *Phytomedicine* 6(4): 217–222.
- Chang, H.M. & But, P.P.H. (1986). *Pharmacology and application of Chinese Materia Medica* (Vol. 1). The Chinese University of Hong Kong.
- Chang, H.M. & But, P.P.H. (1987). *Pharmacology and application of Chinese Materia Medica* (Vol. 2). The Chinese University of Hong Kong.
- Chang, H.M. & But, P.P.H. (2001). *Pharmacology and Applications of Chinese Materia Medica* (Volumes. I & 2). World Scientific, London.
- Chang, H.W., Baek, S.H., Chung, K.W., Son, K.H., Kim, H.P. & Kang, S.S. (1994). Inactivation of phospholipase A2 by naturally occurring biflavonoid, ochnaflavone. *Biochem Biophys Res Commun.* 205(1): 843–9.
- Chang, W.C. & Hsu, F.L. (1992). Inhibition of platelet activation and endothelial cell injury by polyphenolic compounds isolated from *Lonicera japonica* Thunb. *Prostaglandins Leukot Essent Fatty Acids* 45(4): 307–312.
- Chang, W.C., Lin, Y.L., Lee, M.J., Shioh, S.J. & Wang, C.J. (1996). Inhibitory effect of crocetin on benzo(a)pyrene genotoxicity and neoplastic transformation in C3H10T1/2 cells. *Anticancer Res.* 16(6B): 3603–8.
- Chen, K.M., Ge, B.F., Ma, H.P. & Zheng, R.L. (2004). The serum of rats administered flavonoid extract from *Epimedium sagittatum* but not the extract itself enhances the development of rat calvarial osteoblast-like cells in vitro. *Pharmazie.* 59(1): 61–64.
- Chen, Y.C., Shen, S.C., Chen, L.G., Lee, T.J. & Yang, L.L. (2001). Wogonin, baicalin, and baicalein inhibition of inducible nitric oxide synthase and cyclooxygenase-2 gene expressions induced by nitric oxide synthase inhibitors and lipopolysaccharide. *Biochem Pharmacol.* 61(11): 1417–27.
- Chen, Y.F., Tsai, H.Y. & Wu, T.S. (1995). Anti-inflammatory and analgesic activities from roots of *Angelica pubescens*. *Planta Med.* 61: 2–8.
- Chen, Q. & Wei, W. (2003). Effects and mechanisms of glucosides of *Chaenomeles speciosa* on collagen-induced arthritis in rats. *Int. Immunopharmacol.* 3(4): 593–608.
- Chen, Y., Yang, L. & Lee, T.J. (2000). Oroxylin A inhibition of lipopolysaccharide induced iNOS and COX-2 gene expression via suppression of nuclear factor-kappaB activation. *Biochem Pharmacol.* 59(11): 1445–1457.

- Chiou, W.F., Chen, C.F. & Lin, J.J. (2000). Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells by andrographolide. *Br J Pharmacol.* 129(8):1553–60.
- Chiu, H.F., Lin, C.C., Yang, C.C. & Yang, F. (1989). The pharmacological and pathological studies on several hepatic protective crude drugs from Taiwan (II). *Am J Chin Med.* 17(1–2): 17–23.
- Cho, J.Y., Baik, K.U., Jung, J.H. & Park, M.H. (2000). *In vitro* anti-inflammatory effects of cynaropicrin, a sesquiterpene lactone, from *Saussurea lappa*. *Eur. J. Pharmacol.* 398(3): 399–407.
- Cho, J.Y., Kim, A.R., Joo, H.G., Kim, B.H., Rhee, M.H., Yoo, E.S., Katz, D.R., Chain, B.M. & Jung, J.H. (2004). Cynaropicrin, a sesquiterpene lactone, as a new strong regulator of CD29 and CD98 functions. *Biochem. Biophys. Res. Commun.* 313(4): 954–961.
- Chuan, I.C., Chen, K.S., Huang, Y.L., Lee, P.N. & Lin, T.H. (2000). Determination of trace elements in some natural drugs by atomic absorption spectrometry. *Biol Trace Elem Res* 76: 235–244.
- Chung, K.O., Kim, B.Y., Lee, M.H., Kim, Y.R., Chung, H.Y., Park, J.H. & Moon, J.O. (2003). *In vitro* and *in vivo* anti-inflammatory effect of oxyresveratrol from *Morus alba* L. *J. Pharm. Pharmacol.* 55(12): 1695–1700.
- CITES (1997). The Convention on International Trade in Endangered Species of Wild Fauna and Flora. Resolutions of the Conference of the Parties. Conf. 10.19 (Rev. CoP12). <http://www.cites.org/eng/cop/10/E10-Res.pdf>. Last accessed 19 February 2004.
- CITES (2004) The Convention on International Trade in Endangered Species of Wild Fauna and Flora, Appendices I, II and III. <http://www.cites.org/eng/app/appendices.pdf> Last accessed 21 March 2004.
- Corbiere, C., Liagre, B., Bianchi, A., Bordji, K., Dauca, M., Netter, P. & Beneytout, J.L. (2003). Different contribution of apoptosis to the antiproliferative effects of diosgenin and other plant steroids, hecogenin and tigogenin, on human 1547 osteosarcoma cells. *Int J Oncol.* 22(4): 899–905.
- Cuellar M.J., Giner R.M., Recio M.C., Just M.J., Manez S., Rios J.L. (1996) Two fungal lanostane derivatives as phospholipase A<sub>2</sub> inhibitors. *J. Nat. Prod.* 59 (10): 977–979.
- Cuellar, M.J., Giner, R.M., Recio, M.C., Manez, S. & Rios, J.L. (2001). Topical anti-inflammatory activity of some Asian medicinal plants used in dermatological disorders. *Fitoterapia.* 72(3): 221–9.
- Dai, Y., Miki, K., Fukuoka, T., Tokunaga, A., Tachibana, T., Kondo, E. & Noguchi, K. (2000). Suppression of neuropeptides' mRNA expression by herbal medicines in a rat model of peripheral inflammation. *Life Sci.* 66(1): 19–29.

## References

- Dai, M., Wei, W., Shen, Y.X. & Zheng, Y.Q. (2003). Glucosides of *Chaenomeles speciosa* remit rat adjuvant arthritis by inhibiting synoviocyte activities. *Acta Pharm. Sin.* 24 (11): 1161–1166.
- Danz, H., Stoyanova, S., Thomet, O.A., Simon, H.U., Dannhardt, G., Ulbrich, H. & Hamburger, M. (2002). Inhibitory activity of tryptanthrin on prostaglandin and leukotriene synthesis. *Planta Med.* 68(10): 875–80.
- Ding, H.Y., Lin, H.C., Teng, C.M., Wu, Y.C. (2000). Phytochemical and pharmacological studies on Chinese Paeonia species. *J Chin Chem Soc-TAIP* 47: 381–388.
- Duke, J.A. & Ayensu, E.S. (1985). *Medicinal Plants of China*. Volumes I – II. 2nd Edition. Reference Publications, Inc., Algonac, USA.
- Elkayam, O., Yaron, I., Shirazi, I., Judovitch, R., Caspi, D. & Yaron, M. (2003). Active leflunomide metabolite inhibits interleukin-1 $\beta$ , tumour necrosis factor- $\alpha$ , nitric oxide, and metalloproteinase-3 production in activated human synovial tissue cultures. *Ann. Rheum. Dis.* 62(5): 440–443.
- Espinoza, E.O., Shafer, B.S. & Hagey, L.R. (1993). International trade in bear gall bladders: Forensic source inference. *J Forensic Sci.* JFSCA 38(6): 1363–1371.
- Food Regulations (1992). S.I. 1992 No. 496 (ISBN 0 11 023496).
- Fotsing, M.T., Yankep, E., Njamen, D., Fomum, Z.T., Nyasse, B., Bodo, B., Recio, M.C., Giner, R.M. & Rios, J.L. (2003). Identification of an anti-inflammatory principle from the stem bark of *Millettia versicolor*. *Planta Med.* 69(8): 767–770.
- Fukuda, K., Hibiya, Y., Mutoh, M., Koshiji, M., Akao, S. & Fujiwara, H. (1999). Inhibition by berberine of cyclooxygenase-2 transcriptional activity in human colon cancer cells. *J Ethnopharmacol.* 66(2): 227–33.
- Fukutake, M., Miura, N., Yamamoto, M., Fukuda, K., Iijima, O., Ishikawa, H., Kubo, M., Okada, M., Komatsu, Y., Sasaki, H., Wakabayashi, K., Ishige, A. & Amagaya, S. (2000). Suppressive effect of the herbal medicine Oren-gedoku-to on cyclooxygenase-2 activity and azoxymethane-induced aberrant crypt foci development in rats. *Cancer Lett.* 157(1): 9–14.
- Fukutake, M., Yokota, S., Kawamura, H., Iizuka, A., Amagaya, S., Fukuda, K. & Komatsu, Y. (1998). Inhibitory effect of Coptidis Rhizoma and Scutellariae Radix on azoxymethane-induced aberrant crypt foci formation in rat colon. *Biol Pharm Bull.* 21(8): 814–7.
- Giner, E.M., Manez, S., Recio, M.C., Giner, R.M., Cerda-Nicolas, M. & Rios, J.L. (2000). *In vivo* studies on the anti-inflammatory activity of pachymic and dehydrotumulosic acids. *Planta Med.* 66(3): 221–227.
- Giner-Larza, E.M., Manez, S., Giner-Pons, R.M., Recio, M.C. & Rios, J.L. (2000). On the anti-inflammatory and anti-phospholipase A(2) activity of extracts from lanostane-rich species. *J. Ethnopharmacol.* 73(1–2): 61–69.

- Gokhale, A.B., Damre, A.S., Kulkarni, K.R. & Saraf, M.N. (2002). Preliminary evaluation of anti-inflammatory and anti-arthritic activity of *S. lappa*, *A. speciosa* and *A. aspera*. *Phytomedicine*. 9(5): 433–437.
- Goto, H., Shimada, Y., Tanaka, N., Tanigawa, K., Itoh, T. & Terasawa, K. (1999). Effect of extract prepared from the roots of *Paeonia lactiflora* on endothelium-dependent relaxation and antioxidant enzyme activity in rats administered high-fat diet. *Phytother Res*. 13(6): 526–8.
- Guillaume, G. & Chieu, M. (1996). *Rheumatology in Chinese medicine*. USA: Eastland Press Inc.
- Guo, Z., Zhao, H. & Fu, L. (1996). Protective effects of API0134 on myocardial ischemia and reperfusion injury. *J Tongji Med Univ*. 6(4): 193–7.
- Guo, L.Q., Taniguchi, M., Chen, Q.Y., Baba, K. & Yamazoe, Y. (2001). Inhibitory potential of herbal medicines on human cytochrome P450-mediated oxidation: properties of umbelliferous or citrus crude drugs and their relative prescriptions. *Jpn J Pharmacol*. 85(4): 399–408
- Habtemariam, S. (1999). Andrographolide inhibits the tumour necrosis factor- $\alpha$ -induced upregulation of ICAM-1 expression and endothelial-monocyte adhesion. *Phytother Res* 12: 37–40.
- Hao, C.Q. & Yang, F. (1986). Anti-inflammatory effects of total saponins of *Panax notoginseng*. *Acta Pharm. Sin.* 7 (3): 252–255.
- Harada, M., Tenmyo, N., Aburada, M. & Endo, T. (1974). Pharmacological studies of gardeniae fructus. I. Effect of geniposide and genipin on the biliary excretion, the gastric juice secretion, and the gastric contraction, and other pharmacological actions. *Yakugaku Zasshi*. 94(2): 157–62. (Article in Japanese).
- Ho, Y.L. & Chang, Y.S. (2002). Studies on the antinociceptive, anti-inflammatory and anti pyretic effects of *Isatis indigotica* root. *Phytomedicine*. 9(5): 419–24.
- Hocking, G.M. (1997). *A Dictionary of Natural products*. Plexus Publishing, Inc.
- Hong, C.H., Hur, S.K., Oh, O.J., Kim, S.S., Nam, K.A. & Lee, S.K. (2002). Evaluation of natural products on inhibition of inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) in cultured mouse macrophage cells. *J. Ethnopharmacol*. 83 (1–2): 153–159.
- Hsu, H.Y., Shen, S.J., Chen, Y.P., Hsu, C.S., Chen, C.C. & Chang, H.C. (1986). *Oriental Materia Medica: A concise guide*. Keats Publishing, Inc.
- Huang, K.C. (1999). *The Pharmacology of Chinese Herbs* (2nd Ed.). CRC Press.
- Huang, L., Ye, W., Cai, B., Li, D., Liu, J. & Liu, M. (1990). A preliminary study on the pharmacology of the compound prescription huangqin tang and its component drugs. *Zhongguo Zhong Yao Za Zhi*. 15(2): 115–7, 128. (Article in Chinese).

## References

- Huang, Y., Wong, C.M., Lau, C.W., Yao, X., Tsang, S.Y., Su, Y.L. & Chen, Z.Y. (2004). Inhibition of nitric oxide/cyclic GMP-mediated relaxation by purified flavonoids, baicalin and baicalein, in rat aortic rings. *Biochem Pharmacol.* 67(4): 787–94.
- IFAW report (1994). Herbal alternatives to bear bile in Chinese medicine. The Association of Chinese Medicine and Philosophy and Earthcare Society (Hong Kong).
- Iizuka, N., Miyamoto, K., Okita, K., Tangoku, A., Hayashi, H., Yosino, S., Abe, T., Morioka, T., Hazama, S. & Oka, M. (2000). Inhibitory effect of *Coptidis Rhizoma* and berberine on the proliferation of human esophageal cancer cell lines. *Cancer Lett.* 148(1): 19–25.
- Im, E. & Martinez, J.D. (2004). Ursodeoxycholic acid (UDCA) can inhibit deoxycholic acid (DCA)-induced apoptosis via modulation of EGFR/Raf-1/ERK signalling in human colon cancer cells. *J Nutr.* 134(2): 483–6.
- Ingaki, I. & Oida, N. (1970). On the constituents of rhinoceros horn (I). *Nagoya shiritsu Daigaku yakugakubu Kenk Yu Nempo* 18: 57–66 (Article in Japanese).
- Ishihara, T., Kohno, K., Ushio, S., Iwaki, K., Ikeda, M. & Kurimoto, M. (2000). Tryptanthrin inhibits nitric oxide and prostaglandin E(2) synthesis by murine macrophages. *Eur J Pharmacol.* 407(1–2): 197–204.
- Itami, T., Ema, M., Sakamoto, J., Hosoda, K., Noguchi, M. & Kawasaki, H. (1992). Antipyretic effects of traditional Chinese medicines in bacterial endotoxin-induced febrile rabbits. *Yakugaku Zasshi.* 12(2): 129–34 (Article in Japanese).
- Ivanov, A.I. & Romanovsky, A.A. (2004). Prostaglandin E2 as a mediator of fever: synthesis and catabolism. *Front Biosci.* 9:1977–93.
- Jain, D.C., Gupta, M.M., Saxena, S. & Kumar, S. (2000). LC analysis of hepatoprotective diterpenoids for *Andropogon paniculata*. *J Pharm Biomed Anal.* 22: 705–709.
- Jang, S.I., Jeong, S.I., Kim, K.J., Kim, H.J., Yu, H.H., Park, R., Kim, H.M. & You, Y.O. (2003). Tanshinone IIA from *Salvia miltiorrhiza* inhibits inducible nitric oxide synthase expression and production of TNF-alpha, IL-1beta and IL-6 in activated RAW 264.7 cells. *Planta Med.* 69(11): 1057–9.
- Jeong, S.J., Higuchi, R., Ono, M., Kuwano, M., Kim, Y.C. & Miyamoto, T. (2003). cis-hinokiresinol, a norlignan from *Anemarrhena asphodeloides*, inhibits angiogenic response *in vitro* and *in vivo*. *Biol Pharm Bull.* 26(12): 1721–4.
- Kageura, T., Matsuda, H., Morikawa, T., Toguchida, I., Harima, S., Oda, M., & Yoshikawa, M. (2001). Inhibitors from rhubarb on lipopolysaccharide-induced nitric oxide production in macrophages: structural requirements of stilbenes for the activity. *Bioorg Med Chem.* 9(7): 1887–93.
- Kaith, B.S., Kaith, N.S. & Chauhan, N.S. (1996). Anti-inflammatory effect of *Arnebia euchroma* root extracts in rats. *J Ethnopharmacol.* 55(1): 77–80.

- Kang, B.Y., Chung, S.W., Kim, S.H., Cho, D. & Kim, T.S. (2003a). Involvement of nuclear factor-kappaB in the inhibition of interleukin-12 production from mouse macrophages by baicalein, a flavonoid in *Scutellaria baicalensis*. *Planta Med.* 69(8): 687–91.
- Kang, J.H., Park, Y.H., Choi, S.W., Yang, E.K. & Lee, W.J. (2003b). Resveratrol derivatives potently induce apoptosis in human promyelocytic leukemia cells. *Exp Mol Med.* 35(6): 467–74.
- Kang, J.S., Yoon, Y.D., Lee, K.H., Park, S.K. & Kim, H.M. (2004). Costunolide inhibits interleukin-1 beta expression by down-regulation of AP-1 and MAPK activity in LPS-stimulated RAW 264.7 cells. *Biochem. Biophys. Res. Commun.* 313 (1): 171–177.
- Keum, Y.S., Han, S.S., Chun, K.S., Park, K.K., Park, J.H., Lee, S.K. & Surh, Y.J. (2003). Inhibitory effects of the ginsenoside Rg3 on phorbol ester-induced cyclooxygenase-2 expression, NF- $\kappa$ B activation and tumor promotion. *Mut. Res. Fund. Mol. Mech. Mutagen.* 523: 75–85.
- Kim, H.M., An, C.S., Jung, K.Y., Choo, Y.K., Park, J.K. & Nam, S.Y. (1999). *Rehmannia glutinosa* inhibits tumour necrosis factor-alpha and interleukin-1 secretion from mouse astrocytes. *Pharmacol Res.* 40(2): 171–6.
- Kim, Y.S., Jung, E.A., Shin, J.E., Chang, J.C., Yang, H.K., Kim, N.J., Cho, K.H., Bae, H.S., Moon, S.K. & Kim, D.H. (2002c). Daio-Orengedokuto inhibits HMG-CoA reductase and pancreatic lipase. *Biol Pharm Bull.* 25(11): 1442–5.
- Kimura, M., Kimura, I., Luo, B. & Kobayashi, S. (1991). Anti-inflammatory effect of Japanese Sino medicine Keishi-ka-jutsubu-to and its component drugs on adjuvant air pouch granuloma of mice. *Phytother. Res.* 5: 195–200.
- Ko, F.N., Lee, Y.S., Kuo, S.C., Chang, Y.S. & Teng, C.M. (1995). Inhibition on platelet activation by shikonin derivatives isolated from *Arnebia euchroma*. *Biochim Biophys Acta.* 1268(3): 329–34.
- Koh, H.L. & Woo, S.O. (2000). Chinese proprietary medicine in Singapore: regulatory control of toxic heavy metals and undeclared drugs. *Drug Saf.* 23(5): 351–62.
- Kong, L.D., Yang, C., QuiXi, Wu, H.P. & Ye, D.J. (2001). Effects of different processing products of Cortex Phellodendri on scavenging oxygen free radicals and anti-lipid peroxidation. *Zhongguo Zhong Yao Za Zhi.* 26(4): 245–8. (Article in Chinese).
- Kosuge, T., Yokota, M., Sugiyama, K., Yamamoto, T., Mure, T. & Yamazawa, H. (1985). Studies on bioactive substances in crude drugs used for arthritic diseases in traditional Chinese medicine. 2. Isolation and identification of an anti-inflammatory and analgesic principle from the root of *Angelica pubescens* Maxim. *Chem. Pharm. Bull.* 33: 5351–5354.
- Kowdley, K.V. (2000). Ursodeoxycholic acid therapy in hepatobiliary disease. *Am J Med.* 108(6): 481–6.

## References

- Kubo, M., Asano, T., Shiimoto H, Matsuda, H. (1994). Studies on rehmanniae radix. 1. Effect of 50% ethanolic extract from steamed and dried rehmanniae radix on hemorheology in arthritic and thrombotic rats. *Biol. Pharm. Bull.* 17 (9): 1282–1286.
- Kubo, M., Ma, S.P., Wu, J.X. & Matsuda, H. (1996). Anti-inflammatory activities of 70% methanolic extract from cinnamomi cortex. *Biol. Pharm. Bull.* 19 (8): 1041–1045.
- Kuo WH, Wang CJ, Young SC, Sun YC, Chen YJ, Chou FP (2004) Differential induction of the expression of GST subunits by geniposide in rat hepatocytes. *Pharmacology.* 70(1): 15–22.
- Kwak, W.J., Han, C.K., Chang, H.W., Kim, H.P., Kang, S.S. & Son, K.H. (2003). Loniceroside C, an anti-inflammatory saponin from *Lonicera japonica*. *Chem Pharm Bull.* (Tokyo). 51(3): 333–5.
- Laburn, H.P. & Mitchell, D. (1997). Extracts of rhinoceros horn are not antipyretic in rabbits. *J Basic Clin Physiol Pharmacol.* 8(1–2): 1–11.
- Lee, Y.W. (1999). Effect of ursodeoxycholic acid on ischemia/reperfusion injury in isolated rat heart. *Ach Phar Res.* 22 (5): 479–484.
- Lee, G.I., Ha, J.Y., Min, K.R., Nakagawa, H., Tsurufuji, S., Chang, I.M. & Kim, Y. (1995). Inhibitory effects of Oriental herbal medicines on IL-8 induction in lipopolysaccharide activated rat macrophages. *Planta Med.* 61(1): 26–30.
- Lee, S.K. & Kim, Y.E. (1974). Studies on the compositions of hard tissue proteins extracted from bovine horn, water buffalo horn and rhinoceros horn. *Korean Biochem. J.* 7(2): 125–142. (Article in Japanese).
- Lee, J.H., Ko, W.S., Kim, Y.H., Kang, H.S., Kim, H.D. & Choi, B.T. (2001). Anti-inflammatory effect of the aqueous extract from *Lonicera japonica* flower is related to inhibition of NF-kappaB activation through reducing I-kappaB $\alpha$  degradation in rat liver. *Int J Mol Med* 7(1): 79–83.
- Lee, S.J., Lee, I.S. & Mar, W. (2003). Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 activity by 1,2,3,4,6-penta-O-galloyl-beta-D-glucose in murine macrophage cells. *Arch Pharm Res.* 26(10): 832–9.
- Lee, S.M., Li, M.L., Tse, Y.C., Leung, S.C., Lee, M.M., Tsui, S.K., Fung, K.P., Lee, C.Y. & Waye, M.M. (2002). Paeoniae Radix, a Chinese herbal extract, inhibit hepatoma cells growth by inducing apoptosis in a p53 independent pathway. *Life Sci.* 71(19): 2267–77.
- Lee, S.J., Son, K.H., Chang, H.W., Kang, S.S. & Kim, H.P. (1998). Anti-inflammatory activity of *Lonicera japonica*. *Phytother Res.* 12(6): 445–447.
- Li, P.J. (2004). China's Bear Farming and Long-Term Solutions. *J Appl Anim Welf Sci.* 7(1): 71–81.
- Li, X.Y. (2000). Immunomodulating components from Chinese medicines. *Pharm. Biol.* 38: 33–40 Suppl.

- Li, S.H. & Chu, Y. (1999). Anti-inflammatory effects of total saponins of *Panax notoginseng*. *Acta Pharmacol. Sin.* 20 (6): 551–554.
- Li, Y.M., Han, Z.H., Jiang, S.H., Jiang, Y., Yao, S.D. & Zhu, D.Y. (2000). Fast repairing of oxidized OH radical adducts of dAMP and dGMP by phenylpropanoid glycosides from *Scrophularia ningpoensis* Hemsl. *Acta Pharmacol Sin.* 21(12): 1125–8.
- Li, R.W., Lin, G.D., Myers, S.P. & Leach, D.N. (2003). Anti-inflammatory activity of Chinese medicinal vine plants. *J. Ethnopharmacol.* 85(1): 61–67.
- Li, Y.W., Zhu, X.Y., But, P.P.H. & Yeung, H.W. (1995). Ethnopharmacology of bear gall bladder: I. *J Ethnopharmacol.* 47(1): 27–31.
- Lin, D.L., Chang, H.C., Chen, C.P. & Chen, C.Y. (1997). Identification and differentiation of bear bile used in medicinal products in Taiwan. *J Forensic Sci.* 42(5): 817–823.
- Lin, H.C., Ding, H.Y., Ko, F.N., Teng, C.M. & Wu, Y.C. (1999). Aggregation inhibitory activity of minor acetophenones from *Paeonia* species. *Planta Med.* 65(7): 595–9.
- Liu, F. & Ng, T.B. (2000). Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci.* 66(8): 725–35.
- Liu, J.H., Zschocke, S., Reiningger, E. & Bauer, R. (1998a). Comparison of *radix angelicae pubescentis* and substitutes – constituents and inhibitory effect on 5-lipoxygenase and cyclooxygenase. *Pharm. Biol.* 36: 207–216.
- Liu, J.H., Zschocke, S., Reiningger, E. & Bauer, R. (1998b). Inhibitory effects of *Angelica pubescens f. biserrata* on 5-lipoxygenase and cyclooxygenase. *Planta Med.* 64: 525–529.
- Ma, D., Zhang, J., Sugahara, K., Sagara, Y. & Kodama, H. (2001). Effect of sarsasapogenin and its derivatives on the stimulus coupled responses of human neutrophils. *Clin Chim Acta.* 314(1–2): 107–12.
- Maclean, W. & Taylor, K. (2000). *Chinese herbal patent medicines*. Pangolion Press.
- Matsuda, H., Kageura, T., Morikawa, T., Toguchida, I., Harima, S. & Yoshikawa, M. (2000). Effects of stilbene constituents from rhubarb on nitric oxide production in lipopolysaccharide-activated macrophages. *Bioorg Med Chem Lett.* 10(4): 323–7.
- Matsuda, H., Ohta, T., Kawaguchi, A. & Yoshikawa, M. (2001a). Bioactive constituents of chinese natural medicines. VI. Moutan cortex. (2): structures and radical scavenging effects of suffruticosides A, B, C, D, and E and galloyl-oxypaeoniflorin. *Chem Pharm Bull (Tokyo).* 49(1): 69–72.
- Matsuda, H., Morikawa, T., Toguchida, I., Park, J.Y., Harima, S. & Yoshikawa, M. (2001b). Antioxidant constituents from rhubarb: structural requirements of stilbenes for the activity and structures of two new anthraquinone glucosides. *Bioorg Med Chem.* 9(1): 41–50.

## References

- Matsuda H, Toguchida I, Ninomiya K, Kageura T, Morikawa T, Yoshikawa M (2003) Effects of sesquiterpenes and amino acid-sesquiterpene conjugates from the roots of *Saussurea lappa* on inducible nitric oxide synthase and heat shock protein in lipopolysaccharide activated macrophages. *Bioorg. Med. Chem.* 11 (5): 709–715.
- McEvoy, A.N., Bresnihan, B., FitzGerald, O., Murphy, E.P. (2004). Cyclooxygenase 2-derived prostaglandin E2 production by corticotropin-releasing hormone contributes to the activated cAMP response element binding protein content in rheumatoid arthritis synovial tissue. *Arthritis Rheum.* 50(4): 1132–45.
- McKillop, D., Wild, M.J., Butters, C.J. & Simcock, C. (1999). Effects of propofol on human hepatic microsomal cytochrome P450 activities. *Xenobiotica* 28(9): 845–853.
- Ministry of Agriculture, Fisheries and Food, Food Standards Committee (1956). Report on copper. Revised recommendations for limits for copper content of foods. Her Majesty's Stationery Office, London, 5pp.
- Molina, P., Tarraga, A., Gonzalez-Tejero, A., Rioja, I., Ubeda, A., Terencio, M.C. & Alcaraz, M.J. (2001). Inhibition of leukocyte functions by the alkaloid isaindigotone from *Isatis indigotica* and some new synthetic derivatives. *J Nat Prod.* 64(10): 1297–300.
- Moltz, H. (1993). Fever: Causes and consequences. *Neurosci Biobehav Rev.* 17(3):237–69.
- Oh GS, Pae HO, Choi BM, Jeong S, Oh H, Oh CS, Rho YD, Kim DH, Shin MK, Chung HT (2003) Inhibitory effects of the root cortex of *Paeonia suffruticosa* on interleukin-8 and macrophage chemoattractant protein-1 secretions in U937 cells. *J Ethnopharmacol.* 84(1): 85–9.
- Oh, G.S., Pae, H.O., Choi, B.M., Lee, H.S., Kim, I.K., Yun, Y.G., Kim, J.D. & Chung, H.T. (2004). Penta-O-galloyl-beta-D-glucose inhibits phorbol myristate acetate-induced interleukin-8 gene expression in human monocytic U937 cells through its inactivation of nuclear factor-kappaB. *Int Immunopharmacol.* 4(3): 377–86.
- Ohsugi, M., Fan, W., Hase, K., Xiong, Q., Tezuka, Y., Komatsu, K., Namba, T., Saitoh, T., Tazawa, K. & Kadota, S. (1999). Active-oxygen scavenging activity of traditional nourishing-tonic herbal medicines and active constituents of *Rhodiola scabra*. *J Ethnopharmacol.* 67(1): 111–9.
- Ohta Y, Sasaki E, Nishida K, Kongo M, Hayashi T, Nagata M, Ishiguro I (1998) Inhibitory effect of Oren-gedoku-to (Huanglian-Jie-Du-Tang) extract on hepatic triglyceride accumulation with the progression of carbon tetrachloride-induced acute liver injury in rats. *J Ethnopharmacol.* 61(1): 75–80.
- Okuyama, E., Hasegawa, T., Matsushita, T., Fujimoto, H., Ishibashi, M. & Yamazaki, M. (2001). Analgesic components of *Saposhnikovia* root (*Saposhnikovia divaricata*). *Chem. Pharm. Bull.* 49(2): 154–160.

- Ozaki, Y. (1992). Anti-inflammatory effect of tetramethylpyrazine and ferulic acid. *Chem. Pharm. Bull.* 40: 954–956.
- Ozaki, Y., Rui, J., Tang, Y. & Satake, M. (1997). Anti-inflammatory effect of *Forsythia suspensa* Vahl and its active fraction. *Biol Pharm Bull.* 20(8): 861–4.
- Ozaki, Y., Rui, J. & Tang, Y.T. (2000). Anti-inflammatory effect of *Forsythia suspensa* V(AHL) and its active principle. *Biol Pharm Bull.* 23(3): 365–7.
- Paulus, K. & Bauer, R. (2000). Inhibitory Effects from *Salvia miltiorrhiza* on Leukotriene Biosynthesis. In: *Natural Products Research in the New Millennium*. 48th Annual Meeting of the Society for Medicinal Plant Research, Zurich (September 3rd – 7th 2000) Abstract Book. P2A/73.
- Pei-Gen, X. & Nai-Gong, W. (1991). Can ethnopharmacology contribute to the development of anti-fertility drugs? *J Ethnopharmacol.* 32(1–3): 167–77.
- Pham, T.Q., Cormier, F., Farnworth, E., Tong, V.H. & Van Calsteren, M.R. (2000). Antioxidant properties of crocin from *Gardenia jasminoides* Ellis and study of the reactions of crocin with linoleic acid and crocin with oxygen. *J Agric Food Chem.* 48(5): 1455–61.
- Pharmacopoeia of the People's Republic of China (1997). English Ed. (Vol. 1). Compiled by the Pharmacopoeia Commission of PRC.
- Prieto, J.M., Recio, M.C., Giner, R.M., Manez, S., Giner-Larza, E.M. & Rios, J.L. (2003). Influence of traditional Chinese anti-inflammatory medicinal plants on leukocyte and platelet functions. *J Pharm Pharmacol.* 55(9): 1275–82.
- Qi, X.G. (1991). Protective mechanism of *Salvia miltiorrhiza* and *Paeonia lactiflora* for experimental liver damage. *Zhong Xi Yi Jie He Za Zhi.* 11(2): 102–4, 69. (Article in Chinese).
- Rajagopal, S., Kumar, R.A., Deevi, D.S., Satyanarayana, C. & Rajagopalan, R. (2003). Andrographolide, a potential cancer therapeutic agent isolated from *Andrographis paniculata*. *J Exp Ther Oncol.* 3(3): 147–58.
- Ram, V.J. (2001). Herbal preparations as a source of hepatoprotective agents. *Drug News Perspect.* 14(6): 353–63.
- Recio, M.C., Giner, R.M., Manez, S. & Rios, J.L. (1994). Structural considerations on the iridoids as anti-inflammatory agents. *Planta Med.* 60(3): 232–4.
- Rehalison, L., Hamburger, M., Hostettmann, K., Monod, M. & Frenk, E. (1991). A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. *Phytochem Anal.* 2:199–203.
- Resch, M., Steigel, A., Chen, Z.L. & Bauer, R. (1998). 5-lipoxygenase and cyclooxygenase-1 inhibitory active compounds from *Atractylodes lancea*. *J. Nat. Prod.* 61: 347–350.

## References

- Resch, M., Heilmann, J., Steigel, A. & Bauer, R. (2001). Further phenols and polyacetylenes from the rhizomes of *Atractylodes lancea* and their anti-inflammatory activity. *Planta Med.* 67: 437–442.
- Rolo, A.P., Oliveira, P.J., Moreno, A.J. & Palmeira, C.M. (2000). Bile acids affect liver mitochondrial bioenergetics: possible relevance for cholestasis therapy. *Toxicol Sci* 57(1): 177–85.
- Ross, J.S., Kallakury, B.V., Sheehan, C.E., Fisher, H.A., Kaufman, R.P. Jr, Kaur, P., Gray, K. & Stringer, B. (2004). Expression of Nuclear Factor-kappaB and IkappaBalpha Proteins in Prostatic Adenocarcinomas: Correlation of Nuclear Factor-kappaB Immunoreactivity with Disease Recurrence. *Clin Cancer Res.* 10(7): 2466–72.
- Sai, Y., Dai, R., Yang, T.J., Krausz, K.W., Gonzalez, F.J., Gelboin, H.V. & Shou, M. (2000). Assessment of specificity of eight chemical inhibitors using cDNA-expressed cytochromes P450. *Xenobiotica.* 30(4): 327–343.
- Sanchez GM, Re L, Giuliani A, Nunez-Selles AJ, Davison GP, Leon-Fernandez OS (2000) Protective effects of *Mangifera indica* L. extract, mangiferin and selected antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. *Pharmacol Res.* 42(6): 565–73.
- Saraswat, B., Visen, P.K. & Agarwal, D.P. (2000). Ursolic acid isolated from *Eucalyptus tereticornis* protects against ethanol toxicity in isolated rat hepatocytes. *Phytother Res.* 14(3): 163–166.
- Schinella, G.R., Tournier, H.A., Prieto, J.M., Mordujovich, D. & Rios, J.L. (2002). Antioxidant activity of anti-inflammatory plant extracts. *Life Sci.* 70(9): 1023–33.
- Sekiya, N., Kogure, T., Kita, T., Kasahara, Y., Sakakibara, I., Goto, H., Shibahara, N., Shimada, Y. & Terasawa, K. (2002). Reduction of plasma triglyceride level and enhancement of plasma albumin concentration by Oren-gedoku-to administration. *Phytomedicine.* 9(5): 455–60.
- Shen, Y.C., Chen, C.F., Chiou, W.F. (2002). Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. *Br J Pharmacol.* 135(2): 399–406.
- Subbaramaiah, K., Bulic, P., Lin, Y., Dannenberg, A.J. & Pasco, D.S. (2001). Development and use of a gene promoter-based screen to identify novel inhibitors of cyclooxygenase-2 transcription. *J Biomol Screen.* 6(2): 101–10.
- Syrovets, T., Buchele, B., Gedig, E., Slupsky, J.R. & Simmet, T. (2000). Acetyl-boswellic acids are novel catalytic inhibitors of human topoisomerases I and IIa. *Mol Pharmacol.* 58(1): 71–81.
- Tae, J., Han, S.W., Yoo, J.Y., Kim, J.A., Kang, O.H., Baek, O.S., Lim, J.P., Kim, D.K., Kim, Y.H., Bae, K.H. & Lee, Y.M. (2003). Anti-inflammatory effect of *Lonicera japonica* in proteinase-activated receptor 2-mediated paw edema. *Clin Chim Acta.* 330(1–2): 165–71.

- Tanasescu, C. (2004). Correlation between Cholestasis and Infection. *Rom J Gastroenterol*. 13(1): 23–27.
- Tang, W. & Eisenbrand, G. (1992). *Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and use in Traditional and Modern Medicine*. Springer-Verlag.
- Thamlikitkul, V., Dechatiwongse, T., Theerapong, S., Chantrakul, C., Boonroj, P., Punkrut, W., Ekpalakorn, W., Boontaeng, N., Taechaiya, S. & Petcharoen, S. (1991). Efficacy of *Andrographis paniculata* Nees for pharyngotonsillitis in adults. *Med Assoc Thai*. 74(10): 437–442.
- Trivedi, N.P. & Rawal, U.M. (2001). Hepatoprotective and antioxidant property of *Andrographis paniculata* (Nees) in BHC induced liver damage in mice. *Indian J Exp Biol*. 39(1): 41–6.
- Van Den Bogaert, E., Francque, S., Pelckmans, P. & Michielsen, P. (2003). The use of ursodeoxycholic acid in patients with primary biliary cirrhosis: sense or nonsense. *Acta Gastroenterol Belg*. 66(4): 283–7.
- van Loon, I.M. (1997). The golden root: clinical applications of *Scutellaria baicalensis* Georgi flavonoids as modulators of the inflammatory response. *Altern Med Rev*. 2(6): 472–480.
- Wang, W.J., Bai, J.Y., Liu, D.P., Xue, L.M. & Zhu, X.Y. (1994). The anti-inflammatory activity of shikonin and its inhibitory effect on leukotriene B4 biosynthesis. *Yao Xue Xue Bao*. 29(3): 161–5. (Article in Chinese).
- Wang, C.C., Chen, L.G. & Yang, L.L. (1999). Inducible nitric oxide synthase inhibitor of the Chinese herb – I. *Saposhnikovia divaricata* (Turcz.) Schischk. *Cancer Lett*. 145 (1–2): 151–157.
- Wang, S., Zheng, Z., Weng, Y., Yu, Y., Zhang, D., Fan, W., Dai, R. & Hu, Z. (2004). Angiogenesis and anti-angiogenesis activity of Chinese medicinal herbal extracts. *Life Sci*. 74(20): 2467–78.
- Wei, F., Zou, S., Young, A., Dubner, R. & Ren, K. (1999). Effects of four herbal extracts on adjuvant-induced inflammation and hyperalgesia in rats. *J. Altern. Comp. Med*. 5(5): 429–436.
- Wiseman, N. & Ye, F. (1998). *Practical dictionary of Chinese medicine*. Paradigm Publications.
- World Health Organisation (WHO) (2004). Medicinal plants – guidelines to promote patient safety and plant conservation for a US\$ 60 billion industry. <http://www.who.int/mediacentre/news/notes/2004/np3/en/>. Last accessed April 2004.
- 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.

## References

Wu, X., Liu, Y., Sheng, W., Sun, J. & Qin, G. (1997). Chemical constituents of *Isatis indigotica*. *Planta Med.* 63: 55–57.

WWF (2000) [http://www.worldwildlife.org/tigers/natural\\_history.cfm](http://www.worldwildlife.org/tigers/natural_history.cfm) (Last accessed 10 April 2004).

WWF (2002). Asian Rhinos in the Wild: A WWF Species Status Report <http://www.wwf.org.uk/filelibrary/pdf/asianrhinos.pdf>. (Last accessed 10 April 2004).

Xiang, D.B. & Li, X.Y. (1993). Effects of *Achyranthes bidentata* polysaccharides on interleukin-1 and tumor necrosis factor- $\alpha$  production from mouse peritoneal macrophages. *Acta Pharm. Sin.* 14: 332–336.

Xie, T. (1993). Herbal decoction of qingwen baidu yin in treating endotoxic fever in rabbits. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 13(2): 94–97. (Article in Chinese).

Xu, X. (Ed.) (1994). *The English-Chinese encyclopedia of practical traditional medicine: Pharmacology of TCM formulae*. Vol 3. Higher Education Press, Beijing, China.

Xu, G.J. He, H.X. Xu, L.S. & Jin, R.L. (1996). *The Chinese Materia Medica (Vol. I)*. Chinese Medicine and Technology Press, Beijing, China.

Yang, Y. (2002). *Chinese Herbal Medicines*. Churchill Livingstone, London.

Yang, H.O., Ko, W.K., Kim, J.Y. & Ro, H.S. (2004). Paeoniflorin: an antihyperlipidemic agent from *Paeonia lactiflora*. *Fitoterapia*. 75(1): 45–9.

Yankep, E., Njamen, D., Fotsing, M.T., Fomum, Z.T., Mbanya, J.C., Giner, R.M., Recio, M.C., Manez, S. & Rios, J.L. (2003). Griffonianone D, an isoflavone with anti-inflammatory activity from the root bark of *Millettia griffoniana*. *J. Nat. Prod.* 66(9): 1288–1290.

Yao, Q., Zhou, G., Zhu, Y., Pan, Y., Hu, J., Xue, H. & Zhang, Q. (1991). Screening studies on anti-inflammatory function of traditional Chinese herb *Gardenia jasminoides* Ellis and its possibility in treating soft tissue injuries in animals *Zhongguo Zhong Yao Za Zhi*. 16(8): 489–93, 513. (Article in Chinese).

Yasukawa, K., Kaminaga, T., Kitanaka, S., Tai, T., Nunoura, Y., Natori, S. & Takido, M. (1998). 3 $\beta$ -*p*-hydroxybenzoyldehydrotumulosic acid from *Poria cocos*, and its anti-inflammatory effect. *Phytochem.* 48 (8): 1357–1360.

Yokozawa, T., Ishida, A., Cho, E.J. & Nakagawa, T. (2003). The effects of Coptidis Rhizoma extract on a hypercholesterolemic animal model. *Phytomedicine*. 10(1): 17–22.

Yoshimi, N., Matsunaga, K., Katayama, M., Yamada, Y., Kuno, T., Qiao, Z., Hara, A., Yamahara, J. & Mori, H. (2001). The inhibitory effects of mangiferin, a naturally occurring glucosylxanthone, in bowel carcinogenesis of male F344 rats. *Cancer Lett.* 163(2): 163–70.

Yotsumoto, H., Yanagita, T., Yamamoto, K., Ogawa, Y., Cha, J.Y. & Mori, Y. (1997). Inhibitory effects of oren-gedoku-to and its components on cholesteryl ester synthesis in cultured human hepatocyte HepG2 cells: evidence from the cultured HepG2 cells and *in vitro* assay of ACAT. *Planta Med.* 63(2): 141–5.

Zhang, C.Y. & Tan, B.K. (1999). Effects of 14-deoxyandrographolide and 14-deoxy-11, 12-didehydroandrographolide on nitric oxide production in cultured human endothelial cells. *Phytother Res.* 13(2): 157–9.

Zhang, C., Kuroyangi, M. & Tan, B.K. (1998). Cardiovascular activity of 14-deoxy-11, 12-didehydroandrographolide in the anaesthetised rat and isolated right atria. *Pharmacol Res.* 38(6): 413–7.

Zhu, C.H. (1989). *Clinical Handbook of Chinese Prepared Medicines*. Paradigm Publications.

Zou, J. (Ed.) (1989). *The Chinese Materia Medica*. Beijing University of traditional Chinese Medicine.

Zoha, M.S., Hussain, A.H., Choudhury, S.A. (1989). Antifertility effect of *Andrographis paniculata* in mice. *Bangladesh Med Res Counc Bull.* 15(1): 34–7.

# Appendices

## Appendix 1.

Fifty-four herbal alternatives to bear bile (IFAW report, 1994)	
Plant species	Plant part as stated in the IFAW report (1994)
1. <i>Lobelia chinensis</i> Lour.	Whole plant
2. <i>Costus speciosus</i> (Koenig) Smith	Bulb
3. <i>Curcuma zedoaria</i> (Berg.) Rose	Root and stem
4. Radix et Rhizoma Rhei	Root and rhizome
5. Radix Scutellaria	Root
6. Herba Lobelia Chinensis	Whole plant
7. <i>Smilax china</i> L.	Rhizome
8. <i>Andrographitis paniculata</i> (Burm.f) Nees	Whole plant
9. Herba Saururi Chinensis	Whole plant
10. <i>Sarcandra glabra</i> (Thunb.) Nakai	Whole plant
11. <i>Scutellaria barbata</i> Don	Whole plant
12. <i>Cycas revoluta</i> Thunb.	Leaves, flowers, seeds and roots
13. Herba Taraxaci	Whole plant
14. Herba Cirsii Japonici	Aerial parts
15. <i>Selaginella doederleinii</i> Hieron	Whole plant
16. <i>Adiantum flabellutatum</i> Linn.	Whole plant
17. <i>Impatiens balsamina</i> Linn.	Seeds and flowers
18. <i>Osbeckia chinensis</i> Linn.	Whole plant
19. <i>Paris chinensis</i> Fr.	Roots
20. Radix Rhapontici	Roots
21. Herba Sedi Aizoon	Whole plant
22. <i>Chrysanthemum indicum</i> L.	Whole plant or flowers
23. Herba Andrographitis	Aerial parts
24. Herba Sarcandrae	Whole plant
25. Herba Salvia Plebeiae	Aerial parts
26. Herba Hedyotis Diffusae	Whole plant
27. Herba Houttuyniae	Aerial parts
28. Folium Ilieis Chinensis	Leaves
29. <i>Dioscorea bulbifera</i> L.	Tuber and auxiliary sprouts
30. Folium et Ramulus Cephalotaxi	Twig and leaf
31. Cortex Phellodendron	Bark
32. Herba Verbenae	Aerial parts

Appendix 1. Continued	
Plant species	Plant part as stated in the IFAW report (1994)
33. Folium Hibisci Mutabilis	Leaves
34. <i>Catharanthus roseus</i> (L.) G. Don	Whole plant
35. Rhizoma Dioscorae Bulbiferae	Tuber
36. <i>Melothria heterophylla</i> (Lour.) Cogn.	Whole plant or tubers
37. <i>Camptotheca acuminata</i> Decne	Fruits, leaves, branches, bark and root
38. <i>Livistona chinensis</i> R. Br.	Seeds , root and leaf
39. <i>Gardenia jasminoides</i> Ellis	Fruits and roots
40. <i>Hedyotis diffusa</i> Wild.	Whole plant
41. <i>Hedyotis tenelliflora</i> Blume.	Whole plant
42. <i>Duchesnea indica</i> (Andr.) Focke	Whole plant
43. <i>Hedyotis corymbosa</i> (L.) Lamk.	Whole plant
44. <i>Hedyotis auricularia</i> L.	Whole plant
45. <i>Passiflora foetida</i> L.	Whole plant and fruits
46. Caulis Hederae Sinensis	Stem
47. <i>Acanthus ilieifolius</i> Linn.	Root
48. Radix Paeoniae Sinjiagenensis	Root
49. <i>Smilax glabra</i> Roxb.	Root
50. <i>Xanthium sibiricum</i> Patrin.	Whole plant or fruit
51. <i>Houttuynia cordata</i> Thunb.	Whole plant
52. Herba Duchesneae Indicae	Whole plant
53. Herba Catharanthi Rosei	Whole plant
54. Herba Scutellaria Barbatae	Whole plant

## Appendix 2.

Standards covered by the multi-residue pesticide screening in TCM herbs, with their limits of detection (LOD). Ethyl acetate extracts of 16 herbs were analysed using GC-ECD and GC-NPD and GC-MS. For the GC analysis, the relative retention times (relative to an internal standard) of the chromatographic peaks from herbal samples were compared to those of the reference standards (a total of 130) within an acceptable deviation ( $\pm 0.005$ ). Confirmatory analysis was then conducted using GC-MS. None of the pesticide residues identified by GC analysis were confirmed by GC-MS analysis.

Pesticide reference standards	Group	LOD mg/kg
1. Aldrin	Organochlorine	0.01
2. Alpha-HCH	Organochlorine	0.01
3. Atrazine	Organonitrogen	0.05
4. Azinphos-ethyl	Organophosphorus	0.05
5. Azinphos-methyl	Organophosphorus	0.05
6. Azoxystrobin	Strobilurin	0.02
7. Benalaxyl	Organonitrogen	0.05
8. Beta-HCH	Organochlorine	0.01
9. Bifenthrin	Synthetic pyrethroid	0.05
10. Bitertanol	Triazole	0.05
11. Bromophos-ethyl	Organophosphorus	0.01
12. Bromophos-methyl	Organophosphorus	0.01
13. Bromopropylate	Organochlorine	0.01
14. Bupimate	Organonitrogen	0.05
15. Captan	Organochlorine	0.02
16. Carbofuran	Organonitrogen	0.05
17. Carbophenothion	Organophosphorus	0.01
18. Chlorbenzilate	Organochlorine	0.02
19. Chlorfenson	Organochlorine	0.01
20. Chlordane (cis and trans)	Organochlorine	0.01
21. Chlorpyrifos	Organophosphorus	0.01
22. Chlorpyrifos-methyl	Organophosphorus	0.01
23. Chlorthalonil	Organochlorine	0.02
24. Chlorveninphos	Organophosphorus	0.01
25. Crotoxyphos	Organophosphorus	0.01

Appendix 2. Continued		
Pesticide reference standards	Group	LOD mg/kg
26. Cyfluthrin	Synthetic pyrethroid	0.05
27. Cyhalothrin	Synthetic pyrethroid	0.05
28. Cypermethrin	Synthetic pyrethroid	0.05
29. Cyproconazole	Triazole	0.05
30. Cyprodinil	Organonitrogen	0.05
31. Deltametrin	Synthetic pyrethroid	0.05
32. Demeton-S-sulfone	Organophosphorus	0.05
33. Diazinon	Organophosphorus	0.01
34. 3,5-Dichloroaniline (DCA)	Dicarboximides	0.10
35. Dichloran	Organochlorine	0.01
36. Dichlorfluanid	Organochlorine	0.02
37. Dichlorvos	Organophosphorus	0.01
38. Dicofol	Organochlorine	0.02
39. Dieldrin	Organochlorine	0.01
40. Diethofencarb	Organonitrogen	0.20
41. Dimethoate	Organophosphorus	0.05
42. Dioxathion	Organophosphorus	0.05
43. Diphenylamine	Organonitrogen	0.05
44. Disulfoton	Organophosphorus	0.01
45. Disulfoton-sulfone	Organophosphorus	0.20
46. Endosulfan (a and $\lambda$ )	Organochlorine	0.01
47. Endosulfan sulphate	Organochlorine	0.02
48. Endrin	Organochlorine	0.01
49. Ethion	Organophosphorus	0.01
50. Ethoprophos	Organophosphorus	0.05
51. Ethoxyquin	Organonitrogen	0.20
52. Etrimpfos	Organophosphorus	0.01
53. Fenamiphos	Organophosphorus	0.02
54. Fenamirol	Organochlorine	0.01
55. Fenbuconazole	Triazole	0.05
56. Fenchlorphos	Organophosphorus	0.01
57. Fenitrothion	Organophosphorus	0.02
58. Fenpropathrin	Synthetic pyrethroid	0.05
59. Fenthion	Organophosphorus	0.05
60. Fenvalerate	Synthetic pyrethroid	0.01

## Appendices

Appendix 2. Continued		
Pesticide reference standards	Group	LOD mg/kg
61. Fluazifop butyl	Organonitrogen	0.10
62. Flucythrinate	Synthetic pyrethroid	0.05
63. Folpet	Organochlorine	0.03
64. Fonophos	Organophosphorus	0.02
65. Formothion	Organophosphorus	0.05
66. Heptachlor	Organochlorine	0.01
67. Heptachlor epoxide	Organochlorine	0.02
68. Heptenophos	Organophosphorus	0.01
69. Hexachlorobenzene	Organochlorine	0.02
70. Hexaconazole	Triazole	0.05
71. Iodofenphos	Organophosphorus	0.05
72. Iprodione	Dicarboximide	0.50
73. Isofenphos	Organophosphorus	0.02
74. Kresoxim methyl	Organochlorine	0.01
75. Lindane	Organochlorine	0.01
76. Malathion	Organophosphorus	0.01
77. Mercarbam	Organophosphorus	0.01
78. Metalaxyl	Organonitrogen	0.05
79. Methacriphos	Organophosphorus	0.01
80. Methamidophos	Organophosphorus	0.02
81. Methidathion	Organophosphorus	0.02
82. Methoxychlor	Organochlorine	0.01
83. Mevinphos	Organophosphorus	0.01
84. Myclobutanil	Triazole	0.05
85. Omethoate	Organophosphorus	0.01
86. OP-DDE	Organochlorine	0.01
87. OP-DDT	Organochlorine	0.01
88. OP-TDE	Organochlorine	0.01
89. Oxadixyl	Organonitrogen	0.10
90. Oxamyl	Carbamate	0.01
91. Paclobutrazol	Triazole	0.05
92. Parathion-ethyl	Organophosphorus	0.01
93. Parathion-methyl	Organophosphorus	0.02
94. Penconazole	Triazole	0.05
95. Pendimethalin	Organonitrogen	0.05
96. Pentachlorobenzene	Organochlorine	0.01

Appendix 2. Continued		
Pesticide reference standards	Group	LOD mg/kg
97. Permethrin	Synthetic pyrethroid	0.05
98. Phorate	Organophosphorus	0.01
99. Phorate sulfone	Organophosphorus	0.01
100. Phosalone	Organophosphorus	0.01
101. Phosmet	Phthalimide	0.05
102. Phosphamidon	Organophosphorus	0.10
103. Pirimicarb	Carbamate	0.05
104. Pirimiphos methyl	Organophosphorus	0.01
105. pp-DDE	Organochlorine	0.01
106. pp-DDT	Organochlorine	0.01
107. pp-TDE	Organochlorine	0.01
108. Prochloraz	Amide fungicide	0.10
109. Procimydone	Organochlorine	0.02
110. Profenophos	Organophosphorus	0.05
111. Propachlor	Organochlorine	0.03
112. Propiconazole	Triazole	0.05
113. Propoxur	Carbamate	0.05
114. Propyzamide	Benzamide	0.02
115. Pyrazophos	Organophosphorus	0.02
116. Pyrimethanil	Pyrimidine fungicide	0.05
117. Quinalphos	Organophosphorus	0.01
118. Quintozene	Organochlorine	0.01
119. Tecnazene	Organochlorine	0.01
120. Tetrachlorvinphos	Organophosphorus	0.05
121. Tetradifon	Organonitrogen	0.01
122. Thiometon	Organophosphorus	0.01
123. Tolclofos-methyl	Organophosphorus	0.01
124. Tolyfluanid	Organochlorine	0.03
125. Triadimefon	Triazole	0.02
126. Triazophos	Organophosphorus	0.02
127. Trichlorfon	Organophosphorus	0.01
128. Trifluralin	Organochlorine	0.01
129. Vamidothion	Organophosphorus	0.10
130. Vinclozin	Dicarboxides	0.01

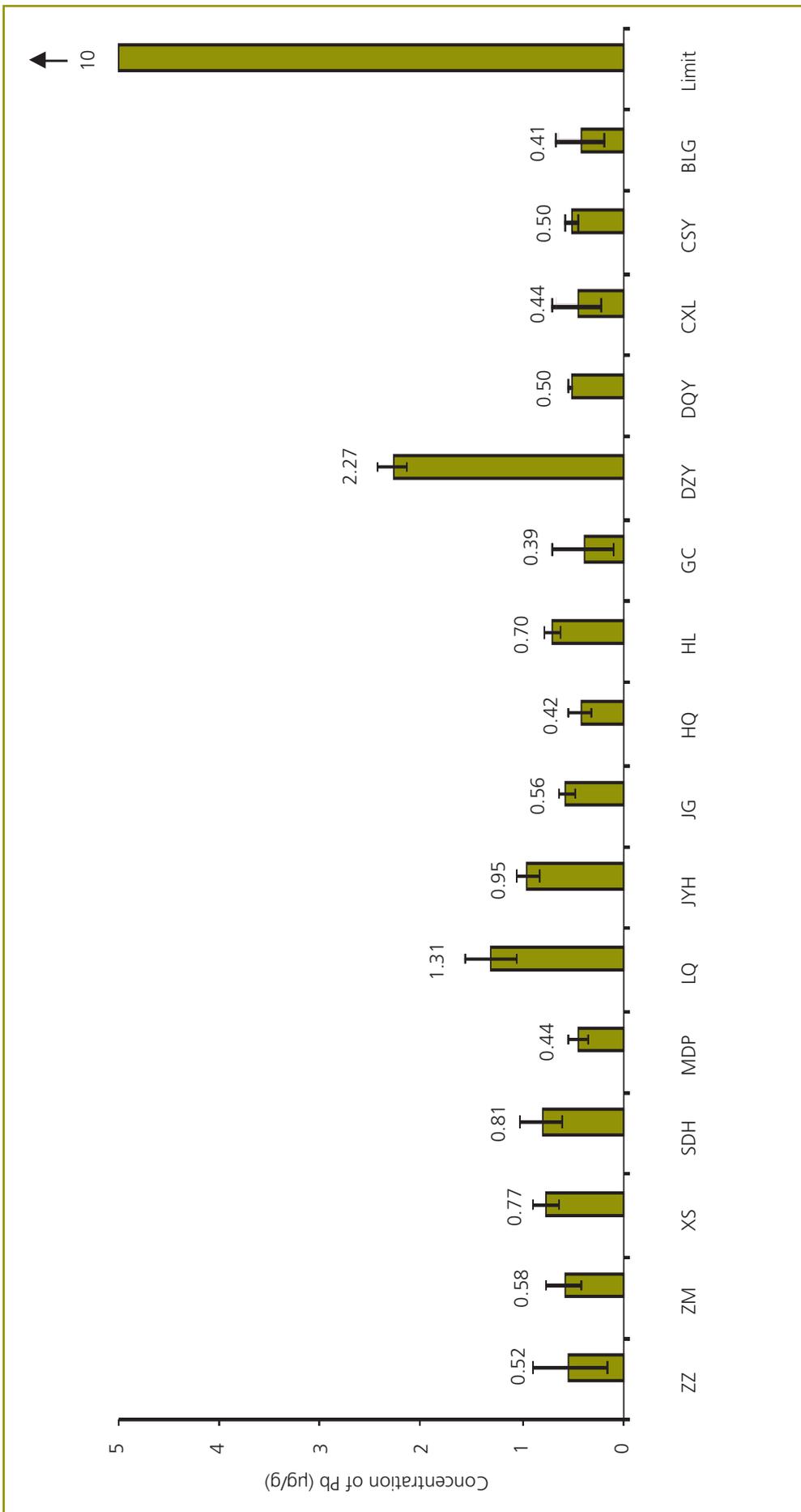
## Appendix 3. Results for metal analyses of extracts of rhino horn and TCM herbs

Appendix 3A. Concentrations of metals in extracts of rhino			
Metal	LOD (µg/g)	Concentration in rhino horn ± SD (µg/g)	Legal limit in food (µg/g)
Ag	0.150	none detected	
V	0.288	none detected	
Sn	0.064	8.3 ± 1.2	200 (Food Regulations, 1992)
Pb	1.052	25.0 ± 3.0	10 (Chuan <i>et al.</i> , 2000)
Cd	0.047	0.6 ± 0.13	0.3 (Chuan <i>et al.</i> , 2000)
Zn	0.033	69.8 ± 0.08	50 (MAFF, 1956)
Cu	0.039	4.0 ± 0.11	20 (MAFF, 1956)
Hg	0.030	2.3 ± 1.4	0.5 (Lide, 1997; Chuan <i>et al.</i> , 2000)
Co	0.040	2.7 ± 0.78	
Be	0.009	0.3 ± 0.00	
Cr	0.024	0.5 ± 0.37	
Ni	0.166	4.7 ± 0.52	
Se	0.047	8.6 ± 2.9	
Mn	0.058	7.6 ± 0.08	
Al	0.164	235.6 ± 5.0	
Fe	0.093	321.4 ± 9.7	

Data represents the mean from six replicate readings ± SD.

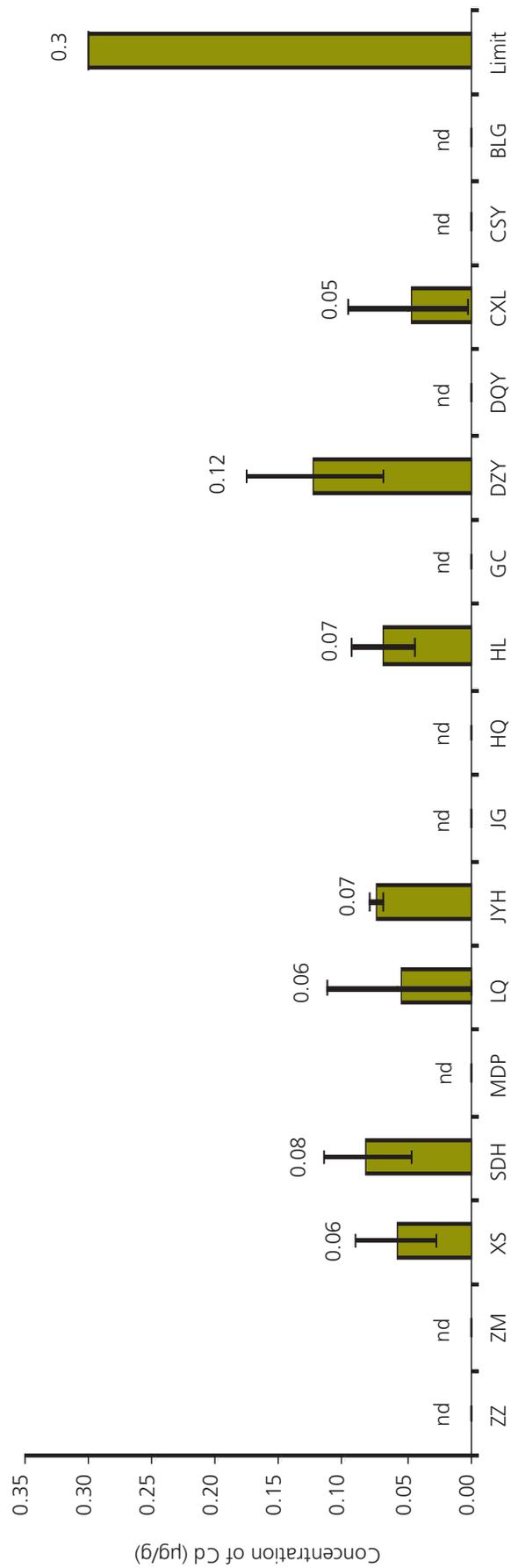
## Appendix 3B. Concentrations of metals in herbal extracts

Appendix 3Bi. The herbs analysed for metals	
Code	Name of herb and plant part
ZZ	<i>Gardenia jasminoides</i> (fruits)
ZM	<i>Anemarrhena asphodeloides</i> (rhizomes)
XS	<i>Scrophularia ningpoensis</i> (roots)
SDH	<i>Rehmannia glutinosa</i> (roots)
MDP	<i>Paeonia suffruticosa</i> (root bark)
LQ	<i>Forsythia suspensa</i> (fruits)
JYH	<i>Lonicera japonica</i> (flower buds)
JG	<i>Platycodon grandiflorum</i> (roots)
HQ	<i>Scutellaria baicalensis</i> (roots)
HL	Coptidis Rhizoma (roots)
GC	<i>Glycyrrhiza uralensis</i> (roots)
DZY	<i>Lophatherum gracile</i> (aerial parts)
DQY	Isatidis Folium (aerial parts)
CXL	<i>Andrographis paniculata</i> (aerial parts)
CSY	Paeonia Rubra Radix (roots)
BLG	Isatidis Radix (roots)

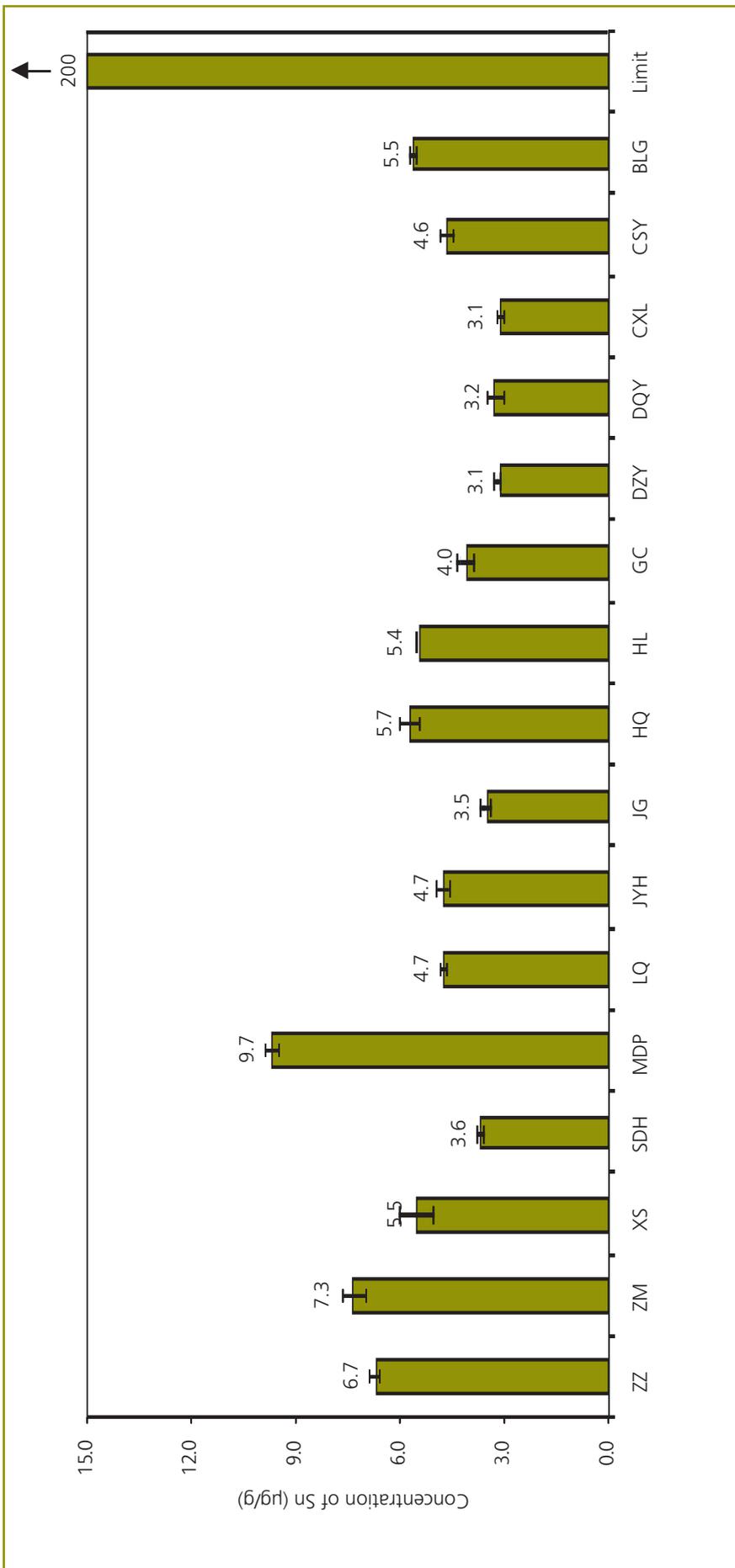


Appendix 3Bii: Average concentrations of Pb in 16 herbs together with standard deviations ( $\pm$  SD). The LOD for Pb in this study was 0.03  $\mu\text{g/g}$ . In China the maximum permitted concentration for Pb (in dried herbs) is 10  $\mu\text{g/g}$  (Chuan *et al.*, 2000). The herbs are describe in the table below.

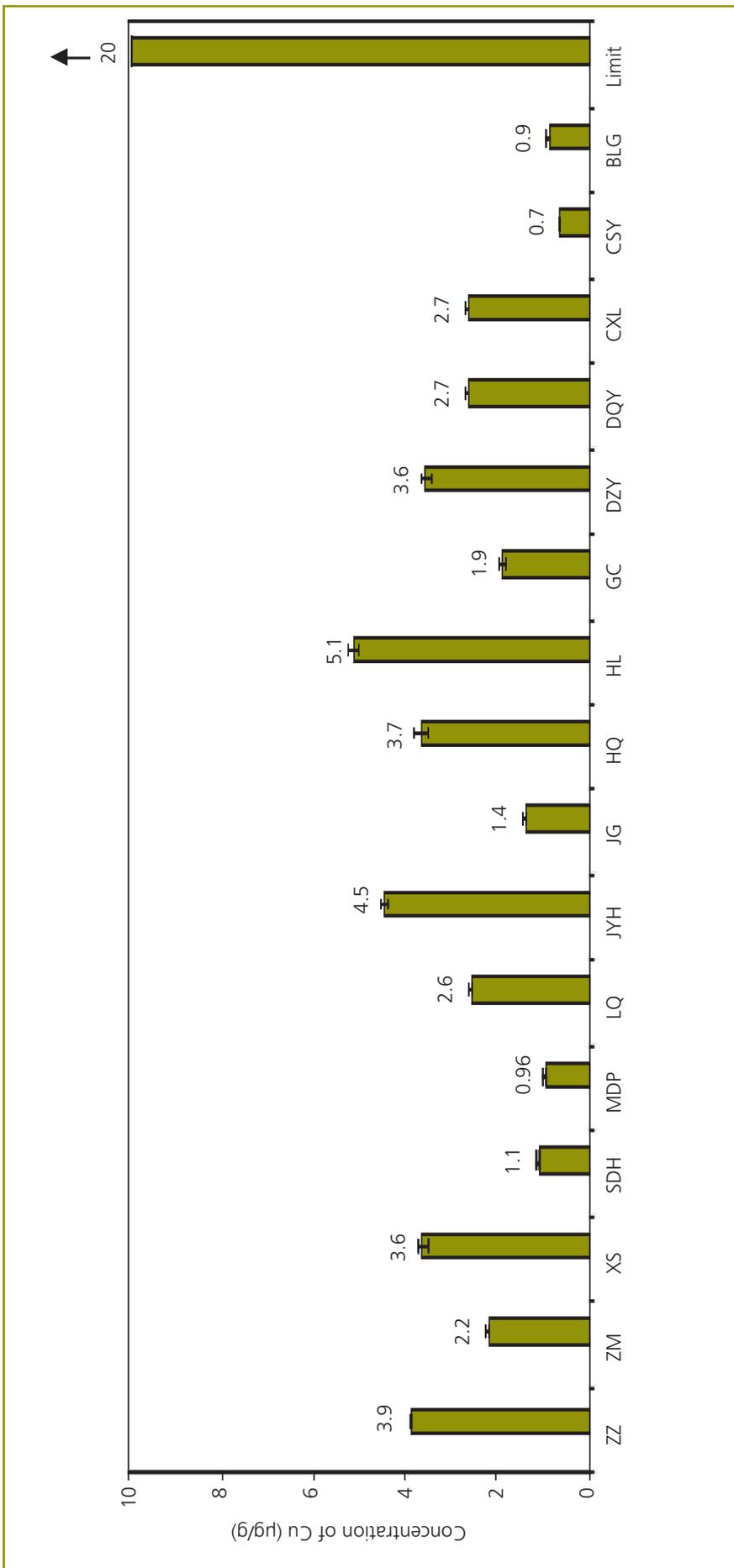
Full names of the herbs are described in Appendix 4Bi.



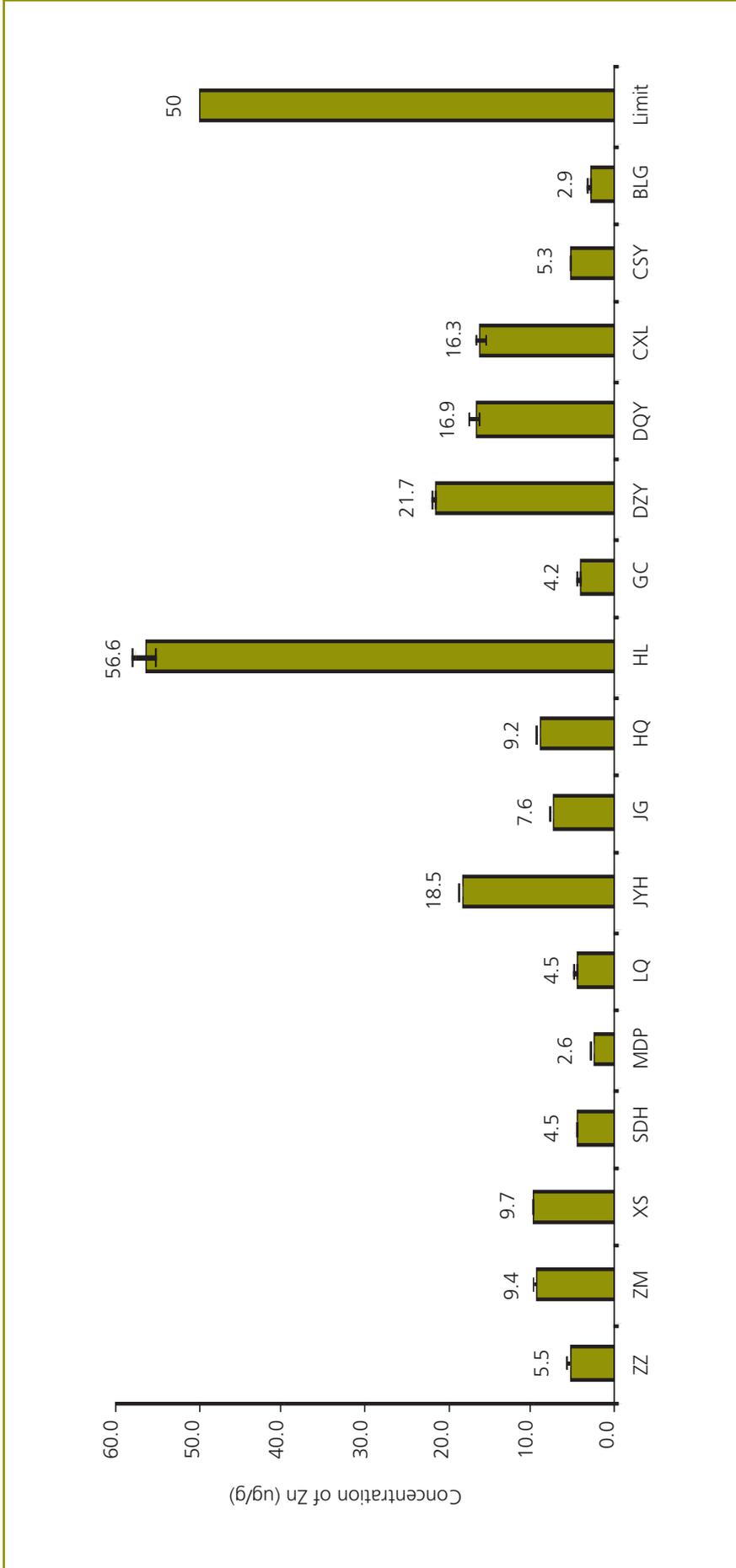
Appendix 3Biii: Average concentrations of Cd in 16 herbs together with standard deviations ( $\pm$  SD). The LOD for Cd in this study was 0.04  $\mu\text{g/g}$ ; nd = not detected above LOD. In China, the current maximum permitted concentration for Cd in food is 0.3  $\mu\text{g/g}$  (Chuan *et al.*, 2000). Full names of the herbs are described in Appendix 4Bi.



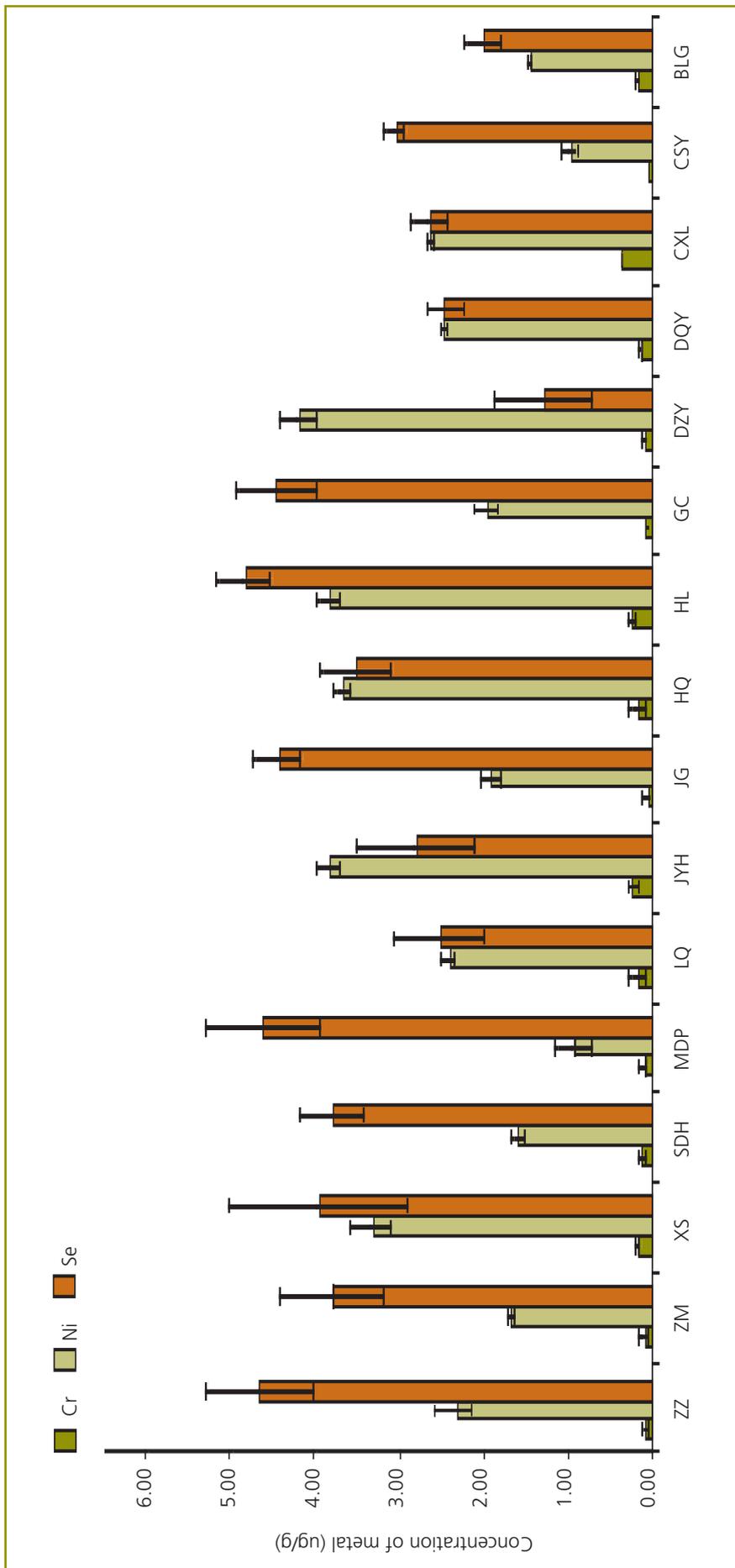
Appendix 3Biv: Average concentrations of Sn in 16 herbs together with standard deviations ( $\pm$  SD). The LOD for Sn in this study was 0.04  $\mu\text{g/g}$ . The UK statutory limit of 200  $\mu\text{g/g}$  for Sn in food was set by the Food Regulations (1992). Full names of the herbs are described in Appendix 4Bi.



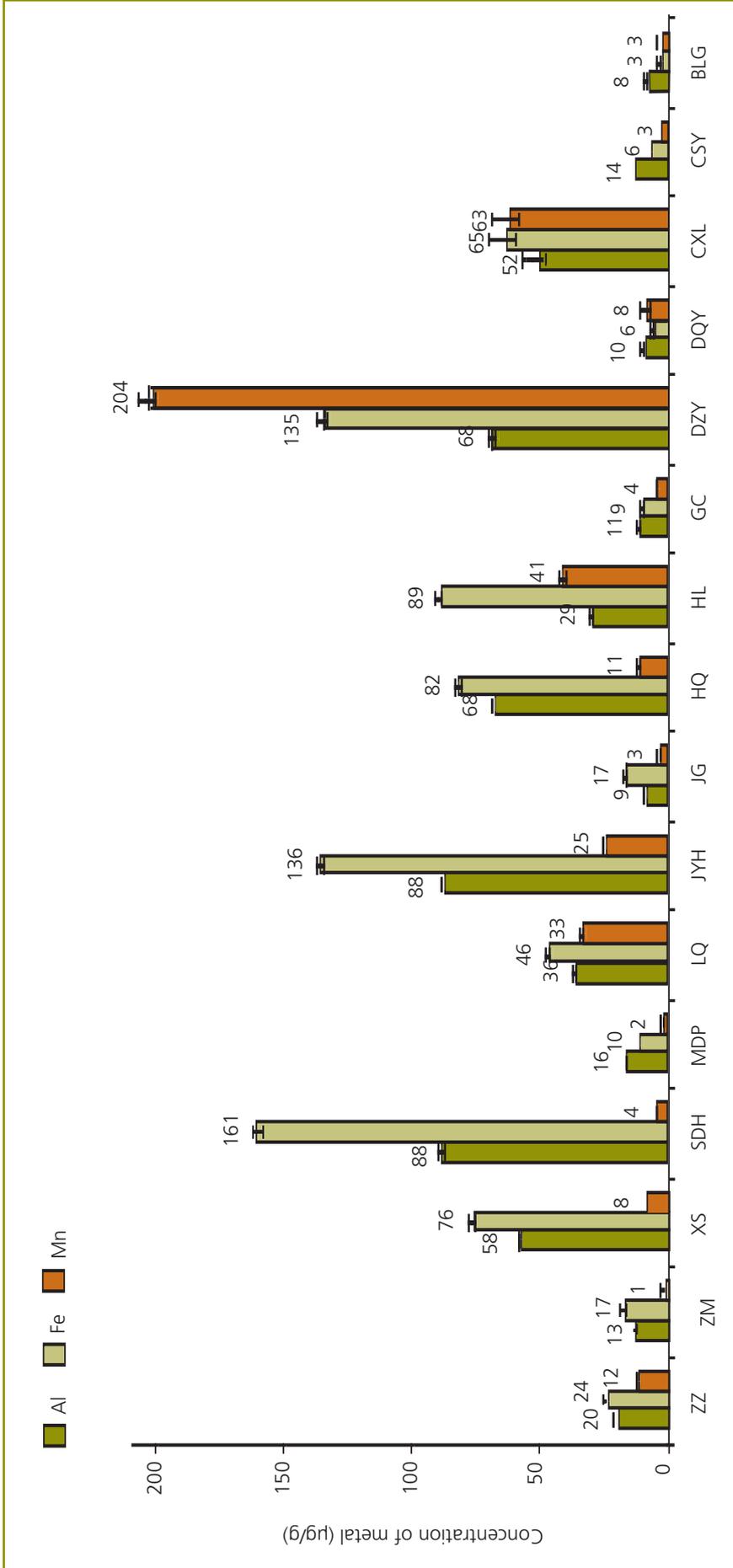
Appendix 3Bv: Average concentrations of Cu in 16 herbs together with standard deviations ( $\pm$  SD). The LOD for Cu in this study was 0.07 µg/g. In the UK, a limit of 20 µg/g of Cu in food was recommended by the Food Standards Committee (MAFF, 1956). Full names of the herbs are described in Appendix 4Bi.



Appendix 3Bvi: Average concentrations of Zn in 16 herbs together with standard deviations (+ SD). The LOD for Zn in this study was 0.11 µg/g. In the UK, the general recommended limit of Zn in food is 50 µg/g (MAFF, 1956; Chuan et al., 2000). Full names of the herbs are described in Appendix 4Bi.



Appendix 3Bvii: Average concentrations of Cr, Ni and Se in 16 herbs together with standard deviations ( $\pm$  SD). The LODs for Cr, Ni and Se in this study were 0.027  $\mu$ g/g, 0.077  $\mu$ g/g and 0.063  $\mu$ g/g, respectively. Full names of the herbs are described in Appendix 4Bi.



Appendix 3Bviii: Average concentrations of Al, Fe and Mn in 16 herbs together with standard deviations ( $\pm$  SD). The LODs for Al, Fe and Mn in this study were 0.058  $\mu\text{g/g}$ , 0.123  $\mu\text{g/g}$  and 0.029  $\mu\text{g/g}$ , respectively. Full names of the herbs are described in Appendix 4Bi.

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