Drug Development for Cardiovascular Diseases from Ayurvedic Plants

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Cardiovascular (CVS) diseases cover a complex range of conditions which result due to several factors. While some of the conditions are a result of defects in the organ itself, others result due to problems with the vascular system, neurological factors (stresses), renal problems, gastro-intestinal and hepatic dysfunctions, endocrine disorders, obesity or diabetes etc.

The oldest ever description of the anatomy and physiology of Heart is given in Rigveda, whereby it is described as an inverted lotus which expands when it awakens and constructs when it sleeps. Ayurveda looks at the cardiac problems under its own perspective, from a multidimensional viewpoint of Prakruti (constitution) of the individual, Tridoshas (body humours) prevailing at the given point of time as well as the condition of Srotases (channels) affecting the CVSystem.

While the modern western system of medicine also accepts the role of different organs and systems (renal, GI and hepatic, and endocrine etc.), including obesity and diabetes, in CVS disorders, the drugs for the management of distortions in these organs (or systems) are at best given the importance of the associated organs requiring attention, as the case may be. However, in the Ayurvedic approach to management of CVS disorders, very often the primary action is importantly sought at the associated organs and their dysfunctions, as the situation may demand. Thus, toning of the entire body is given its due importance as per the basic Ayurvedic philosophy of 'Holistic' treatment.

This can be easily explained by looking at the cardiac as well as extra cardiac etiological factors responsible for a given cardiac condition, *viz.* palpitation, as described in Table 1. Obviously, the management of a patient of palpitation has to be dependent on the etiology of the specific patient. Thus, use of a medicinal plant for the specific condition will be decided accordingly to the extrinsic or intrinsic factors responsible for increased palpitation in the individual.

Table 1: Etiological Factors of Palpitation

(1) Extrinsic or Extra Cardiac

(A) Psychosomatic factors
- Emotional excitement
- Fear or injury

(B) Neurocirculatory
- Asthenia
- During convalescence

(C) Physiological
- Exertion
- Excitement
- Fever

(D) Drug / Addictions
- Tea/ Coffee/ Tobacco/Alcohol/ Ephedrine/Digitalis/Thyroid/ Atropine, Adrenaline

(E) Pollution
- Coal gas
- Car fumes

(F) Abdominal
- Gastric flatulence
- Chronic Amoebiasis
- Diaphragmatic Hernia
- Ascites
- Tympanitis
- Pregnancy

(G) Intrathoracic
- Lung collapse / fibrosis
- Pneumothorax

Pleural effusion
Aortic aneurysm
Mediastinal tumour

(H) Hyperthyroidism

(I) Blood related

Anaemia
Polycythaemia

(2) Intrinsic or Intra Cardiac

(A) Arrythmias

Extra systoles
PAT (Paroxysmal atrial tachycardia)
Atrial fibrillation
Atrial flutter

(*B) Stokes Adams syndrome*

(C) Hypertension

(D) Valvular diseases

Mitral
Aortic

(E) Congenital Heart Disease

A large number of medicinal plants which are used in Ayurveda, depending on the Doshas affecting the Hrd Rog (cardiac disorder), or to reduce obesity (Medorog) or inflammation (Shoth) are described in Table 2. All these plants are used in Ayurved for the management of CVS disorders as per the specific etiology of the patient according to Ayurvedic principles.

Table 2: Major Ayurvedic Medicinal Plants Useful in Cardiac Diseases (Hrid Rog)

Sanskrit Name	Botanical Name
Vatik Condition	
Ajamoda	*Trachyspermum ammi*
Amlaki	*Emblica officinalis*
Haritaki	*Terminalia chebula*
Nagarmotha	*Cyperus rotundus*
Pippali	*Piper longum*
Shunthi	*Zingiber officinale*
Vacha	*Acorus calamus*
Vibheetaka	*Terminalia bellerica*
Paittik	
Draksha	*Vitis vinifera*
Tiktaka rohini	*Picrorhiza kurroa*
Yeshtimadhu	*Glycyrrhiza glabra*
Kaphaj	
Nimba	*Azadirachta indica*
Pippali	*Piper longum*
Shunthi	*Zingiber officinale*
Vacha	*Acorus calamus*
General/Sannipataj	
Abhaya	*Terminalia chebula*
Arjuna	*Terminalia arjuna*
Bala	*Sida cordifolia*
Chitrak	*Plumbago zeylanica*
Darbha	*Ergostrostis cynosuroides*
Duralabha	*Fagoria cretica*
Jeevak	*Macrostylis walichii*
Jeevanti	*Leptadenia reticulata*
Krishna	*Piper nigrum*
Kustha	*Sausarrea lappa*
Meda	*Litsea glutinosa*
Nagbala	*Grewia hirsute*
Palash	*Butea monosperma*
Patha	*Cissampelos pareira*
Pushkarmula	*Inula racemosa*
Riddhi	*Habenaria intermedia*
Rishbhak	*Macrostylis walichii*
Shatavar	*Asparagus racemosus*
Sthira	*Desmodium gangeticum*
Ugragandha	*Acorus calamus*
Vidanga	*Embelia ribes*
Visha	*Aconitum heterophyllum*
A-Obesity (Medorog)	
Adoosa	*Adhatoda vasica*
Agnimantha	*Premna integrifolia*
Amlaki	*Emblica officinalis*
Amruta	*Tinospora cordifolia*
Bakuchi	*Psoralea corylifolia*
Bilva	*Aegle marmelos*
Chabya	*Piper chaba*
Gudmar	*Gymnema sylvestre*
Guggulu	*Commiphora weightii*
Isabgol	*Plantago ovata*
Kantakari	*Solanum xanthocarpum*
Katuki	*Picrorrhiza kurroa*
Lason	*Allium sativum*
Nagarmotha	*Cyperus rotundus*
Punarnava	*Boerhavia diffusa*
Shalmali	*Bombax malabaricum*
Shigru	*Moringa olifera*
Shireesh	*Albizia lebbeck*
Shyamak	*Echinochloa frumentaceae*
Ela	*Elettaria cardamomum*
Vidanga	*Embelia ribes*
Anti inflammatory (Dashmool)	
Vrihad Panchmool	
Bilva -	*Aegle marmelos Corn*

Agnimanth	-	*Premna integrifolia Roxb*.
Syonaka		*Oroxylum indicum Linn.*
Gambhari	-	*Gmelina arborea Roxb*.
Patali	-	*Stereospermum suaveolens DC*.
Laghu		*Panchmool*
Salaparni		*Desmodium gangeticum DC*.
Prsnaparni		*Uraria picta*
Goksuru		*Tribulus terresris*
Brhati		*Solanum indicum*
Kantakari		*Solanum surattense*

While some of the plants, *viz. Acorus calamus, Emblica officinalis, Piper longum, Picroshiza kurroa, Terminalia chebula* and *Zingiber officinale* are used under multiple cardiac conditions only some of these are considered as Hridya (Cardiotonic). Thus, these are important drugs for tuning up the body as a whole. However, if one selects a few important plants which have been shown in modern studies to possess activities for management of CVS disorders, these are shown in Table3. Many of these drugs are also important for efficacy on other associated organs or physiological actions.

Table 3: Major Ayurvedic Medicinal Plants with Modern Studies

S. No.	Plant Name	Part	Ayurvedic Properties	ModernStudies
1	*Aegle marmelos (Bilva)*	fruit	Paachak, Tikta, Kashaya, Balya, Laghu; Ushna; Snigdha	Anti-diarrhoeal, IBS, Hypoglycemic
		root bark	Shotha Har (Vata Har)	Inhibits Palpitation
2	*Berberis aristata (Daruharidra)*	root	Lekhaniya, Paachak, Katu-tikta, Ushna; kapha-shamak; Rasayana	Anti-anginal; A-hypertensive; A-arrythmic; Prevents M-I A-inflammatroy; Immuno-potentiation
3	*Celastrus paniculatus (Jyotishmati)*	fruit	Katu-Tikta; Kpha-vata, Shamaka; Sara; Ushna; Tikshna; Rasayana	Tranquiliser; Anti-inflammatory; Anti-fatigue; Hypolipaedemic; A-atherosclerotic; LDL HDL Hypotensive (ACE)
4	*Curcuma longa (Haridra)*	rhizome	Katu-Tikta; Ruksha; ushna, Kapha-vata shamak; Rasayana;	A-inflammatory; A-obesity; Fibrinolytic, a-atherosclerotic a-thrombotic; Hypolipidaemic; A-oxidant
5	*Emblica officinalis (Aamlaki)*	fruit	Vata,Pitta-Kapha Shamak; Vrshya & Rasayana; Rakta-Pittaghn	Anti-inflammatory; A-fatigue; Rejuvenator (Rasayana); a-oxidant; Cardioprotective, Adaptogenic; Hypolipidaemic;
6	*Inula racemosa (Pushkarmoola)*	root	Katu-Tikta; shotha-Har; Parshva Shoola; Jvaraghna	A-anginal; Ischemic heart disease; A-inflammatory Immunopotentiator

7	*Nardostachys jatamansi (Mansi)*	rhizome	Tikta-kashaya; Balya;	Hypotensive; Tranquilising; Anti-arrythnic;
8	*Nelumbo nucifera (Kamalam)*	rhizome	Sheetal; Vrishya;	Hypotensive;Anti-arrythmic; PAF (aggregation¯); Ca-antagonist; Tranquiliser; Anti-inflammatory
9	*Punica granatum (Dadimba)*	seeds	Hridya; Kashaya; Balya Snigdha; Tarpana	A-atherosclerotic; anti-oxidant; Immunopotentiator
10	*Rauvolfia serpentina (Sarpagandha)*	root	Kapha-vatahar; Hrdavsaadini, Nidraprad;	Anti-hypertensive; Tranquiliser, a-arrythmic; Tachycardia; Anti-atherosclerotic; Anti-obesity
11	*Terminalia arjuna (Arjuna)*	stem bark	Sheeta, Hrdya, Kashaya; Kapha-pitta Shamak	Anti-oxidant; Cardioprotective, Hypolpidaemic; Anti-atherosclerotic;
12	*Terminalia chebula (Haritaki)*	fruit-rind	Ruksha, Ushana,Laghu, Shothahar; Rasayana, Dipana, Hrdya	Anti-inflammatory; a-oxidant anti-atherosclerotic; Hypocholesterolaemic; ¯ACE hypotensive
13	*Tribulus terrestris (Gokshur)*	fruit	Hrd Rog; Balya; Sheetal; Deepana; Vrshya	Diuretic; Anti-hypertensive; Anti-anginal (CVS); Anti-inflammatory
14	*Zingiber officinalis (Shunthi)*	rhizome	Laghu; Paachan; Vrshya; Katu; Snighdha; Ushna; Hrdya;	Digestive; anti-oxidant, A-inflammatory; Cardioprotective (antiplaque); angina (CAD); a-obesity; Hypocholesterolemic

There are a few more important plants which have been shown to possess properties important in CVS disorders. These are shown in Table 4. While many of these plants are primarily important for their action on associated organs but their action to regulate Vata, pitta or Kapha doshas eventually helps in the management of CVS disorders due to aggravation of these doshas. Obviously, many of these plants are shown in modern studies to possess properties to treat CVS disorders.

Table 4: Other Ayurvedic Medicinal Plants with Potential Cardiovascular Properties

Plant Name	CVS Properties
Acorus calamus (Vacha)	Tranquiliser, Heart Disease; Immunomodulator; Hypolipidemic;
Allium sativum (Lason)	Anti-obesity
Andrographis panniculata (Yavtikta)	Anti-inflamatory; Anti-oxidant; Immunostimulant; Hypotensive; Anti-thrombotic; anti-atherosclerotic; □ PAF induced aggregation (Myocardial ischaemia)

Feature

Azadirachta indica (Nimba)	Anti-inflammatory; Immunopotentiating; hypotensive; CAD; anti-arrythmic; Diuretic
Boerhaavia diffusa (Punurnava)	Diuretic; A-inflammatory; cardiotonic; Ca-channel; Immunosuppressant
Commiphora weightii (Guggulu)	Platelet Aggregation (anti-thrombotic) Hypocholesterolemic; Hypolipidaemic, anti-oxidant; anti-atheresclerotic
Desmodium gangeticum (Shalparni)	Anti-oxidant; Anti-inflammatory;
Fumaria indica (Parpata)	Anti-inflammatory; Anti-platelet (anti-thrombotic); anti-arrythmic; Anti-psychotic
Glycyrrhiza glabra (Yeshtimadhu)	Anti-inflammatory; Anti-thrombotic; Antilipaemic Immunosuppresive
Ocimum sanctum (Tulasi)	A-oxidant; Anti inflammatory; Immunomodulator; Anti-stress; Adaptogenic; Cardioprotective (MI);
Phyllanthus fraternus (Bhuamlaki)	Hypolipidaemic; A-inflammatory; ACE □;
Piper longum (Pippali)	Anti-inflammatory; Immunomodulatory; Coronary vasodilator
Plumbago zeylanica (Chitrak)	A-obesity,
Psoralea corylifolia	(A-obesity also) Anti-inflammatory; anti-oxidant; Immunopotentiation; coronary vasodilator; □ Platelet aggregation
Pueraria tuberosa (Vidari)	Coronary vasodilator; anti-angina, □ platelet aggregation; M.I.
Terminalia bellirica (Vibheetaka)	Immunopotentiator; Hypotensive; Smooth muscle relaxant; A-inflammatory; A-oxidant
Withania somnifera (Ashwagandha)	Anti-oxidant; Immunomodulatory Cardioprotective (Prevents MI); Hypotensive; A-oxidant; Antidepressant; Hypocholesterolemic;

If we compare major CVS activities found in some of the important Ayurvedic medicinal plants, it is found that many of the plants which are not primarily in the CVS category in Ayurveda, these are also having several very important activities Table 5. While 19 of these 51 plants possess two type of CVS activities, 9 plants are positive for three or more activities.

Table 5: Reported Cardiovascular Activities of Major Ayurvedic Plants

Plant Name	A-anginal	A-arrythmic	A-atherosclerotic	Hypotensive	A-PAF	Cardioprotective	Hypocholesterolenic	Others
Acacia catechu	-	-	+	+	-	-	-	
Acorus calamus	-	-	-	-	-	-	+	Sedative
Aegle marmelos	-	+	-	-	-	+	-	Sedative

Feature

Aloe vera	-	-	-	-	-	-	+	
Andrographis paniculata	-	-	+	+	+	+	-	A-thrombotic
Azadirachta indica	-	+	-	+	-	+	-	Diuretic
Berberis aristata	+	+	-	+	-	-	-	
Boerhavia diffusa	-	-	-	-	-	+	-	Diuretic, Ca-antagon. A-fibrinol.
Cassia absus	-	-	-	+	-	-	-	
Cassia tora	-	-	-	+	+	-	+	
Cedrus deodara	-	-	-	+	-	-	+	
Celastrus paniculatus	-	-	-	+	-	-	+	
Cissus quadrangularis	-	-	-	+	-	-	-	
Commiphora weightii	-	-	-	-	-	-	+	
Convolvulus microphyllus	-	-	-	+	-	-	-	
Crocus sativus	-	-	-	+	-	-	+	Sedative
Curcuma longa	-	-	-	+	-	-	+	A-thrombotic
Cyperus rotundus	-	-	-	+	-	-		
Emblica ribes	-	-	-	-	-	-	+	A-oxidant
Emblica officinalis	-	-	-	-	-	+	+	A-oxidant
Eugenia jambolana	-	-	-	-	-	-	+	
Fumaria indica	-	+	-	-	+	-	-	
Glycyrrhiza glabra	-	-	-	-	-	-	+	A-oxidant, a-thrombotic
Hemidesmus indicus	-	-	-	-	-	-	+	A-thrombotic A-oxidant
Holarrhaena antidysenterica	-	-	-	+	-	-		–
Inula racemosa	+	-	-	-	-	-	-	B-Adr.blocker
Leptadinea reticulata	-	-	-	+	-	-	-	
Litsea glutinosa	-	-	-	+	+	-	-	A-oxidant, B-adren.block
Momordica charantia	-	-	-	-	-	-	+	Immuno-suppr.
Moringa oleifera	-	-	-	+	-	-	+	A-oxidant

Feature

Nardostachys jatamansi	-	-	-	+	-	-	-	Sedative
Nelumbo nucifera	-	+	-	+	+	-	-	Sedative, A-oxidant, ca-channel antag,
Nigella sativa	-	-	-	+	+	-	-	A-oxidant, Ca-channel antag,Diuretic
Ocimum sanctum	-	-	-	-	-	+	+	A-oxidant
Phyllanthus fraternus	-	-	-	+	-	-	+	ACE,
Picrorrhiza kurroa	-	-	+	-	+	-	-	A-oxidant
Plumbago zeylanica	-	-	+	-	-	-	+	
Psoralea corylifolia	-	-	-	-	+	+	-	A-oxidant
Pterocarpus marsupium	-	-	-	+	-	-	+	
Pueraria tuberosa	+	-	-	-	+	-	-	Vasodilator
Punica granatum	-	-	+	-	-	-	-	A-oxidant
Rauvolfia serpentina	+	+	+	+	-	-	-	A-Adren. block Diuretic
Rubia cordifolia	-	-	-	-	+	-	-	A-oxidant
Semecarpus anacardium	-	-	+	-	-	-	+	A-oxidant
Terminalia arjuna	+	-	+	+	-	+	+	A-oxidant
Terminalia bellirica	-	-	-	+	-	-	-	A-oxidant
Terminalia chebula	-	-	+	+	-	+	+	ACE, A-oxidant
Tribulus terrestris	+	-	-	+	-	-	-	ACE, Diuretic
Trigonella foenum graecum	-	-	-	-	-	-	+	A-oxidant
Withania somnifera	-	-	-	-	-	-	-	Sedative, A-oxidant, Diuretic

Feature

Zingiber officinale	-	-	+	-	+	+	+	A-oxidant

Several of these plants have been shown to contain active principles which have been identified over the years. These are described in Table 6. However, for many of the plants, active principles are yet to be identified. Moreover, there is need for understanding the mechanism of action of many of these plants.

Table 6: CVS Active Principles from Selected Ayurvedic Plants

Plant Name	Active Principles
Aegle marmelos	Aurapten -Palpitation inhibitor (Mouse) (Comparable to Ca-antagonist, Verapamil)
Berberis aristata	Berberine- Hypotensive (Human Trials) Berbamine - Prevents MI, A-anginal, a-arrythmic (Rats, Rabbits)
Celastrus paniculatus	Himacholol-Hypotensive; Femoral blood flow (Rabbits) Ethanolic extract-Tranquiliser, Hypolipidaemic & a-atherosclerotic (Rats, Rabbits) Methanolic Extract- A-Oxidant; Hypotensive (ACE)
Curcuma longa	Curcumin- A-Oxidant (ar-turmarone; turmerin); Platelet aggregation-Fibrinolytic (A-atherosclerotic; a-thrombolytic; Hypolipidaemic & Triglycerides (Human trials) Immunopotentiation (Ukonan A & D also)-Mice
Emblica officinalis	Emblicanin A & B (Fresh juice fraction) - Prevents Ischaemia-reperfusion induced oxidative stress (Rats); Cardioprotective; A-Oxidant (Rats) Immunomodulator-Adaptogenic (Rats); Fresh Juice - Hypolipidaemic; Hypocholesterolaemic (Rabbits; Human)
Inula racemosa	Root powder- Anti-anginal (Ischemic Heart Disease (Humans)- Beta blocker
Nardostachys jatamansi	Jatamansone- Hypotensive (Rats, Cats, Dogs & Humans); A-arrythmic (Rats, Rabbits); Sedative, tranquiliser (Humans)
Nelumbo nucifera	Liensemine & Neferine- Hypotensive and a-arrythmic (Ca-antagonist)-Rats, Rabbits; Platelet aggregation □ (Rabbits);
Punica granatum	Anthocyanins, flavones, Tannins - A-atherosclerosis, (A-Oxidant); □Lipooxygenase & Cyclo-oxygenase (Mice)
Rauvolfia serpentina	Ajmaline- A-arrythmic; Ventricular tachycardia (Humans); Atherosclerotic, PAF (Rats), Hypotensive, Diuretic (Humans, Animals) Reserpine, Ajmalicine-Tranquiliser, A-psychotic (Human, Animals)
Terminalia arjuna	Arjunolic acid- Cardioprotective (Humans); Hypotensive; Bark Powder A-atherosclerotic; (Rabbit, Humans) A-oxidant

Terminalia chebula	Casuarinin, chebulanin, chebulinic acid- A-oxidant (Rat) Gallic acid-A-inflammatory; Immunosuppressant Ellagic acid & chebulin- Hypotensive (ACE)
Tribulus terrestris	Saponins - Coronary Heart diseases (Humans); Coronary circulation (artery dilation); ACE ; Diuretic (Rats)
Zingiber officinale	Gingerols (A-emetic; A-inflammatory- Cox & lipoxygenase (Diarylheptanoids also); Cardioprotection; A-platelet; A-anginal; (E)- 8 b-17, epoxylabd-12-ene-15,16 dial- hypochlesterolimic

Thus, it is the need of the day to extensively study these Ayurvedic plants important for CVS disorders. While some plants may provide leads for developing ethical drugs in the modern western medicine mode, there is a lot of scope for developing standardizes of single drugs as well as combinations of specific drugs to tackle some of the CVS disorders. This is particularly important in light of the fact that there are better chances of getting more effective drugs which are acting at multiple targets. Ayurvedic drugs are an important example of this approach which needs through examination.

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Indian Herbal Medicines

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For centuries people have used plants for healing, to cure and prevent diseases. Herbal medicines are the medicinal products that contain plant materials as their pharmacologically active components. These generally consist of complex mixtures of one or more plants and plant materials. The plant products include botanical materials such as leaves, flowers, fruits, seeds, stems, wood, bark, roots, rhizomes, or other plant parts. The plant materials also include gums, essential oils, resins etc.

The twentieth century became a triumph for the synthetic chemistry dominated pharmaceutical industry, which replaced the natural extracts with synthetic molecules. The spectacular rise of the pharmaceutical industry had tremendous impact on disease management. There is no doubt that in extreme situations, the treatments devised by conventional/modern medicine can offer an unparalleled opportunity to relieve symptoms and save lives.

Today herbal medicines are coming back into prominence because of decreasing efficacy of the modern medicines such as antibiotics, which once had universal effectiveness against serious infections. Over the years infectious organisms have developed resistance to synthetic drugs. Besides, many synthetic drugs are also causing serious side effects.

Ayurveda, the most ancient medicinal tradition of the world is derived from the word Ayur (Life) and Veda (knowledge or science), hence means the science of life. Ayurveda is a holistic system based on the interaction of body, mind and spirit. It aims to bring about a union of physical, emotional and spiritual health. The cosmic energy is manifested in the five elements-ether, air, fire, water and earth - which together form the basis of all matter. The five elements combine to form three basic forces, known as *tridoshas* - which influence all mental and physical processes. From ether and air, the air principle *vata* is created, fire and water yield the fire principle *pitta;* and the earth and water produce the water principle *kapha.*

According to Ayurveda, we are all born with a particular constitution (prakruti), referring to a balance of doshas. These proportions are largely influenced by the balance of doshas in our parents at the time of conception. Our body type, temperament, and susceptibility to illness are largely governed by the predominant doshas. In this way we inherit our basic constitution, called the prakruti, which remains unaltered throughout life.

First requirement of health in Ayurveda is a proper balance of doshas. If the balance is upset, *vyadhi* or disease results. The disruption of this balance may be manifested in physical discomfort, and pain in mental and emotional sufferings.

Our lifestyle has strong effect on overall health, and it easily disrupts the dosha balance.

Medicines, food, and lifestyle activities are all classified according to their effects on the three doshas--*vata, pitta* and *kapha*. An Ayurvedic practitioner prescribes herbal remedies on the basis of their attributes, and aims to balance the patient's doshas, the principles that regulate sickness and health.

For example if a health problem is associated with an excess of *kapha*, it is characterized by catarrh, excess weight, fluid retention and lethargy. The physician prescribes the consuption of warm, dry and light foods, because the quality of kapha is cool and damp. Avoidance of cold, damp foods (such as wheat, sugar, and milk products), which increase *kapha.* would also be advised. Herbal medicines would include warming medicines like ginger, cinnamon, turmeric, etc., thereby restoring the imbalance created by the aggravated *kapha*. In India several Ayurvedic medicines are used routinely as spices and foods in Indian Kichens (Box 1).

BOX-1

Asafoetida (*Ferula asafoetida*)
Cardamom (*Eletteria cardamomum*)
Cinnamon (*Cinnamomum vercum*)
Cloves (*Eugenia caryophyllata*)
Cumin (*Cuminum cyminum*)
Fenugreek (*Trigonella foenum-graecum*)
Garlic (*Alium sativum*)
Ginger (*Zingiber officinale*)
Holy basil (*Ocimum sanctum*)
Lemon (*Citrus limon*)
Nutmeg (*Myristica fragrans*)
Onion (*Allium cepa*)
Pepper (*Piper nigrum*)
Promegranate (*Punica granatum*)
Red pepper (*Capsicum annuum*)
Turmeric (*Curcuma longa*)

Important herbal medicines commonly used in Ayurveda are included in Table-1.

Table1: Important Herbal Medicines in Ayurveda

Plant Name	Medicinal Properties
Achyranthus aspera	pungent, purgative, diuretic, used in piles, boils, skin eruptions, colic and snake bite
Acorus calamus	nervinetonic, antispasmotic, sedative, stomachic, expectorant, emetic, laxative, diuretic
Adhatoda zeylanica	respiratory disorders, asthma and fever
Aloe vera	stomach purgative, anthelmintic, cooling, useful in fevers,
Alpinia galanga	rheumatism, fevers, respiratory troubles, oral antiseptic
Alstonia scholaris	bitter tonic, febrifuge, in malaria, diarrhoea, dysentry
Andrographis panniculata	antihelminthic, useful in debility, dyspepsia and fever
Asparagus racemosus	galactagogue, antispasmodic, antidiarrhoeal, demulcent
Azadirachta indica	skin disease, blood disorders, antibacterial
Bacopa monnieri	nervine tonic, diuretic and sedative
Berberis aristata	skin diseases, menorrhagia., diarrhoea,. Jaundice, and afflictions of the eye
Boerhavia diffusa	diuretic, expectorant, laxative
Boswellia serrata	antiarthritic, analgesic, anti-inflammatory
Buchanania lazan	skin diseases, laxative
Butea monosperma	diarrhoea, flatulence, anthelminthic
Callicarpa macrophylla	rheumatic joint pain, skin disease, blood disease
Calotropis gigantea	antiphlogistic, bronchitis, diarrhoea, tonic, cancer
Cannabis indica	tonic, intoxicant, antispasmodic, insomnia, cachexia, dysmenorrhea , analgesic, narcotic, sedative
Capsicum annuum	rubefacient, stimulant, soar throat, hoarseness , tonic
Carum carvi	flatulence, stomachic, lactogogue
Carum copticum	spastic bowel, flatulence, dyspepsia ,
Cassia angustifolia	laxative, purgative, liver disease, joint pain

Cassia fistula	ringworm, constipation, fever, antibacterial
Cedrus deodara	fever, diarrhea, urinary disorders, rheumatism, piles, gravels in kidney, antidote in snake bite
Celastrus panniculatus	laxative, emetic,aphrodisiac, in rheumatism, leprosy, gout, various fevers, paralysis
Centella asiatica	tonic in diseases of skin, leprosy, nerves and blood
Cichorium intybus	emmenogogue, digestive hepatoprotective
Cinnamomum camphora	tonic in fever, diarrhoea, vomiting, and enlargement of spleen
Cinnamomum zeylanicum	stimulant, carminative used in rheumatism dyspepsia, flatulence, diarrhoea, menorrhagia
Cissampelos pareira	antiperiodic, diuretic, purgative, bitter, in dyspepsia, diarrhoea, and urinary troubles
Cissus quadringularis	In digestive troubles, irregular menstruation, fractured bones, asthma
Clitoria ternatea	purgative, laxative, diuretic
Cocos nucifera	aphrodisiac, diuretic, fever, loss of hair, uterine diseases
Coleus aromaticus	kidney stones, spastic colon, carminative
Cordia myxa (obliqua)	expectorant, colic, dyspepsia, ulcers, cough
Coriandrum sativum	flatulence, colic, joint pain, antiseptic
Crinum deflexum (asiaticum)	emetic, inflammatory conditions
Crocus sativus	nervine sedative, emmenogogue, aphrodisiac
Cuminum cyminum	diarrhea, dyspepsia, antiseptic, hookworm
Curculigo orchiodes	hemmorrhoids, asthma, kidney stones, skin
Curcuma longa	arthritic pain, antiinflammatory, skin disease
Curcuma zedoaria	cough, asthma, leucorrhea, tonsillitis
Cynodon dactylon	diuretic, styptic, hematuria, hemmorrhoids
Cyperus rotundus	anti-inflammatory, flatulence, fever, estrogenic
Datura metal	antispasmodic, joint pain, asthma, dysmenorrhea
Daucus carota	blood purifier, nervine tonic, jaundice
Dolichos biflorus	edema, kidney stone, asthma, dysmenorrhea, tumors,
Eclipta Alba	hepatic deobstruent and tonic, alterative, emetic, purgative, antiseptic, antiviral
Elettaria cardamomum	bronchitis, flatulence, dyspepsia, hemorrhoids
Emblica officinalis	cooling, laxative, stomachic, tonic, diuretic
Evolvulus alsinoides	anxiety, diarrhea, bronchitis, memory loss, fever, tonic
Ferula foetida	flatulence, cough, constipation, palpitations, aphrodisia
Ficus racemosa	diarrhoea, hemorrhoids, bleeding disorders, antiseptic
Ficus religiosa	ulcers, skin diseases, diabetes, constipation
Foeniculum vulgare	cough, flatulence, dysmenorrhea, hookworm, edema
Gmelina arborea	general tonic, to increase strength, antiviral, indigestion
Grewia hirsuta	diarrhoea, wounds, heart disease, fever
Gymnema sylvestre	diuretic, astringent, hypoglycemic, refrigerant, stomachic
Hemidesmus indicus	excellent alterative, to increase appetite, cough, skin
Holarrhena antidysenterica	diarrhea, dysentery, amoebiasis, anthelminthic
Hyoscyamus niger	chronic dementia, hysteria, palpitations, asthma, sedative
Hyssopus officinalis	cough, asthma, bronchitis, amenorrhea
Ipomoea digitata	cough, hoarseness, respiratory stimulant, tonic
Justicia adhatoda	bronchitis, asthma, jaundice, antispasmodic
Linum usitatissimum	cystitis, bronchitis, boils, expectorant, demulcent
Luffa acutangula	splenomegaly, emetic, excellent for skin disease, expectorant
Madhuca longifolia	tonsillitis, cough, rheumatic joints, diabetes, appetizer
Michelia champaca	gastritis, chronic arthritis, emmenagogue, diuretic, colic

Mimosa pudica	menorrhagia, hemorrhoids, skin wounds, diarrhoea
Mimusops elengi	tonic, cardiotonic, urogenital disease, snakebite, skin sores
Morinda citrofolia	acne, eczema, hyperlipidemia, brochitis, diarrhoea
Moringa oleifera	source of vitamin C, colds, boils, fever, joint pain, gout
Mucuna pruriens	nervine tonic, aphrodisiac, parkinsonism, hypercholesterolemia
Nardostachys jatamansi	nervousness, anxiety, dysmenorrhea, insomnia, hair tonic
Nelumbo nucifera	refrigerant, sedative, demulcent
Nyctanthes arbor-tristis	liver diseases, constipation, anthelminthic, antihistaminic
Ocimum sanctum	demulcent, expectorant, anticatarrhal, antispasmodic, anthelminthic
Paederia foetida	rheumatic joint pain, edema, bladder stones, inflammation
Papaver somniferum	anxiety, diarrhoea, aphrodisiac, sedative
Peucedanum graveolens	flatulence, colic, abscesses, digestive
Phyllanthus fraternus(amarus)	jaundice, liver disease, fever, genitourinary disease, edema
Picrorhiza kurroa	hepatitis, asthma, anorexia
Piper nigrum	dyspepsia, cough, pharyngitis, headache, diarrhea
Plantago ovata	constipation, colitis, irritable bowel, cystitis
Plumbago zeylanica	abortifacient, warts, rheumatic joint pain
Premna integrifolia	flatulence, fever, arthritis, liver deobstruent
Prunus amygdalus	mental energy, general tonic esp. nerve & kidney, semen,
Pterocarpus santalinus	skin tonic, liver disorders, fever
Punica granatum	anthelminthic (esp. tapeworm), diarrhoea, dyspepsia
Randia dumentorium	fruit and rind are emetic, diaphoretic, and antispasmodic; bark is sedative and nervine calmative.
Rauwolfia Serpentina	hypertension, anxiety, insomnia, colic
Rubia cordifolia	Skin diseases and disorders of spleen
Sausserea lappa	respiratory disorders and rheumatism
Swertia chirata	chronic fevers and liver disorders
Terminalia arjuna	treatment of cardiac disorders, ulcers and diarrhoea
Terminalia bellirica	gastrointestinal disorders,cardiotonic, antitussive
Terminalia chebula	Tonic, restorative, antiageing, purgative
Tinospora cordiflora	cure for diseases of liver
Tribulus terrestris	Potent aphrodsic, treatment of urinary sones
Vitex negundo	Parasitic infections, antidote to poisioning and treatment of rheumatism
Withania somnifera	restorative and anabolic agent
Zingiber officinale	digestive stimulant, antiseptic for throat disorders
Abutilon indicum	demulcent, astringent, diuretic
Aegle marmelos	gastric stimulant, treatment of chronic dysentery and diarrhoea
Albizza lebbeck	snake bite and scorpion sting, and diarrhoea, tonic, restorer
Bauhinia variegata	Tonic, useful in skin diseases, ulcers, dysentery, Piles, antidote for snake poison
Bergia odorata	cleaning teeth and applied to broken bones, leaves as poultice for sores.
Caesalpenia bonduc	bitter leaves used as emmenogoguge and roots prescribed in dysentry
Commiphora wigheti	anti-inflammatory, hypolipaedermic
Crataeva nurvala	demulcent,stomachic, laxative, diuretic, antipyritic
Curcuma Amanda	carminative, stomach cooling, applied over contusions and sprains
Desmodium gaganticum	astringent, in diarrhoea, tonic, diuretic, chronic fever, biliousness, cough, vomiting, asthma, snake bite and

	scorpion bite
Ephedra vulgaris	for controlling asthmatic paroxysms.Tincture of ephedra is cardiac and circulatory stimulant
Fumaria parviflora	antihelminthic, diuretic, diaphoretic,aperient, in low fever, blood purifier in skin diseases
Garcinia indica	antiscorbutic, cooling, cholagogue, emollient, demulcent, astringent
Gentiana kurroa	tonic, for stomach problems, urinary infections,
Gloriosa superba	purgative, cholagogue, anthelminthic, given internally in gonorrhoea, in snake bite and scorpion sting, abotifacient
Glycyrrhiza glabra	tonic, laxative, demulcent, cough and sore throat
Inula racemosa	used as expectorant and as resolvent. In indurations
Nigella sativa ,	carminative, diuretic, useful in mild cases of puerperal fevers, used in external applications of skin erruptions
Piper longum	used in bronchitis,cough and coldantidot to snake bite, and scorpion bite,
Pluchea lanceolata	aperient, substitute for senna,
Pongamia glabra	As external application in skin diseases, in bleeding piles
Pterocarpus marsupium	good astringent in diarrhoea and purosis, used for tooth ache
Ricinus communis	Purgative, counter irritant, in scorpion biting, leaves applied to head for relieving headache,
Santalum album	for headache, fevers, local inflammation, and stomachic, skin diseases allay heat and puritis
Saraca asoca	used in uterine affections
Semicarpus anacardium	To procure abortion,vermifuge,used externally in rheumatism and leprous nodules
Sida cordifolia	facial paralysis and sciatica, oral administration for relief of frequent micturation
Solanum indicum	carminative, expectorant, useful in asthma and cough
Solanum nigrum	chronic enlargement of liver, blood spitting piles, psoriasis, fevers, diarrhoea, dysemtery
Solanum surattense	asthma, eosinophilia, cough
Syzygium aromaticum	stimulant, aromatic, carminative, used in flatulance
Tephrosia purpurelaxative	antihelminthic for children,blood purifirea
Tylophora indica	useful in the overloaded states of the stomach, emetic, diaphoretic, expectorant
Urarica picta	applied to thesore mouths of children, antidot for snake bite,
Vetiveria zizanioides	cooling, aphrodisiac, tonic
Viola odorata	antipyretic, emetic, used in bilousness and lung troubles
Woodfordia fruticosa	astringent, used in dysentery, menorrhagia, in derangements of liver, disorders of mucus membrane
Writia tinctoria	

Herbal medicines often complement the conventional modern treatments providing safe, well-tolerated remedies for many chronic diseases like rheumatoid arthritis, diabetes, obesity cardiovascular and neurological disorders and asthma.

The ability of herbal medicines to affect the body systems depends on the chemical constituents they contains. The contents include--phenols, tannins, coumarins, flavonoids, saponins, cardiac glycosides and alkaloids etc.

Research into the isolated plant constituents is of great importance. Many of today's medicines are either obtained directly from a natural source or were developed from a lead compound originally obtained from a natural source. Morphine, cocaine,

digitalis, quinine, tubocurarine, nicotine, muscaline are some of the many lead compounds, useful as drugs in themselves or the basis for synthetic drugs. Some of the active principles obtained from Ayurvedic plants are given in Table 2.

Table2: Drugs and Lead Molecules from Ayurvedic Herbal Medicines

Name of plant	Therapeutic use in Ayurveda	Pharmacologically active compound (s)	Pharmacological effects
Rauvolfia serpentina	Hypertension, insomnia, insanity	Reserpine, Ajmaline, Ajmalicin, and Deserpidine	Treatment of hypertension
Psoralea corylifolia	Vitiligo, inflammatory and infective conditions of skin	Psoralene and Bakuchiol	Vitiligo, stimulate the formation of melanin, antimicrobial, used in mouth wash
Commiphora weightii	Rheumatoid arthritis, obesity, skin diseases	E-Guggulsterone Z-Guggulsterone	Antihyperlipoproteinemic
Cedrus deodara	Cough, bronchitis, inflammation, skin diseases	Himacholol Alpha-Himachalene Beta- Himachalene	Spasmolytic Anti-inflammatory
Cannabis sativa	Psychoactive	Cannabinoids	Psychotropics
Berberis aristata	Afflictions of eyes, ear and mouth	Berberine	Antiprotozoal (Leishmania), antimalarial, anti-bacterial, antidiarrheal
Adhatoda vasica	Antispasmodic and cough supressant	Vasicin	Lead molecule for bromhexin and ambroxol
Papaver somniferum	Analgesic, antitussive	Morphine Codeine Papavarine	Analgesic(narcotic) Analgesic, antitussive Cerebral vasodilator
Nicotina species	Sedative narcotic, emetic	Nicotine	Smoking cessation therapy
Cassia angustifolia	Laxative, purgative	Sennosides A and B	Laxative
Digitalis lantana	Cardiac stimulant and tonic	Digoxin and digitoxin	Cardiotonic

Modern allopathic medicine usually aims at developing a patentable single compound to treat specific conditions of a disease. Traditional medicines often aim at restoring the body balance by using plants which contain thousands of chemical compounds, or mixing together several different plants in order to maximize a synergistic effect or to improve the likelihood of an interaction with a relevant molecular target or a series of targets in the pathway. In most of the societies today, allopathic and traditional medicines are used side by side in a complementary way. The former

treates serious acute conditions while the latter is used for chronic illness, to reduce symptoms and improve the quality of life in a cost effective way.

Although it is important to understand the actions of individual active constituents, herbal medicines, unlike conventional medicines are concerned, ultimately about the use and action of whole plants or their extracts. Separating a medicinal herb into its constituents cannot often explain exactly the way in which it works in the natural form. The whole herb is worth more than the sum of its components. A plant contains hundreds of chemical constituents that interact in a complex way to produce therapeutic effects of the remedy.We may not understand the detailed mechanism, in which a particular herb works – even though its medicinal benefits are well established.

New biochemical studies/researches are showing that the active constituents of many herbs, interact in a complex way to produce therapeutic effect of the remedy as a whole. For example tea (*Camellia sinensis*) and coffee (*Caffea arabica*) contain approximately the same levels of caffeine. Tea, however, contains a much greater quantity of tannins. The tannins present in the tea reduce the amount of nutrients and drugs that are absorbed from the intestines into the blood-stream and consequently less caffeine is absorbed. As a result tea is less stimulating than coffee.

There is another fundamental fact about herbal medicines. Evolutionarily, we have evolved side-by-side with plants over thousand of years and our digestive system and physiology as a whole are geared to digesting and utilizing plant-based remedies.

For Further Reading:

The Encyclopedia of Medicinal Plants; Edi. Penny Warren; Published: Droling Kindersley Limited, London.

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Editor

Feature

Combinatorial Biosynthesis of Medicinal Plant Secondary Metabolites

1. Introduction

The approach to combine genes from different microorganisms for the production of new and interesting metabolites has become known as combinatorial biosynthesis. Recent achievements with the polyketide biosynthesis from microorganisms, especially in *Streptomyces*, prove the potential of combinatorial biosynthesis. It also showed that this approach can be used to improve the biosynthesis capacity of known producing microorganisms like *Escherichia coli*, *Bacillus subtilis* or *Saccharomyces cerevisiae*. The heterologous expression of human genes in microorganisms is well known for more than 30 years now.

Fundamental work on the expression of plant genes from biosynthetic pathways, performed since the 1980s, opens a way to similar research that may even be extended in the future by directed evolution. It is now possible to combine these genes and extend the realm of combinatorial biosynthesis far beyond the polyketide biosynthesis. The diversification of products will increase dramatically when genes of very different origins are used. However, there is no need to concentrate on new compounds only; there are many interesting natural products, of which the application (e.g. as a drug or fine chemical) is hampered by its availability. This problem might be solved by using alternative production systems yet to be discovered, that are based on enzymes from other biosynthetic pathways.

Nature and its huge biodiversity harbor an endless source of compounds containing unique chemical structures. Even on a species level a given biosynthetic pathway adapts through the continuous selection pressure of its surrounding. Only those compounds that are highly favorable for the producing organism are accumulated, which is a delicate balance between energy cost and physiological/ecological benefit. There are many speculations about how evolution diverges biosynthetic pathways. Often the result is that specific compounds are produced by specific organisms. There are certainly products that will not be produced because they cost too much energy to synthesize, their activity is not beneficial enough or the organism lacks the enzyme machinery to perform a specific chemical reaction. In other words, the biodiversity is endless and there are still possibilities to enlarge the diversity from a chemical point of view, by combining genes and products from different sources that in nature would never meet. This strategy will deliver compounds that are not influenced by selection pressures, by a habitat, or the biochemical limitations of an organism (such as compartmentalization or storage). These compounds can be selected for a specific pharmaceutical mode of action or an activity can be adjusted to a more specific pharmaceutical demand.

There are several pharmaceuticals in the market that are highly expensive, due to the fact that these compounds are only found in rare plants and often in extreme low concentrations. Podophyllotoxin and paclitaxel are clear examples of pharmaceuticals that can only be produced through the isolation from plants. To achieve a sustainable source of such compounds scientists all over the world have been experimenting with biotechnological approaches aiming at the development of an alternative production system. With this aim in mind, combinatorial biosynthetic

strategies are expected to yield interesting alternatives in the near future. With regard to the production of podophyllotoxin it has been shown that plant cell cultures of *Linum flavum* L. can be used to convert deoxypodophyllotoxin, a major lignan of *Anthriscus sylvestris* L. into 6-methoxypodophyllotoxin. The combination of the product of one species and the enzymes of another species to yield a desired product is a good example of combinatorial biosynthesis.

Not only can the expression of a single gene be of interest. The reconstruction of complete biosynthetic pathways by combining genes of the desired pathway in host organisms is the current aim of actual research projects. There are many papers describing the functional heterologous expression of single genes from biosynthetic pathways. Still in contrast the coupling of more genes and the controlled expression of genes encoding biosynthetic enzymes for metabolizing precursors is a challenging approach. Thus far, the biosynthesis of flavonoids in *E. coli* is the only total heterologous biosynthesis of a plant compound that has been described, but promising results have been reported already for the biosynthesis of artemisinin paclitaxel and srictosidine. The authors discuss here the biosynthesis of specific natural products in detail, and we want to give insight in the basic understanding of the concept of combinatorial biosynthesis of other natural products, which is gaining more and more interest.

2. Definition of Combinatorial Biosynthesis

The definition of combinatorial biosynthesis has been changed and is still changing because of the rapid developments in molecular biological techniques and innovative strategies applied in this research area. From the past, combinatorial biosynthesis is defined on the metabolic level, using different precursors or further modification of a structural scaffold.

The concept of combinatorial biosynthesis has been introduced from the work with polyketides and oligopeptides. These natural products were model compounds showing that repeated use of the same type of reaction with different precursors like acetyl-CoA units or amino acids can lead to a combined biosynthetic product. The finished peptide or polyketide scaffold can be post-translational structurally modified. Also this step has been accepted as part of combinatorial biosynthesis. An important example of combinatorial biosynthesis on the metabolic level is the development erythromycin analogues, which are impossibly obtained by synthetic organic chemistry. The scope of combinatorial biosynthesis and the number of structural variants, which can be generated by manipulation of biosynthetic modules, is limited by the specificity of different domains and modules for initiating, extending and terminating the growing chain of the polyketide or the nonribosomal peptide, or even by combinations thereof. To date, combinatorial biosynthesis of natural products has to be defined wider, not focusing the metabolic level only. With the current knowledge of molecular biology, it has become possible to combine genes (thus also the resulting enzymes) and products of different organisms. This can yield a further diversification of both chemical and natural product libraries. Because these strategies have also become known as combinatorial biosynthesis, we define combinatorial biosynthesis has been defind also as the approach to combine genes from different organisms to produce bioactive compounds.

Current research in this field still focuses mainly on the polyketide biosynthesis in microorganisms. But a careful examination of the literature on plant biotechnology reveals that several studies have already been carried out in the past twenty years that can now be called combinatorial biosynthesis as per the new definition. Due to the strategy of combinatorial chemistry at the beginning of the eighties, which uses a random approach to synthesize novel polymeric or oligomeric chemical entities from uniform monomers (e.g. amino acids), the term combinatorial biosynthesis since the 1990s suggested a random approach and combination of genes in the polyketide or terpenoid biosynthetic pathways using also biosynthetic monomers (e.g.

isoprenes, acetyl and propionyl units) from natural origin. Today, one would like to add to this definition the possibility to have directed and controlled combination of genes to produce a desired single compound. At the moment combinatorial biosynthesis of plant secondary metabolites focuses on the reconstruction of the basic pathways into microbial hosts. This review gives a survey of the use of genes and products from plants in combination with genes and products from other organisms. It emphasizes the potential of plant combinatorial biosynthesis for drug discovery and its future importance for pharmaceutical sciences.

3. Bioconversion Capacity of Plant Cells

Bioconversion can be defined as the transformation of one chemical into another using a biocatalyst. The biocatalyst can be a cell (e.g. microorganism, plant or animal cell), a vital extract from such cells, or a (partly) purified enzyme. The biocatalyst may exist free, in solution, immobilized on a solid support or entrapped in a matrix. In bioconversions by whole cells or extracts one single enzyme or several enzymes may be involved.

In the past 20 years the use of enzymes as catalysts for the preparation of novel organic molecules has received increasing attention. Enzymes can catalyze a wide range of reactions; it is likely that nearly all existing compounds can react with an appropriate enzyme. Even persistent environmental pollutants such as pesticides, raw oil, and halogenated hydrocarbons can be degraded by certain bacterium species.

Each individual cell contains a plethora of enzymes that can display different catalytic properties depending on the conditions to which they are exposed. From genome studies on different organisms it is well known that approximately 30,000 up to 35,000 genes are present in a plant organism. Not all genes are involved in the biosynthesis of secondary metabolites, but a number of proteins/enzymes are capable of catalyzing more than one reaction, which will compensate the above assumption. This means that in a higher plant around 30,000 low molecular weight products are biosynthesized and should be present. Today, from no plant species the metabolic profile is completely known, and because of the insufficient analytical techniques and the broad range of polarities and quantities of natural products it seems to be an unrealistic endeavour. Some of the best studied plants are *Arabidopsis thaliana* and *Nicotiana tabacum* (tobacco), but here the number of known constituents does not exceed 3000.

Since each enzyme has its own specific active site, enzymes usually show selectivity towards their precursor. However, a number of enzymes with broad specificity are known. There are two specific properties of enzymes that most chemical catalysts do not possess, making them especially interesting for combinatorial biosynthesis: stereo- and regiospecificity. Stereospecificity: enzymes are chiral catalysts and often able to produce molecules of high optical purity, which characteristic can be of influence on the biological activity. This makes the production of such compounds by enzymes especially interesting as pharmaceuticals. For example, the active stereoisomer of the antimalarial artemisinin is biosynthesized in *Artemisia annua* L. plants while 128 (27) stereoisomeric forms are theoretically possible. Two areas of bioconversions that have great relevance for organic synthesis involve the use of hydrolase enzymes on the one hand and oxidoreductase enzymes on the other, e.g. in cells of *Papaver somniferum.* The stereocontrolled formation of carbon–carbon bonds is the heart of organic synthesis and this reaction is performed by aldolase enzymes. The search for and the employment of lipases, esterases and amidases for the preparation of chiral compounds of high optical purity will also be continued in the future.

General reaction types catalyzed by enzymes like oxidation, reduction, hydroxylation, methyl-lation, demethylation, acetylation, isomerization, glycosylation, esterification, epoxidation and saponification can all be used in the diversification of compounds and are therefore relevant in combinatorial biosynthesis.

The yield of a plant biosynthetic pathway leading to important or expensive products can be improved by feeding precursors or intermediates. This may be an economically interesting strategy when the precursors are cheap and easily obtainable. The enzyme-catalyzed modification of added precursors, into more valuable products has been performed with plant cells, either freely suspended or in an entrapped state, or with enzyme preparations, sometimes from a heterologous host organism. These biocatalytic systems are mostly able to perform stereo- and regiospecific reactions on a sometimes surprisingly broad precursor range, even including cell-foreign, chemically prepared compounds. From a pharmaceutical point of view, hydroxylations and glycosylations are considered to be particularly useful bioconversions. They can yield new drugs and existing drugs can be improved as to increased activity and decreased toxicity. The biological availability, meaning the overall blood concentration a drug reaches after administration and resulting in a therapeutic action, can be enhanced by the introduction of hydrophilic moieties in the pharmacological active molecule. The therapeutic action can either be prolonged by the introduction of protecting groups resulting in so-called prodrugs, or be increased when the new moieties enhance the affinity for target cells or receptors involved. Furthermore, side-effects can be reduced and the stability increased by modification of the parent drug. In the following sections of this chapter we concentrate on the role of plant cells and their enzymes as biocatalysts for the production of plant secondary metabolites and related compounds with special attention to (potential) pharmaceuticals. The sections are ordered by their biosynthetic pathways.

4. Combinatorial Biosynthesis of Terpenoids

Terpenoids represent a large and important class of natural products with more than 30,000 different structures. Terpenoids (consisting of C5 (isoprene) “building blocks”) are known for their wide commercial applications, such as flavour and fragrance additives. Essential oil constituents are predominantly monoterpenoids (C10) and sesquiterpenoids (C15). From a pharmaceutical point of few the sesquiterpenoids are of high relevance. In this group artemisinin, gossypol, and zingiberene are of high medicinal and economic interest. Furthermore diterpenoids (C20) are of high interest and paclitaxel as a major representative of this group is a blockbuster drug. Carotenoids with a C40 backbone show important functions in photosynthesis, pigmentation and as antioxidants. From a pharmaceutical point of view the most important class of terpenoids is without doubt formed by the sterols, which are derived from a C30 backbone and are used as starting material in the organic synthesis of synthetic drugs like steroid hormones and contraceptives.

Terpenoids are biosynthesised via the mevalonate (MVA) pathway or the deoxyxylulose phosphate (DOXP) pathway. Both pathways are described elsewhere in an excellent way.It is important to mention that the upstream biosynthetic steps are genetically well mapped out. In contrast to other pathways, such as alkaloids or phenolics, the current knowledge allows transfer of the steps from the terpenoid pathways into microbial hosts and to hook on with extended pathways for higher terpenoids. The MVA pathway has recently been established in *E. coli*, harbouring the DOXP pathway itself, and an efficient production was shown for the terpenoids amorpha-4,11-diene .

For the production of artemisinin or paclitaxel the presence of a terpene cyclase is a prerequisite. The conversion of a linear isoprenoid precursor (e.g. farnesyl diphosphate or geranylgeranyl diphosphate) to a cyclic terpene such as amorphadiene or taxadiene for artemisinin or paclitaxel, respectively, is still considered as a rate limiting step in the overall biosynthesis. Terpenoid cyclisation involves the generation of a reactive carbocation and moving of the ions over the isoprene backbone and correct dimensional coupling to the desired cyclic form. Terpene cyclases have been described for microorganisms and plants, which often contain multiple cyclase genes as has been shown for *Arabidopis thaliana* with 40 different genes.

4.1. Artemisinin

Artemisinin is an antimalarial drug isolated from *A. annua*, Asteraceae. The drug is especially used in those areas where resistance of *Plasmodium falciparum* against the commonly used antimalarials is often found. Until now the costs for artemisinin treatment are much too high for most people in low income countries suffering from (life threatening) malaria. Several initiatives have been undertaken to lower the costs for this treatment. The selection of plants yielded varieties containing 0.5–0.8% of artemisinin in the aerial parts based on dry weight. Alternatives could be the production via transgenic plants or engineering the biosynthetic pathway into less complex host cells. This implies that the full elucidation of the biosynthetic pathway is required. Although several biosynthetic pathways have been postulated, until now only the genes encoding the enzymes for the synthesis of the first specific intermediate amorpha-4, 11-diene by amorphadiene synthase and for artemisinic acid by the cytochrome P450 enzyme, CYP71AV1 have been isolated and identified. The cDNA encoding amorphadiene synthase has been expressed in *E. coli* and characterized. The recently discovered enzyme, CYP71AV1, has been shown to be able to catalyse the regioselective oxidation of amorpha-4,11-diene into artemisinic alcohol. Besides this metabolic action the enzyme has been shown to be able to oxidize the precursors artemisinic alcohol and artemisinic aldehyde yielding artemisinic acid. Nevertheless, more effort should be invested in the elucidation of the subsequent steps of the pathway leading to artemisinin.

Despite the lack of knowledge of the entire biosynthetic pathway, research already achieved some progress in the metabolic engineering of host cells for the production of amorpha-4, 11-diene. An artificial fusion of the proteins farnesyl diphosphate synthase from *A. annua* and 5-epi-aristolochene synthase from *N. tabacum* yielded a bifunctional enzyme producing the sesquiterpenoid structure from isopentenyl diphosphate (IDP) and geranyl diphosphate (GDP) . The same technique could be applicable for amorphadiene synthase.

The concept of *E. coli* as a host cell producing sesquiterpenoids out of the endogenous pool of farnesyl diphosphate (FDP) has been investigated.This work resulted in the production of 10.3 μg of (+)-δ-cadinene, 0.24 μg of 5-epi-aristolochene, or 6.4 μg vetispiradiene per liter of bacterial culture. Furthermore, the authors concluded that the poor expression of the plant terpene cyclases was limiting for the synthesis of sesquiterpenes and not the endogenous supply of FDP. This has been confirmed in their further work by coexpressing the *E. coli dxs* gene, which did not result in an increase of sesquiterpenoids produced where it did result in an increase of lycopene production in *E. coli.*

To overcome the low enzyme levels, the expression of amorphadiene synthase has been optimized by constructing a synthetic amorphadiene synthase gene completely optimized for the expression in the bacterial host. This strategy has been combined with engineering of genes from the mevalonate dependent isoprenoid pathway, which resulted in an *E. coli* strain producing 24 μg/ml amorpha-4, 11-diene (calculated as caryophyllene equivalent) from acetyl-CoA after supplementation of 0.8% glycerol.

Recently, attempts to use *S. cerevisiae* for the production of artemisinin precursors have been described. The expression of the amorphadiene synthase gene in yeast using plasmids and chromosomal integration led to the production of respectively 600 and 100 μg/ml amorpha-4,11-diene after 16 days batch cultivation. Using a *S. cerevisiae* strain containing an engineered MVA pathway coupled with the genes encoding amorphadiene synthase and CYP71AV1 the production of artemisinic acid up to 100 mg/l has been reported. This strain transported the artemisinin precursor outside the yeast cell, which makes purification of the product less complex. Artemisininc acid can be used for the semi-synthesis of artemisinin, but to lower the costs for production of the drug bioprocessing must be optimized.

4.2. Paclitaxel

Paclitaxel , mostly described by the tradename Taxol®, is a diterpenoid that can be found in the bark and needles of different *Taxus* trees. Paclitaxel was first isolated from *Taxus brevifolia* (pacific yew tree) Taxaceae in the sixties from last century, and its derivative Taxotere® was clinically introduced 30 years later for the treatment of mainly ovarian and breast cancers. Isolation from the *T. brevifolia* bark is a problem, because of the low yield (500 mg kg^{-1}). Facing the high demand, various *Taxus* species are endangered in China and India. Paclitaxel has a complex chemical structure. Its total synthesis has been established, but the complexity and low yield of this alternative for natural sources made it commercially inapplicable. Semisynthetic approaches have been more successful, since the more easily available intermediate taxoids, like 10-deacetylbaccatin III, can be isolated from the green needles of various *Taxus* species and used as starting material. Nevertheless, the production of paclitaxel still relies on the yew species or on cell culture systems derived from these plants. *Taxus* cultures elicitated by methyl jasmonate showed an increased biosynthesis of paclitaxel.

The biosynthesis of paclitaxel starts with the cyclisation step from geranylgeranyldiphosphate (GGDP) to taxadiene. Most of the 19 known enzymatic steps in the biosynthesis are related to hydroxylation and other oxygenation reaction of the taxadiene skeleton. Croteau and coworkers isolated and identified several genes from differrent *Taxus* species that are responsible for steps in the biosynthesis and built a basis for today's combinatorial biosynthesis in a heterologous microorganism. Today, all genes have been cloned into *E. coli* and activity screening confirmed the function of isolated enzymes , . The first intermediate, taxadiene can now be produced in *E. coli*. Co-expression of the taxadiene synthase from *T. brevifolia* with a geranylgeranyl diphosphate synthase isolated from *Erwinia herbicola,* isopentenyl diphosphate synthase from *Schizosaccharomyces pombe,* and the endogenous deoxyxylulose 5-phosphate synthase from *E. coli* resulted in a production of 1.3 mg taxadiene per liter of cell culture. This non-optimized system proved the principle of genetically engineering *E. coli* for the heterologous production of taxanes by combining enzymatic biosynthetic steps derived from several different organisms.

Recently, the genetic engineering of *S. cerevisiae* for the production of taxadien-5α-acetoxy-10β-ol and the implement-ation of 8 of the 19 genes in two plasmids provides an overview of the biosynthetic pathway of paclitaxel, including the genes that have been transferred to *S. cerevisiae*. The use of *S. cereviseae* seems to tackle a physiological problem in the combinatorial production. *E. coli* does not have an efficient isoprenoid biosynthetic pathway. This is the main reason to clone this pathway into *E. coli* as discussed before for artemisinin. A second problem with *E. coli* is the limited supply of complementary NADPH: cytochrome P450 reductase that is also essential for the correct function of reconstituted plant cytochrome P450 enzymes. Yeast does have endogenous microsomal cytochrome P450 enzymes and energy supporting systems, what is a major advantage for this host system.

The taxadiene synthase encoding gene has also been expressed in *A. thaliana***.** Constitutive expression of the gene led to taxadiene accumulation, but the *A. thaliana* plants showed growth retardation and decreased levels of photosynthetic pigment. The negative effects may have been caused by the toxicity of taxadiene, but more likely they are a result of the disturbance of the endogenous geranylgeranyldiphosphate pool. The use of an inducible expression system resulted in an increase of taxadiene accumulation. These findings clearly show that only the expression of heterologous genes results in the production of the desired compound, but the influence on the metabolic network has to be taken into account as well.

4.3. Carotenoids

Carotenoids are tetraterpenoids (C40 compounds) and produced in many plants and microorganisms. Their main biological function is

the protection against oxidative damage and some are used as warning colours in plant defense system. The commercial interest for carotenoids can be explained mainly by their use as colorant, nutraceutical, or antioxidant in food and cosmetics. Next to that, it has been suggested that carotenoids could possibly play an important role as anticarcinogenic drug and in the prevention of chronic diseases. The carotenoid β-carotene is the primary source of Vitamin A in the human diet. The biosynthesis of carotenoids starts with the tail-to-tail coupling of two molecules of the general precursor GGDP by phytoene synthase (CrtB) resulting in the colorless carotenoid phytoene. Desaturation reactions inserting four additional double bonds in the molecule give eventually lycopene, the main carotenoid in tomato fruit, from which different cyclic and acyclic structures can be synthesized depending on the producing organism. Lycopene cyclase (CrtY) catalyses the cyclization at both ends of the lycopene molecule, resulting in two β-rings at the molecule β-carotene. Several other enzymes involved in the carotenoid biosyntheses have been identified, responsible not only for cyclization, but for glycosylation and diverse oxygenations as well.

More than 600 different naturally occuring carotenoids have been identified so far. The three main carotenoids β-carotene, asthaxantin, and lycopene are produced by chemical synthesis and fermentation for commercial purposes. However, for carotenoids combinatorial biosynthesis in microorganisms is also described. Several carotenoid producing plants have been genetically modified to increase the production of the desired compounds. This review does not describe this research topic in detail, but the use of transgenic medicinal plants of *Lycopersicum esculentem*, *Daucus carota*, *Solanum tuberosum*, and *Brassica napus* has been reported. To overcome the problems with Vitamin A deficiencies in the third world, the biosynthetic pathway to β-carotene engineered in rice (*Oryza sativa*) has led to the production of Golden Rice providing β-carotene, also referred to as pro-Vitamin A. Here, the use of microorganisms for the production of carotenoids has been discussed.

The production of carotenoids by fermentation of carotenoid producing microorganisms such as *Xanthophyllomyces dendrorhous*, *Haematococcus pluvialis*, and *Blakeslea trispora* has been investigated. *X. dendrorhous* produces 200–400 $\mu g\ g^{-1}$ astaxanthin (85% of total carotenoid content). Engineering of *X. dendrorhous* by random mutagenesis led to an increase of 1.5–9 fold of the astaxanthin production in mutant strains. As a disadvantage of this approach growth inhibition and a decrease of biomass have been observed. More sophisticated recombinant DNA techniques introducing multiple copies of genes encoding a bifunctional phytoene synthase/ lycopene cyclase and a phytoene desaturase also showed an increase in carotenoid production, but unexpectedly mostly other carotenoid structures than the desired astaxanthin. Apparently, the hydroxylating enzyme became limited by overexpressing the mentioned enzymes. Several groups used gene clusters of *Erwinia* sp. for the expression in other hosts. In the last years several non-carotenoid producing organisms have been explored for the production of carotenoids. This heterologous production is dependent on efficient expression systems for the carotenoid gene clusters, but increasing the supply of precursors in the host organisms is of importance as well. The yeasts *Candida utilis* and *S. cereviseae* have been engineered for the production of lycopene, β-carotene, and astaxantin. The prokaryote *E. coli* is most elaborated as a heterologous host, because most of the genes were already expressed in the strain for functional analysis.

The production of carotenoids in a host requires the biosynthesis of the intermediate GGDP. *E. coli* produces the C15 precursor FDP for endogenous terpenoid molecules. The extension of the prenyl chain to C20 has been performed by the expression of the CrtE gene encoding geranygeranyl diphosphate synthase from *Erwinia* sp. This prenyltransferase catalyses the production of GGDP from FDP. The GGDP synthase encoding gene *gps* from *Archaeoglobus fulgidis* has been expressed as well. Expression of this gene

is more efficient, because the enzyme catalyses the three chain elongation reactions starting from the C5 precursors to the C20 molecule.

One way to increase the heterologous production is to increase the pool of precursors in the host. Overexpression of several genes upstream in the isoprenoid biosynthesis resulted in the identification and overcome of bottlenecks in this pathway. Where the expression of a carotenoid gene cluster in *C. utilis* resulted in a lycopene production of 1.1 mg g^{-1} (dry weight) of cells, the overexpression of the catalytic domain of the HMG-CoA enzyme, involved in the isoprenoid biosynthesis via the mevalonate pathway, resulted in a 4-fold increase. Following disruption of the ergosterol biosynthetic gene *ERG9* encoding squalene synthase yielded even more lycopene (7.8 mg g^{-1} (dry weight) of cells).

To increase the isoprenoid flux in *E. coli* several genes of the DOXP pathway have been overexpressed. This resulted in a maximum increase of 10 times of the total carotenoid production. Overexpression of genes encoding enzymes involved in a biosynthetic pathway is not always the solution for higher production levels, because they often cause an imbalance in the metabolic system of a host cell. Regulation of the supply of precursors and expression levels can contribute to the heterologous biosynthesis systems as well. The negative effects of overexpressing a rate limiting protein have been demonstrated for the deoxyxylulose phosphate synthase gene (*dxs*). The use of a multicopy plasmid containing a *tac* promoter resulted in a decrease of growth and lycopene production when expression was induced by IPTG where the *dxs* gene constructed on a low copy plasmid did not show these negative effects. Instead of plasmids the strong bacteriophage T5 promoter has been used to replace native promoters in *E. coli*. As a consequence the increased expression of isoprenoid genes led to improved production of lycopene (6 mg g^{-1} of dry cell weight) in *E. coli.*This production yield is comparable to the levels produced by carotenoid producing microorganims. Another approach to regulate the metabolic flux towards specific carotenoids has been observed by using a construct containing mRNA stability control elements. Variation of the mRNA stability modulated the flux of carotenoid production 300 fold towards β-carotene relative to lycopene.

The balance of the starting precursors of the DOXP pathway has been investigated. Overexpression of several central metabolic genes redirected the flux of pyruvate towards glyceraldehyde 3-phosphate, resulting in an increase of lycopene in the heterologous *E. coli* strain. The same group also tried to design a controlled expression system for limiting enzymatic steps using an artificial intracellular loop. Since most carotenoid genes of different origin can function together in a host, combining several enzymatic combinations led to the production of new carotenoid structures not isolated from nature before.

The use of host cells gives the opportunity to use directed evolution techniques for the modification of enzymes as well. Schmidt-Dannert et al. shuffled phytoene synthases of different bacterial species, which has resulted in a fully conjugated carotenoid containing six instead of four double bonds. The combination with shuffled lycopene synthases has shown production of the monocyclic carotenoid torulene. Extension of these pathways with other carotenoid modifying enzymes led to the production of novel structures in *E. coli.* Directed evolution has been used to create carotenoid-like molecules with different amounts of carbon atoms (C30, C35, C45 and C50) as well.

Out of the group of terpenoids, the carotenoids have been most investigated in the production by naturally non-producing microorganisms and the production of new structures by combinatorial biosynthesis strategies. In contrast to the commercial interest, the pharmaceutical relevance of these compounds seems not to be of high importance at the moment. However, the knowledge out of this work can be applied for the heterologous production of other valuable terpenoid drugs like the mentioned artemisinin or

paclitaxel. Although the availability of carotenoid gene clusters and promiscuity of the enzymes involved in the carotenoid biosynthesis are not present for structures of other terpenoids, the progress made, especially in engineering the upstream pathway creating a higher flux of general isoprenoid precursors, can be useful for all terpenoid structures as counts for the directed evolution techniques as well.

5. Combinatorial Biosynthesis of Alkaloids

By definition alkaloids contain nitrogen which is usually derived from amino acids. Because of the presence of a nitrogen atom, alkaloids react mostly alkaline and are able to form soluble salts in aqueous environments. In plants however they can occur in the free state, as a salt or as an N-oxide and they are accumulated in the plant vacuole as reservoir or often coupled to phenolic acids like chlorogenic acid or caffeic acid. Alkaloids can be classified in terms of their biological activity, their chemical structure, or more accepted according their biosynthetic pathway. In plants over 12,000 alkaloids are known and several are used medicinally with a world market volume of 4 billion US$. Alkaloids are usually divided into five major groups dependent on the amino acid of origin in the biosynthesis (amino acid in brackets):

I. tropane-, pyrrolidine- and pyrrolizide-alkaloids ornithine),
II. benzylisoquinoline (tyrosine),
III. indolequinoline (tryptophane),
IV. pyridine (pyridine), and
V. quinolizidine- and piperidine-alkaloids (lysine).

Combinatorial biosynthesis of alkaloids is known for a few examples like vincristine, vinblastine, ajmaline and morphine from plants and rebeccamycin and staurosporine from *Streptomyces albus.* The compounds mentioned have in common that a rather long biosynthetic pathway (30 enzymes for monoterpenoide indole alkaloids like vincristine and more than 17 enzymes for morphine) has to be elucidated and transferred into a heterologous host. This review discusses in detail the combinatorial biosynthesis of morphine as benzylisoquinoline alkaloid and *Vinca* alkaloids as monoterpenoide indole alkaloids as examples for recent research strategies.

5.1. Benzylisoquinoline alkaloids

Morphine is the most important member of the group of benzylisoquinoline alkaloids and is a natural product with high medicinal significance. Also other benzylisoquinoline alkaloids are pharmaceutical important. Like morphine, codeine is used as an analgetic. Berberine and sanguinarine are used as antimicrobials and others as muscle relaxants like papaverine and (+)-tubocurarine.

The morphine biosynthesis consists of 17 steps in *P. somniferum*, Papaveraceae, and has almost completely been elucidated. In the biosynthesis a key intermediate is (*S*)-norcoclaurine, that is biosynthesised by condensation of dopamine and 4-hydroxy-phenylacetaldehyde (4-HPAA). The catalysing enzyme (*S*)-norcoclaurine synthase has recently been identified from *Thalictrum flavum*, Ranunculaceae, and cloned in *E. coli.* Further key enzymatic steps towards (*S*)-reticulin include three NADPH oxidoreductases and cytochrome P450 and an acetyl-CoA dependent acetyltransferase. Recently the last step reducing codeine to morphine by codeinone reductase has been elucidated and the gene expressed in insect cells and/or in *E. coli.*

Berberine as a second representative for benzylisoquinoline alkaloids is known from different plants, bust is mostly associated with *Chelidonium majus*, Papaveraceae, and responsible for the colour and the antimicrobial activity of the yellow latex and the plant extract. With the exception of the oxidase leading to the quartenary nitrogen all enzymes are known like the key step for bridging from (*S*)-reticulin to (*S*)-scoulerin , the introduction of a methylgroup to give (*S*)-tetrahydrocolumbamin, and the building of a methylenedioxy ring . Therefore one can expect that in the near future the successful combinatorial biosynthesis of berberine in a heterologous host will be tested.

5.2. Vinca alkaloids

Vinblastine and vincristine are monoterpenoid indole alkaloids from *Catharanthus roseus*, Apocyanaceae, and are used in medicine as

antineoplastic drugs. Because of the high importance and the extreme low yield from plants (3 mg kg^{-1}) these could be considered as trace compounds. For the production of 3 kg of *Vinca* alkaloids, which is the annual need worldwide around, 300 tonnes of plant material has to be extracted. Production of *Vinca* alkaloids in plant cell cultures did not lead to a significant improvement and today is accepted that biotechnological approaches in plant cell culturing may not provide an instant solution to this problem.

The biosynthesis of vincristine and vinblastine is complex and is shown in for the early phase starting from geraniol to strictosidine. In the early phase tryptophan and secologanin as terpenoid precursors are condensed to form strictosidine as an important branching intermediate for also other alkaloids. In this short part of the entire route already seven enzymes and corresponding genes are involved. From these seven genes, four of these have been cloned in *E. coli.* For the whole biosynthesis at least 30 biosynthetic and two known regulatory genes are involved, which encode around 35 intermediates. Furthermore, intracellular trafficking of intermediates between 7 compartments must also be considered, what can be considered as major challenge in combinatorial biosynthesis.

The tryptophan decarboxylase and strictosidine synthase (STR) were the first two genes from *C. roseus* cloned from the monoterpenoid indole pathway into *S. cerevisiae*. In the past single genes of the biosynthesis have been expressed in different heterologous organisms. The cDNA coding for STR from *R. serpentina* has previously been expressed in *E. coli* and in insect cells and was found to convert secologanin and tryptamine into strictosidine . Recently, after feeding the precursors tryptamine and secologanin, strictosidine and its aglycon were biosynthesised in *S. cereviseae* as a new heterologous host. When strictosidine glucosidase was additionally overexpressed in the recombinant host *S. cereviseae* carrying the tryptophan decarboxylase and strictosidine synthase gene a sufficient amount of strictosidine was formed.

Besides in microbial hosts the mentioned genes of the early biosynthesis have also been cloned into *Nicotina tabacum*. The major drawback however, is the disability to hydrolyse strictosidine glucoside because *N. tabacum* does not posses specific glucosidases. Later, strictosidine glucosidase has also been successfully inserted and expressed in suspension cultured tobacco cells. The strictosidine glucosidase protein in *N. tabacum* was present in the same high molecular weight complexes as known before in *C. roseus*.

The late biosynthesis of vindoline and related monoterpenoide indole alkaloids is only partly known and comprehensively summarized. Starting point in this phase is the strictosidine aglycon and its transformation via an unknown route to catheamine and tabersonine. From this precursor vindoline is biosynthesised in at least six enzyme reactions and in multiple cellular compartments. All enzymes have been cloned in expressed in *E. coli* and in part in *S. cerevisiae.*

6. Combinatorial Biosynthesis of Phenolic Natural Products

6.1. Flavonoids

Flavonoids represent a very important group of plant natural products. They are considered as health promoting substances in the human diet for their antioxidant, antiasthmatic, anti-blood-clotting, and anticancer activities. Flavonoids are exclusively produced in plants and found in almost all studied species in the plant kingdom. Flavonoids are produced via the so-called phenylpropanoid pathway, in which phenylalanine ammonia lyase (PAL) deaminates phenylalanine or tyrosine yielding cinnamic acid . The biosynthetic route on the enzymatic and genetic level has been elucidated in the past and can be reconstructed in detail. The biosynthesis starts with L-phenylalanine that is metabolised to cinnamic acid derivatives, which condensates with malonyl-CoA to a chalcone. In the biosynthesis cinnamic acid is hydroxylated by cinnamic-4-hydroxylases (C4H) to *para*-4-hydroxy-cinnamic acid, activated by 4-coumarate/cinnamate coenzyme A, coupled with 3

malonyl-CoA units and converted by chalcone synthase (CHS) to a chalcone derivate as first committed precursor for the flavonoid biosynthesis. Chalcones are converted to flavonoids by a ring closing step forming the heterocyclic C ring by chalcone isomerases. Naringenin is a chalcone and key intermediate leading to isoflavones, to condensed tannin precursors and, via different hydroxylation, glycosylation, prenylation and alkylation steps, to more than 600 known flavonoids .

Recent publications have documented the production of pinocembrin, naringenin and chrysin, apigenin, galangin, kaempferol and dihydrokaempferol in recombinant *E. coli* BL21 (DE3). Because the main genes for flavonoids are missing in *E. coli*, recombinant plasmids (pUC, pET) containing the genes of interest have been constructed . These artificial gene clusters contain up to three genes from microorganisms or plant origin (*Glycyrrhiza echinata*, *Petroselinum crispum* and *Citrus sinensis*). Expression of all genes encoding the flavonoid biosynthesis up to the level of naringenin was successful, but only limited amounts of flavonoids were detected. To overcome this problem, the production of the essential precursor malonyl-CoA was increased by overexpression of the acetyl-Coa carboxylase from *Corynebacterium glutamicum*.

In recent publications, further biosynthetic genes have been introduced to modify the oxygenation pattern of flavonoids leading to kaempferol and apigenin. The published work is of high interest, because for the first time a nearly complete biosynthetic pathway from plants was established in a heterologous microorganism. In the future it would be of interest to investigate whether further enzymes modifying flavones and flavonols like glycosylation, prenylation or *O*-methylation, can be integrated.

7. Conclusion

The concept of expressing genes from biosynthetic pathways in heterologous organisms has dramatically extended the possibilities for combinatorial biosynthesis. The purpose of this review is to highlight these recent developments in genetic engineering of heterologous microorganisms to reconstituate biosynthetic pathways from plants. At the start of this development only single genes were expressed for single enzyme characterisation. By the development of genetic techniques multi gene expression systems in hosts organisms, like *E. coli*, *B. subtilis* or *S. cerevisiae*, became realistic to engineer whole pathways.

Today the flavonoid pathway has been fully transferred to *E. coli* as a host, and the early isoprenoid pathway up to farnesyl diphosphate has been fully reconstructed up to level of linear precursors for the cyclisation by different terpene cyclases. The example of heterologous biosynthesis of flavonoids and taxadien-5α-acetoxy-10β-ol documents clearly the development to construct multigene vectors and to express more then one gene.

The strategy for the future will be to identify a microbial host in which basic primary pathways can be exploited for the production of biosynthetic precursors for further secondary pathways. An advantage is that no transfer of genes and promoter sequences for a primary pathway is necessary – as known for the MVA and DOXP pathway – and that genes and expressed enzymes for the desired secondary pathway can just hook on. These systems can then be used for the production of valuable compounds or for further engineering strategies.

A major problem is still the gap of knowledge about genes and their regulation in plants of interest. Only a minority of plants has been genetically sequenced, and most of them are crops with a high economic value. Only a few specific medicinal plants like *A. annua* and *Taxus baccata* are currently under investigation, for others the elucidation of pathways is more or less a prediction and enzymes and intermediates have to be confirmed by genomics and proteomics. However, the number of genes that has been identified from biosynthetic pathways is steadily increasing and, as shown, combinations from different genes of

different organisms can be a viable approach. This makes the realm of combinatorial biosynthesis probably the most exciting new area for plant biotechnology.

(Based on the article written by Mattijs K. Julsing et al. in Biomolecular Engineering Vol. 23, No. 6 Dec., 2006 p. 265-279)

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News & Views

Government Urged to Hold IPRs on Ayurvedic Formulations

The Association of Ayurveda Practioners (India) has suggested the Centre to hold patents on ayurvedic formulations. This would be the first step towards getting the nearly Rs 8,000 crore industry to standardise. Standardisation is critical if Ayurveda is to survive, hence the government should also bring in intellectual property rights protection for formulation. "We need to standardize ayurvedic formulations in India. We do not have protection for formulations hence individual Ayurvedic institutes and practitioners prefer to keep their formulations secret. So, the government must also bring in an IPR protection regime for formulations which will ensure that the practitioners get their royalties," observed JB Abhyankar, newly-appointed president of the Maharastra Ayurvedic Aushadhi Utpadak Sangh.

The organisation covers the entire value chain in the sector, from the farmers who grow the *aushadhi* (medicinal plants), to its suppliers and *vaids*-(Ayurvedic doctors). It has over 150 members across the state and its first priority is to set up a centralised Ayurvedic formulations testing laboratory. All practitioners would be able to test all their products here, Mr Abhyankar said. The government laboratory in Mumbai is not capable of handling the volumes, it was pointed out. They will seek the help of the Pune Municipal Corporation (PMC) and the state government to set up such a lab in Pune, which can be accessed by the Rs 2,000 crore ayurveda industry in Maharashtra.

(The Economic Times, 25.5.2006)

Indian and Chinese Doctors to Prove Efficacy of Herbal Drugs

Indian and Chinese doctors will collaborate to prove through clinical trials the efficacy of some traditional herbal drugs that have for generations been known to cure but for which there is no scientific evidence.

"The protocol of the first such collaboration has been approved by the board members and the final draft report is soon to be submitted to the government for seeking funding," said Ranjit Roy Chaudhury, chair of the Indian Clinical Epidemiology Network (INCLEN) board of trustees and a US-based non-profit organization.

(The Economic Times, 19.5.2006)

Emami Eyes Ayurvedic Market

The Indian FMCG company Emami has finalized up plans to enter the Ayurvedic products market and expects the business to contribute as much as Rs 50 crore over the next three years. Emami has planed to introduce several products in the Ayurvedic segment, primarily in competition with Dabur and various other domestic brands, according to company vice-president Amalendu Ghose.

Himani Ayurvedic Health Care, the new division, has already launched five products which were test marketed in regions of Uttar Pradesh, Madhya Pradesh, Chhattisgarh and Uttaranchal. The products include a memory tonic, a cough syrup, a vapour rub, a laxative and digestive pills, all based on ayurvedic formulations.

(The Economic Times, 22.5.2006)

Himachal Pradesh to Formulate Policy for Medicinal Plants

The Himachal Pradesh government has formulated a comprehensive policy for turning itself into a major herbal destination in the country by 2025. The-forestry sector medicinal plants policy was focussing on conservation and augmentation of medicinal plant resource in its natural habitat through adaptive and participative management with linkages to sustainable use for commerce and research purposes. Under the new policy, a system would be developed for pricing of wild harvest of medicinal herbs, reflecting the conservation cost, and community benefits and organic cultivation of commercially important species on private lands, it has been learned.

The policy would also promote the use of commercially viable medicinal plants available in

the state by the state owned and private pharmaceutical units and subsidiaries engaged in value addition.

(Business Standard, 1.6.2006)

Varsity Study Gives Clean Chit to Indian Herbal Medicines

Use of the Indian system of medicine is not harmful and it is quite safe, claims Victor Rajamanickam, Dean, Centre for Advanced Research in Indian System of Medicine, SASTRA Deemed University, Thanjavur.

Prof. Rajamanickam questioned the scientific validity of an article that appeared in the *Journal* of *American Medical Association* (JAMA) in December 2004. The authors had analysed 14 Ayurvedic formulations manufactured in India and concluded that they contained heavy metals such as mercury, arsenic and lead.

Prof. Rajamanickam said the authors had failed to analyse the different forms by which the elements are bound but have projected only the quantum of elemental distribution. This was critical since these elements could be chelated in the formulation and will be safe to use.

Moreover, the final product in Bhasmas and Rasa yogas are different from the raw materials since they would be transformed to therapeutic compounds by different processes like detoxification, titration, heating, etc. Hence it is unlikely that free elements would be present in these products that may cause damage as claimed by the authors.

ICMR, NIF Ink Pact on Developing Herbal Drugs

The Indian Council of Medical Research (ICMR) and the National Innovation Foundation (NIF) have signed a memorandum of understanding (MoU) for development of drugs from medicinal plants or multi-herb formulations.

Under the five-year MoU, the ICMR will work towards validating the safety and efficacy of the practices claimed to have therapeutic value. Such a step will also serve to recognise, respect and reward the knowledge rich but economically poor indigenous people.

The MoU was signed by N.K. Ganguly, Director General of ICMR, and Anil K. Gupta, executive vice president of the NIF. CSIR chief R.A. Mashelkar is the chairperson of the NIF, an autonomous body sponsored by the Department of Science and Technology. The NIF - through its country wide network - has compiled a huge database of 30,000 medical practices dealing with herbal remedies that were never codified and validated by any medical research agency. These are not even mentioned in classical Ayurveda, Siddha or Unani literature.

(The Hindu, 25.6.2006)

India Hopes TRIPS to Extend GI Protection

The council for Trade Related Intellectual Property Rights (TRIPS) at the World Trade Organisation (WTO) is going to take up crucial issues, including extension of geographical indications to products beyond wines and spirits and incorporating certain provisions on bio-diversity in the TRIPS agreement. The issues are of great importance to India as it is championing both these causes at the WTO. India wants GI protection to be extended to products, including basmati rice and Darjeeling tea, to ensure that the names are not misused by producers of other varieties of tea and rice. GIs are indications that are attributed to the geographical location from which a particular product originates.

India is also fighting for including stringent disclosure norms in the TRIPS Agreement to protect its biological resources and traditional knowledge. The disclosure norms would make it mandatory for patent-applicants to divulge the source of origin of the product they want to protect. India is also insisting on sharing benefits arising from the patent with the origin country.

(The Economic Times, 9.6.2006)

Patent Granted for Herbal Drug to Cure Rheumatoid Arthritis

A herbal medicine immunepathy-I, invented by a Chennai based company 21st Century Medical

Science Immunepathy, as a cure for Rheumatoid arthritis has received Indian patent from the Indian Patent Office.

The drug is claimed to have the ability to give permanent cure and immediate relief from Rheumatoid arthritis, without any side effects, according to the claims of the company. The medicine is entirely a combination of herbal and Homeopathy ingredients, which helps in secretion of synovial fluids, bone metabolism, and bone regeneration, along with removing the genetic defects, according to the inventor Dr Malsiraj Samuel.

The combination of the nutritive herbals include Emblica officinalis (Amla), Ishabgul (Plantago-ovata), Cymbopogon flexuosus (lemon grass), *Azadirachta indica* (neem), Centratherum, *Cosanium fenestratum* (Gaertn Coleb), *Euphorbia hirta*, Amukra, *Holarrhena antidysenterica* and *Allium cepa* mother tincture, in various quantities. The patent application was filed in 2004 and was publicised for pre grant opposition last week.

According to the inventor, the 21st Century Medical Science "immunopathy" has invented the cause and medicines for the Rheumatoid Arthritis. The causes are due to genetic, excessive loss of synovial fluids, decreased secretion of synovial fluids, loss of bone metabolism and regeneration.

(Pharmabiz, 14 June 2006)

Indian Move on Bio-piracy at WTO

India is leading a group of nine developing countries including Brazil, China and Pakistan in an effort to frame multilateral rules to check bio-piracy. The group has submitted a proposal to the TRIPS council at the World Trade Organisation (WTO) demanding that the TRIPS agreement should incorporate provisions making it mandatory for patent applicants to disclose the origin of the biological resource being patented. The patent applicant should also share benefits arising out of the patent with the origin country.

Developed countries, especially the US, have made several efforts to patent the properties of products like neem and turmeric, which Indians have been using for centuries. The US government revoked patents on certain uses of neem and turmeric when India challenged the decision. Despite the small victories, the developed countries have been extensively using products growing in the developing world in their patented products without paying royalty. In the submission, the group pointed out that bringing a parity between the agreement and the Convention on Biological Diversity 'which provides for disclosure of origin was an outstanding implementation issue which needed to be addressed as part of the on-going Doha round of multilateral trade talks.

(The Economic Times, 14.7.2006)

Chinese Herbal Medicine may Help Fight Diabetes

A traditional Chinese herbal medicine may help fight Type-2 diabetes and obesity, scientists said. Berberine, a bitter-tasting yellow chemical found in some roots and barks, has been documented in Chinese literature as being able to lower glucose levels in diabetics. To investigate its reported glucose-lowering effects, an international team examined the effect of berberine on mice and rats. The scientists of the team found that a dose of the compound, given orally, caused blood sugar levels to go down. It also led to fewer fats circulating in the bloodstream, made insulin work better and lowered the animals' body weights.

Writing in the Diabetes journal, the scientists added that berberine reduced the animals' body weight, suggesting it could also be used to treat obesity. Berberine is found in several plants, including goldenseal, the Oregon grape and barberry. It has been used in many cultures, including India, for medicinal purposes, most commonly to treat diarrhoea.

(www.economictimes. co.in, 14.8.2006)

Government may Permit Other Countries to Access Traditional Knowledge Library

The department of AYUSH is planning to permit other countries to use India's knowledge on alternative systems of medicine following the Cabinet decision last month to allow access by international patent offices to India's Traditional

Knowledge Digital Library (TKDL). The department, which comes under the Ministry of Health and Family Welfare is in the final round of talks with patent offices of the European, Union (EU) and the UK to sign mutual non-disclosure agreements, it has been learnt. The non disclosure agreement will allow access to the knowledge library database on a secured portal. Patent offices of other countries can access it for patent search. They, however, will not be allowed to make any third party disclosure other than what is required for patent search and examination.

At present negotiations were on regarding whether to use an international search engine for the access or not. Access by international patent offices to TKDL would mean an end to disputes relating to patents by foreign companies on medicines and remedies, which had been a part of Indian traditional knowledge for ages.

(The Economic Times, 14.7.2006)

IICT Scouts for Industry Partners to Develop Anti-Gastric Ulcer Drug

The Indian Institute of Chemical Technology (IICT) has isolated an anti-gastric ulcer molecule from a known Indian medicinal plant. The molecule code named OA-5 showed potent anti-gastric ulcer activity against different types of ulcers such as chemical, stress induced, pylorus ligated and ethanol induced ulcers.

The activity against all the types of ulcers is on par with the known drugs in the market and in some cases much higher. IICT has tied up with Gulbarga University, with the university carrying animal experimentation and testing. The research on this molecule had been initiated three years ago.

As of now, *in vivo* studies have been completed. A patent has been filed. IICT team is ready to initiate pre-clinical toxicology studies. IICT is actively looking for collaborative partners - from industry to take the molecule further into clinical trial, Dr: JS Yadav, Director, IICT, said.

(Chronicle Pharmabiz, 10.8.2006, p. 4)

CSIR Gets US Patent for Using Indian Green Mussel to Cure Diabetes

The scientists at the Council of Scientific and Industrial Research (CSIR) and Department of Biotechnology have received a United States Patent for inventing a new process to cure diabetes mellitus from Indian green mussel (*Perna viridis*) extract. The US patent for an application filed four years ago was granted on July 11, 2006.

According to the inventors, the process for the cure and control of diabetes mellitus using natural products from *Perna viridis* is non-toxic and cyto-protective. The extract also possesses islet neogenesis activity as evidenced by the reversal of the experimental diabetes in mice. The invention consists of a method for treatment of a patient with diabetes mellitus by administering a pharmaceutically effective amount of a mussel extract to the patient, mussel extract being a hydrolysate of meat and mantle fluid from *Perna viridis.*

The extract prepared from the *Perna viridis* has previously been found to be active against all influenza, herpes and hepatitis viral strains. The extract is also found to possess not only prophylactic efficacy for protection from several viral diseases but it also shows a high therapeutic activity against these diseases. Indian scientists Ramesh Ramachandra and Anil Chatterjee have now come out with its properties to cure diabetes.

(Chronicle Pharmabiz, 10.8.2006, p. 2)

Sami Labs Gets DCGI Nod for Glaucoma Drug

Sami Labs has received approval from the Drugs Controller General of India to manufacture and market a new drug for glaucoma. The drug, Forskolin 1 % Ophthalmic Solution with the brand name Ocufors, has been developed from the herb *Coleus forskohlii*.

(The Economic Times, 10.8.2006)

BARC Researchers Find Triphala as Potential Cancer Treatment

The traditional health booster and ayurvedic formulation ‘Triphala' has found its use in cancer

treatment, according to Radiation biologists of Bhabha Atomic Research Centre (BARC) in Mumbai. Triphala has been shown to be toxic to cancer cells and non-toxic to normal cells, Dr. K.P. Mishra, Head, Radiation Biology, said recently at a symposium on 'Advances in Radiation research and their impact on Bio-medical sciences".

The studies were conducted on leukemic cells (blood cancer cells - HELA cells) and breast cancer cells (MCF-7), Mishra said. Triphala is a three-in-one powder constituting amla or Indian goosberry (*Emblica officinalis*), Harada (*Terminalia chebula*), Haritaki and Bibitaki or Bahera (*Terminalia belerica*).

"The cytotoxic effects of aqueous extract of Triphala were investigated on human breast cancer cell line (MCF-7) and a transplantable mouse thymic lymphoma (barcl-95). The viability of treated cells was found to decrease with the increasing concentrations of Triphala", he said adding it was published in the international journal "Cancer letters" in May this year.

Dr. Mishra and his colleages pointed out that these results suggest that Triphala possessed ability to induce cytotoxicity in tumor cells but spared the normal cells. The differential effect of Triphala on normal and tumor cells seem to be related to its ability to evoke differential response at intracellular level, they said. "The differential response of normal and tumour cells to Triphala *in vitro* and the substantial regression of transplanted tumour in mice fed with Triphala points to its potential use as an anti-cancer drug for clinical treatment", researchers said.

(Chemical Weekly, 5.9.2006)

US-FDA Bans Dietary Supplements Containing Ephedrine Alkaloids

The Court of Appeals for the Tenth Circuit in Denver upheld the Food and Drug Administration's (FDA) final rule declaring all dietary supplements containing ephedrine alkaloids adulterated, and therefore illegal for marketing in the United States, reversing a decision by the District Court of Utah.

The Tenth Circuit Court of Appeals' ruling demonstrates the soundness of FDA's decision to ban dietary supplements containing ephedrine alkaloids, consistent with the Dietary Supplement Health and Education Act (DSHEA) of 1994.

FDA conducted an exhaustive and highly resource intensive evaluation of the relevant scientific data evidence on ephedrine alkaloids before issuing its final rule, which became effective in 2004. The court found that FDA supports the agency's findings that dietary supplements containing ephedrine alkaloids pose an unreasonable risk of illness or injury to users, especially those suffering from heart disease and high blood pressure. No dosage of dietary supplements containing ephedrine alkaloids is safe and the sale of these products in the United States is illegal and subject to FDA enforcement action.

(IDMA Bulletin, XXXVII(32), 30.8.2006, p. 41)

China to Standardize Traditional Drugs

China's State Administration of Traditional Chinese Medicine is to draw up standardised procedures, terms and quality measures for 500 traditional Chinese medicines (TCMs) over the next five years as part of efforts to boost the international acceptance of such therapies. The administration is also supporting research into side-effects and efficacy, including through clinical trials, to generate more scientific data. Concerns over improperly characterised ingredients, consistency and heavy metal/pesticide residues have been major concerns elsewhere, but China is promoting international discussions on regulatory frameworks, the official Xinhua news agency states.

(Scrip, No. 3202, 20.10.2006, p. 19)

R & D Highlights

A computational approach to botanical drug design by modeling quantitative composition-activity relationship.

Wang Y *et al.*

Chem Biol Drug Des. 2006 Sep;**68(3):**166-72.

Herbal medicines have been successfully applied in clinical therapeutics throughout the world. Following the concept of quantitative composition-activity relationship, the study proposes a computational strategy to predict bioactivity of herbal medicine and design new botanical drugs. As a case, the quantitative relationship between chemical composition and decreasing cholesterol effect of Qi-Xue-Bing-Zhi-Fang, a widely used herbal medicine in China, was investigated. Quantitative composition-activity relationship models generated by multiple linear regression, artificial neural networks, and support vector regression exhibited different capabilities of predictive accuracy. Moreover, the proportion of two active components of Qi-Xue-Bing-Zhi-Fang were optimized based on the quantitative composition-activity relationship model to obtain new formulation. Validation experiments showed that the optimized herbal medicine has greater activity. The results indicate that the presented method is an efficient approach to botanical drug design.

Berberine alters the processing of Alzheimer's amyloid precursor protein to decrease Abeta secretion.

Asai M *et al.*

Biochem Biophys Res Commun. 2007 Jan 12;**352(2):**498-502

Berberine's biological activity includes antidiarrheal, antimicrobial, and anti-inflammatory effects. Recent findings show that berberine prevents neuronal damage due to ischemia or oxidative stress and that it might act as a novel cholesterol-lowering compound. The accumulation of amyloid-beta peptide (Abeta) derived from amyloid precursor protein (APP) is a triggering event leading to the pathological cascade of Alzheimer's disease (AD). Therefore, the inhibition of Abeta production should be a rational therapeutic strategy in the prevention and treatment of AD. Here, authors report that berberine reduces Abeta levels by modulating APP processing in human neuroglioma H4 cells stably expressing Swedish-type of APP at the range of berberine concentration without cellular toxicity. These results indicate that berberine would be a promising candidate for the treatment of AD.

Antimicrobial and general toxicity activities of *Gymnosperma glutinosum:* A comparative study.

Canales M *et al.*

J Ethnopharmacol. 2006 Oct 13;

Gymnosperma glutinosum (Spreng.) Less (Asteraceae) is an important, and an effective herbal medicine which is widely used for the treatment of diarrhoea in Mexico. Authors examined and compared the antibacterial and antifungal activities through the dilution method and for general toxicity activity by the brine shrimp lethality assay of two samples of *Gymnosperma glutinosum* from two localities of Mexico: San Rafael-Coxcatlan (Puebla State) and Tepeji del Rio (Hidalgo State). In addition, two bioactive compounds (-)-17-hydroxy-neo-clerod-3-en-15-oic acid(1) and 5,7-dihydroxy-3,6,8,2',4',5'-hexa-methoxyflavoneh(2) were isolated. From the hexane extract from both places was obtained a MeOH partition M(2). M(2) of Tepeji del Rio showed the least MICs (<125mug/ml) in the majority of the bacterial strains. *Sarcina lutea* was the most sensitive bacteria (MIC< 125mug/ml). The hexane extract of both localities showed antifungal activity against all tested fungi. San Rafael's hexane extract has significantly more activity than Tepeji del Rio. *Aspergillus niger* (IC(50)=23.79mug/ml) and *Trichophyton mentagrophytes* (IC(50)=90.25mug/ml) were the more sensitive fungus strains. The strongest general toxicity activity was observed with the M(2) partition from Tepeji del Rio (LC(50)=503.7mug/ml). The results obtained in

this investigation, showed differences between the antimicrobial activities of the samples of plants collected in San Rafael (Puebla) and Tepeji del Rio (Hidalgo).

Stimulative activity of *Drynaria fortunei* (Kunze) J. Sm. extracts and two of its flavonoids on the proliferation of osteoblastic like cells.

Li F, Meng F *et al.*

Pharmazie. 2006 Nov;**61(11):**962-5.

The osteoblastic activity of extracts of *Drynaria fortunei* (Kunze) J. Sm. rhizome was assayed in the UMR106 cell line cultured *in vitro*. An ethanol extract and its fractions were added to the cell culture at different concentrations. Osteoblastic proliferation stimulating activity was determined using the MTT method. The ethanol extract, and its ethyl acetate and n-butanol fractions exhibited stimulating activity. Two active constituents were isolated from n-butanol fraction by bioassay-directed isolation, and identified as naringin and neoeriocitrin. The latter is reported for the first time from this herbal medicine.

Inhibitory effects of herbal drugs on the growth of human ovarian cancer cell lines through the induction of apoptosis.

Zhu, Kun *et al.*

Gynecologic Oncology, **97(2),** 405 (May 2005)

In order to develop and search for more effective and safe treatments for early and advanced stages of ovarian cancer, authors examined the direct effects of four extracts of Chinese herbal drugs on ovarian cancer cells *in vitro*. The growth inhibition of four herbal drugs on a total of six cell lines of human ovarian cancer cells was determined by a Cell Counting Kit-8 by counting viable cells. Apoptotic cells induced by herbal drugs were detected by using MEBCYTO Apoptosis Kit. All experiments were performed in triplicate. The significance of the difference was analyzed with a two-sided Student's t test. The P value less than 0.05 was accepted as statistically significant. The MN, A2780, and KF cell lines exhibited significant growth inhibition in the presence of Sho-saiko-to concentrations of 150 [mu]g/ml, 300 [mu]g/ml, and 500 [mu]g/ml, respectively, and at the concentration of 1000 [mu]g/ml, Sho-saiko-to demonstrated a significant apoptotic induction effect on all six kinds of ovarian cancer cell lines. This concentration is the same as the blood concentration attained when 7.5 g of Sho-saiko-to per day is orally administered and all absorbed. Sho-saiko-to exhibited significant growth inhibition of ovarian cancer cell lines, and the mechanisms of the inhibitory effects can be attributed, in part, to apoptosis induced by Sho-saiko-to.

The *in vitro* cytotoxic and apoptotic activity of Triphala--an Indian herbal drug.

Kaur, Swayamjot *et al.*

Journal of Ethnopharmacology, **97(1),** 15(Feb., 10, 2005)

A study on cytotoxic effect of acetone extract of "Triphala" whose antimutagenicity has already been tested was extended to test its cytotoxic effects on cancer cell-lines using Shionogi 115 (S115) and MCF-7 breast cancer cells and PC-3 and DU-145 prostate cancer cells as models. The results revealed that acetone extract of "Triphala" showed a significant cytotoxic effect on these cancer cell-lines and the effect was similar on all cancer cell lines used in this study. The major phenolic compounds in the most potent acetone extracts were isolated and purified. Structural analysis was conducted using spectroscopic techniques including mass spectroscopy, nuclear magnetic resonance (NMR) and infrared (IR) which showed gallic acid as the major component. The suppression of the growth of cancer cells in cytotoxic assays may be due to the gallic acid--a major polyphenol observed in "Triphala".

The antidiabetic activity of the herbal preparation ADD-199 in mice: a comparative study with two oral hypoglycaemic drugs.

Okine, L.K.N. *et al.*

Journal of Ethnopharmacology, **97(1),** 31 (Feb., 10, 2005)

The antidiabetic and antioxidant effects of the herbal preparation ADD-199 were investigated in STZ-induced diabetic C3H mice and results were compared with two allopathic hypoglycaemic drugs, glibenclamide and metformin. Plasma

glucose, insulin and lipids as well as liver glycogen, lipids and lipid peroxidation were measured following treatment for 8 weeks. The results indicated that plasma insulin levels in normal controls at termination were about 76 [mu] mol/L compared to trace levels in untreated diabetic mice. Glibenclamide and ADD-199 increased insulin levels in diabetic mice up to 70% of levels in untreated non-diabetic mice whilst metformin had no effect. Basal plasma glucose levels in diabetic controls (18.8 mM) were reduced to 14.0 mM by 100 mg/kg ADD-199 in <2 weeks compared to 4 and 6 weeks for glibenclamide and metformin, respectively. This hypoglycaemic effect of ADD-199 appeared to be associated with the alkaloidal content of the extract. Treatment with ADD-199 or the hypoglycaemic agents reversed the observed elevation in plasma lipids but increased hepatic glycogen, triacylglycerol and cholesterol levels. Treatment also increased glucose uptake by isolated diaphragms and attenuated hepatic lipid peroxidation. These antihyperglycaemic and antioxidant actions of ADD-199 at a dose of 100 mg/kg/day are comparable to those of the maximum daily therapeutic doses of glibenclamide (0.25 mg/kg) and metformin (50 mg/kg). These could explain the basis for use of this plant extract to manage diabetes mellitus (DM).

Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction.

Izzo, Angelo A. *et al.*

International Journal of Cardiology, **98(1),** 1 (Jan., 19,2005)

Use of herbal medicines among patients under cardiovascular pharmacotherapy is widespread. In this paper, authors have reviewed the literature to determine the possible interactions between herbal medicines and cardiovascular drugs.

Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria.

Fazeli, Mohammad Reza *et al.*

Food Control, **18(6),**646 (June 2007)

Food poisoning originating from contaminated foods by both Gram-positive and Gram-negative bacteria causes concern to society and to the industry. Spices have been used safely since ancient times as food flavoring agents and also as herbal medicines and are now mainly considered "generally regarded as safe" (GRAS). Antimicrobial effects of two spices used in Iranian traditional medicine were investigated against some pathogenic food-borne bacteria.

Hydroalcoholic extracts of *Rhus coriaria* L. (sumac) and *Zataria multiflora* Boiss (avishan-e shirazi or zaatar) obtained from Tehran botanicals market were prepared by cool percolation method using 80% (v/v) aqueous alcohol. Antimicrobial activities of the extracts were tested against several Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli, Salmonella typhi, Proteus vulgaris,* and *Shigella flexneri*. All the extracts were primarily screened for their possible antimicrobial effects using disc and well diffusion methods. Both *R. coriaria* L. and *Z. multiflora* Boiss showed considerable antibacterial effects. The potential antibacterial activities of the two herbal extracts were further investigated at concentrations of 0.05%, 0.1%, 0.2%, 0.4%, 0.8% and 1.6% (w/v) and minimum inhibitory concentration (MICs) as well as minimum bactericidal concentrations (MBCs) of the extracts were elucidated. Sumac showed better activity against the tested bacteria compared to avishan-e shirazi inhibiting *Bacillus cereus* and *Staphylococcus aureus* at concentrations of 0.05% and 0.1%, respectively while Gram-negative bacteria were affected by higher concentrations of sumac ranging 0.1-2%. The MICs of avishan-e shirazi against the tested bacteria ranged from 0.4% to 0.8%. *Salmonella typhi* was found to be the most resistant showing the MICs of 0.2% and 0.8% with sumac and avishan-e shirazi, respectively. Both popular Iranian spices which are traditionally used as astringent agents have promising inhibitory effects on food-borne bacteria and could be considered as natural food preservatives.

Preventive effect of kami-untan-to on performance in the forced swimming test in thiamine-deficient mice: Relationship to functions of catecholaminergic neurons.

Nakagawasai, Osamu *et al.*

Behavioural Brain Research, **177(2),**315 (feb., 27, 2007)

The kampo (Japanese herbal) medicine "kami-untan-to" (KUT) has been used for a long time in the treatment of neuropsychiatric disorders. We have recently reported that mice put on a thiamine-deficient (TD) diet exhibit a depressive behavior and impairment in avoidance learning after 20 days, and that this impairment was reversed by the chronic administration of KUT. In the study, authors investigated the effect of KUT on the depressive behavior observed in TD mice by using the forced swimming test.

The results show that oral administration of KUT from the 1st day of TD feeding prevented the increased duration of immobility in TD mice. Administration of KUT from the 10th day of TD feeding also had a beneficial effect on depressive behavior. To examine the relationship between the potential effects of KUT on monoaminergic neuronal functions and the depressive behavior observed in TD mice, the immunohistochemical distribution of tyrosine hydroxylase (TH) in the brain using microphotometry was measured. The fluorescence intensity of TH decreased in the limbic cortex and brainstem in TD mice compared with pair-fed mice as the control group, while KUT treatment protected against these decreases. These results suggest that KUT treatment may prevent a sign of depressive behavior, the animal immobility time, induced by TD feeding through a mechanism that involves the decrease of TH in some brain areas of TD mice.

Antipyretic effect of Mao-to, a Japanese herbal medicine, for treatment of type A influenza infection in children.

Kubo, Tomohiro *et al.*

Phytomedicine, **14(2-3),** 96 (Feb., 19, 2007)

Mao-to is a Japanese traditional herbal medicine which has been used since ancient times for the treatment of influenza-like illness. This study was conducted to evaluate the effect of oral Mao-to administration in children with type A influenza, in comparison to Oseltamivir. Authors performed a controlled trial of 60 children, from 5 months through 13 years of age, with fever and influenza-like symptom of up to 48 h duration. Diagnosis of influenza type A was performed by virus isolation or detection of a viral gene by RT-PCR. Patients were assigned into the following 3 groups: oral Mao-to powder (TJ-27) 0.06 g/kg body wt./dose three times daily (n=17), Oseltamivir 2 mg/kg body wt./dose twice daily (n=18) or both oral Mao-to plus Oseltamivir (n=14).

The median duration of fever after treatment was significantly shorter in the Mao-to and Mao-to plus Oseltamivir groups, compared with the Oseltamivir only group (15 h [95%CI 13.2-22.1] $p<0.01$; 18 h[15.2-27.7] $p<0.05$; 24 h[23.5-43.0], respectively). Oral Mao-to administration was effective in the control of fever due to type A influenza infection in children.

***Ligusticum chuanxiong* prevents rat pheochromocytoma cells from serum deprivation-induced apoptosis through a protein kinase A-dependent pathway.**

Lin, Yun-Lian *et al.*

Journal of Ethnopharmacology, **109(3),** 428 (Feb., 12, 2007)

Ligusticum chuanxiong (LC) is a traditional Chinese herbal medicine used to treat various cardiovascular diseases. In this study, the butanol extract of LC was found to protect neuronal-like pheochromocytoma cells from serum deprivation-induced apoptosis. Both a serine/threonine kinase inhibitor and a specific protein kinase A (PKA) inhibitor blocked the protective effect of LC. A transcription inhibitor (actinomycin D) and a protein synthesis inhibitor (cyclohexamide) also attenuated the protective effect of LC, suggesting the requirement of gene expression for the protection of LC. On the other hand, LC increased both the formation of cyclic-AMP and the phosphorylation of the cyclic-AMP response element-binding protein (CREB), a downstream target of PKA and a nuclear transcription factor

known for neuroprotective mechanism. Furthermore, LC-induced CREB phosphorylation and protective effect could be blocked by a PKA inhibitor and overexpression of the dominant negative CREB, respectively. Taken together, the protective mechanism of LC in antagonizing serum deprivation-induced PC12 cell apoptosis might be mediated through a PKA/CREB-dependent pathway.

Herbal medicine Gamgungtang down-regulates autoimmunity through induction of TH2 cytokine production by lymphocytes in experimental thyroiditis model.

Sa, Eun-Ho *et al.*

Journal of Ethnopharmacology, **109(3),** 472 (Feb., 12, 2007)

The crude herbal formulation, Gamgungtang (GGT), has been shown to protect animals against a wide range of spontaneously developing or induced autoimmune diseases. Authors have previously reported that GGT shows marked down-regulation of several experimental autoimmune diseases. Although very effective at preventing thyroid infiltrates in mice immunized with mouse deglycosylated thyroglobulin and complete Freund's adjuvant and in spontaneous models of thyroiditis, it completely failed to modify experimental autoimmune thyroiditis (EAT) induced in mice immunized with mouse thyroglobulin and lipopolysaccharide.

In this study, in an effort to elucidate the mechanisms by which GGT suppresses EAT, and autoimmunity in general, authors investigated the *in vivo* effects of this drug on the Th1/Th2 lymphocyte balance, which is important for the induction or inhibition of autoreactivity. Naive SJL/J mice were treated orally for 5 days with GGT (80 mg/(kg day)). Spleen cells were obtained at various time points during the treatment period and were stimulated in vitro with concanavalin A. Interleukins IL-4, IL-10 and IL-12, transforming growth factor-[beta] (TGF-[beta]) and interferon-[gamma] (IFN-[gamma]) cytokine production was evaluated at the protein levels of the cytokines in the medium and mRNA expressions. A significant upregulation of IL-4, IL-10 and TGF-[beta] was observed following treatment with GGT, which peaked at day 5 (IL-10) or day 10 (IL-4). On the other hand, IL-12 and IFN-[gamma] production were either unchanged or decreased. It seems therefore that GGT induces in vivo a shift towards Th2 lymphocytes which may be one of the mechanisms of down-regulation of the autoimmune reactivity in EAT. These observations indicate that down-regulation of TH1 cytokines (especially IL-12) and enhancement of Th2 cytokine production may play an important role in the control of T-cell-mediated autoimmunity. These data may contribute to the design of new immunomodulating treatments for a group of autoimmune diseases.

Stimulative effects of *Ulmus davidiana* Planch (Ulmaceae) on osteoblastic MC3T3-E1 cells.

Suh, Seok-Jong *et al.*

Journal of Ethnopharmacology, **109(3),** 480(Feb.,12,2007)

Ulmus davidiana Planch (Ulmaceae) has long been known to have anti-inflammatory and protective effects on damaged tissue, To treat rheumatoid arthritis (RA), a herbal medicine, *Ulmus davidiana* Planch (Ulmaceae) extract (UD) is being used in traditional oriental medicine. The effect of UD on the proliferation and osteoblastic differentiation in non-transformed osteoblastic cells (MC3T3-E1) was studied. UD dose-dependently increased DNA synthesis (significant at 5-20 [mu]g/ml). UD increased alkaline phosphatase (ALP) activity and prolyl hydroxylase activity of MC3T3-E1 cells (5-20 [mu]g/ml). Antiestrogen tamoxifen eliminated the stimulation of proliferation and ALP activity of MC3T3-E1, which was induced by UD. UD at concentrations ranged from 30 to 100 [mu]g/ml inhibited prostaglandin E2 production in MC3T3-E1. These results indicate that UD directly stimulates cell proliferation and differentiation of osteoblasts. These results also suggest and UD is effective for bone anti-resorptive action in bone cells.

Assessment of antidiarrhoeal activity of the methanol extract of *Xylocarpus granatum* bark in mice model.

Rouf, Razina *et al.*

Journal of Ethnopharmacology, **109(3),** 539 (Feb., 12, 2007)

The methanol extract of *Xylocarpus granatum* bark was studied for its antidiarrhoeal properties in experimental diarrhoea, induced by castor oil and magnesium sulphate in mice. At the doses of 250 and 500 mg/kg per oral, the methanol extract showed significant and dose-dependent antidiarrhoeal activity in both models. The extracts also significantly reduced the intestinal transit in charcoal meal test when compared to atropine sulphate (5 mg/kg; i.m.). The results showed that the extracts of *Xylocarpus granatum* bark have a significant antidiarrhoeal activity and supports its traditional uses in herbal medicine.

Effect of Mongolian plants on *in vivo* insulin action in diabetic rats.

Khookhor, Oyun *et al.*

Diabetes Research and Clinical Practice, **75(2),** 135 (Feb., 2007)

In the present study, authors performed a preliminary investigation on the acute effect of Mongolian medicinal plant extracts (*Dryopteris* species, Aspidiaceae) on the glucose tolerance in rats, evaluated by an oral glucose tolerance test (OGTT). Then, the effect of the same extracts on *in vivo* insulin action in streptozotocin (50 mg kg-1 BW, i.v.)-induced diabetic rats by means of the euglycemic clamp was investigated. In diabetic rats, the glucose metabolic clearance rate (MCR) during 3.0 (low-dose) and 30.0 mU kg-1 min-1 (high-dose) insulin infusion was significantly higher in animals administered with plant extracts (500 mg kg-1 BW, p.o.), compared with saline-administered animals The results suggest that a single administration of plant extracts can improve glucose utilization and insulin resistance in diabetic rats.

Abrogation of streptococcal pyrogenic exotoxin B-mediated suppression of phagocytosis in U937 cells by *Cordyceps sinensis* mycelium via production of cytokines.

Kuo, Chih-Feng *et al.*

Food and Chemical Toxicology, **45(2),** 278 (Feb., 2007)

Streptococcal pyrogenic exotoxin B (SPE B) is a virulent factor in group A streptococcal infection. In the present study the mycelium extract of *Cordyceps sinensis* (CS), a Chinese immunomodulatory herbal medicine, showed increased phagocytosis in U937 cells. Neither heat nor trypsin pretreatment prevented CS extract from causing this increase. Further studies indicated that SPE B-mediated suppression of U937 cell phagocytic activity was abrogated by CS extract. Factors in the conditioned medium from CS-extract-treated U937 cells were responsible for blocking the SPE B-mediated suppression of phagocytosis. Heating the conditioned medium eliminated the increase, which suggested that the U937-cell protein products augmented phagocytosis. Analyzing cytokine mRNA expression of U937 cells revealed increases in interferon-[gamma] (IFN-[gamma]), interleukin (IL)-12 p35 and p40, and tumor necrosis factor-[alpha] (TNF-[alpha]), but not in IL-1[beta], IL-6, or IL-8. Treating U937 cells with anti-IFN-[gamma], IL-12, and TNF-[alpha] antibodies also eliminated the conditioned medium-induced increase in phagocytosis. Taken together, SPE B inhibited phagocytosis, but CS mycelium extract abrogated this inhibition by causing cytokine production.

Enhancement of learning and memory by a medicinal formulation, Saenhyetang, in mice.

Jin, Un-Ho *et al.*

Journal of Ethnopharmacology, **109(2),** 271 (Jan.,19, 2007)

The effects on memory and learning ability of the Korean herbal medicine, Saenhyetang (SHT), which is a combination of nine herbs, were investigated. Hot water extracts (HWE-SHT) and ethanol extracts (EE-SHT) were used for the studies. It was shown that N-methyl-d-aspartate (NMDA) receptor 2B (NR2B) was increased in the forebrains of SHT-administrated mice (HWE-SHT), leading to enhanced activation of NMDA receptors, facilitating synaptic potentiation in response to stimulation at 10-100 Hz.

These HWE-SHT-treated mice exhibit superior ability in learning and memory in various

behavioral tasks, showing that NR2B is enhanced by HWE-SHT treatment and also is critical in gating the age-dependent threshold for plasticity and memory formation. NMDA receptor-dependent modifications, which were mediated in part by HWE-SHT administration, of synaptic efficacy, therefore, represent a mechanism for associative learning and memory. Results suggest that oriental medical enhancement of NR2B attributes such as intelligence and memory in mammals is feasible. On the other hand, to examine the effects of EE-SHT on the learning and memory in experimental mice, the passive and active avoidance responses were studied.

The EE-SHT ameliorated the memory retrieval deficit induced by ethanol, but not other memory impairment in mice. EE-SHT (10, 20 mg/100 g, p.o.) did not affect the passive avoidance responses of normal mice in the step through and step down tests, the conditioned and unconditioned avoidance responses of normal mice in the shuttle box and lever press performance tests, and the ambulatory activity of normal mice in normal condition. However, EE-SHT was shown to significantly decrease the spontaneous motor activity during the shuttle box test, and also to prolong the sleeping time induced by pentobarbital in mice at 20 mg/kg. These results suggest that EE-SHT has an ameliorating effect on memory retrieval impairment and a weak tranquilizing action.

Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam.

Chuang, Ping-Hsien *et al.*

Bioresource Technology, **98(1),** 232 (Jan., 2007)

Investigations were carried out to evaluate the therapeutic properties of the seeds and leaves of *Moringa oleifera* Lam as herbal medicines. Ethanol extracts showed anti-fungal activities *in vitro* against dermatophytes such as *Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum,* and *Microsporum canis.* GC-MS analysis of the chemical composition of the essential oil from leaves showed a total of 44 compounds. Isolated extracts could be of use for the future development of anti-skin disease agents.

Protective effect of *Ugni molinae* Turcz against oxidative damage of human erythrocytes.

Suwalsky, M. *et al.*

Food and Chemical Toxicology, **45(1),** 130 (Jan., 2007)

Ugni molinae Turcz, also known as "Murtilla", is a plant that grows in the south of Chile. Infusions of its leaves have long been used in traditional native herbal medicine. The chemical composition of the leaves indicates the presence of polyphenols, which have antioxidant properties. In the present work, the antioxidant properties of U. molinae were evaluated in human erythrocytes exposed *in vitro* to oxidative stress induced by HClO. The experiments were carried out by scanning electron microscopy (SEM) and hemolysis measurements.

The SEM observations showed that HClO induced a morphological alteration in the red blood cells from a discoid to an echinocytic form. According to the bilayer couple hypothesis, the formation of echinocytes indicates that HClO was inserted in the outer leaflet of the erythrocyte membrane. However, a concentration as low as 10 [mu]M gallic acid equivalents (GAE) U. molinae aqueous extract neutralized the shape change effect of HClO applied in a concentration as high as 0.25 mM. The significant protection of U. molinae aqueous extract was also shown in the hemolysis experiments. In fact, very low concentrations of the extract considerably reduced the deleterious capacity of HClO to induce hemolysis in red blood cells.

It is concluded that the location of the extract components into the membrane bilayer and the resulting restriction on its fluidity might hinder the diffusion of HClO and its consequent damaging effects. This conclusion can also imply that this restriction could apply to the diffusion of free radicals into cell membranes and the subsequent decrease of the kinetics of free radical reactions.

Effects of *Butea superba* on reproductive systems of rats.

Manosroi, Aranya *et al.*

Fitoterapia, **77(6),** 435 (Sept., 2006)

The effects of *Butea superba* on the reproductive system in male Wistar rats were investigated. The animals were fed daily with the powdered crude drug suspended in distilled water by a gastric tube at the dose of 2, 25, 250 and 1250 mg/kg body weight for 8 weeks. Rats fed with 1 ml of distilled water were used as a negative control. The weights of all vital organs in all treated groups were not different from the control. The percentage weight ratios of body weights of seminal vesicles and prostate glands were not different from the control, except that the testis of the group fed with 1250 mg/kg was significantly different from the control and the other treated groups. In addition, the sperm counts in this group showed about 16% more than the control group.

Hematology as well as the liver and kidney function of all treated groups showed no difference from the control. B. superba, drug at 250 mg/kg which was 100 times more than the Thai FDA recommended dose for humans appeared to be safe in rats. The crude drug has demonstrated an increase tendency on testis weight and sperm counts in rat. The information from the present study can be used to explain the Thai folklore application of this plant in Thailand.

Saikokaryukotsuboreito, a herbal medicine, prevents chronic stress-induced dysfunction of glucocorticoid negative feedback system in rat brain.

Mizoguchi, Kazushige *et al.*

Pharmacology Biochemistry and Behavior (In Press)

Disruption of the hypothalamo-pituitary-adrenal (HPA) axis characterized by dysfunction of the glucocorticoid negative feedback system is frequently observed in human depressives and is thought to involve a reduction in glucocorticoid receptor (GR) function in the feedback sites including the brain. Recently, it was found that chronic stress in rats induces similar HPA disruption that is caused by abolishment of feedback ability in the prefrontal cortex (PFC) and hippocampus, which involves decreased cytosolic GRs or increased nuclear GRs, respectively. Also, the authors found that Saikokaryukotsuboreito (SRBT), a herbal medicine, prevents the chronic stress-induced HPA disruption. The authors, therefore, examined here the effects of this drug on the chronic stress-induced changes in GRs in the PFC and hippocampus. Chronic stress was induced in rats by water immersion and restraint (2 h/day) for 4 weeks. SRBT significantly prevented decreased cytosolic GRs in the PFC and increased nuclear GRs in the hippocampus in the chronically stressed rats. Moreover, SRBT significantly prevented the abolishment of feedback ability in both regions. These results suggest that the beneficial effects of SRBT on the GR level are involved in its ameliorating actions on the HPA disruption. This finding provides information important for the prevention and treatment of depression.

***Pelargonium sidoides* preparation (EPs(R) 7630) in the treatment of acute bronchitis in adults and children.**

Matthys, H. *et al.*

Phytomedicine (In Press)

Acute bronchitis, although mostly caused by viral infections, is commonly treated with antibiotics. As antibiotics should only be prescribed upon strict indication, treatment options like a liquid herbal drug preparation from the roots of *Pelargonium sidoides* (EPs(R) 7630) gain more and more interest. To evaluate the efficacy and safety of treatment with EPs(R) 7630 in patients with acute bronchitis, a multi-centre, prospective, open observational study was conducted in 440 study sites located in Germany. A total of 2099 patients aged 0-93 years with productive cough for less than six days without indication for treatment with antibiotics were given EPs(R) 7630-solution in an age-dependent dosage for 14 days.

The primary outcome criterion was the mean change of the Bronchitis Severity Score (BSS: cough, sputum, rales/rhonchi, chest pain at cough, dyspnoea) from baseline to patient's individual last observation. During treatment, the mean BSS of all

patients decreased from 7.1+/-2.9 points at baseline to 1.0+/-1.9 points at patients' individual last visit. Subgroup analysis for children showed a decrease of mean BSS from 6.3+/-2.8 points to 0.9+/-1.8 points and analysis of children younger than three years showed a decrease of mean BSS from 5.2+/-2.5 points to 1.2+/-2.1 points. Adverse events occurred in 26/2099 (1.2%) patients. Serious adverse events were not reported. In conclusion, EPs(R) 7630 is an effective and well tolerated treatment of acute bronchitis in adults, children and infants outside the strict indication for antibiotic treatment.

Is the yin-yang nature of Chinese herbal medicine equivalent to antioxidation-oxidation?

Szeto, Yim-Tong *et al.*

Journal of Ethnopharmacology, **108(3),** 361 (Dec., 6, 2006)

It has been suggested that yin-yang theory described in traditional Chinese medicine is somewhat equivalent to the modern theory of antioxidant-oxidant balance. Some yin-tonic Chinese herbal medicines possess antioxidant properties. In this context, the DNA protective effect of 12 yin-tonic and 13 yang-tonic herbs were tested using the single cell gel electrophoresis (comet) assay. Lymphocytes from three healthy subjects were pre-incubated with aqueous herb extract, and the comet assay was performed on treated, untreated, challenged and unchallenged cells in parallel, oxidant challenge being induced by 5 min exposure to hydrogen peroxide.

Results using this *ex vivo* cellular assay showed protection by some herbs. Seven out of 12 yin-tonic Chinese herbs demonstrated decreased DNA damage after treatment while 10 out of 13 yang-tonic herbs showed protection. Among 25 herbs tested, rhizome of *Ligusticum sinensis* Oliv and aerial part of *Artemisia annua* L demonstrated greatest DNA protective effect. Results indicated that the yin nature of herbs may not be necessarily associated with superior antioxidative effect to yang-tonic herbs, at least in terms of DNA protection against oxidant challenge.

***In vitro* anti-microbial and *in vivo* cytokine modulating effects of different prepared Chinese herbal medicines.**

Lin, Shyh-Jye *et al.*

Food and Chemical Toxicology, **44(12),** 2078 (Dec., 2006)

The toxicity, antimicrobial and cytokine modulating effects of herbal medicines in treating periodontal diseases were evaluated in this study. Using the broth dilution method and disc agar diffusion test, in individual and combined decocted preparations, different concentrations of Ching-Wei-San and its individual herbal components, *Coptidis rhizoma, Angelicae sinensis* radix, Rehmanniae radixet rhizom, *Moutan radicis cortex,* and *Cimicifuga foetida,* were tested for *in vitro* inhibitory effects on three well-known plaque-causing bacteria, Porphyromonas gingivialis, Streptococcus sanguis, and Streptococcus mutans, and two common pathogens, Staphylococcus aureus and Escherichia coli. The cytokine modulating effects were evaluated in Balb/c mice.

The results suggested that one milliliter Ching-Wei-San at the 25,000 mg/mL concentration daily for the mice had significantly high levels in the liver function indexes in the 3-day acute toxicity test and in both the liver and kidney function indexes in the 28-day subacute toxicity test ($P < 0.01$). The 250 mg/mL Ching-Wei-San is comparable to 250 mg/mL of tetracycline, and had similar inhibitory effects on the tested bacteria. *Coptidis rhizoma* (62.5 mg/mL) was the only individual herbal component to show 100% inhibitory effects. The mean cytokine ratios of IL-2, IL-4, IFN-[gamma], and TNF-[alpha] in Balb/c mice treated with individual herbal components were shown to be different from each other. Ching-Wei-San modulated the immunity of mice, up-regulated IL-2, IL-4 and TNF-[alpha], but down-regulated IFN-[gamma]. The effects of none of the individual herbal components alone can substitute for the cumulative effect of Ching-Wei-San.

Curcumin inhibits human colon cancer cell growth by suppressing gene expression of epidermal growth factor receptor through reducing the activity of the transcription factor

Egr-1.

A Chen *et al.*

Oncogene (2006) **25,** 278–287.

High expression of epidermal growth factor receptor (EGFR) is found in a variety of solid tumors, including colorectal cancer. EGFR has been identified as a rational target for anticancer therapy. Curcumin, the yellow pigment of turmeric in curry, has received attention as a promising dietary supplement for cancer prevention and treatment. The authors recently reported that curcumin inhibited the growth of human colon cancer-derived Moser cells by suppressing gene expression of cyclinD1 and EGFR. The aim of the present study was to explore the molecular mechanisms underlying curcumin inhibition of gene expression of EGFR in colon cancer cells. The generality of the inhibitory effect of curcumin on gene expression of EGFR was verified in other human colon cancer-derived cell lines, including Caco-2 and HT-29 cells. Promoter deletion assays and site-directed mutageneses identified a binding site for the transcription factor early growth response-1 (Egr-1) in *egfr* promoter as a putative curcumin response element in regulating the promoter activity of the gene in Moser cells. Electrophoretic mobility shift assays demonstrated that curcumin significantly reduced the DNA-binding activity of the transcription factor Egr-1 to the curcumin response element. In addition, curcumin reduced the *trans*-activation activity of Egr-1 by suppressing *egr-1* gene expression, which required interruption of the ERK signal pathway and reduction of the level of phosphorylation of Elk-1 and its activity.

Taken together, these results demonstrated that curcumin inhibited human colon cancer cell growth by suppressing gene expression of EGFR through reducing the *trans*-activation activity of Egr-1. These results provided novel insights into the mechanisms of curcumin inhibition of colon cancer cell growth and potential therapeutic strategies for treatment of colon cancer.

Herbal remedy magnolol suppresses IL-6-induced STAT3 activation and gene expression in endothelial cells.

Shih-Chung Chen *et al*.

British Journal of Pharmacology (2006) **148,** 226–232.

Magnolol (Mag), an active constituent isolated from the Chinese herb Hou p'u (*Magnolia officinalis*) has long been used to suppress inflammatory processes. Chronic inflammation is well known to be involved in vascular injuries such as atherosclerosis in which interleukin (IL)-6 may participate. Signal transducer and activator of transcription protein 3 (STAT3), a transcription factor involved in inflammation and the cell cycle, is activated by IL-6. In this study, we evaluated whether Mag can serve as an anti-inflammatory agent during endothelial injuries. The effects of Mag on IL-6-induced STAT3 activation and downstream target gene induction in endothelial cells (ECs) were examined. Pretreatment of ECs with Mag dose dependently inhibited IL-6-induced Tyr705 and Ser727 phosphorylation in STAT3 without affecting the phosphorylation of JAK1, JAK2, and ERK1/2. Mag pretreatment of these ECs dose dependently suppressed IL-6-induced promoter activity of intracellular cell adhesion molecule (ICAM)-1 that contains functional IL-6 response elements (IREs). An electrophoretic mobility shift assay (EMSA) revealed that Mag treatment significantly reduced STAT3 binding to the IRE region.

Consistently, Mag treatment markedly inhibited ICAM-1 expression on the endothelial surface. As a result, reduced monocyte adhesion to IL-6-activated ECs was observed. Furthermore, Mag suppressed IL-6-induced promoter activity of cyclin D1 and monocyte chemotactic protein (MCP)-1 for which STAT3 activation plays a role. In conclusion, our results indicate that Mag inhibits IL-6-induced STAT3 activation and subsequently results in the suppression of downstream target gene expression in ECs. These results provide a therapeutic basis for the development of Mag as an anti-inflammatory agent for vascular disorders including atherosclerosis.

Effects of natural anthraquinones on neuronal survival in rat brain neuron.

Hsin-Hsueh Lee *et al*.

Journal of Cerebral Blood Flow & Metabolism (2005) **25,** S449.

Recently, the herbal medicine was widely used to treat the neurodegenerative diseases. Neuroinflammation is a characteristic of pathologically affected tissue in several neurodegenerative disorders. These changes are particularly observed in affected brain areas of Alzheimer's disease (AD) and neuronal injury. In the central nervous system, ischemic insult-induced neuronal injury is believed to result from glutamate toxicity and glucose deprivation. Rhubarb– the root of *Rheum officinale* is one of the famous Chinese herbs as astringent bitters in gastric constipation and in diarrhea. It is used to treat blood stasis and cathartic by traditional medicine. Rhubarb has a very broad spectrum of biological activities and pharmacological functions, such as laxative, antiphlogistic, and homeostatic in the treatment of constipation, diarrhea, jaundice, and gastro-intestinal hemorrhage, etc.

In this study, five natural anthraquionones of *R. officinale* were investigated the neuroprotective effects against glutamate/NMDA (Glu/NMDA) stimulation in primary cultured rat brain cortical neurons. Cell death was accessed by lactate dehydrogenase (LDH) release assay for necrosis, and mitochondrial activity was accessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction activity assay. Among the five anthraquionones tested, it was found aloin, emodin and aloe-emodin decreased MTT reduction activity, whereas sennoside A and B significantly reduced Glu/NMDA-increased LDH release in cultured neurons. These results suggest that Rhubarb extract contain both neuroprotective and neurotoxic anthraquionones

Herbal care for reproductive health: Ethno medicobotany from Uttara Kannada district in Karnataka, India.

Hegde HV *et al.*

Complement Ther Clin Pract. 2007 Feb;**13(1):**38-45.

Traditional herbal medicine is predominantly practiced by the rural people of India, especially remote areas such as the Uttara Kannada District in Western Ghats of Karnataka. Local traditional healers play an important role in the management of reproductive health problems of the native population due to socio-economical and geographical factors. In the present study, 92 traditional medicine practitioners/healers from various regions of Uttara Kannada district were interviewed to collect information on the use of herbal treatments for a range of female and male reproductive disorders. Information was also collected on the method of preparation, dose and duration along with the botanical names, family and local names of the medicinal plants. The plants were then collected and identified. A total of 18 formulations from 25 plant species belonging to 17 families were identified, which are commonly used to treat 12 different reproductive ailments. This study identifies herbal remedies not previously documented, that are used by indigenous people in the treatment of reproductive disorders. Additionally, the paper highlights the need to retain and explore the rich biodiversity associated with Indian rain forests that may result in the discovery of new medical treatments. Finally, this paper notes the continuing reliance on herbal medicines and healing traditions by local people in remote areas. Understanding and working with local healers and tribes provides a unique opportunity to learn about the use of potentially new herbal and plant medications.

The psychopharmacology of European herbs with cognition-enhancing properties.

Kennedy DO *et al.*

Curr Pharm Des. 2006;**12(35):**4613-23.

Extensive research suggests that a number of plant-derived chemicals and traditional Oriental herbal remedies possess cognition-enhancing properties. Widely used current treatments for dementia include extracts of *Ginkgo biloba* and several alkaloidal, and therefore toxic, plant-derived cholinergic agents. Several non-toxic, European herbal species have pan-cultural traditions as treatments for cognitive deficits, including those associated with ageing. To date they have not received research interest commensurate with their potential utility.

Particularly promising candidate species include sage (*Salvia lavandulaefolia/officinalis*), Lemon balm (*Melissa officinalis*) and rosemary (*Rosmarinus officinalis*). In the case of sage, extracts possess anti-oxidant, estrogenic, and anti-inflammatory properties, and specifically inhibit butyryl- and acetyl-cholinesterase. Acute administration has also been found to reliably improve mnemonic performance in healthy young and elderly cohorts, whilst a chronic regime has been shown to attenuate cognitive declines in sufferers from Alzheimer's disease. In the case of Melissa officinalis, extracts have, most notably, been shown to bind directly to both nicotinic and muscarinic receptors in human brain tissue. This property has been shown to vary with extraction method and strain. Robust anxiolytic effects have also been demonstrated following acute administration to healthy humans, with mnemonic enhancement restricted to an extract with high cholinergic binding properties. Chronic regimes of aromatherapy and essential oil respectively have also been shown to reduce agitation and attenuate cognitive declines in sufferers from dementia. Given the side effect profile of prescribed cholinesterase inhibitors, and a current lack of a well tolerated nicotinic receptor agonist, these herbal treatments may well provide effective and well-tolerated treatments for dementia, either alone, in combination, or as an adjunct to conventional treatments.

Traditional herbal remedies for gynecological disorders in women of Bidar district, Karnataka, India.

Vidyasagar GM *et al.*

Fitoterapia. 2007 Jan;**78(1):**48-51.

A survey of medicinal plants used by rural population in Bidar district, Karnataka, India is reported. Eighteen plant species belonging to 13 families and 18 genera were included. Their botanical and vernacular names, plant part used, popular medicinal use, forms of preparation and application of the herbal remedies are given.

Use of complementary and alternative medicine in epilepsy.

Ricotti V *et al.*

Curr Neurol Neurosci Rep. 2006 Jul;**6(4):**347-53.

Complementary and alternative medicine (CAM) has become much in vogue, and CAM practitioners have increased in tandem with this. The trend of using CAM for treating epilepsy does not differ from that in other medical conditions, with nearly one half of patients using CAM. In the article authors have reviewed the major complementary and alternative medicines used for treatment of epilepsy. They include mind-body medicines such as reiki and yoga; biologic-based medicine such as herbal remedies, dietary supplements, and homeopathy; and manipulative-based medicine such as chiropractic.

Consumption of historical and current phytotherapeutic agents for urolithiasis: a critical review.

Gurocak S *et al.*

J Urol. 2006 Aug;**176(2):**450-5.

Recent years have shown a dramatic expansion in the knowledge of molecular mechanism of phytotherapeutic agents used to treat urolithiasis. The discovery and elucidation of the mechanism of action, in particular the clinical role of these herbal remedies, has made an important contribution to treatment for urinary stone disease as an alternative or adjunct therapy. Thus, the potential clinical application of these folk medicines to urolithiasis is discussed.

Safety and hypoglycaemic properties of aqueous leaf extract of *Ocimum gratissimum* in streptozotocin induced diabetic rats.

Egesie UG *et al.*

Niger J Physiol Sci. 2006 Jun-Dec;**21(1-2):**31-5.

The claim by Nigerian traditional herbal medicine practitioners that *Ocimum gratissimum* leaves have antidiabetic properties was investigated. Diabetes mellitus was induced with streptozotocin and graded doses of the aqueous leaf extract were administered orally to the experimentally diabetic rats for 28 days. Administration of the aqueous leaf extract caused a statistically significant reduction in plasma glucose level in streptozotocin induced diabetic rats. The extract appeared nontoxic as evidenced by normal serum levels of AST, ALT, ALP, TPT, ALB and bilirubin. These data appear to agree with claimed hypoglycaemic effects of *Ocimum gratissimum.*

A double-blind placebo-controlled randomized study of Chinese herbal medicine as complementary therapy for reduction of chemotherapy-induced toxicity.

Mok T *et al.*

Ann Oncol. 2007 Jan 17

Chinese herbal medicine (CHM) is a common complementary therapy used by patients with cancer for reduction of chemotherapy-induced toxic effects. This study applied the highest standard of clinical trial methodology to examine the role of CHM in reducing chemotherapy-induced toxicity, while maintaining a tailored approach to therapy. Patients with early-stage breast or colon cancer who required postoperative adjuvant chemotherapy were eligible for the study. Enrolled patients were randomly assigned to one of three Chinese herbalists who evaluated and prescribed a combination of single-item packaged herbal extract granules. Patients received either CHM or placebo packages with a corresponding serial number. The placebo package contained nontherapeutic herbs with an artificial smell and taste similar to a typical herbal tea. The primary end points were hematologic and non-hematologic toxicity according to the National Cancer Institute Common Toxicity Criteria Version 2. One hundred and twenty patients were accrued at the time of premature study termination. Patient characteristics of the two groups were similar. The incidence of grade 3/4 anemia, leukopenia, neutropenia, and thrombocytopenia for the CHM and placebo groups were 5.4%, 47.3%, 52.7%, and 1.8% and 1.8%, 32.2%, 44.7%, and 3.6%, respectively (P = 0.27, 0.37, 0.63, and 0.13, respectively). Incidence of grade 2 nausea was the only non-hematologic toxicity that was significantly reduced in the CHM group (14.6% versus 35.7%, P = 0.04). Traditional CHM does not reduce the hematologic toxicity associated with chemotherapy. CHM, however, does have a significant impact on control of nausea.

Herbal medicine for low back pain: a Cochrane review.

Gagnier JJ *et al.*

Spine. 2007 Jan 1;**32(1):**82-92.

Low back pain is a common condition and a substantial economic burden in industrialized societies. A large proportion of patients with chronic low back pain use complementary and alternative medicine (CAM) and/or visit CAM practitioners. Several herbal medicines have been purported for use in low back pain. Randomized controlled trials (RCTs), using adults (>18 years of age) suffering from acute, subacute, or chronic nonspecific low back pain. Types of interventions included herbal medicines defined as a plant that is used for medicinal purposes in any form. Ten trials were included in this review. Two high-quality trials utilizing *Harpagophytum procumbens* (Devil's claw) found strong evidence for short-term improvements in pain and rescue medication for daily doses standardized to 50 mg or 100 mg harpagoside with another high-quality trial demonstrating relative equivalence to 12.5 mg per day of rofecoxib. Two moderate-quality trials utilizing *Salix alba* (White willow bark) found

moderate evidence for short-term improvements in pain and rescue medication for daily doses standardized to 120 mg or 240 mg salicin with an additional trial demonstrating relative equivalence to 12.5 mg per day of rofecoxib. Three low-quality trials using *Capsicum frutescens* (Cayenne) using various topical preparations found moderate evidence for favorable results against placebo and one trial found equivalence to a homeopathic ointment. *Harpagophytum procumbens, Salix alba,* and *Capsicum frutescens* seem to reduce pain more than placebo. Additional trials testing these herbal medicines against standard treatments will clarify their equivalence in terms of efficacy. The quality of reporting in these trials was generally poor; thus, trialists should refer to the statement in reporting clinical trials of herbal medicines.

Prevalence of use of alternative and complementary medicine in patients with irritable bowel syndrome, functional dyspepsia and gastroesophageal reflux disease.

Carmona-Sanchez R *et al.*

Rev Gastroenterol Mex. 2005 Oct-Dec;**70(4):**393-8.

The prevalence of use of complementary and alternative medicine (CAM) is high in developed countries and is believed to be even higher in developing countries. There are no data on the use of CAM in patients with gastrointestinal tract disorders in Mexico. To determine the prevalence of CAM use in Mexican patients with gastrointestinal disorders. A survey was applied to all first-time patients presenting at the gastroenterology service between December 2003 and November 2004. Patients with irritable bowel syndrome (IBS, defined by Rome II), functional dyspepsia (FD, defined by Rome II), and gastroesophageal reflux disease (GERD, defined as erosive esophagitis or abnormal pH intraesophageal monitoring) were included. The use of CAM to relieve gastrointestinal symptoms was examined and the demographic data, the disease history and healthcare use 12 months prior to the study were recorded. The survey was answered by 413 patients (61% IBS, 22% FD y 17% GERD). A total of 181 users of CAM were identified (44% of the total group). The use of CAM was most frequent for IBS (51% IBS, 36% FD, 27% GERD). Herbal medicine was the type of CAM most often used by all groups. The factors associated with CAM use were IBS, history of abdominal surgery, emergency room visits, disabilities and prescription of benzodiazepines, and >or = 3 medical visits for gastrointestinal tract disorders within the previous year. Prevalence of CAM use is high in general and significantly higher for IBS. Herbal medicine is the most frequently used modality.

Genipin suppresses subconjunctival fibroblast migration, proliferation and myofibroblast transdifferentiation.

Kitano A *et al.*

Ophthalmic Res. 2006;**38(6):**355-60. Epub 2006 Oct 13.

Inchin-ko-to is a herbal medicine which has therapeutic effects in ameliorating liver fibrosis or cholestatic liver diseases. Its main bioactive component is genipin, which is an intestinal bacterial metabolite of this medication. Accordingly, we determined whether or not Inchin-ko-to suppresses in a wound healing model subconjunctival fibroblast (SCF) migration proliferation and myofibroblast transdifferentiation since an inhibitory effect could be of value in improving trabeculotomy outcome. Effects of genipin on SCF cell migration were examined subsequent to wounding confluent monolayer cultures. Alamar blue staining evaluated the effects of genipin (0-50 microg/ml) on fibroblast cell proliferation. Immunostaining determined alpha-smooth muscle actin (alphaSMA) expression. Western blotting evaluated (alphaSMA) expression and phospho-Smad2 formation. Real-time RT-PCR evaluated TGFbeta1 and collagen I alpha2 mRNA expression. Enzyme-immunoassay determined culture medium collagen I content. Genipin suppressed wound-induced cell migration and proliferation. It also decreased collagen type I TGFbeta1 and alphaSMA mRNA and protein expression. Smad2 signaling was inhibited by genipin in a dose-dependent manner. Genipin suppresses injury-induced fibrogenic responses in

SCFs. This result suggests that the herbal medicine Inchin-ko-to might have therapeutic value following trabeculotomy.

Inchinkoto, an Herbal Medicine, and Its Ingredients Dually Exert Mrp2/MRP2-Mediated Choleresis and Nrf2-Mediated Antioxidative Action in Rat Livers.

Okada K *et al.*

Am J Physiol Gastrointest Liver Physiol. 2006 Oct 12

Inchinkoto (ICKT), an herbal medicine, has been recognized in Japan and China as a "magic bullet" for jaundice. To explore potent therapeutic agents for cholestasis, the effects of ICKT or its ingredients on multidrug resistance-associated protein 2 (Mrp2/ MRP2)-mediated choleretic activity, as well as on antioxidative action, were investigated using rats and chimeric mice with livers that were almost completely repopulated with human hepatocytes. Biliary excretion of Mrp2 substrates and the protein mass, subcellular localization, and mRNA level of Mrp2 were assessed in rats after 1-week oral administration of ICKT or genipin, a major ingredient of ICKT. Administration of ICKT or genipin to rats for 7 days increased bile flow and biliary excretion of bilirubin conjugates. The Mrp2 protein and mRNA levels and the Mrp2 membrane densities in the bile canaliculi and the renal proximal tubules were significantly increased in the ICKT- or genipin-treated rat livers and kidneys. ICKT and genipin, thereby, accelerated the disposal of intravenously infused bilirubin. The treatment also increased the hepatic levels of heme oxygenase-1 and GSH by a nuclear factor-E2-related factor (Nrf2)-dependent mechanism. Similar effects of ICKT on MRP2 expression levels were observed in the humanized livers of chimeric mice. In conclusion, these findings provide the rationale for therapeutic options of ICKT and its ingredients that should potentiate bilirubin disposal *in vivo* by enhancing the Mrp2/MRP2-mediated secretory capacities in both livers and kidneys, as well as Nrf2-mediated antioxidative action, in the treatment of cholestatic liver diseases associated with jaundice. Key words: Multidrug resistance-associated proteins, bilirubin, gluthathione, heme oxygenase-1, chimeric mice with humanized liver.

Enhancing the efficacy of photodynamic therapy by a chinese herbal medicine for hepatocellular carcinoma.

Juan M *et al.*

Cancer Biol Ther. 2006 Sep;**5(9):**1117-9. Epub 2006 Sep 23.

Hepatocellular carcinoma (HCC) ranks the sixth among the most common malignancies, with chronic HBV infection being the most common cause. HCC is more common in Africa, China and south-east Asia, but its incidence in the USA, Canada and Australia is rising. Current treatment modalities for HCC are not effective, and only a small percentage of patients are suitable for surgical resection and liver transplantation. Thus other treatment options and improvement of available modalities are badly in need. Photodynamic therapy (PDT) may have some therapeutic benefit for patients with HCC. The study has implicated that coupled with Pheophorbide a (Pa), PDT may offer therapeutic benefit for patients with HCC. Inhibition of cell proliferation and induction of apoptosis by Pa may be mechanistically responsible for Pa-PDT. As Pa is an extract from a Chinese herbal medicine *Scutellaria barbata,* which is widely available, less toxic and less expensive, such a combination may find a better clinical usage in the treatment of HCC patients. More studies are mandatory to fully elucidate the efficacy and mechanisms of Pa-mediated PDT.

Quality of life in patients treated with Kampo medicine: a complementary alternative to modern medicine.

Yamada K.

J Altern Complement Med. 2006 Oct;**12(8):**799-803.

Kampo (Japanese traditional herbal medicine) is widely used as a complementary medicine for improving the quality of life (QOL) of patients in Japan. Authors investigated the efficacy of kampo therapy in improving QOL of patients with various diseases and disorders, using the World Health Organization Quality of Life Brief Scale. One

hundred and sixty-seven outpatients with various diseases or disorders wishing to receive kampo therapy and who had already been treated with modern medicines were recruited into the study. Patients were offered kampo formulas and modern medicines for 3 months. Eleven patients were "very much improved," 46 were "much improved," 59 were "minimally improved," 42 showed "no change," 7 were "minimally worse," and one was "much worse" in the CGI Global Improvement scale. The mean WHOQOL-BREF score improved significantly from 3.05 +/- 0.54 at baseline to 3.14 +/- 0.53 after 3 months ($p = 0.002$). Patients presenting with various diseases and disorders were successfully treated with kampo therapy. These results suggest that kampo therapy as a complementary medicine may improve the QOL of patients with various diseases and disorders.

Potential drug-herb interaction with antiplatelet/anticoagulant drugs.

Saw *JT et al*

Complement Ther Clin Pract. 2006 Nov;12(4):236-41.

A cross-sectional survey evaluating the use of herbal medicines in medical wards patients that may interfere with the effect of antiplatelet or anticoagulant therapy. Among the 250 patients participated, 42.4% (n=106) were taking herbs with 76 patients (71.7%) using herbs for the past 12 months. Overall, almost 31% (n=23, N=76) of patients were taking one or more of the specified herbal medicines [ginseng (*Panax ginseng*), garlic (*Allium sativum*), ginkgo (*Gingko biloba*) thought to interact with antiplatelet or anticoagulant therapy. The study showed that 21% (n=16, N=76) of patients co-ingested specified herbs with antiplatelet or anticoagulant therapy, of which half of them were at risk of potential drug-herb interactions. A large proportion of respondents involved in potential drug-herb interaction were elderly people (62.5%, n=5). However, more than 90% of herbal users did not disclose the use of herbal medicine to their health professionals. It is thus prudent for all care givers to be aware of the possibility of drug-herb interaction and inquire about herbal use from patients.

Efficacy of the kampo medicine xiong-gui-jiao-AI-tang, a traditional herbal medicine, in the treatment of threatened abortion in early pregnancy.

Ushiroyama T *et al.*

Am J Chin Med. 2006;**34(5):**731-40.

The study was carried out to evaluate the clinical efficacy of Xiong-gui-jiao-ai-tang (Kyuki-kyogai-to), a traditional Chinese herbal medicine, in the treatment of threatened abortion in early pregnancy. 72 women diagnosed with threatened abortion at Osaka Medical College Hospital and assigned them at random to the following two groups: a group of 36 women who received Xiong-gui-jiao-ai-tang at a dose of 7.5 g/day and another group of 36 women who received human chorionic gonadotropin (hCG)(control group). We found that in the Xiong-gui-jiao-ai-tang group (2.9 +/- 3.5 days), the number of days required before hemostasis was reached in the uterus was significantly shorter than in the control group (10.8 +/- 8.2 days, $p < 0.0001$). Furthermore, the number of days required for retroplacental hematoma in the vicinity of the gestational sac to disappear was significantly shorter in the Xiong-gui-jiao-ai-tang group (9.9 +/- 7.1 days) than in the control group (23.2 +/- 12.8 days) ($p < 0.0001$). In retroplacental hematoma size, significant rates of reduction were obtained in both major and minor axis measurements at the 7^{th} day of treatment for the Xiong-gui-jiao-ai-tang group compared to the control group (control vs Xiong-gui-jiao-ai-tang: major axis: 7.5 +/- 3.8% vs 42.3 +/- 10.5%; minor axis: 15.3 +/- 16.8% vs 71.5 +/- 48.2%)($p <$ 0.0001, each case). The results of this study demonstrated the beneficial effects of Xiong-gui-jiao-ai-tang in stabilizing early pregnancy. Xiong-gui-jiao-ai-tang can be expected to improve unstable early pregnancy with uterine bleeding and to prevent abortion.

The efficacy of *Silybum marianum* (L.) Gaertn. (silymarin) in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial.

Huseini HF *et al.*

Phytother Res. 2006 Dec;**20(12):**1036-9.

Oxidative stresses are increasingly implicated in the pathogenesis of diabetic complications which may either cause direct pancreatic beta-cell damage or lead to metabolic abnormalities that can induce or aggravate diabetes. The valuable effect of antioxidant nutrients on the glycemic control of diabetic patients has been reported in experimental and clinical studies. The present study was designed to investigate the effects of the herbal medicine, *Silybum marianum* seed extract (silymarin), which is known to have antioxidant properties on the glycemic profile in diabetic patients. A 4-month randomized double-blind clinical trial was conducted in 51 type II diabetic patients in two well-matched groups. The first group (n = 25) received a silymarin (200 mg) tablet 3 times a day plus conventional therapy. The second group (n = 26) received the same therapy but a placebo tablet instead of silymarin. The patients were visited monthly and glycosylated hemoglobin (HbA(1)c), fasting blood glucose (FBS), insulin, total cholesterol, LDL and HDL, triglyceride, SGOT and SGPT levels were determined at the beginning and the end of the study. The results showed a significant decrease in HbA(1)c, FBS, total cholesterol, LDL, triglyceride SGOT and SGPT levels in silymarin treated patients compared with placebo as well as with values at the beginning of the study in each group. In conclusion, silymarin treatment in type II diabetic patients for 4 months has a beneficial effect on improving the glycemic profile.

Comparison of effects of vitamin E and Wen-jing-tang (unkei-to), an herbal medicine, on peripheral blood flow in post-menopausal women with chilly sensation in the lower extremities: a randomized prospective study.

Ushiroyama T *et al.*

Am J Chin Med. 2006;**34(6):**969-79.

Authors examined the association between blood flow and chilly sensation in the lower extremities, comparing the changes in blood flow induced by the vitamin E and herbal therapy (Wen-jing-tang) in perimenopausal women with chilly sensation. One hundred sixty-one perimenopausal women aged 42-61 years (mean: 50.4 +/- 3.8 years) with chilly sensation in the lower extremities participated in the study. The participants were randomized for treatment with Wen-jing-tang or a vitamin E preparation containing 600 mg tocopherol nictinate per day for 8 weeks. Blood flow measurement was performed by laser Doppler fluxmetry to determine tissue under the jaw, in the middle finger, and in the third toe. Wen-jing-tang significantly increased the peripheral blood flow in the skin surface in the tiptoe (12.8 +/- 8.8, p = 0.0068) from basal levels (6.0 +/- 5.1), although no significant change was observed in the blood flow in fingertip or under the jaw during treatment. The rate of increase of blood flow in the skin surface of in the lower extremities was significantly higher in the Wen-jing-tang treating group (116.4 +/- 46.5%) than in the vitamin E group (39.8 +/- 21.3%) (p < 0.0001). When the effects of herbal treatment and vitamin E treatment were compared in the subjects with baseline upper extremity blood flow above the mean + 1.5 SD, mean blood flow through the upper extremities was found to have been significantly decreased after Wen-jing-tang treatment (from 57.7 +/- 4.8 to 43.1 +/- 4.2, p = 0.0277), whereas it remained unchanged after treatment with vitamin E. Classical monographs described Wen-jing-tang as being particularly useful in curing chilly sensation in lower extremities. The present study using a laser Doppler fluxmeter demonstrated that treatment with this herbal medicine significantly increased blood flow through the periphery of lower extremities in patients with chilly sensation. It also showed that this herbal medicine suppresses excessive blood flow through the upper half of the body and thus stimulates restoration of physiological distribution of blood flow throughout the entire body.

Gastroprotective effect of a traditional Chinese herbal drug "Baishouwu" on experimental gastric lesions in rats.

Shan, Lei *et al.*

Journal of Ethnopharmacology, **107(3),** 389(Oct.,11, 2006)

"Baishouwu" is an appellative name of dried root tubers from three Asclepiadaceae plants: *Cynanchum auriculatum* Royle ex Wight,

Cynanchum bungei Decne and *Cynoctonum wilfordii* Maxim. In order to establish the pharmacological basis for the ethnomedicinal use of Baishouwu in gastric disorders, this study examined the effects of ethanol extracts and fractions from root tubers of *Cynanchum auriculatum, Cynanchum bungei and Cynoctonum wilfordii* on ethanol-, indomethacin-induced gastric lesions and histamine-induced gastric acid secretion in rats. Plant materials were collected from various areas of China. Oral administration of ethanol extract and chloroform fraction of *Cynoctonum wilfordii* collected from Changbai Cordillera at doses of 150 and 68 mg/kg, respectively, significantly inhibited the development of ethanol- and indomethacin-induced gastric lesions and also caused significant decrease of gastric acid secretion after histamine-induced gastric lesion. Oral administrations of ethanol extract and chloroform fraction of *Cynanchum auriculatum* collected from Binhai at the doses of 300 and 69 mg/kg, respectively, significantly inhibited ethanol- and indomethacin-induced gastric lesions. This study demonstrates the gastroprotective property of Baishouwu for the first time.

Complexities of the herbal nomenclature system in traditional Chinese medicine (TCM): lessons learned from the misuse of Aristolochia-related species and the importance of the pharmaceutical name during botanical drug product development.

Wu, K.M. *et al.*

Phytomedicine(In Press)

Herbs used in traditional Chinese medicine (TCM) have diverse cultural/historical backgrounds and are described based on complex nomenclature systems. Using the family Aristolochiaceae as an example, at least three categories of nomenclature could be identified: (1) one-to-one (one plant part from one species): the herb guan mutong refers to the root of *Aristolochia manshuriensis;* (2) multiple-to-one (multiple plant parts from the same species serve as different herbs): three herbs, madouling, qingmuxiang and tianxianteng, derived respectively from the fruit, root and stem of *Aristolochia debilis*; and (3) one-to-multiple (one herb refers to multiple species): the herb fangji refers to the root of either *Aristolochia fangchi*, *Stephania tetrandra* or *Cocculus trilobus*; in this case, the first belongs to a different family (Aristolochiaceae) than the latter two (Menispermaceae), and only the first contains aristolochic acid (AA), as demonstrated by independent analytical data provided in this article. Further, mutong (*Akebia quinata*) is allowed in TCM herbal medicine practice to be substituted with either guan mutong (*Aristolochia manshuriensis*) or chuan mutong (*Clematis armandii*); and mu fangji (*Cocculus trilobus*) by guang fanchi (*Aristolochia fangchi*) or hanzhong fangji (*Aristolochia heterophylla*), thereby increasing the risk of exposing renotoxic AA-containing Aristolochia species to patients. To avoid these and other confusions, the importance of a pharmaceutical name is emphasized, which defines the species name, the plant part, and sometimes the special process performed on the herb, including cultivating conditions. The pharmaceutical name as referred to in this article is defined, and is limited to those botanicals that are intended to be used as drug. It is hoped that by following the pharmaceutical name, toxic herbs can be effectively identified and substitution or adulteration avoided.

Natureceuticals (natural products), nutraceuticals, herbal botanicals, and psychoactives: drug discovery and drug-drug interactions.

Pal, Dhananjay *et al.*

Nutraceuticals, Herbals and Related Products Volume II:

According to recent epidemiological reports, almost 40% of American population use complimentary and alternative medicine (CAM) during their lifetime. Patients detected with HIV or cancer often consume herbal products especially St. John's wort (SJW) for antidepressants in combination with prescription medicines. Such self-administered herbal products along with prescribed medicines raise concerns of therapeutic activity due to possible drug-herbal interactions. P-

glycoprotein (P-gp) and cytochrome P450 3A4 (CYP3A4) together constitute a highly efficient barrier for many orally absorbed drugs. Available literature, clinical reports and *in vitro* studies from our laboratory indicate that many drugs and herbal active constituents are substrates for both P-gp and CYP3A4. Results from clinical studies and case reports indicate that self-administered SJW reduce steady state plasma concentrations of amitriptyline, cyclosporine, digoxin, fexofenadine, amprenavir, indonavir, lopinavir, ritonavir, saquinavir, benzodiazepines, theophyline, irinotecan, midazolan and warfarin. This herbal agent has been also reported to cause bleeding and unwanted pregnancies when concomitantly administered with oral contraceptives. Most of these medicinal agents and SJW are substrates for P-gp and/or CYP3A4. *In vitro* studies suggest that short-term exposure with pure herbal agents such as hypericin, kaempferol and quercetin or extract of SJW resulted in higher uptake or influx of ritonavir and erythromycin. Hypericin, kaempferol and quercetin also caused a remarkable inhibition of cortisol metabolism with the percent intact cortisol values of 64.58%, 89.6% and 90.1%, respectively, during short-term *in vitro* experiments. Conversely, long-term exposure of herbal agents (hyperforin, kaempferol and quercetin) showed enhanced expression of CYP3A4 mRNA in Caco-2 cells. In another study, we observed that long-term exposure of hypericin, kaempferol, quercetin and silibinin resulted in higher MDR-1 mRNA expression in Caco-2 cells. Therefore, herbs can pharmacokinetically act as inhibitors or inducers. Medicinal agents that are substrates P-gp-mediated efflux and/or CYP-mediated metabolism are likely to be potential candidates for drug-herbal interactions. The duration of exposure of cells/healthy volunteers/animals to herbals appears to be critical for drug-herbal interaction. An increase in plasma drug concentration is possible during concomitant administration of SJW and prescribed drugs. In contrast, prolonged intake of herbal supplement followed by drug administration may result in subtherapeutic concentrations. Therefore, clinical implications of such drug herbal interactions depend on a variety of factors such as dose, frequency and timing of herbal intake, dosing regimen, route of drug administration and therapeutic range. *In vitro* screening techniques will play a major role in identifying possible herb-drug interactions and thus create a platform for clinical studies to emerge. Mechanisms of drug-herbal interaction have been discussed in this review article.

Evidence-based drug-herbal interactions

Chavez, Mary L. *et al.*

Life Sciences, **78(18),** 2146 (Mar., 27, 2006)

Due to the growing use of herbals and other dietary supplements healthcare providers and consumers need to know whether problems might arise from using these preparations in combination with conventional drugs. However, the evidence of interactions between natural products and drugs is based on known or suspected pharmacologic activity, data derived from *in vitro* or animal studies, or isolated case reports that frequently lack pertinent information. The usefulness of such information is questionable. More recently an increasing number of documented case reports, *in vivo* studies, and clinical trials have evaluated herbal-drug interactions. Results have sometimes been contradictory and more research is needed. Since there is a lack of rigorous studies that can establish the clinical significance of herb-drug interactions, an evidence-based evaluation of the current literature concerning commonly used herbal-drug interactions, as well as other dietary supplements, was conducted.

Herbal, prescribed, and over-the-counter drug use in older women: prevalence of drug interactions.

Yoon, Saunjoo L. *et al.*

Geriatric Nursing, **27(2),** 118(2006)

Older adults are at particular risk for drug and herbal interactions because they have multiple health problems that require treatment and are generally more susceptible to adverse drug effects. This study used a database containing self-reported herbal, prescription, and over-the-counter (OTC) drugs concurrently taken by a sample of 58 women who were aged 65 years or older. Drug-drug interactions (DDIs) were identified using a Web-

based pharmaceutical program. At least 1 moderate or high-risk DDI was identified in 74% of participants, with 136 total DDIs identified. Fifty-two percent (71) of total DDIs were between prescribed and OTC or herbals, with 63% (45) of these involving nonsteroidal antiinflammatory drugs (NSAIDs). It is imperative that health care providers identify all prescribed, OTC, and herbal drugs taken by their patients and assess all interactions in order to avoid the possibility of adverse drug effects.

Improvement of accessory symptoms of hypertension by Tsumura Orengedokuto extract, a four herbal drugs containing Kampo-Medicine Granules for ethical use: A double-blind, placebo-controlled study.

Arakawa, K. *et al.*

Phytomedicine, 13**(1-2),** 1(Jan., 5, 2006)

A double-blind, placebo-controlled study was conducted to evaluate the efficacy, safety, and utility of Tsumura Orengedokuto Extract Granules for Ethical Use (TJ-15) as a treatment for the accessory symptoms of hypertension. Two capsules of the study drug were administered orally 3 times daily (i.e., before meals) for 8 weeks. Among 265 patients enrolled in the study, 134 were assigned to the TJ-15 group and 131 were assigned to the placebo group, of whom 204 patients (103 in the TJ-15 group and 101 in the placebo group) were included in the efficacy and utility analyze and 251 patients (128 in the TJ-15 group and 123 in the placebo group) were included in the safety analysis. Efficacy was significantly higher in the TJ-15 group based on the total score for the accessory symptoms of hypertensions which was the primary efficacy endpoint (Wilcoxon's rank sum test, p=0.013). When each accessory symptom of hypertension was assessed separately, efficacy was higher for hot flushes and facial suffusion in the TJ-15 group (Wilcoxon's rank sum test, p=0.034, and 0.022, respectively). There were no significant differences between the TJ-15 and the placebo groups with respect to the decrease of blood pressure or the antihypertensive effect. There was also no significant difference between the two groups with regard to the overall safety rating. The utility rating was significantly higher in the TJ-15 group than in the placebo group (Wilcoxon's rank sum test, p=0.016). In conclusion, TJ-15 was superior to placebo with respect to efficacy, safety, and utility for the treatment of accessory symptoms of hypertension.

Actions of Ya-hom, a herbal drug combination, on isolated rat aortic ring and atrial contractions.

Suvitayavat, W. *et al.*

Phytomedicine, **12(8),** 561 (Aug., 2, 2005)

The effect of the Thai popular medicine Ya-hom on cardiovascular function was studied in isolated rat aortic ring and atrium by comparison with norepinephrine (NE). Water extraction of Ya-hom at concentrations of 0.83, 1.67, 8.33 and 16.67 mg/ml stimulated aortic ring contraction dose-dependently. The maximum contraction, at 16.67 mg/ml, was about 14% that of NE. This stimulatory effect of Ya-hom was inhibited partially by phentolamine, which indicated that the effect of Ya-hom was partially dependent on the [alpha] receptor, similar to NE. Administration of Ya-hom with NR decreased the force of aortic ring contraction as compared to the effect of NE alone, indicating that Ya-hom may have a partial [alpha]-agonist activity. Ya-hom at concentrations of 1.67, 8.33 and 16.67 mg/ml showed a dose-dependent, positive inotropic and negative chronotropic effects. Ya-hom increased the force of isolated atrial contraction with a slow onset and prolonged action. In contrast to norephinephrine, which acted on [beta]1 receptor, causing positive inotropic and chronotropic effects, propranolol did not alter the effect of Ya-hom on the atrial contraction. This shows that the action of Ya-hom on atrial contraction does not involve [beta] receptor. This study demonstrated that the selected Ya-hom preparation increased vascular smooth muscle contraction, and increased the force but decreased the rate of atrial contraction.

Ultrasound-assisted extraction methodology as a tool to improve the antioxidant properties of herbal drug Xiao-chia-hu-tang.

Liu, Chu-Ting *et al.*

Journal of Ethnopharmacology, **99(2),** 293

(June 3, 2005)

Xiao-chai-hu-tang (XCHT) is an important Chinese herbal prescription for curing many types of liver diseases. The contents of bioactive constituents (saikosaponins a, c and d, baicalin, baicalein, and glycyrrhizic acid), and antioxidant properties of XCHT extracts prepared with ultrasound-assisted (US) extraction in combination with ethanol (up to 95%) as extraction modifier were studied. The results showed that the US extraction significantly increased the bioactive constituents concentrations and antioxidant properties of XCHT extracts when compared with the XCHT prepared with traditional boiling-water extraction. Among the XCHT extracts made with US extraction, the sample prepared with 95% ethanol showed the highest bioactive constituent concentrations and the best antioxidant functionalities. The results suggest that US extraction of XCHT is feasible to replace the traditional time-consuming and low efficiency preparation procedure in the future modernized and commercialized manufacture of this highly valuable Chinese herbal medicine.

Development of a fingerprint of *Salvia miltiorrhiza* Bunge by high-performance liquid chromatography with a coulometric electrode array system.

Lijuan, Ma *et al.*

Journal of Chromatography B, **846(1-2),** 139 (Feb., 1, 2007)

To standardize and control herbal medicines, a feasible approach and control system is necessary. In this paper, a high-performance liquid chromatography with a coulometric electrode array detector (HPLC-CEAD) system was applied to fingerprint *Salvia miltiorrhiza* Bunge (*S. miltiorrhiza* Bunge), a popular herbal medicine, for the first time. pH of mobile phase, working potentials and sample preparation were included in our research. Twenty-five common peaks were obtained from extracts of *S. miltiorrhiza* Bunge (Shandong province), more than that obtained in previous report. Fingerprints of *S. miltiorrhiza* Bunge from different locations were also studied. The content of main components varied in different samples. Overlapping ratio of peaks (ORP) in 10 batches of *S. miltiorrhiza* Bunge (Shandong province) was not less than 72.46%. In method validation, relative standard deviation (RSD) of relative retention times and relative peak areas were of not more than 3%. It was concluded that HPLC-CEAD system can be applied in fingerprinting herbal medicines.

Effect of gosha-jinki-gan, a blended herbal medicine, on bladder activity in rats.

Nishijima, Saori *et al.*

The Journal of Urology, **177(2),** 762 (Feb., 2007)

Authors have investigated the effect of the blended herbal medicine Gosha-jinki-gan on bladder activity and the autonomic nervous system in rats. A total of 42 female rats were divided into a control diet group of 21 and a Gosha-jinki-gan diet group of 21. Rats in the control diet group were fed a standard diet, while animals in the Gosha-jinki-gan were fed a special diet containing 1.08% Gosha-jinki-gan. After 4 weeks 28 rats, including 14 in the control and 14 in the Gosha-jinki-gan group, underwent continuous cystometry with physiological saline or 0.1% acetic acid solution and bladder activity was recorded. The remaining 14 rats were anesthetized with halothane, and body weight, serum amino acid (glutamate and glycine) and plasma monoamine (noradrenaline, adrenaline, dopamine and serotonin) levels were measured. The amplitude of bladder contraction on continuous cystometry with physiological saline was lower in the Gosha-jinki-gan diet group than in the control diet group, and plasma dopamine and serotonin levels were also lower in the Gosha-jinki-gan group. When cystometry was done with 0.1% acetic acid, the interval between bladder contractions was shortened in the control and Gosha-jinki-gan groups. However, the interval and duration of bladder contractions were longer in the Gosha-jinki-gan than in the control group. These results suggest that Gosha-jinki-gan inhibits bladder activity by maintaining the balance of the sympathetic and parasympathetic nervous systems

Effects of three Chinese herbal medicines on plasma and liver lipids in mice fed a high-fat

diet.

Nakayama, Tohru *et al.*

Journal of Ethnopharmacology, **109(2),** 236(Jan., 19, 2007)

Chinese herbal medicines, Inchinko-to, Bofu-tsusho-san and Dai-saiko-to, containing 3, 18 and 8 components, respectively, have since long been used as an anti-inflammatory, antipyretic, choleretic and diuretic agent for liver disorders and jaundice, as an anti-obesity agent, a hypocholesterolemic agent for liver disorders and a therapeutic and/or preventive agent for cholesterol gallstone disease with hypertriglycerid-emia in China and Japan, respectively. In the present study, we investigated the effects of these three herbal medicines in young male mice fed a high-fat diet. Plasma levels of lipids and the numbers of the fatty droplets in the liver cytoplasm were markedly lowered by the diets supplemented with three herbal medicines. The liver weights and the body growth were reduced by the diet supplemented with Dai-saiko-to, which slightly affected the concentrations of total protein, albumin, creatinine or calcium, and the activity of lactate dehydrogenase. Thus, Dai-saiko-to, besides Bofu-tsusho-san, seems effective in the activities of anti-obesity, anti-hyperlipidemia and anti-hyperlipids in liver cytoplasm, when used carefully.

Combinative method using HPLC quantitative and qualitative analysis for quality consistency assessment of a herbal medicinal preparation.

Xie, Ying *et al.*

Journal of Pharmaceutical and Biomedical Analysis, **43(1),** 204 (Jan., 4, 2007)

A selective and efficient quality consistency assessment system was developed for monitoring the manufacturing processes of a Chinese herbal preparation, Qingfu Guanjieshu (QFGJS) capsule, and for assessing its stability over time. This system is based on quantitative determination of four marker compounds, i.e., sinomenine, paeoniflorin, paeonol, and curcumin, and on qualitative fingerprinting analysis of QFGJS using high-performance liquid chromatography-photodiode array detection (HPLC-DAD) method. The separation was performed on a Phenomenex ODS column by gradient elution with acetonitrile and aqueous phase (containing 0.1% phosphoric acid, adjusted with triethylamine to pH 3.5 +/- 0.2) at a flow-rate of 1.0 ml/min. In fingerprinting analysis, the chemical characteristics of four herbs present in QFGJS (excluding *Radix aconiti and Lateralis preparata*) were present in the HPLC chromatographic file. In addition, quantitative determination of hypaconitine was carried out with our published HPLC method as a supplement for quality control of the *Radix aconite and Lateralis preparata* in QFGJS. The results showed that the contents of these five marker compounds and HPLC fingerprint profiles of three batches of QFGJS products collected at 3 months after production in the stability testing were relatively consistent. This well-developed method could be used for quality assessment of the complex preparations of herbal medicine.

Analysis of phenolic compounds in rhubarbs using liquid chromatography coupled with electrospray ionization mass spectrometry.

Ye, Min *et al.*

Journal of the American Society for Mass Spectrometry, **18(1),** 82 (Jan., 2007)

Rhubarb is an important herbal medicine for the treatment of constipation, inflammation, and cancer. In this study, a facile method based on liquid chromatography coupled with electrospray ionization tandem mass spectrometry has been established for the analysis of bioactive phenolic compounds in rhubarbs. From six rhubarb species, official (*Rheum officinale, R. palmatum,* and *R. tanguticum)* and unofficial (*R. franzenbachii, R. hotaoense,* and *R. emodi*), a total of 107 phenolic compounds were identified or tentatively characterized based on their mass spectra. These compounds include sennosides, anthraquinones, stilbenes, glucose gallates, naphthalenes, and catechins. Ion chromatograms for the identified compounds of different rhubarbs were then compared. Consistent with previous reports, sennosides and rhein were only detected in official rhubarbs. It was found that *R. officinale* contained very different phenolic compounds from the other

two official species. Sennoside A, which has been considered as the major purgative component of rhubarb, was only detected in *R. officinale*, while its close isomers were observed in *R. palmatum* and *R. tanguticum*. In addition, the predominant anthraquinone glycosides in *R. officinale* were found to be rhein 8-O-glucoside and emodin 1-O-glucoside, whereas those in *R. palmatum* and *R. tanguticum* were rhein 1-O-glucoside and emodin 8-O-glucoside. Stilbenes, which are the major constituents of unofficial rhubarbs, were also different among the species. These results clarify the chemical composition of rhubarbs comprehensively for the first time. Due to the significant differences in chemical components of rhubarbs, authors suggest that different *Rheum* species be used separately in clinical practice.

Quality assessment of South African herbal medicines by means of HPLC fingerprinting.

Springfield, E.P. *et al.*

Journal of Ethnopharmacology, **101(1-3),** 75(Oct.,3, 2005)

An estimated 70% of South Africans regularly use traditional plant medicines. Incorporation of these medicines within the formal health care system, which is the stated intention of the Health Ministry, requires the establishment of standards for quality control. Except in the case of a handful of South African plant species, such standards are lacking. Of central importance with respect to quality control is correct identification of the species concerned, whether in the fresh, dried or powdered state. In cases where botanical identification is impossible, high performance liquid chromatography (HPLC) with diode array detection (DAD), offers an alternative qualitative profile and is being increasingly used for the authentication of crude drugs or their extracts. As a contribution to establishing quality standards for South African plant species used as traditional medicines, HPLC-DAD "fingerprints" of 60 commonly-used species have been generated in our laboratory. One of these species is presented here, together with UV spectra of individual components represented by major peaks in the HPLC profiles.

Comparison of petal of *Crocus sativus* L. and fluoxetine in the treatment of depressed outpatients: A pilot double-blind randomized trial.

Akhondzadeh Basti, Afshin *et al.*

Progress in Neuro-Psychopharmacology and Biological Psychiatry (In Press)

Depression is one of the most common neuropsychiatric conditions, with a lifetime prevalence approaching 17%. Although a variety of pharmaceutical agents is available for the treatment of depression, psychiatrists find that many patients cannot tolerate the side effects, do not respond adequately, or finally lose their response. On the other hand, many herbs with psychotropic effects have far fewer side effects. They can provide an alternative treatment or be used to enhance the effect of conventional antidepressants. A number of recent preclinical and clinical studies indicate that stigma and petal of *Crocus sativus* have antidepressant effect. Our objective was to compare the efficacy of petal of *C. sativus* with fluoxetine in the treatment of depressed outpatients in an 8-week pilot double-blind randomized trial. Forty adult outpatients who met the DSM- IV criteria for major depression based on the structured clinical interview for DSM-IV participated in the trial. Patients have a baseline Hamilton Rating Scale for Depression score of at least 18. In this double-blind and randomized trial, patients were randomly assigned to receive capsule of petal of *C. sativus* 15 mg bid (morning and evening) (Group 1) and fluoxetine 10 mg bid (morning and evening) (Group 2) for a 8-week study. At the end of trial, petal of C. sativus was found to be effective similar to fluoxetine in the treatment of mild to moderate depression ($F = 0.03$, d.f. = 1, $P = 0.84$). In addition, in the both treatments, the remission rate was 25%. There were no significant differences in the two groups in terms of observed side effects. The present study is supportive of other studies which show antidepressant effect of *C. sativus.*

Sunflower therapy for children with specific learning difficulties (dyslexia): A randomised, controlled trial.

Bull L.

Complement Ther Clin Pract. 2007 Feb;**13(1):**15-24.

The aim of the study was to determine the clinical and perceived effectiveness of the Sunflower therapy in the treatment of childhood dyslexia. The Sunflower therapy includes applied kinesiology, physical manipulation, massage, homeopathy, herbal remedies and neuro-linguistic programming. A multi-centred, randomised controlled trial was undertaken with 70 dyslexic children aged 6-13 years. The research study aimed to test the research hypothesis that dyslexic children 'feel better' and 'perform better' as a result of treatment by the Sunflower therapy. Children in the treatment group and the control group were assessed using a battery of standardised cognitive, Literacy and self-esteem tests before and after the intervention. Parents of children in the treatment group gave feedback on their experience of the Sunflower therapy. Test scores were compared using the Mann Whitney, and Wilcoxon statistical tests. While both groups of children improved in some of their test scores over time, there were no statistically significant improvements in cognitive or Literacy test performance associated with the treatment. However, there were statistically significant improvements in academic self-esteem, and reading self-esteem, for the treatment group. The majority of parents (57.13%) felt that the Sunflower therapy was effective in the treatment of learning difficulties. Further research is required to verify these findings, and should include a control group receiving a dummy treatment to exclude placebo effects.

Herbal remedy in the treatment of malaria: cross sectional survey of residents of Lagos State, Nigeria.

Idowu ET *et al.*.

Afr J Med Med Sci. 2006 Jun;**35(2):**149-53.

Semi structured questionnaires. designed to capture information on the type. composition, method of preparation. dosage, mode of administration. and frequency of use of herbal preparations in malaria treatment, were administered to 1,593 adults of the 3 main ethnic groups and a forth group comprising other smaller ethnic groups designated as "others", all resident in Lagos metropolis in a cross sectional survey. The 1,593 respondents were made up of 892 males and 701 females and their ages ranged from 19 to 60 years. A high percentage in all the ethnic groups especially the Yorubas admitted to the use of herbs in treating malaria [Yoruba (69%), Hausa (47%). others (32%) and Igbo (30%)1. Effectiveness of herbs in treating malaria episodes featured as the major factor for their use. as claimed by the majority (>50%) of the respondents in each of the ethnic groups, while cost consideration was the next most important factor. Other factors mentioned included the absence of side effect in herbal use. to avoid the itchy side effect and ineffectiveness of chloroquine and some other anti-malarials. An appreciable percentage across the ethnic groups had no idea of the constituents of the herbal remedies they use for treating their malaria episodes since they buy these from traditional herbalists. Varied combinations of these herbs in combination with different types of fruits and other substances are claimed to be used, the main ones of which are *Azardiracha indica* and pineapple. A large majority of respondents in all the ethnic groups claimed to use the same herbs for the treatment and prevention of malaria and great improvement is experienced after use [Hausas (90%). Igbos (83%). Yorubas (77%) and the others (88%)]. There is usually no specific dose or dose regimen however a high proportion in all the ethnic groups use herbal preparation thrice a day and a few of the respondents take unspecified measures at arbitrary intervals. The lack of standards in the use of these herbal preparations needs to be urgently addressed especially as use continued until the malaria symptoms and signs are deemed to have disappeared. There is also need to standardize the usage of herbs if they are to play a significant role in malaria prevention and treatment.

Traditional management of ear, nose and throat (ENT) diseases in Central Kenya.

Njoroge GN *et al.*

J Ethnobiol Ethnomedicine. 2006 Dec 27;**2:**54.

Diseases of ear, nose and throat (ENT) often have serious consequences including hearing impairment, and emotional strain that lower the quality of life of patients. In Kenya, upper respiratory infections are among the most common infections encountered in outpatient facilities. Some of these infections are becoming difficult to control because some of the causing microorganisms have acquired antibiotic resistance and hence the need to develop new drugs with higher efficacy. Ethnobotanical studies have now been found to be instrumental in improving chances of discovering plants with antimicrobial activity in new drug development. In Kenya the majority of local people are turning to herbal remedies for primary health care needs. In most cases the sources of these remedies are undocumented and the knowledge about them passed orally form generation to generation, hence under threat of disappearing with current rates of modernisation.This study explored the traditional remedies used in managing various ENT diseases in seven districts of the Central Province of Kenya. The most common ENT conditions managed using traditional therapies include: common cold, cough, tonsillitis, otitis-media, chest pains and asthma. The results indicate that 67 species belonging to 36 plant families were utilized in this region. These plants were of varying habits; herbs (37.3%), shrubs (34.4%), trees (25.4%) as well as some grasses and sedges (3%). The traditional preparations were found to be made mainly from leaves (49%), roots (20.5%) and barks (12.5%). For each of the ENT conditions multiple species are utilized mainly as individual preparations but occasionally as polyherbal concoctions. In the case of common cold for example, 30 different species are used. Plants reported in this survey are important candidates for antimicrobial tests against ENT disease causing micro-organisms, especially those with antibiotic resistance.

A pharmaco-metabonomic study on the therapeutic basis and metabolic effects of *Epimedium brevicornum* Maxim. on hydrocortisone-induced rat using UPLC-MS.

Li F *et al.*

Biomed Chromatogr. 2007 Jan 19

This paper describes the pharmaco-metabonomic study on *Epimedium brevicornum* Maxim. treated rats with a pathologic condition similar to the 'kidney deficiency syndromes' in traditional Chinese medicine and its therapeutic basis. UPLC-MS technique was used for the development of chemical profile of *Epimedium brevicornum* Maxim. and endogenous metabolite profiles of rats pre- and post-hydrocortisone interfered and treated with this herbal medicine. The comparison among profiles was performed with a statistical technique, principle component analysis (PCA). Significant difference in endogenous metabolite profiles was observed in the intervention rats and the abnormality of metabolism recovered towards the normal level after administration with *Epimedium brevicornum* Maxim. extract. Four active constituents of *Epimedium brevicornum* Maxim. were found into the blood circulation of kidney-deficient rats and two of their metabolites in the urine. This work suggests that the metabonomic approach is a potentially powerful tool to explore the therapeutic basis and to clarify the possible action mechanism of traditional Chinese medicine.

Studies on the effect of extracts of several Chinese herbal medicines and other medicines on alcohol dehydrogenase activity.

Li WZ *et al.*

Zhong Yao Cai. 2006 Aug; **29(8):**816-8.

To study the effects of water and alcohol extracts of several Chinese herbal medicines and other medicines on alcohol dehydrogenase (ADH) activity in order to provide enzymology basis on new medicine was carried out. Water or alcohol extracts of Chinese herbal medicine and other medicine were tested on the effects of alcohol dehydrogenase activity by Valle and Hoch method. Among them, 8 were found to have the effect of activation on alcohol dehydrogenase. They were water extracts of Amomum kravanh and Pueraria flowers, the alcohol extracts of Pueraria flowers, compound hepatcare Chinese medicine and compound Pueraria medicine, L-cysteine, notoginseng saponin. Others had inhibiting action.

To decrease alcohol concentration in the body through activating the activity of ADH may be one of the mechanisms for some traditional Chinese herbal medicine in neutralizing the effect of alcohol drink.

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Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice.

Li XM *et al.*

J Ethnopharmacol. 2006 Dec 28;

Oxidative damage of biomolecules increases with age and is postulated to be a major causal factor of various physiological function disorders. Consequently, the concept of anti-ageing by antioxidants has been developed. *Lycium barbarum* fruits have been used as a traditional Chinese herbal medicine and the data obtained in *in vitro* models have clearly established the antioxidant potency of the polysaccharides isolated from the fruits. In the present study, the age-dependent changes in the antioxidant enzyme activity, immune function and lipid peroxidation product were investigated and effect of *Lycium barbarum* polysaccharides on age-induced oxidative stress in different organs of aged mice was checked.

Lycium barbarum polysaccharides (200, 350 and 500mg/kg b.w. in physiological saline) were orally administrated to aged mice over a period of 30 days. Aged mice receiving vitamin C served as positive control. Enzymatic and non-enzymatic antioxidants, lipid peroxides in serum and tested organs, and immune function were measured. Result showed that increased endogenous lipid peroxidation, and decreased antioxidant activities, as assessed by superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and total antioxidant capacity (TAOC), and immune function were observed in aged mice and restored to normal levels in the polysaccharides-treated groups. Antioxidant activities of *Lycium barbarum* polysaccharides can be comparable with normal antioxidant, vitamin C. Moreover, addition of vitamin C to the polysaccharides further increased the *in vivo* antioxidant activity of the latter. It is concluded that the *Lycium barbarum* polysaccharides can be used in compensating the decline in TAOC, immune function and the activities of antioxidant enzymes and thereby reduces the risks of lipid peroxidation accelerated by age-induced free radical.

Antihyperglycemic activity of herb extracts on streptozotocin-induced diabetic rats.

Jung CH *et al.*

Biosci Biotechnol Biochem. 2006 Oct;**70(10):**2556-9.

Authors have investigated the effects of herb extracts, *Rhus verniciflua, Agrimonia pilosa, Sophora japonica*, and *Paeonia suffruticosa,* on the lowering of blood glucose levels and thiobarbituric acid reactive substances (TBARS) in streptozotocin (STZ)-induced diabetic rats. After 4 weeks, oral administration of *Rhus verniciflua* extract (50 mg/kg) exhibited a significant decrease in blood glucose levels in diabetic rats ($P<0.05$). Blood TBARS concentrations, the products of glucose oxidation in blood, were also lowered by Rhus verniciflua extract supplementation. In addition, *Sophora japonica* and *Paeonia suffruticosa* extracts significantly reduced TBARS levels versus diabetic controls. Serum concentrations of liver-function marker enzymes, GOT and GPT, were also restored by *Rhus verniciflua* (50 mg/kg) supplementation in diabetic rats.

Stimulative effects of *Ulmus davidiana* Planch (Ulmaceae) on osteoblastic MC3T3-E1 cells.

Suh SJ *et al.*

J Ethnopharmacol. 2007 Feb 12;**109(3):**480

Ulmus davidiana Planch (Ulmaceae) has long been known to have anti-inflammatory and protective effects on damaged tissue, inflammation and bone among other functions. To treat rheumatoid arthritis (RA), a herbal medicine, *Ulmus davidiana* Planch (Ulmaceae) extract (UD) is being used in traditional oriental medicine. The effect of UD on the proliferation and osteoblastic differentiation in non-transformed osteoblastic cells (MC3T3-E1) was studied. UD dose-dependently increased DNA synthesis (significant at 5-20mug/ml). UD increased alkaline phosphatase (ALP) activity and prolyl hydroxylase activity of MC3T3-E1 cells (5-20mug/ml).

Antiestrogen tamoxifen eliminated the stimulation of proliferation and ALP activity of MC3T3-E1, which was induced by UD. UD at concentrations ranged from 30 to 100mug/ml inhibited prostaglandin E2 production in MC3T3-E1. These results indicate that UD directly stimulates cell proliferation and differentiation of osteoblasts. These results also suggest and UD is effective for bone anti-resorptive action in bone cells.

A study of the neuroprotective effect of the phenolic glucoside gastrodin during cerebral ischemia *in vivo* and *in vitro*.

Zeng X *et al.*

Planta Med. 2006 Dec;**72(15):**1359-65.

The phenolic glucoside gastrodin (Gas) is themain component extracted from the rhizome of *Gastrodia elata*, a Chinese herbal medicine, which has long been used for treating dizziness, epilepsy, stroke and dementia. In this study, authors investigated the neuroprotective effects of Gas on cerebral ischemic injury in rats caused by transient middle cerebral arterial occlusion (MCAO), oxygen/glucose deprivation (OGD) and glutamate-induced injury in cultured rat hippocampal neurons. Additionally, the effects of Gas on the extracellular glutamate level and changes in intracellular Ca (2+) and the generation of nitric oxide (NO) were examined in cultured hippocampal neurons subjected to OGD *in vitro*. The results showed that the high dose of Gas (100 mg/kg) markedly decreased the infarct volume and edema volume, and improved the neurological functions after MCAO. Gas treatment (15 mug/mL, 30 mug/mL) also significantly inhibited OGD- and glutamate-induced neuronal cell death and reduced the extracellular glutamate level following OGD. Moreover, Gas treatment significantly inhibited the OGD-induced Ca (2+) and NO increases. In conclusion, the present study indicates that Gas has a neuroprotective action.

Antipyretic effect of Mao-to, a Japanese herbal medicine, for treatment of type A influenza infection in children.

Kubo T *et al.*

Phytomedicine. 2006 Nov 30

Mao-to is a Japanese traditional herbal medicine which has been used since ancient times for the treatment of influenza-like illness. This study was conducted to evaluate the effect of oral Mao-to administration in children with type A influenza, in comparison to Oseltamivir. Authors performed a controlled trial of 60 children, from 5 months through 13 years of age, with fever and influenza-like symptom of up to 48h duration. Diagnosis of influenza type A was performed by virus isolation or detection of a viral gene by RT-PCR. Patients assigned into the following 3 groups: oral Mao-to powder (TJ-27) 0.06g/kg body wt./dose three times daily (n=17), Oseltamivir 2mg/kg body wt./dose twice daily (n=18) or both oral Mao-to plus Oseltamivir (n=14). The median duration of fever after treatment was significantly shorter in the Mao-to and Mao-to plus Oseltamivir groups, compared with the Oseltamivir only group (15h [95%CI 13.2-22.1] $p<0.01$; 18h[15.2-27.7] $p<0.05$; 24h[23.5-43.0], respectively). Oral Mao-to administration was effective in the control of fever due to type A influenza infection in children.

Gypenoside XLIX, a naturally occurring gynosaponin, PPAR-alpha dependently inhibits LPS-induced tissue factor expression and activity in human THP-1 monocytic cells.

Huang TH *et al.*

Toxicol Appl Pharmacol. 2007 Jan 1;**218(1):**30-6.

Tissue factor (TF) is involved not only in the progression of atherosclerosis and other cardiovascular diseases, but is also associated with tumor growth, metastasis, and angiogenesis and hence may be an attractive target for directed cancer therapeutics. *Gynostemma pentaphyllum* (GP) is widely used in the treatment of various cardiovascular diseases including atherosclerosis, as well as cancers. Gypenoside (Gyp) XLIX, a dammarane-type glycoside, is one of the prominent components in GP. It has been reported that Gyp XLIX is a potent peroxisome proliferator-activated receptor (PPAR)-alpha activator. Here authors demonstrate that Gyp XLIX (0-300 muM) concentration dependently inhibited TF promoter activity after induction by the inflammatory stimulus lipopolysaccharide (LPS) in human

monocytic THP-1 cells transfected with promoter reporter constructs pTF-LUC. Furthermore, Gyp XLIX inhibited LPS-induced TF mRNA and protein overexpression in THP-1 monocyte cells. Its inhibition of LPS-induced TF hyperactivity was further confirmed by chromogenic enzyme activity assay. The activities of Gyp XLIX reported in this study were similar to those of Wy-14643, a potent synthetic PPAR-alpha activator. Furthermore, the Gyp XLIX-induced inhibitory effect on TF luciferase activity was completely abolished in the presence of the PPAR-alpha selective antagonist MK-886. The present findings suggest that Gyp XLIX inhibits LPS-induced TF overexpression and enhancement of its activity in human THP-1 monocytic cells via PPAR-alpha-dependent pathways. The data provide new insights into the basis of the use of the traditional Chinese herbal medicine *G. pentaphyllum* for the treatment of cardiovascular and inflammatory diseases, as well as cancers.

***In vitro* evaluation of antibacterial and immunomodulatory activities of *Pelargonium reniforme, Pelargonium sidoides* and the related herbal drug preparation EPs(R) 7630**

Kolodziej, Herbert *et al.*

Phytomedicine (In Press)

The importance of *Pelargonium* species, most notably *Pelargonium reniforme* and *Pelargonium sidoides,* in traditional medicine in the Southern African region is well documented. Nowadays, a modern aqueous-ethanolic formulation of the roots of *P. sidoides* (EPs(R) 7630) is successfully employed for the treatment of ear, nose and throat disorders as well as respiratory tract infections. To provide a scientific basis of its present utilization in phytomedicine, EPs(R) 7630, extracts and isolated constituents of the titled Pelargoniums with emphasis on *P. sidoides* were evaluated for antibacterial activity and for their effects on nonspecific immune functions. The samples exhibited merely moderate direct antibacterial capabilities against a spectrum of Gram-positive and Gram-negative bacteria. Functional bioassays including an *in vitro* model for intracellular diseases, a fibroblast-lysis assay (tumour necrosis factor (TNF) activity), a fibroblast-virus protection assay (IFN activity) and a biochemical assay for nitric oxides revealed significant immunomodulatory properties. Gene expression experiments (iNOS, IFN-[alpha], IFN-[gamma], TNF-[alpha], Interleukin (IL)-1, IL-10, IL12, IL-18) not only confirmed functional data, they also clearly showed differences in the response of infected macrophages when compared to that of noninfected cells. ELISA confirmed the protein production of TNF-[alpha], IL-1[alpha] and IL-12, while FACS analyses reaffirmed the cytokines IL-1[alpha] and IL-12 at the singular cell level. The current data provide convincing support for the improvement of immune functions at various levels, hence, validating the medicinal uses of EPs(R) 7630. Despite considerable efforts, the remedial effects cannot yet be related to a chemically defined principle.

Prostaglandin-H-synthase (PGHS)-1 and -2 microtiter assays for the testing of herbal drugs and *in vitro* inhibition of PGHS-isoenzyms by polyunsaturated fatty acids from *Platycodon radix.*

Reininger, E.A. *et al.*

Phytomedicine, **13(3),** 164 (Feb., 13, 2006)

In order to test inhibition of prostaglandin-H-synthase-1 and -2 (PGHS-1 and -2) by plant extracts, authors have established two enzyme based *in vitro* assays with enzyme immunoassay (EIA) evaluation. The assays have been evaluated with known synthetic inhibitors and with plant extracts. In a screening of traditionally used Chinese herbs for anti-inflammatory activity, a series of n-hexane and dichloromethane extracts showed significant inhibitory effect in comparison with the known specific PGHS-2 inhibitors NS-398 (IC50=2.6 [mu]M) and nimesulide (IC50=36 [mu]M). The lipophilic extracts of the Chinese drug Jiengeng, the dried roots of *Platycodon grandiflorum* (Jacq.) A. DC. (Campanulaceae), showed good inhibitory activity against both PGHS isoenzymes. The directly prepared DCM-extract exhibited better activity against PGHS-2 (IC50=4.0 [mu]g/ml) than against PGHS-1 (IC50=17.6 [mu]g/ml). We identified fatty acids as main active

constituents and quantified them. Linoleic acid showed the highest content (ca. 20% of the dried extract) and a high and preferential PGHS-2 inhibitory activity (IC50 (PGHS-1)=20 [mu]M; IC50 (PGHS-2)=2 [mu]M). The comparison of the concentration of linoleic acid and the inhibitory activity of the direct DCM-extract showed, that linoleic acid is mainly responsible for the *in vitro* activity of the extract on PGHS-2.

Skin penetration behaviour of sesquiterpene lactones from different *Arnica* preparations using a validated GC-MSD method.

Wagner, Steffen *et al.*

Journal of Pharmaceutical and Biomedical Analysis, **43(1),**32(Jan.,4, 2007)

Preparations of *Arnica montana* L. are widely used for the topical treatment of inflammatory diseases. The anti-inflammatory activity is mainly attributed to their sesquiterpene lactones (SLs) from the helenalin and 11[alpha],13-dihydrohelenalin type. To study the penetration kinetics of SLs in Arnica preparations, a stripping method with adhesive tape and pig skin as a model was used. For the determination of SLs in the stripped layers of the stratum corneum (SC), a gas chromatography/mass spectrometry method was developed and validated. Thereby the amount of helenalin derivatives was calculated as helenalin isobutyrate, and 11[alpha],13-dihydrohelenalin derivatives as 11[alpha],13-dihydrohelenalin methacrylate. This GC-MSD method is suitable also to determine low amounts of SLs in Arnica preparations. The penetration behaviour of one gel preparation and two ointment preparations was investigated. The SLs of all preparations show a comparable penetration in and a permeation through the stratum corneum, the uppermost part of the skin. Interestingly, the gel preparation showed a decrease of the penetration rate over 4 h, whereas the penetration rate of ointments kept constant over time. Moreover, we could demonstrate that the totally penetrated amount of SLs only depends on the kind of the formulation and of the SLs-content in the formulation but not on the SLs composition or on the used extraction agent.

Ginseng and Salviae herbs play a role as immune activators and modulate immune responses during influenza virus infection.

Quan, Fu Shi *et al.*

Vaccine, **25(2),** 272 (Jan., 4, 2007)

Authors have investigated the adjuvant roles of common herbal medicines (Ginseng, Salviae) and their effects on early immune responses during influenza virus infection in a mouse model. Intranasal co-administration with inactivated influenza virus A (PR8) and ginseng or Salviae extract increased the levels of influenza virus specific antibodies and neutralizing activities compared to immunization with PR8 alone, and provided protective immunity. Salviae co-administration significantly enhanced IFN-[gamma] and IL-2 cytokine producing splenocytes while ginseng induced high levels of IL-4 and IL-5 cytokine producing cells after challenge infection. Cells expressing an early activation marker CD69 and levels of a pro-inflammatory cytokine IL-6 were highly elevated in lungs from naive mice during challenge virus infection, which might be a mechanism in lung inflammation leading to death. In contrast, immunized mice that were co-administered ginseng or Salviae modulated CD69 expressing immune cells, did not produce IL-6, and showed significant enhancement of influenza virus specific IgA antibody in lungs after challenge virus infection. Therefore, these results indicate that both ginseng and Salviae play a role as mucosal adjuvants against influenza virus as well as immuno-modulators during influenza virus infection.

***Antrodia camphorata* extract induces replicative senescence in superficial TCC, and inhibits the absolute migration capability in invasive bladder carcinoma cells.**

Peng, Chiung-Chi *et al.*

Journal of Ethnopharmacology, **109(1),** 93 (Jan., 3, 2007)

The *Antrodia camphorata* crude extract (ACCE), an extract obtained from a precious traditional Chinese folkloric herbal medicine Zhan-Ku (a camphor tree mushroom) since the 18th century, has showed rather significant inhibitory

effects on the growth and proliferation of the transitional cell carcinomas (TCC) cell lines RT4, TSGH-8301, and T24. On treatment with ACCE at 100 [mu]g/mL, the p53-independent overexpression of p21 with simultaneous down alteration of pRb was observed in RT4, which was thus speculative of proceeding through a mechanism of replicative senescence. On the contrary treatment with ACCE, at 50 [mu]g/mL, resulting in simultaneous down-regulations of Cdc2 and Cyclin B1, with suppression of the absolute migrating capability of the two cell lines TSGH-8301 and T24, and eventually the cell deaths. These findings suggest that ACCE can be rather effective and beneficial in suppression of both the superficial cancer cell line RT4 and the metastatic cell lines (TSGH-8301 and T24) through different mechanisms.

Protective effects of echinacoside on carbon tetrachloride-induced hepatotoxicity in rats.

Wu, Yu *et al.*

Toxicology (In Press)

The study was carried out to investigate the possible protective effects of echinacoside, one of the phenylethanoids isolated from the stems of *Cistanches salsa*, a Chinese herbal medicine, on the free radical damage of liver caused by carbon tetrachloride in rats. Treatment of rats with carbon tetrachloride produced severe liver injury, as demonstrated by dramatic elevation of serum ALT, AST levels and typical histopathological changes including hepatocyte necrosis or apoptosis, haemorrhage, fatty degeneration, etc. In addition, carbon tetrachloride administration caused oxidative stress in rats, as evidenced by increased reactive oxygen species (ROS) production and MDA concentrations in the liver of rats, along with a remarkable reduction in hepatic SOD activity and GSH content. However, simultaneous treatment with echinacoside (50 mg/kg, intraperitoneally) significantly attenuated carbon tetrachloride-induced hepatotoxicity. The results showed that serum ALT, AST levels and hepatic MDA content as well as ROS production were reduced dramatically, and hepatic SOD activity and GSH content were restored remarkably by echinacoside administration, as compared to the carbon tetrachloride-treated rats. Moreover, the histopathological damage of liver and the number of apoptotic hepatocytes were also significantly ameliorated by echinacoside treatment. It is therefore suggested that echinacoside can provide a definite protective effect against acute hepatic injury caused by CCl4 in rats, which may mainly be associated with its antioxidative effect.

Flavonoids with anti-Helicobacter pylori activity from *Cistus laurifolius* leaves.

Ustun, Osman *et al.*

Journal of Ethnopharmacology, **108(3),** 457 (Dec., 6, 2006)

Cistus laurifolius flower buds are used traditionally in folk medicine against gastric ailments. The prior studies have shown that the chloroform extract of *Cistus laurifolius* had a potent anti-ulcer activity. It has been known that there is a causal relationship between peptic ulcer and *Helicobacter pylori* infection. In a previous study, it was found that chloroform extract of *Cistus laurifolius* possessed a significant anti-*Helicobacter pylori* activity. Authors designed this study to isolate and define the active component(s) involved in the anti-*Helicobacter pylori* activity of the extract through activity-guided fractionation procedures. The chloroform extract was fractionated by using various chromatography techniques, i.e., Sephadex LH-20 column chromatography and preparative thin layer chromatography and six compounds were isolated (1-6). Each of these six compounds' anti-*Helicobacter pylori* activity was tested *in vitro* and was measured as minimum inhibition concentration (MIC) values by using agar dilution method. The compound 2 had the highest activity against *Helicobacter pylori* (MIC 3.9 [mu]g/mL). Its chemical structure was elucidated as quercetin 3-methyl ether (isorhamnetin) by various spectroscopic techniques. These observations are indicative that the therapeutic effect of *Cistus laurifolius* in ulcer is at least partially related to its effect on *Helicobacter pylori.* Authors hope that the isolated flavonoid having anti-Helicobacter pylori activity ultimately can be utilized as an

alternative or additive agent to the current therapy.

Inhibition of P-glycoprotein-mediated transport by terpenoids contained in herbal medicines and natural products.

Yoshida, Naoko *et al.*

Food and Chemical Toxicology, **44(12),** 2033 (Dec., 2006)

Terpenoids form a large and structurally diverse family of natural products and are ingredients of various herbal medicines. Authors have investigated possible interactions between herbal medicines and conventional medicines, and recently reported that monoterpenoids contained in *Zanthoxyli fructus* can be potent inhibitors of P-glycoprotein (P-gp). In the present study, the influence of 70 kinds of terpenoids present in natural products on P-gp-mediated efflux transport was investigated. LLC-GA5-COL150 cells transfected with human MDR1 cDNA encoding P-gp were used to screen the terpenoids. Large increases in the intracellular accumulation of [3H]digoxin were observed in the presence of (R)-(+)-citronellal, (S)-(-)-[beta]-citronellol, [alpha]-terpinene, terpinolene, (-)-[beta]-pinene, abietic acid, ophiobolin A, cucurbitacin I, and glycyrrhetic acid. A study of the concentration-dependency revealed that the IC50 of ophiobolin A, glycyrrhetic acid, (R)-(+)-citronellal, abietic acid, and cucurbitacin I was smaller than that of verapamil. The transcellular transport of [3H]digoxin across Caco-2 cell monolayers was then examined in the presence of (R)-(+)-citronellal, abietic acid, and glycyrrhetic acid. Significant increases in the apical-to-basolateral transport and decreases in the basolateral-to-apical transport and efflux ratio were demonstrated. These findings suggest that some natural products containing these terpenoids may inhibit P-gp-mediated transport and interact with P-gp substrates in the intestinal absorption process.

Recent advances in coumarins and 1-azacoumarins as versatile biodynamic agents.

Kulkarni MV *et al.*

Curr Med Chem. 2006;**13(23):**2795-818.

Coumarins, also referred as benzopyran-2-ones, and their corresponding nitrogen counterpart, 1-azacoumarins also referred to as carbostyrils, are a family of nature-occurring lactones and lactams respectively. The plant extracts containing coumarin-related heterocycles, which were employed as herbal remedies in early days, have now been extensively studied for their biological activities. These investigations have revealed their potentials as versatile biodynamic agents. For example, coumarins with phenolic hydroxyl groups have the ability to scavenge reactive oxygen species and thus prevent the formation of 5-HETE and HHT in the arachidonic pathway of inflammation suppression. Recent *in vivo* studies have revealed the role of coumarins in hepatotoxicity and also in depletion of cytochrome P450. Similarly 1-azacoumarins which is part of quinoline alkaloids, are known for their diverse biological activity and recently, a 6-functionalized 1-aza coumarins are undergoing human clinical trials as an orally active anti-tumor drug in view of its farnesyl protein-inhibiting activity in the nanomolar range. Furthermore, several synthetic coumarins with a variety of pharmacophoric groups at C-3, C-4 and C-7 positions have been intensively screened for anti-microbial, anti-HIV, anti-cancer, lipid-lowering, anti-oxidant, and anti-coagulation activities. Specifically, coumarin-3-sulfonamides and carboxamides were reported to exhibit selective cytotoxicity against mammalian cancer cell lines. The C4-substituted aryloxymethyl, arylaminomethyl, and dichloroacetamidomethyl coumarins, along with the corresponding 1-azacoumarins, have been demonstrated to be potential anti-microbial and anti-inflammatory agents. To expand the structural diversity of synthetic courmarins for biological functions, attempts have also been made to attach a chloramphenicol side chain at C-3 position of courmarin. In addition, the bi- and tri-heterocyclic coumarins and 1-azacoumarins with benzofuran, furan and thiazole ring systems along with biocompatible fragments like vanillin have shown remarkable potency as anti-inflammatory agents in animal models. Photobiological studies on pyridine-fused polycyclic coumarins have highlighted their potential as thymine dimer photosensitisers and the structurally related

compounds of both coumarin and carbostyrils have also been found to act via the DNA gyrase pathway in their anti-bacterial activity. Apart from the above works, the present review also addresses the potential roles of coumarins and carbostyrils as protease inhibitors, or fluorescent probes in mechanistic investigation of biochemical pathways, and their application for QSAR in theoretical studies. Though 1-Azacoumarins have received less attention as compared to coumarins in the literature, an attempt has been made to compare both the systems at various stages, so that it can spark new thoughts on synthetic methodologies, reactivity pattern and biological activities.

Discrimination of Herbal Medicines by Molecular Spectroscopy and Chemical Pattern Recognition

1. Introduction

Historically, herbal medicines have played an important role in clinic therapy in China. They are widely used and cultivated in Asian countries such as China, Japan and Korea. Unlike synthetic drugs, herbal medicine is a complicated system of mixtures. The quality and efficacy of these kinds of botanical drugs are somewhat different according to geographical origin and processing methods even the plants are from the same species. Traditionally, the identification of herbal medicines mainly depends on the difference of the appearance of the plants, which could only be figured out by the experienced herbalist doctors. Some modern analytical techniques, such as high performance liquid chromatography, gas chromatography, gas chromatography–mass spectrometry and thin layer chromatography, have been also adopted for this purpose. Because of time-consuming and laborious preparation of samples made these methods unpopular. Therefore a rapid, simple and accurate analytical method is essentially required for the qualitative analysis of herbal medicines. For this purpose molecule spectroscopic analysis was applied to evaluating herbal medicines. In this study, chemical pattern recognition technique (clustering analysis) combined with molecular spectroscopic data, including Raman spectra, IR spectra and near infrared (NIR) spectra, was employed to clustering ginsengs according to origin and processing methods. The identification between ginsengs and pseudo-ginsengs was also carried out to test the efficiency of this method.

2. Chemometric Theory

Clustering analysis can be considered the most important unsupervised learning problem. A loose definition of clustering analysis could be "the process of organizing objects into groups whose members are similar in some way". A cluster is therefore a collection of objects, which are "similar" between them and are "dissimilar" to the objects belonging to other clusters. Hierarchical clustering method is usually employed in clustering analysis. Given a set of *N* items to be clustered, the basic process of hierarchical clustering is this:

1. Start by assigning each item to a cluster, so that if you have *N* items, you now have *N* clusters, each containing just one item. The distances (similarities) between the clusters are the distances (similarities) between the items they contain.
2. Find the closest (most similar) pair of clusters and merge them into a single cluster, so that now you have one cluster less.
3. Compute distances (similarities) between the new cluster and each of the old clusters.
4. Repeat steps 2 and 3 until all items are clustered into a single cluster of size *N*. A clustering tree will be gotten finally after these steps are carried out.

As we can see from Step 2, the similarity criterion is distance. An important component of a clustering algorithm is the distance measure between data points. Usually one could use Mahalanobis distance or Euclidean distance to measure the distance between two clusters. However, there are no general theoretical guidelines for selecting the kind of distance in many given applications. Euclidean distance was employed in this presented article. The Euclidean distance between clusters *i* and *j* is:

Step 3 can be done in different ways, which makes the differences between hierarchical clustering methods such as single-link clustering, complete-linkage, average-linkage clustering, error sum of squares clustering method, etc. In single-link clustering (also called the minimum method), we consider the distance between one cluster and another cluster to be equal to the shortest distance from any member of one cluster to any member of the other cluster. In complete-link clustering (also called maximum method), one considers the distance between one cluster and another cluster to be equal to the longest distance from any member of one cluster to any member of the other cluster. In average-link clustering, one considers the distance between one cluster and another cluster to be equal to the average distance from any member of one cluster to any member of the other cluster.

3. Results and Discussion

The main components of ginsengs, which are much different with pseudo-ginsengs, are polysaccharides, starch, amino acids and some trace elements, etc. In general ginsengs could be classified as two clusters according to geographic origin: Ginseng from Northeast Asia, mainly including China, Japan, and Korea and Radix Panacis Quinquefolii from North America, which now has been cultivated successfully in China. The components, such as contents of polysaccharides, starch, amino acids, etc., between Asia and American ginsengs are different due to different origin. Red ginseng (RGR) and white ginseng (RG) all come from Asia ginseng and the only difference between them is the processing method, which leads to the differences in the components. Maltol and its glycoside are peculiar components in red ginseng and the content of argininyl–fructosyl–glucoside in red ginseng is three times higher than that of white ginseng. It's proved that those differences between red ginseng and white ginseng are mainly caused by Maillard reaction during the processing of ginsengs.

Those differences among red ginseng, white ginseng, American ginseng and pseudo-ginsengs in components lead to the differences of their spectra. That is the basis of spectra analysis and clustering analysis.

3.1. Spectra analysis results

It can seen from Raman and IR spectra of all samples, the non-ginsengs (RP and RC) could be identified with ginsengs (RG, RGR, KRGR, and RPQ) directly because their spectra are much different with others. But there are no significant differences among the ginsengs in the characteristic absorption bands and fingerprint region by visual recognition.

Because of the similarity of all samples NIR spectra and all the Raman and IR spectra of ginsengs, chemometric analysis is applied to cluster samples.

3.2. Clustering analysis results

Clustering analysis was carried out using the normalized spectra for all the spectra in the selected region, which was 8640–4100 cm^{-1} in NIR diffuse reflectance spectra and both 3430–2800 and 1720–180 cm^{-1} for DRIFT spectra and FT-Raman spectra. All spectra were pretreated by 2nd derivative, which can accentuate sharp spectral features and help resolve overlapping bands. Further more, derivatization can also minimize the effects of scattering by removing additive offsets that are independent of wavelength (1st derivatization) or by removing additive offsets that change linearly with wavelength (2nd derivatization).

The cluster trees of samples from FT-Raman spectra, DRIFT spectra and NIR diffuse reflectance spectra. In cluster trees all herbal medicines were clustered into four items: white ginsengs (RG), red ginsengs (RGR), America ginsengs (RPQ) and pseudo-ginsengs (RC and RP).

In clustering tree of FT-Raman spectroscopic analysis, the pseudo-ginsengs were excluded from the ginsengs (RG, RGR, and RPQ) at first. Then red ginsengs including Chinese red ginseng (RGR) and Korean red ginseng (KRGR) were clustered into one item called cluster of red ginseng. The rest samples (white ginsengs and American ginsengs) were classified correctly according species. In

clustering tree of FT-IR spectroscopic analysis, most samples were classified correctly except the sample RG2, which was mistaken as red ginseng. All samples were also classified correctly in clustering tree of NIR diffuse reflectance spectroscopic analysis. The results showed that samples could be exactly identified by clustering analysis with FT-Raman and NIR diffuse reflectance spectra. It could not only figureout pseudo-ginsengs but also classify different kinds of ginsengs, such as white ginsengs, red ginsengs, and American ginsengs, etc. However, the accuracy of DRIFT spectroscopic analysis was not as good as that of FT-Raman and NIR diffuse reflectance spectroscopic analysis. The reasons may be the absorption intensity of IR spectra of samples is stronger compared with NIR and Raman spectra so that a very little change, such as the content of water in samples, the influence of environment and the instability of instruments, etc., would make the IR spectra fluctuate greatly. Sometimes even two different spectra were obtained with the same sample just because of the change of exterior conditions, especially the influence the absorption of water. In such a situation the result from chemometric analysis may show that these two spectra represent different species.

The intensity of FT-Raman scattering is weaker so many times accumulation is necessary to obtain a good spectrum, which could remove occasional errors. The effect of water on FT-Raman spectra is also much weaker than that on IR. All these facts made FT-Raman spectra are not easy fluctuating with minor change of exterior conditions. And the near-infrared is dominated by overtone and combination bands of fundamental vibrations occurring in the mid-infrared, which means that other effects, such as the exterior conditional changes, have litter effects on this region. Therefore the FT-Raman and NIR spectra are less affected by other factors, which result in the most correct result from chemometric analysis.

4. Conclusion

The results showed that molecular spectroscopic analysis combined with chemometric analysis was very effective and reliable for identification of herbal medicines. All samples could be clustered into four items correctly: white ginseng, red ginseng, American ginseng, and pseudo-ginseng. It's a simple, quick, nondestructive (for NIR spectroscopic analysis) method compared with chromatography methods. It's believed that it could replace traditional and chromatographic methods in further.

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DNA-based Authentication of Plant Extracts

1. Introduction

DNA analysis has become routine technique to identify raw materials of food. An advantage of these methods is their possible use throughout the food supply chain, which is essential for example in the traceability of genetically modified organisms (GMO's) in the whole food production chain. Therefore, DNA analysis of processed food has received much attention although DNA degradation during processing steps may hamper the analysis. Coneflower (Echinacea sp., Asteraceae) and German chamomile (Matricaria chamomilla, Asteraceae) are botanicals used in herbal medicines, in (functional) food and food supplements due to their immunostimulating effect (Echinacea) or for the symptomatic treatment of digestive ailments (chamomile) (WHO, 1999). Chamomile is additionally used for flavoring food. Both botanicals are either applied dried, powdered or as extracts. Since plant extracts are traded goods it is of importance for the customer to be able to control identity and quality of an extract. For the authentication of an extract several chemical methods are established like color and precipitation reactions (which are in most cases only group specific), isotopic ratios and mainly chromatographic methods. Additionally the specific gravity is analysed but gives more information about the correct use of the solvent than about the species used for the extraction. In this paper we communicate an approach to isolate DNA from commercial plant extracts suitable for DNA authentication of these extracts.

2. Materials and Methods

Extracts and reference plant material:

The following commercial extracts were analysed: spissum ethanol extract of Echinacea tincture and fluid extract of chamomile (Matricaria chamomilla).

Since the botanical identification of Echinacea species is difficult, no reference samples were collected for this plant. For chamomile, reference plant material was taken from the botanical garden of the University of Veterinary Medicine, Vienna, Austria (Matricaria chamomilla cv. 'Manzana').

Purification and separation:

Twenty-five milliliter of each extract was diluted with 10 ml of 30% ethanol (v/v), shaken for 15 min and centrifuged (4000 rpm, 5 min). 10 ml of the supernatant was removed and replaced with 10 ml of 30% ethanol (v/v). The procedure was repeated five times with removal and replacement of 30 ml each time using increasing ethanol concentrations from 30% to 70% (v/v). The residue was filled up to 50 ml with ddH_2O, dissolved by vortexing, and centrifuged (4000 rpm, 5 min). The supernatant was discarded and the washing step with ddH_2O was repeated. The residue now consisted of three fractions, a small brown level between two light colored levels. The residues were examined under the microscope. Intact plant cells were found in the brown residue, which was separated with a spatula for further processing.

DNA extraction and quantification:

The brown residue was collected in a 50 ml Falcon tube, autoclaved sea sand 'extra pure' (Merck, Darmstadt, Germany) was added and the plant fragments were grinded with a pestle. A DNA extraction in 50 ml Falcon tubes was carried out, according to the manufacturers protocol. DNA from the dried reference material from chamomile was extracted and measured according to the same protocols as the DNA from the residue.

PCR primer development:

Primers were developed for Echinacea with the program 'PRIMER3' on the basis of an ITS1 sequence of Echinacea purpurea published in Genbank. For the chamomile extracts and reference, primers for the internal transcribed spacer 2 (ITS2, forward primer: GCATCGATGAAGAACGTAGC, reverse primer: TCCTTCCGCTTATTGATATGC) were selected from the proposed primers

PCR reaction and visualisation of amplification products:

The 15 ll PCR reaction was carried out in 1· enzyme buffer (160 mM (NH4)2SO4, 670 mM Tris–HCl pH 8.8, 15 mM MgCl2, 0.1% Tween 20) reaction mixtures containing 0.6 lM of each of the primers, 0.1 mM of each dNTP, 0.5 U Taq Biotherm DNA polymerase and 5 ll of the DNA extract. The PCR reaction was performed in a GeneAmp PCR System 9700. The program for the Echinacea extract started at 94°C for 1 min, followed by 30 cycles of 1 min at 94°C, 45 s at 59° C, 30 s at 72°C, and a final extension step of 72°C for 5 min. The program for the chamomile extracts started at 95°C for 3 min, 55°C for 30 s and 72°C for 45 s, followed by 34 cycles of 30 s at 95°C, 30 s of 55°C, 45 s of 72°C and a final extension step of 72°C for 7 min. A second PCR reaction was carried out in 50 ll reaction mixtures using 5 ll of the PCR mix of the first PCR-run (instead of the whole genomic DNA) following the procedure described above. The amplification product was separated in a 1.4% agarose gel, stained with ethidium bromide and visualized under UV light. The size of the fragment was determined using a standard 100 bp ladder

Fragment isolation and DNA sequencing:

About 45 ll of the amplification products were purified by electrophoresis in a 1% agarose gel and the subsequent use of the QIAGEN QIAEX II Gel extraction kit. Sequencing was performed in duplicate at IBL with the forward primers as sequencing primer. The two sequences were checked for ambiguous bases using the program Chromas. The sequence of the reference sample of chamomile was submitted to EMBL.

Sequence comparison and multiple alignments:

The sequence obtained from the Echinacea extract was compared to sequences deposited in Genbank. The sequences of the chamomile extracts were compared to the reference sequence. Comparison was done by aligning the sequences using the ClustalW algorithm of the program Megalign.

3. Results and Discussion

DNA was successfully extracted from minor amounts of remaining intact plant cells from three differently prepared plants extracts, a spissum extract of Echinacea sp., a tincture and a fluid extract of chamomile. As plant secondary compounds interfere with the DNA amplification in the PCR, the remaining plant fragments were first purified with ethanol/water mixtures in increasing ethanol concentrations. This step was introduced before DNA extraction to eliminate plant secondary compounds in a simple and efficient way. A further advantage of this purification process was that the solids centrifuged from the solution could easily be evaluated and the layer containing plant fragments could be isolated after visual inspection under the microscope.

The extracted DNA was not quantifiable due to its low concentration. To authenticate the plant extracts, a short DNA fragment from nuclear DNA-regions often used in molecular plant taxonomy (ITS1 for Echinacea and ITS2 for chamomile) was amplified using the same primers in two consecutive PCR reactions. The amplification products were purified, sequenced and the identification was based on sequence comparisons to reference sequences.

3.1. Echinacea

The sequence comparison to published sequences revealed the unambiguous identification of the plant DNA in the extract as originating from the genus Echinacea. Significant differences could be found to sequences from Asteraceae species of the same tribe (Heliantheae) like Rudbeckia sp., Sanvitalia fruticosa, Oblivia mikanioides and

Idiopappus quitensis. The sequence of the plant extract differed from E. purpurea in only one nucleotide at position 12. Compared to E. paradoxa and E. pallida the plant extract was different in only two nucleotides at positions 99 and 173 (E. paradoxa) and at positions 173 and 178 (E. pallida). These small differences between the Echinacea spp. do not justify an unambiguous determination of the species in the extract up to now. However, increasing sequence information from additional DNA-regions will enable a better differentiation capacity.

3.2. Chamomile

Both extracts (the tincture as well as the fluid extract) showed an amplification product equal in size to the reference sample of approx. 400 bp. From the fluid extract, however, an additional fragment of 420 bp length was amplified ('upper band'). The DNA fragments equal in size to the reference sample were completely homologous to the reference, while the sequence of the upper band of the fluid extract showed only a similarity of 78%, which would indicate an impurity in the plant raw material used for producing the fluid extract. The identity of the impurity could not be determined. A Genbank search resulted in resemblances to Perovskia abrotanoides (89% similarity), Salvia davidsonii (87%), Rosmarinus officinalis (83%) and Salvia pachyphylla (82%) (all from the Lamiaceae family). The similarity is too low to identify the DNA fragment as belonging to any of these species mentioned above. In this example, the impurity could easily be detected due to DNA inserts which resulted in a larger DNA-fragment easily separable from the chamomile DNA-fragment. DNA authentication was possible, because plant cells could be isolated from the extract. The success of this approach is therefore strongly dependent on the technology (filtration, etc.) used in producing the plant extract. Usually a small portion of very fine vegetal material manages to pass though the filtering system of the extractor. Typical sizes of plant cells are between 5 and 100 lm, sizes that would already require microfiltration, a technique not applicable to extracts especially when they contain mucilaginous or polymeric substances which hinder filtration. Unambiguous plant identification is of primary concern to guarantee quality, safety and efficacy of a drug or an extract. DNA analysis is able to complement or substitute existing authentication methods of extracts.

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Editor

The Application of DNA Micro-Arrays (Gene Arrays) to the Study of Herbal Medicines

Introduction

The DNA array (micro-array; gene array) is a new methodology developed for the purpose of analyzing the effects of various agents on cellular gene expression. Thousands of genes can be analyzed in a single experiment. The application of this technique to the fields of cancer research, inflammatory diseases, and others, has led to significant advances in our understanding of the mechanisms underlying disease and its treatment Consequently, it is believed that similar advances could be made in understanding how herbal medicines work.

In this review an attempt as been made to explain the methodology, as well as its limitations and pitfalls, and to critically evaluate the attempts made so far to apply the technique to the study of herbal medicines, including the experience of the authors with *Echinacea*. One must keep in mind, however, that the technique is new and is still evolving, especially in connection with the methods used for data correlation and analysis (bio-informatics).

The term DNA (gene) array refers to a solid surface to which is attached numerous small pieces of DNA representing short sequences of specific genes. The arrays may be macro-arrays, which are usually fabricated membranes of nylon, or other relatively inert materials, impregnated with scores or hundreds of pieces of DNA in the form of discrete spots. In contrast the micro-array is usually a siliconized glass slide to which thousands of pieces of DNA fragments or oligonucleotides are precisely spotted by a robot.

The macro-arrays, available in a number of commercial kits, are perhaps more amenable to routine laboratory use, but are restricted by the relatively small number of genes represented on a membrane, whereas up to 30,000 genes can be placed on a single micro-array slide. However the latter requires sophisticated equipment for analysis. In practice there is probably no significant difference in cost to a laboratory that has access to the appropriate expertise and equipment. Needless to say, the more commercialization involved, the greater the total costs.

A disadvantage of the macro-array, is the fact that restricting the number of genes for analysis keeps one in the reductionist mental framework, in which one becomes interested in examining only a small number of related genes, according to pre-conceived hypotheses, whereas the micro-array opens up the possibility of looking at the entire genome, and perhaps revealing novel genes and pathways not previously thought to be involved in the actions of the test agent. In fact, recent studies with viruses and other microbes have revealed that in some infections hundreds of host genes, representing a multitude of different functions, can be switched on or off, and the authors' experience with *Echinacea* (discussed below) has indicated numerous interactions between virus-induced changes and herbal modulation, many of which had not been pre-conceived.

One believes that it is worthwhile to explore the potential of this new tool, to give it a trial run so to speak, and see what it can reveal. One does not believe that it is ready to replace traditional ethnopharmacology research; but it could at least augment it, and perhaps help to guide its progress. In addition, of even greater possible significance and impact, this approach might mollify the critics who claim that there is no scientific basis for

claims made about herbal medicines.

Studies Involving Herbal Medicines

For this review a total of 18 studies have been selected (published in the period 2001–2005) in which some form of herbal medicine has been evaluated in cell cultures or in animals, by means of DNA arrays. There have been important studies on gene-expression analysis, in response to herbal preparations, which involved examination of selected genes, but the present review is restricted to the use of gene arrays containing thousands of genes. Many of the array studies utilized Affymetrix or other commercial form of array, while the others employed custom prepared micro-arrays on glass slides, with a wide variety of genes represented.

Animal Studies

Gingko biloba: Leaf extracts of *Gingko biloba* have been advocated for many years as dietary supplements to ameliorate symptoms associated with various brain and circulatory disorders, including memory problems, and are currently among the most popular items sold in health food stores. Some specific bioactive components have been examined, such as the gingkolides and flavonoids, although it has often been assumed that the whole leaf extract is more useful because of synergistic actions of many bioactive constituents decided to examine the effect of *Gingko biloba* leaf extract (a standardized commercial extract, EGb 761, which has been used in clinical trials) for neuro-modulatory effects on gene expression in mouse cortex and hippocampus. Mice were maintained for a month on a low flavonoid diet, or one supplemented with *Gingko biloba* extract, 300 mg/kg of food pellets. They were then sacrificed, and the cortex and hippocampal separately removed and frozen. RNA was extracted from pooled tissues by standard "Trizol" methodology, amplified and converted into biotinylated probes for hybridization to Affymetrix chips, containing oligonucleotides representing 6000 discrete mouse genes plus 6000 ESTs (expressed sequence tags).

They presumably used standard Affymetrix array analysis and interpretation equipment and software, and they recorded reproducible changes of a least two-fold between control and treated tissues. Forty-three genes were significantly affected in the cortex and 13 in the hippocampus (<1%), although only four were activated in both tissues. Various functional groups were represented, including growth factors, transcription factors and signaling pathway components, and a few specific ion channels and cytoskeletal proteins. Some of the more conspicuous changes were verified by RT-PCR measurements on the same RNA preparations. The most impressive change was in hippocampal transthyretin (increased 16-fold), which is considered to play an important function in hormone transport in the brain, and possibly in amyloid protein production.

Obviously there are numerous limitations in the model used, such as the use of pooled heterogeneous tissues, and particularly the choice of a single time point during the 1-month administration of extract. However a more detailed analysis of gene changes over time would require a lot more work, in addition to the substantial expense incurred in using numerous Affymetrix chips. Subsequently described results from a study of the effect of the same *Gingko biloba* extract on cultures of human bladder carcinoma cells, the line T24. Following a 72 h incubation with extract, treated and control cells were analyzed for changes in gene expression by means of a 2000 gene chip. Presumably the protocols were the same as in the previous report (described above). One hundred and fifty-five genes were affected by two-fold or greater (approximately 8% of total genes tested), mostly increases, and these represented a variety of functional groups, including some that could be important in anti-oxidant defences. These could be significant to the long term human consumption of the herb.

St. John's wort (Hypericum perforatum, SJW) and hypericin: SJW is one of the most popular herbal medicines in Europe and North America. Its principal application is for people who suffer from seasonal mood disorders and other types of depression. Clinical studies have been

conducted, with both positive and negative outcomes. As is the case for most herbal medicines, the nature of the SJW preparation has probably contributed significantly to its apparent success or lack of. Alcoholic extracts/tinctures, and water-based teas, have been promoted as anti-stress and relaxation drinks.

Among the many bio-active ingredients, hypericin and its related analogues, and hyperforin, have been incriminated in its beneficial actions, although extracts usually contain many other phenolic compounds, and the composition varies between different *Hypericum* species . Some of the bio-active compounds, such as hypericin and its analogues, are also photosensitizers, a fact which has not always been taken into account in laboratory studies. Hypericin itself, and some extracts, possess antiviral and antibiotic activities, which might have accounted for some of the uses of this plant in Ayurvedic medicine.

A potential drawback in the use of SJW has been popularised recently, namely the fact that its interaction with CYP's could lead to interference with the metabolism of drugs that are consumed simultaneously. Such claims have led to a negative press on SJW, although one could turn the argument around and suggest that people taking SJW or hypericin should avoid certain synthetic drugs that require CYP metabolism. Used DNA arrays to study the effects of SJW extract, in comparison with the synthetic anti-depressant drug imipramine, on gene expression in the hypothalamus of rats. Hypothalamus was chosen because of its known importance in mood altering functions, and it was suggested that the SJW and imipramine could be expected to share some common pathways leading to their similar beneficial effects. Previous studies had indicated that anti-stress effects in rats required a period of several weeks for the test materials to show their effects. Accordingly, following administration of a partly characterized SJW (by daily gavage) or imipramine (daily intra-peritoneal injection), and appropriate controls, rats were sacrificed after 8 weeks and their hypothalami frozen. Subsequently RNAs were extracted from these tissues (Trizol followed by "RNeasy"), one hypothalamus for each array, and the RNAs reverse-transcribed and used to prepare biotylinated RNA for use with Affymetrix chips.

The arrays contained 8799 rat genes plus EST's. Following hybridization and processing, the chips were scanned and analyzed by means of Affymetrix software.

The results were disappointing: SJW differentially regulated a total of only 66 genes/EST's, representing <1%, in comparison to imipramine, which affected 74 genes/EST's. A variety of different pathways were represented by these genes, but the surprising finding was that only six genes were common to the treatments, in spite of the similar physiological effects on the animals. In addition to these small numbers, the actual magnitude of the changes was generally small, less than two-fold, although they were claimed to be significant.

These relatively minor effects could have been due to the use of heterogeneous tissues (unavoidable in animal experiments) in which real significant effects in certain cell types could have been masked by non-responding (or differently-responding) cells. It is also conceivable that the animals may have adapted their metabolism during 8 weeks of daily exposure to the test materials. Another drawback was the use of a pure compound to compare with a crude extract, since the latter may have induced different responses from different ingredients.

It would be useful to examine the effects of SJW/hypericin in appropriate cell lines, where one could anticipate a more significant response, although the choice of appropriate cell type is obviously open to discussion.

Cannabinoids: The subject of cannabis, and its well known major constituents the cannabinoids, need no introduction. According to the myriad of anecdotal observations and studies, based on both traditional and modern experiences, various preparations derived from the plant *Cannabis sativa* (Fam. Cannabaceae) are used primarily for two purposes, the psycho-active one,

due principally to delta-9-THC (tetrahydrocannabinol), and with which we personally cannot profess to be experts, and the applications of so-called "medical marijuana", which has been claimed to possess benefits to many patients suffering from different kinds of chronic disorders, such as cancer, AIDS, pain, multiple sclerosis, epilepsy, and others . In general the psycho-active preparations are rich in delta-9-THC, whereas the medically applied preparations are low in THC but contain substantial quantities of cannabindiol and cannabinol, plus other cannabinoids. However both types of preparation also contain numerous other phytochemicals, some of which probably contribute to the overall bioactivities.

Studies performed to date with pure compounds have revealed the presence of receptors, CB1, CB2, and likely additional ones, that bind different cannabinoids to different degrees, and thereby activate various signaling pathways, with a variety of gene expression changes, depending upon the nature of the cannabinoid and the cell type . It has also become clear that there are several endogenous compounds, chemically unrelated to the cannabinoids, which share these receptors and which can mimic some of the bioactivities of the cannabinoids.

Since the cannabinoids, even individual compounds, clearly possess multifunctional attributes, then a DNA micro-array analysis might prove fruitful. Attempted this in rats and mice, respectively. At the outset there are potential problems associated with such an approach. First of all is the obvious one that mice and rats are not necessarily good models for humans, and we really cannot say if animals respond to THC in the same way that humans do; even individual humans differ widely in their responses. Secondly there is the problem of tissue heterogeneity, as mentioned already; consequently if there is a specific target site (e.g. cell type) in the brain for a compound such as THC, then its effects would be diluted by inclusion of unaffected tissues/cells. Furthermore different cell types might respond in opposite ways to the compound, and analysis of the whole tissue could not reveal this. On the positive side, the study was at least *in vivo*, which should make it theoretically more relevant, although the method of administration of THC in animal studies is unlikely to reflect human usage; we are not aware of any reports of intra-peritoneal inoculations of cannabinoids or extracts in humans.

Rats, which were given daily intraperitoneal injections of delta-9-THC. Animals were sacrificed after 1, 7, or 21 days, and hypothalamic were removed and frozen at −80 C for subsequent RNA extractions. Commercial kits were used to extract and purify the mRNAs, which were then reverse-transcribed to yield ^{32}P-labelled cDNAs. They produced rat gene arrays in - house from a library of more than 24,000 cloned cDNAs, and spotted these on two sets of nylon membranes. Following standard hybridization procedures, they analyzed the membranes by means of scanning and software programs. About a quarter of the cloned cDNAs hybridized to test RNA, but only 28 of them (<1%) showed alterations in response to delta-9-THC. However the significance of these changes is open to question since most of them were less than two-fold and the authors did not indicate if they made allowance for differential ^{32}P-labelling between preparations. Made their analysis in the brain tissues of THC treated mice. They administered delta-9-THC, or the DMSO vehicle alone, or in some cases a synthetic agonist, intraperitoneally in mice, and after 12 h killed the mice, removed brains and extracted RNA. The cDNAs were labelled with Cy3 or Cy5, for controls and test compound respectively, and hybridized to slides containing 11,200 ESTs derived from a mouse brain library. The data were scanned and analyzed by commercial software to give ratios of Cy3:Cy5. Averages were taken from three separate experiments, which in retrospect might be considered dubious, although to qualify for acceptance the ratios had to exceed 1.8 in all three experiments.

A total of 86 genes (<1%) were affected by one or both agents, mostly decreases by two to three-fold, but only 20 genes in common. A variety of functionally different genes were affected,

although they pointed out that their results were not comparable to those of Kittler et al. (above). This might be explicable by the use of different animal species and experimental protocols, but it could just as easily reflect differences in the array systems used and their processing and analyses.

It has been previously studied the effect of a cannabinoid CB2 receptor agonist in a human promyelocytic cell line, HL-60. This cell line has been the object of many investigations in connection with cell differentiation and signaling pathways in the immune system. It has the theoretical advantage of a "homogeneous" cell culture, in which significant changes in gene expression should be more obvious than in animal tissue models. Nevertheless this apparent "homogeneity" could be less real in a cell line that undergoes continuous differentiation in culture. In other words, its attraction as a model of differentiation mitigates against its value in the analysis of gene expression changes induced by an agent.

The likelihood of seeing receptor-mediated changes by transecting multiple copies of the receptor gene into these cells. They then treated the transfected cells with a synthetic cannabinoid agonist (CP 55,940), or synthetic antagonist (SR 144,528), for various times. RNA was extracted from these different cell populations, converted into biotinylated cRNAs and used with standard Affymetrix DNA arrays (huGene FL chips containing 6800 genes). These were processed and analyzed by means of the usual Affymetrix prescribed equipment and software.

Ten genes (1%) were concluded to be significantly altered (fold ratios, treated to control, in the range (2.2–4.7), mostly stimulated, following a 1 h exposure to the receptor agonist, and these represented genes involved in cytokine, transcription, and cell cycle processes. The changes were confirmed by combinations of Northern blots and ELISAs. The authors were able to relate these changes to a demonstrable translocation of activated NFkB transcription factor, frequently implicated in immune activation and differentiation related events. They suggested that this finding could reflect an involvement of the receptor in normal differentiation; but clearly the system bears no relationship to the animal studies described above. However, considering the abundance of genes involved in differentiation processes and immune activation, it is surprising that so few genes in the HL-60 cells were altered.

Catalposide (Catalpa ovata), Boswellia serata, and inflammatory bowel disease (IBD): Extracts of both of these plants have been used traditionally in Korea and India, respectively, to treat inflammatory conditions. People with IBD often have raised levels of certain pro-inflammatory cytokines, such as IL-6, IL-8, and TNFa, in colonic tissues. This has led to the prospect of therapy by means of anti-inflammatory compounds and herbals. Combination of cell culture and mouse models to study the effect of catalposide, an iridoid isolated from *Catalpa ovata*, on gene expression. They had previously shown that this iridoid inhibited the activation of NFkB, and hence the secretion of several cytokines, in a line of mouse macrophage-like cells.

In this study, authors incubated the HT-29 human intestinal cell line with TNFa (to stimulate pro-inflammatory cytokine secretion) ±catalposide (500 ng/mL) for 16 h, extracted RNA, and converted the RNAs into Cy3 and Cy5 labelled cDNAs, by means of a commercial kit. These were hybridized to cDNAs on a commercial human 8000 cDNA chip, and the results were analyzed by means of standard imaging and processing programs. The data were not presented, although the authors stated that a number of cytokine and chemokine-related genes were down regulated, as expected in view of the anti-inflammatory effects. In some instances individual transcripts or proteins were measured, for confirmation, by means of various Northern blots, western blots and histochemical staining procedures.

The chemically induced mouse IBD model was also analyzed, by a similar combination of techniques. Mice received two rectal doses of trinitrobenzene sulfonic acid (TNBS), with or

without intra-colonic catalposide given before and after the TNBS. However it appears that DNA micro-array studies were not done on these tissues. The authors stressed the inhibition of activated NFkB as the focus of the anti-inflammatory effect of catalposide.

The *Boswellia serrata* tree gives rise to a resinous gum, which has been used traditionally as an anti-inflammatory agent in Ayurvedic medicine. Among the potential active ingredients is a class of terpenoids called boswellic acids, which have themselves been shown to possess anti-inflammatory activity in cell culture and animal models.

In the study, colitis was induced in mice by either dextran sulfate in drinking water or trinitrobenzene sulfonic acid administered rectally. Two different kinds of *Boswellia* extract were tested for protective effects, a hexane extract and a methanol extract. But no protective effects were observed. Liver RNA was isolated from the various treated mice and converted into biotin-labelled cRNA for hybridization to Affymetrix mouse array chips, containing 13,672 genes. They searched for genes reproducibly affected by three-fold or more. A total of 58 genes (<1%) were modulated only by the hexane fraction, 20 genes only by the methanol fraction; and in addition 24 were common to both. The differences could be anticipated since the fractions were chemically distinct and probably contained a variety of different bioactive compounds. Among the genes affected were several CYP genes and glutathione *S*-transferase, suggestive of hepatotoxic reactions, and this conclusion accorded with the histologic findings, i.e., rather than protection, the extracts were in fact hepatotoxic.

In the human intestinal cell line $CaCo_2$, the six individual boswellic acids either inhibited experimentally induced NFkB levels, or had no effect on them. Perhaps the boswellic acids operate through the mediation of alternative transcription factors and pathways. It is unfortunate that investigators in general have become pre-occupied with explaining inflammatory responses by means of effects solely on NFkB.

Herbal glycosides: Various recipes of herbal glycosides, including baicalein and dioscin, have been used for a long time in Chinese medicine to enhance memory problems in stroke victims. Mouse model of surgically induced cerebral ischemia to investigate the spatial memory abilities of mice with and without glycoside treatment, which was given regularly before and after surgery until the animals were sacrificed. Their hippocampi were frozen, and subsequently used for the extraction and purification of RNAs. ^{32}P-labelled cDNAs were produced and hybridized to arrays of 1176 mouse genes in the Clontech Atlas system. About half of the array genes reacted with transcripts in the different RNA preparations, and of these 33–46 (approximately 8%) showed significant (>1.8-fold change) increases or decreases compared with sham or vehicle-treated animals. However, although these gene changes correlated with the performance of the animals in spatial memory tests, there was little overlap among the nature of the genes affected by three different doses of the glycoside formulations, which makes complete interpretation of the data difficult.

Cell Culture Studies

Genistein-treated prostate carcinoma cells : Genistein, a major isoflavone isolated from soybeans, was already known to inhibit the growth of cultured PC3 prostate carcinoma cells (human origin), and to inhibit NFkB and AKT signaling pathways. The investigators wanted to determine which genes were involved in this process. They therefore treated the cells with 50 umol/L of genistein for 6, 36, or 72 h (to examine early and delayed responding genes, an important parameter to consider), extracted RNA by means of standard commercial kits and prepared the labelled probes according to the Affymetrix protocols, followed by hybridization to chips containing more than 12,500 human genes (cDNAs). Results were obtained by using the prescribed software and tools. Unfortunately it is not clear if the relative cell numbers of treated and untreated cultures were

taken into account; presumably they were.

The authors found what they considered to be significant alterations, i.e. a change by two-fold or more, in 832 genes (about 6.7%), of which 774 were decreased and 58 were increased. The majority of these changes were observed at the 6 h time point, with further decreases noticeable at the later times. Alterations in transcription levels were confirmed in 26 cases by means of RT-PCR measurements. A large number of the genes affected involved players in signaling pathways, transcription factors, protein kinases, apoptosis, and cell cycle functions, which is not surprising in view of the original rationale of the study.

This study is interesting because of the large number of genes affected, in contrast to some of the others described below, and also this represents the activities of a single component of soybean extracts. It would be interesting to learn whether these activities are modulated by other components of soybean. This is clearly relevant to prospective applications of such extracts, or genistein itself, in the treatment of prostate and other cancers.

PC-SPES and prostate carcinoma : This crude mix of eight different herbs has been advocated for some years in the prevention and treatment of cancer, especially for prostate carcinoma. The usual formulation comprises *Scutallaria baicalensis, Glycyrrhiza glabra, Ganoderma lucidum, Isatis indigotica, Panax pseudo-ginseng, Dendranthema morifolium, Rabdosia rebescens*, and *Serenoa repens*. Needless to say, many members of the medical and scientific establishments are suspicious of such a "witches' brew", although many of these individual extracts are known to contain bioactive ingredients, and in fact some trials have shown decreases in blood PSA (prostate-specific antigen) levels over time with daily consumption of the mixture.

It would seem to be an ambitious task to analyze gene responses to such a mixture of uncharacterized phytochemicals; nevertheless this group has attempted to do this, and the data are spread over the two manuscripts cited. Of the three prostate carcinoma cell lines available to them, PC-3, DU-145 and LNCaP, the latter was selected for the gene array analysis. The authors used glass micro-arrays spotted with 3000 cDNAs obtained from their library of prostate cDNA clones. The authors compared each RNA preparation (PC-SPES-treated or solvent control treated) with a reference prostate RNA, one labelled with Cy3 the other with Cy5. The authors also did comparisons with the dye labels reversed, essential to compensate for possible differences in dye-dUTP incorporation rates.

The authors used their own combination of processing and analytical tools, and searched for genes that were reproducibly increased or decreased by a factor of 1.5, which is lower than most investigators would want to use. The number and intensity of changes increased with time up to 48 h of treatment, resulting in 144 genes increased and 175 genes decreased, approximately 10% of the genes represented. A variety of genes with different functions were affected, including numerous cytoskeletal functions, which the authors followed up with further studies. Other functions included apoptosis, stress, cell cycle and proliferation, as well as androgen-regulated genes, all of which could be anticipated from the objectives of the study.

Paeoniae radix and apoptosis : The root of *Paeoniae lactiflora pallas* has been used traditionally in China to treat liver diseases, and various other disorders. This plant is often found as a constituent of multi-herb formulations, and extracts of PRE (*P. radix* extract) have been shown to induce apoptosis in two human hepatoma cell lines.

In this study Lee et al., prepared a hot water extract of PR and used the filtered and lyophilized preparation, in 5–10 mg/mL concentrations, to treat HepG2 cells. RNA was used to make Cy3 and Cy5 labelled cDNAs, which were hybridized to 374 oligomer spots on the Operon human apoptosis array, with the usual collection of control spots. The most remarkable thing about this study is that only four genes were affected (one increased, three decreased, a total of ~1%), even though the array

was heavily biased toward apoptosis genes. On this basis, apart from the confirmation by other tests that apoptosis was induced by such treatment, it would be difficult to conclude anything from the array analysis.

Coptidis rhizoma and berberine : This plant, and one of its major constituents berberine, has been shown to possess anti-proliferative effects on pancreatic carcinoma cell lines. The authors attempted to compare ID_{50} values for both extract and berberine against the eight cell lines with corresponding effects on specific gene expression. The extracted RNAs (from cells treated with berberine or hot water extract) were purified and processed according to Affymetrix protocols, and hybridized to 11,000 oligomers on the huU95A chips, along with various controls.

Comparisons between ID_{50} and a number of specific genes was made, although it was not really clear what exactly was being measured in terms of gene expression. Out of 33 genes apparently altered (ratios or levels of significant changes were not given), some correlated with both extract and berberine ID_{50}, but others only correlated with one or the other ID_{50}. Among the genes allegedly altered were the usual mixture of functionally diverse entities such as signaling, transcription, DNA repair, cell cycle genes.

In a more recent publication, the same group refined their analyses, using the original data, by concentrating on 27 specific genes affected by several specific compounds, including berberine, isolated from the extract.

Tripterygium alkaloids and apoptosis : *Tripterygium hypoglaucum* root has been used traditionally in Chinese medicine to treat a number of inflammatory diseases. An alkaloid fraction, prepared by organic solvent extractions, has been shown to cause apoptosis in some cultured cells.

In this study, the human promyelocytic leukemia cell line, HL-60, was treated for 8 h with 40 ug/mL of the alkaloid fraction, and the RNA extracted and processed to produce Cy3 and Cy5 (control and treated) labelled probes, which were hybridized to 3000 spots derived from a commercial human leukocyte cDNA library. Unfortunately it was not clear what controls were used, whether background values were subtracted, or whether the labels were reversed. However 16 (<1%) genes were reported to show ratios of more than two-fold change, and these included apoptosis, signaling pathways, cell cycle and differentiation functions. Thus the array data really did not add to the results obtained by the other techniques described in the report.

Anoectochilus formosanus and plumbagin : The anti-cancer extract from *Anoectochilus formosanus* and the pure phytochemical plumbagin (a naphthoquinone from *Plumbago rosea*) were compared with respect to their effects on gene expression in MCF-7 cells, a line of breast adenocarcinoma origin.

RNA extracted from the various treated and control cells was converted into biotinylated cDNA probes, which were hybridized to a collection of 9600 genes on nylon membranes. Various equipment and software programs were used in the analysis and interpretation of the results.

Data for individual genes were not shown, although the authors concluded that 59 genes (only <1%) were significantly affected (>3-fold change) by the extract, whereas plumbagin modulated 80 genes. It was not clear to what extent the effects of the two agents had related effects, if at all.

Propolis and differentiation: Propolis has been advocated for innumerable purposes, in health and disease. Its major drawback is the very nature of the product, which, because of its high variability in composition, is almost impossible to standardize in terms of phytochemistry and bioactivities. Consequently a meaningful DNA array analysis of its effects on gene expression is, to say the least, ambitious.

In this study, the scenario was made even more complex by evaluating two different extracts of propolis in a cell line undergoing differentiation, the promyelocytic leukemia HL-60 cells. The investigators were then faced with the challenge of deciding which genes are affected by the extracts themselves, and which are affected secondarily as a

consequence of the process of differentiation. The results were compared with the effects of ATRA (all-trans retinoic acid), which is known to induce differentiation in these cells.

The RNAs extracted from the various treated cells were converted to Cy3 and Cy5 labelled cDNAs and hybridized to AceGene Human oligo chips, containing 10,000 genes (all in the form of 50-mers). Appropriate controls and dye-reversal reactions were carried out. One hundred and eighteen genes (~1%) were affected >2-fold by ATRA (all-trans retinoic acid), 79 by aqueous extract of propolis, and only 6 by an ethanolic extract. In general there were more decreases than increases, which might be expected in the face of a differentiation process. However correlations were difficult because of the different degrees of differentiation (and possibly the type of differentiation?) shown by the different agents.

Echinacea and Gene Array Analysis: TheExperience with a System in Evolution

Different species and parts of *Echinacea* (Asteraceae) have been used traditionally in North America for the treatment of various symptoms of "colds" and "flu", as well as other skin applications such as wound healing. The taxonomy of the genus has been revised recently, based on new data derived from genetic and phytochemical profiling. A number of well known marker compounds have been characterized, including polysaccharides, specific caffeic acid derivatives and alkyl amides, and these have all demonstrated biological activities in various bioassay tests, such as immune-modulating activities, anti-viral activities, and anti-fungal activities. Some of these activities have been attributed to photosensitizers, which are common among members of the Asteraceae, a fact that could explain some of the efficacy of extracts in skin applications.

Many clinical trials have been conducted, in individuals suffering from natural or experimentally induced rhinovirus infections, but with variable results, many of which were undoubtedly due to the unfortunate use of source materials that had not been adequately characterized. Thus the question of clinical efficacy remains unresolved. Meanwhile additional studies have been conducted with the objectives of identifying the principal immuno-modulating compounds and anti-viral compounds, and their mechanisms of action.

There is also controversy about the timing of *Echinacea* consumption, in relation to cold or 'flu symptoms, and what kind of formulation should be consumed. Thus, should it be consumed at the "first sign of a cold", or as a preventative measure? Should one take an ethanolic tincture/extract, or a water based formulation? These same issues are of course relevant to any "anti-cold" herbal or drug preparation, since, contrary to commercial advertising claims, we have *a priori* no idea how these formulations are supposed to work.

In order to shed light on these problems, while at the same time testing *Echinacea* as a model for evaluating the novel DNA array approach, we decided to analyze host gene expression in a line of human bronchial epithelial cells (BEAS-2B), infected with rhinovirus type 14, or uninfected, and with or without one of two characterized preparations of *Echinacea purpurea* (*Echinacea purpurea* (L.) Moench (Heliantheae: Asteraceae)). Rhinovirus was chosen because of its frequent association with colds and other upper respiratory infections, and because it has often been implicated in exacerbation of asthmatic and allergic symptoms . The *Echinacea* preparations used were an aqueous extract of mixed *Echinacea purpurea* aerial plant parts, enriched in polysaccharides (labelled E1), and a 55% ethanol extract of the roots of *Echinacea purpurea* (E2), which contained alkyl amides and various caffeic acid derivatives. Both preparations have been shown to have pronounced effects on cytokine and chemokine secretion in uninfected and rhinovirus-infected cells

Previous studies had shown that rhinovirus infection in cultured epithelial cells, and in nasal epithelial tissues in vivo, resulted in low levels of virus replication and cytopathology, but substantial induction of secretion of certain pro-inflammatory

cytokines and chemokines, including IL-6, IL-8, and TNFα. Thus the typical symptoms of a common cold, such as sneezing, coughing, runny nose, stuffed nasal passages, etc., are not the direct result of viral pathology, but rather the indirect stimulation of pro-inflammatory cytokines and chemokines.

RNA was extracted from the six different types of culture (uninfected cells with or without extract E1 or E2, and rhinovirus-infected cells with or without E1 or E2), reverse transcribed the RNAs into Cy3 or Cy5 labelled cDNAs, and hybridized with arrays containing a total of 13,500 human gene-specific oligomers.

A commercially available Universal Reference RNA, prepared from a collection of 10 different human tumor cell lines with various transcription programs was used. The advantage of this commercial product is that it is available readily to investigators and is a consistent product.

The arrays were scanned after hybridization by means of the ScanArray Analyzer (Perkin-Elmer), and image intensities and background correction were quantitated using the computer program Imagene. Only these experiments were selected in which the ratio of signal to noise was higher than 3.0 in at least 40% of the total number of spots.

Gene expression was evaluated with the aid of Gene Spring 6.2 Program. Every spot intensity in the micro-array from the experimental sample was compared against the intensity of the universal control reference (two color experiments) in order to obtain a signal ratio as working value. Normalization was used to adjust the individual hybridization intensities to balance them appropriately so that meaningful biological comparisons can be made. There are a number of reasons why data must be normalized, including unequal quantities of starting RNA, differences in labeling or detection efficiencies between the fluorescent dyes used, and systematic biases in the measured expression levels.

Normalized data were subjected to statistical analysis of variance (ANOVA), which statistically compares mean expression levels between two or more groups of samples. The object was to find the set of genes for which the specified comparison showed statistically significant differences in the mean normalized expression levels. Clustering algorithms were used to divide genes into groups with similar expression patterns.

Additional processing of the data (details to be published) resulted in a table of ratios expressing differential expression between any two treatments and controls for each individual gene. The authors constructed these so as to show the effects of each extract (E1, E2) on control uninfected cells, the effects of rhinovirus infection on the cells, and the modulating effects of E1 and E2 on the infected cells. The stimulatory effects of the virus, and the modulatory effect of both *Echinacea* extracts, are clearly evident in these figures. Similar charts can be prepared for any other specific gene on the array.

Analysis by means of the Ingenuity Pathway Network has emphasized the importance of immune function genes in responses to the virus, and has also revealed other groups of genes modulated in uninfected cells by either one or both *Echinacea* extracts.

Discussion

What are the lessons from the analysis of the individual studies described? It is not possible to draw general conclusions because the plant sources and the methods used, including the array techniques, differed so much between the studies. In many cases a relatively small number of genes appeared to be affected by treatment. This is not surprising in the animal studies, in which heterogeneous whole tissues were compared between treated and control animals. Therefore important and dramatic changes in specific cell types could have been obscured. But in some of the cell culture studies also only small numbers of genes were affected, and in these cases the magnitude of the changes was often unimpressive. In contrast, in the *Echinacea* studies, it was observed significant changes in hundreds of genes as a consequence of rhinovirus infection, in line

with other DNA array studies showing the effects of viruses on cell gene expression. Furthermore, the *Echinacea* preparations modulated these virus-induced changes for many genes, and in addition showed equally impressive changes in uninfected cells.

These latter observations accord with the general concept of the cell transcriptome being a very flexible entity, responsive to numerous challenges from the micro-environment. It was demonstrated in the pioneering studies on DNA micro-arrays that a relatively simple manipulation of cultured cells (mammalian or yeast) such as addition of fresh serum and medium could result in profound changes in the transcription profile, both qualitatively and quantitatively. This is obviously relevant to the use of herbal medicines, since such mixtures comprise numerous potentially bio-active molecules, to all of which individual cells of superficial tissues might be exposed and hence modulated. Thus, an apparently simple exercise of consuming orally an alcoholic tincture of *Echinacea* would likely result in mucosal epithelial cells being exposed to a myriad of compounds, such as polysaccharides, caffeic acids and flavonoids and many other phenolic compounds, alkyl amides, polyacetylenes, and many of these could interact with numerous receptors on the cell surfaces, and thereby activate various signaling pathways, transcription factors and eventually changes in specific gene transcription. There could also be more immediate effects on various pathways not involving transcription. In addition one cannot ignore the potential bioactive contribution of the ethanol, which is frequently present in concentrations of up to 50–60%. The point of this argument is to illustrate the potential complexity of such interactions. Yet at the same time, if one anticipates specificity in the actions of different herbal preparations, then one has to presume that consumption of a different herbal mixture, e.g. St. John's wort or a ginseng formulation for example, would produce equally complex but different interactions because the chemical composition of the preparation would be substantially different from *Echinacea*. Some common chemicals would be present, and this could account for a certain amount of overlap in possible activities, such that several apparently unrelated herbs could help alleviate some common symptoms, but the total picture, i.e. the resulting transcription profile, would be different. In view of this we are surprised that some of the studies reported here observed so few changes. What could be the explanation for this apparent disparity? We have no answer, other than the obvious differences in technical details due to the use of different array analyses. We may find that the newer and more sophisticated bio-informatic analyses that are in development could overcome these limitations. Of course changes in transcription do not necessarily correlate with changes in corresponding proteins. Transcription is normally followed by many translation and post-translation steps, and some of these can act as regulators. The science of proteomics is rapidly approaching the same level of analytical capability as genomics, and soon one will be able to correlate transcription changes with protein changes for a given herbal preparation.

In regard to the type of changes observed, there is more agreement between the studies. In many cases, alterations in transcription factors, signaling pathway components, apoptosis factors, cell cycle genes, and cyto-skeletal products, were seen, not surprisingly, but nevertheless clear indications of profound changes in the cells.

Experts in Ethnopharmacology will have to examine the results of such studies in order to assess their relevance and impact. We may conclude in another decade or so that all these endeavors will have been a complete waste of time and money; alternatively, one might be on the verge of a real breakthrough in unraveling the detailed mechanisms of action of specific herbal medicines.

(Based on the article written by Jim Hudson; and Manuel Altamirano in the J. of Ethnopharmacology Vol. 108 No. 1 Nov., 2006, p.2-15)

Patents

Herbal pharmaceutical compositions for prophylaxis and/or treatment of cardiovascular diseases and the method of preparing the same

Sheu, *et al*

Sun Ten Phytotech Co., Ltd. Taipei County, Taiwan

US Patent 7,150,887 December 19, 2006 Appl. No.:10/875,812 June 25, 2004

The invention provides an herbal pharmaceutical compositions comprising the root of scutellaria, the rhizome of coptis, the root and rhizome of rhubarb, and the dry powders of the root of ginseng (or American ginseng) or the rhizome of ginger. The herbal pharmaceutical compositions are effective in preventing patients from developing or treating patients with cardiovascular diseases, which include, but are not limited to, hypertension, coronary heart disease, cerebrovascular disease, peripheral vascular disease, heart failure, rheumatic heart disease, congenital heart disease, and cardiomyopathies. The present invention also provides methods for preparing and using the herbal pharmaceutical compositions.

Compositions for removal of toxins.

Riley , *et al*.

US Patent 7,147,876 December 12, 2006 Appl. No.: 10/486,817 August 5, 2002

A medicinal or cosmetic composition comprising *Aloe vera* in combination with at least one vitamin, a minerals concentrate, an organic oils concentrate, at least one Chinese Herb, at least one essential oil and at least one spice. The composition may be administered orally or topically.

Method for chromatographic finger printing and standardization of single medicines and formulations.

Dadala , *et al*.

Council of Scientific and Industrial Research, New Delhi, India

US Patent 7,144,740 December 5, 2006 Appl. No.:09/779,377 February 8, 2001

The invention provides a method for the chromatographic fingerprinting, chemical and therapeutic standardization, bar-coding of the fingerprints and preparation of a data base for Enterprise Resource Planning (ERP) and Customer Relationship Management (CRM) machines and applications of medicines in general and traditional medicines in particular; this invention includes a software based instrumental method and a novel method of fingerprinting and standardization is proposed for the above purpose and the said method for the chromatographic finger printing which facilitates to correlate the traditional therapeutic standardization methods with the chemical properties of the medicines and humors and provides a rational basis to understand the methods used for the said purpose.

Preventive, alleviative or remedy for hypertension.

Okawa , *et al*.

Kao Corporation, Tokyo, Japan

US Patent 7,125,573 October 24, 2006 Appl. No.: 10/192,075 July 11, 2002

Provided is a preventive, ameliorant or remedy for hypertension, excellent in both blood pressure lowering action and blood pressure-rise suppressing action and having high safety.This preventive, ameliorant or remedy for hypertension comprises a coffee bean extract.

Drynaria extractions for treating osteo-porosis and their extraction process.

Xie; Yanming *et al.*

Qihuang Pharmaceutical Technology and Investment Co., Ltd. Beijing, China

US Patent 7,122,214 October 17, 2006 Appl. No.: 10/451,966 December 29, 2000

Rhizoma Drynariae extract is used as therapeutical agent or in the preparation for Osteoporosis, which is characterized in containing over 30 percent of total flavonoids. Furthermore naringin contained in flavonoids is more than 30 percent and less than 100 percent. The RDE is applied for the therapy of osteoporosis or used for producing drugs treating osteoporosis. And

methods of extracting the same are also related.

Pyranocoumarin derivatives.

Yoon , *et al.*

Elcom Biotechnology, Co. Ltd. Seoul, Korea

US Patent 7,115,655 October 3, 2006 Appl. No.: 10/510,923 April 9, 2002

The invention relates to compounds and pharmaceutically acceptable salts thereof. The present invention also relates to use for a cognitive-enhancing agent of compounds of the following formula (I) or pharmaceutically acceptable salts thereof and to a process for preparing the same. Further, the present invention relates to use for a congnitive-enhancing agent of decursin of the following formula (II) or pharmaceutically acceptable salts thereof. In addition, the present invention relates to extracts of Angelica gigantis Radix comprising decursin of the following formula (II), having cognitive-enhancing effects.

Immune system reconstructor composition.

Shoemake; *et al*.

McMinnville, Tennessee US

US Patent 7,112,343 September 26, 2006 Appl. No.: 10/852,718 May 24, 2004

The invention discloses an all natural composition comprising herbs for the use of restoring the immune system and subsequent medicinal value. The formulization of the present invention utilizes the benefits of herbal medicine for the use of restoring the immune system to protect and cure the body from infectious disease and various allergies. The composition formula consists of nineteen herbal ingredients in powder form that carrot powder, pau d' arco bark, gingko leaf, myrrh bark, echinacea augusta folia, slippery elm bark, heal-all-herb, celery stalk, celery leaf, red clover blooms, burdock root, goldenseal root, poke root, garlic bulb powder, beet root powder, yellow dock root, dandelion root, St. John's wort, and capscium in predetermined quantities.

Kavalactone profile.

Gow ,R *et al*

Herbal,Science, LLC, Naples, Florida, USA

US Patent 7,105,185 September 12, 2006 Appl. No.: 10/273,943 October 18, 2002

A method of producing a processed kava product involves using an extraction solvent, such as liquid CO.sub.2, to preferentially extract different kavalactones from the source material at different rates. By controlling the extraction parameters and stopping the extraction before all of the kavalactones have been extracted or allowing the extracted kavalactones to be preferentially precipitated in one or more collection environments, a processed kava product can be produced that has a kavalactone distribution profile that can differ substantially from that of the source material. As a result, roots from a less desirable kava cultivar can be used to produce a processed kava product which has a kavalactone distribution profile that is similar to that of a highly desired cultivar. A material having a preferred kavalactone distribution profile is also disclosed. The kava paste can be further processed to produce a dry flowable powder suitable for use in, e.g., a tableting formula. A rapid dissolve tablet formulation for use in the delivery of kavalactones is also disclosed.

Synergistic composition for the treatment of diabetes mellitus.

Bhaskaran , *et al.*

Indus Biotech Pvt. Ltd. Pune, India

US Patent 7,141,254 November 28, 2006 Appl. No.: 10/846,299 May 14, 2004

The invention relates to a synergistic composition for the treatment of diabetes in a subject in need thereof, said composition-comprising Trigonelline of concentration ranging between 20 to 30%, amino acids of concentration ranging between 20 to 60%, and soluble fiber of concentration ranging between 10 to 60%, optionally along with pharmaceutically acceptable additives, a process thereof and also, a method of treating diabetes.

Treatment of skin conditions

Raman , *et al.*

BTG International Limited, London, UK

US Patent 7,122,561 October 17, 2006 Appl. No.: 10/630,901 July 31, 2003

The invention provides piperine and analogues or derivatives thereof for the treatment

of skin conditions treatable by stimulation of melanocyte proliferation, such as vitiligo, and also for treating skin cancer. The piperine and analogues or derivatives thereof may also be used to cosmetically promote or enhance the natural coloration of the skin.

Physiologically active composition and process for producing the same.

Goindo Tadashi

Goindo Todashi, Japan

Publication number: EP1736206 27.12.2006 (16.12.2005)

An object of the present invention is to provide a mixed composition of a herbal medicine with an effective physiological activity. The composition comprises an extract component from any one of a carpophore, a mycelium and a culture from more than two kinds of the Basidiomycete fungi selected from a group comprising a fungus belonging to Basidiomycetes Aphyllophorales Ganoderma Ganodermaceae, a fungus belonging to Basidiomycetes Polyporaceae Coriolus, a fungus belonging to Basidiomycetes Agaricales Agaricaceae Agaricus, and a fungus belonging to Basidiomycetes Agaricales Hymenochaetaceae Phellinus and an extract component from a root of a plant belonging to Araliaceae, and has an oxidation-reduction potential sufficient to express the antitumor effect and hypoglycemic effect.

Hair Restorer

Meng, Yuehua et al.

Meng, Yuehua and Zhaw Chuanshui, China.

WO/2006/108319 Application No.:PCT/CN 2005/ 000473 19.10.2006

The invention relates to an externally usable hair restorer as one kind of Chinese traditional medicine preparation. The hair restorer is prepared from alcohol and 14 Chinese herbal medicinal materials including Radix Notoginseng, Radix Ginseng, Radix Angelicae Sinensis, Rhizoma Gastrodiae, and Herba Cynomorii etc., through stir-frying with liquid, washing, sieving, baking, Ooling, micronizing, mixing, stirring and filtrating. The hair restorer can be used to treat alopecia areata, universal alopecia and seborrheic alopecia with total effective rate of higher than 95%, as well as with no toxic side effect.

Herbal medical preparation for the treatment of arthritis.

LIU, Liang *et al.*

Hong Kong Jockey Club Institute of Chinese Medicine Limited, China

WO/2006/056117 Application No.:PCT/CN 2005/001675 01.06.2006

An oral preparation containing components extracted from Caulis Sinomenii, Radix Aconiti Praeparata, Radix Paeoniae Alba, Cortex Moutan Radicis and Rhizoma Curcumae Longae has effects of anti-arthritis, anti-inflammation and analgecizing. It is suitable for the treatment of arthritis and its relating symptoms, as well as other similar symptoms.

Herbal formulations for modulating blood lipids.

Yang, Dajian *et al.*

Hong Kong Jockey Club Institute of Chinese Medicine Limited ,China

WO/2006/021149 Application No.:PCT/CN 2005/001319 02.03.2006

The invention concerns herbal formulations for the modification of the levels of blood lipids. Aspects of the invention include the preparation of herbal formulations and methods for their use.

Herbal formulations for modulating blood lipids.

Yang, Dajian

Hong Kong Jockey Club Institute of Chinese Medicine Limited , China

WO/2006/001319 Application No.: PCT/CN 200502.03.2006

The present invention concerns herbal formulations for the modification of the levels of blood lipids. Aspects of the invention include the preparation of herbal formulations and methods for their use.

Herbal composition phy906 and its use in chemotherapy.

Cheng, Yung-Chi *et al.*

Two Whitney Avenue, New Haven, Connecticut, US

WO/2006/053049 Application No.:PCT/US

2005/ 040605 18.05.2006

This invention provides herbal compositions useful for increasing the therapeutic index of drugs, including those used in the treatment of disease, especially viral infections and neoplasms of cancer. This invention provides methods useful for improving the quality of life of an individual undergoing chemotherapy. Furthermore, this invention improves the treatment of disease by increasing the therapeutic index of chemotherapy drugs by administering the herbal composition PHY906 to a person undergoing such chemotherapy.

Method of providing an essential oil extract of capsicum.

Torres L. *et al.*

New Mexico Tech Research Foundation, Socorro NM

US Patent 6919095, July 19,2005 (20.09.2002)

Essential oil extract of capsicum or the preparation of capsicum extract are described. Extract contain mainly capsaicinoid and terpene. Capsicum devoid of seeds and stems is mixed with pentane solvent where in pentane predominantly dissolve capsicum and when the temperature is maintained at around 64°C essential oil extract of capsicum results. Further advantage is the efficacy and compatability of the extract with wide range of formulation.

Cosmetic/dermatological compositions: A tetrahydrocurcuminoid and an amide oil.

Seyer, N *et al*.

L'Oreal, Paris, France

US Patent 6872401, March 29, 2005 (28.03.2003)

The invention refer to cosmetic or determatological composition containing a carrier with at least one fatty phase and tetrahydrocurcumin, charaterised in that it contains the derivative or mixture of 4,7-diphenyl 3,5-heptanedione and one oil having one amide unit. It is reported that in an emulsion, solubilizing the mixture of tetrahydrocurcuminoids in an oil having, in its structure at least one amide unit in accordance, makes it possible to obtain good stability over time.

Methods and compositions for rapid *in vitro* propagation of *Swertia chirata*.

Ahuja A *et al.*

CSIR Depatment of Biotechnology, New Delhi, India

US Patent 6855547, February 15,.2005 (30. 11. 2001)

The patent relates to methods and compositions of *in vitro* cultivation of species of *Swertia chirata.* It provides culture media comprising MS basal culture medium, plant hormones, gibberellic acid, auxins and other additives. In vitro cultivation of Swertia chirata comprising auxillary bid and shoot apex explants with a basal culture Medium, BAP, IAA, IBA, and NAA, to produce primary explant, contacting the primary explant with a shoot propagation medium, comprising, a modified MS basal culture medium. BAP, GA Sub3, and IAA to produce secondary explant. The methods and compositions of the invention are inducing extra-ordinary rapid *in vitro* propagation of *Swertia chirata* (Gentianaceae)

Pesticidal activity of plant essential oils and their constituents.

Bessettee SM *et al.*

Ecosmart Technologies Incorporation, US

US Patent 6841577, January11, 2005 (18.12.2001)

A pestiside for cockroaches and larvae based on natural substances viz. eugenol, phenethyl propionate, methyl salicylate, pulegone, carvacrol, thymol, thyme oil, citronellal etc., different blends consisting of the natural substances have been used as pesticides. Methods of use of the pesticides are disclosed.

Process for the extraction of valerian root.

Michael JA *et al*.

Ancile Pharmaceuticals, San Deigo, USA

US Patent 6913770, July 05,2005 (11.04.2005)

A pharmaceutically active extract from the root of *Valeriana officinalis* has been described. The process involves the steps of adding the roots to an extract of approximately 50% (v/v) (alcoholic

mixture) to 100% (v/v) in a remainder of water. The mixture is heated to 70-80°C for at least 2 hours. The mixture is useful in the formulation of sedative/muscle relaxant and/or anxiolytic activity.

Composition useful as heptoprotectants of plant *Cryptolepsis buchnani* and a method.

Qazi GN *et al.*

CSIR New Delhi, India

US Patent 6913772, July 05, 2005 (11.12.2003)

A method is described by making use of active constituent from the plant *Cryptolepsis buchnani* for the preparation of heptoprotectants. The clinically acceptable method of treatment have been dealt-with.

Herbal healing lotion for veterinary use.

Rafkin S *et al.*

Rafkin S, New York, USA

US Patent 6844014, January18, 2005

A herbal healing lotion for application in the form of topical composition of relieving dermatological skin conditions encountered by the animals has been described. The lotion combines natural ingredients in a topical composition to successfully manage dermatological conditions.

Process for the preparations of a extract rich in bacosides from the *Bacopa monniera.*

Kahol AP *et al.*

CSIR, New Delhi, India

US Patent 6833143, April 21, 2004 (26.03.2003)

The invention provides a novel process for the preparation of the bacosides enriched fraction in a non hydroscopic fraction from the plant. The step involves drying of freshly harvested herb in a hot air oven 37-42° C, pulverizing and sieving of the dried herb to obtain powder (30-40 mesh size)

Herbal-based composition for treating acute and chronic myloid leukemia.

Bandyopadhya S *et al.*

CSIR, New Delhi, India

US Patent 6967034, November 22, 2005 (30.05.2003)

A new herbal-based composition for treating chronic myloid leukemia by making use of Piper betle (Piperaceae) jeaf extracts and the process for the isolation of the active fractions from leaves have been described.

Herbal medicaments for the treatment of neurovascular disorders.

Ray Madhur *et al.*

CSIR, New Delhi, India

US Patent 6991814, January 31, 2006, Appl. No. 10/319373, (13.12.2002).

The invention relates to a composition obtained from the lipid soluble extract of rhizomes and leaves of Curcuma species of Zingiberaceae family, useful for the treatment of neurocerebrovascular disorders, said composition comprising fraction A consisting of an turmerone formula I, and turmerone of formula II, and /or along with fraction B consisting of curcumene and zingiberine, and/or curcumerone, and curione, and/or pharmaceutically acceptable additives and a method of treating neurocerebrovascular disorders in animals including humans using said composition by administering therapeutically effective amount of lipid soluble extract.

Isolation of figogenin pentaglycoside from *Chlorophytum nimonni.*

Vijay Lakshmi *et al.*

CSIR, New Delhi, India

US Patent 7160866, January 9, 2007, Appl. No. 10/806065, (22.03.2004)

The invention provides a novel saponins tigigenin glycoside isolated from the aerial parts of Chlorophytum nimonii and aprocess for the isolation thereof as well as its use in anti-hyperglycemic and hypolipidemic activities.

Patents

Drugs and Pharmaceuticals

Current R & D Highlights

Title	*Volume*	*No.*	*Month*
Analgesic, Antipyretic Drugs	18	1	Jan.,-Mar., 1995
Immunomodulators	18	2	Apr.,- June 1995
Vaccines	18	3	July-Sept., 1995
Diagnostics	18	4	Oct.,-Dec., 1995
Acquired Immunodeficiency Syndrome	19	1	Jan.,Mar., 1996
Drugs for Cardiovascular System	19	2	Apr.,-June 1996
Traditional Medicine	19	3	July-Sept., 1996
Skin Disorders	19	4	Oct.,-Dec., 1996
Gastrointestinal Disorders	20	1	Jan.,-Mar., 1997
Vector Borne Disorders	20	2	Apr.,-June 1997
Hormonal Disorders	20	3	July-Sept., 1997
Quantitative Structural Activity Relation	20	4	Oct.,-Dec., 1997
Lipid Lowering Agents	21	1	Jan.,-Mar., 1998
High Throughput Screening	21	2	Apr.,-June 1998
Combinatorial Chemistry	21	3	July-Sept., 1998
Respiratory Disorders	21	4	Oct.,-Dec., 1998
Chiral Synthesis	22	1	Jan.,-Mar., 1999
Musculoskeletal Disorders	22	2	Apr.,-June 1999
Wound Healing Agents	22	3	July-Sept., 1999
Herbal Products	22	4	Oct.,-Dec.,1999
Biotherapeutics	23	1	Jan.,-Mar., 2000
Marine Based Bioactives	23	2	Apr.,-June 2000
Peptidomimetics	23	3	July-Sept., 2000
Drug Targets	23	4	Oct.,-Dec., 2000
Immunotherapeutics	24	1	Jan.,-Mar., 2001
Gene Therapy	24	2	Apr.,-June 2001
Neurologicals	24	3	July-Sept., 2001
Bioinformatics	24	4	Oct.,-Dec., 2001
Protein Structure & Drug Discovery	25	1	Jan.,-Mar., 2002
Stem Cell Research & Health Care	25	2	Apr.,-June 2002
Chemoinformatics	25	3	July-Sept., 2002
Pharmacogenomics	25	4	Oct.,-Dec., 2002
Emerging &Re-emerging Diseases	26	1	Jan.,-Mar., 2003
Recombinant Pharmaceutical Proteins	26	2	Apr.,-June 2003
Ophthalmic Disorders	26	3	July-Sept., 2003
Proteomics	26	4	Oct.,-Dec., 2003
Musculoskeletal Disorders	27	1	Jan.,-Mar., 2004
Proteomics	27	2	Apr.,-June., 2004
Pharmacogenomics	27	3	July-Sept., 2004
Cerebrovascular Disorders	27	4	Oct.,-Dec., 2004
Heart failure	28	1	Jan.,-Mar.,2005
Osteoporosis	28	2	Apr.,-June 2005
Asthma	28	3	July-Sept.,2005
Obesity	28	4	Oct.,-Dec., 2005
Ageing	29	1	Jan.,-Mar., 2006
Cancer	29	2	Apr.,-June 2006
Tuberculosis	29	3	Jully-Sept., 2006
Malaria	29	4	Oct.,-Dec., 2006