Immunomodulatory and Antimicrobial Effects of Some Traditional Chinese Medicinal Herbs: A Review

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Abstract: The current practice of ingesting phytochemicals to support the immune system or to fight infections is based on centuries-old tradition. We review reports on seven Chinese herbs, (*Aloe vera* Mill. (Aloaceae)*, Angelica species* (Umbelliferae)*, Astragalus membranaceus* Bunge. (Leguminosae)*, Ganoderma lucidum* (Fr.) Karst. (Ganodermataceae), *Panax ginseng* C.A Mey. (Araliaceae)*, Scutellaria species* (Lamiaceae) and *Zingiber officinale* Rosc. (Zingiberaceae) with emphasis to their immunomodulatory and antimicrobial activities. While some of these herbaceous plants have a direct inhibitory effect on microbial organisms, we observe that each plant has at least one compound that selectively modulates cells of the immune system. The successful derivation of pure bioactive compounds from *Ganoderma lucidum, ginseng* and *Zingiber officinale* supports the traditional practice of using these plants to stimulate the immune system. As many modern drugs are often patterned after phytochemicals, studying the influence of each compound on immune cells as well as microbes can provide useful insights to the development of potentially useful new pharmacological agents.

Keywords: Anti-bacterial, anti-fungal, anti-microbial, Chinese herbs, immune system, phytochemicals, TCM, herbal toxicology.

INTRODUCTION

The world we live in is one full of microbes. Our body temperature and wealth of nutrients provide an ideal home for these micro-organisms to thrive. The human immune system has the essential function of protecting the body against the damaging effects of microbial agents that are pathogenic. The system comprises innate (non-specific) and acquired (specific) immunity. Natural killer (NK) cells, complement system, macrophages, antigen presenting cells (APCs) and neutrophils make up the innate immune system, and mounts an immediate non-specific response to foreign microbial agents. If microbes by-pass this primary defence, the acquired immune response, comprising humoral and cell-mediated components, will then act to contain the invaders. The type of antigen (fungi, virus, bacteria, toxin) processed and presented by APCs to the $CD4^+$ T cell determines the type of cytokines secreted, which in turn, determine the differentiation of helper T (T_H) cells into T_H1 or T_H2 cells, and B-cells to give immunoglobulin subtypes. T_H1 response involves the activation of macrophages, which contain and destroy mycobacteria and fungal pathogens. T_H1 pathway also activates cell-mediated immunity. T_H2 cells, on the other hand, effect immunoglobulin differentiation and antibody secretion, and therefore mediate humoral immunity. CD8+ cytotoxic T cells induce apoptosis in antigen-laden cells [1].

Apart from these natural mechanisms, there are additional factors that stimulate and suppress host immunity. Immunostimulants enhance the overall immunity of the host, and present a non-specific immune response against the microbial pathogens. They also work to heighten humoral and cellular immune responses, by either enhancing cytokine secretion, or by directly stimulating B- or T-lymphocytes.

The use of plant products as immunostimulants has a traditional history. However, the isolation of the active principals involved did not gain momentum till the 19th century [2]. A study in 1990 showed that 64% of the world's population use botanic drugs to combat health problems [3]. Currently, it is estimated that almost 50% of the synthetic medicines are derived from, or patterned after, phytochemicals [4]. Plants synthesize chemicals as part of their defense against pathogens. Many such compounds occur in nature as anti-feedant and anti-infectant chemicals, and are found effective against microbes. Flavonoids and hydroxylated phenols, for example, are naturally synthesized by plants in response to infection [5]. Flavones and flavanones, being bitter, also have natural anti-feedant effects. Alkaloids are the most common plant metabolites [6]. An alkaloid derivative, nicotine, for example, has been shown to have insecticidal activities [7]. Quinine, another alkaloid isolated from the bark of the *Cinchona* tree, was the first effective anti-malarial drug [8].

TRADITIONAL CHINESE MEDICINE

The concepts relating to Traditional Chinese Medicine (TCM) had already been well accepted by their practitioners, when scientific proof of medical concepts emerged. For instance, the theory that blood circulates in the body, and is not static originated more than 1000 years before William Harvey described it in 1628 [9]. TCM encompasses 4 disciplines of practice: Herbalism, food cures, acupuncture

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and manipulative therapy [10]. In this review, we focus on the herbal component of TCM.

The use of Chinese herbs is believed to have originated during the Xia dynasty, more than 4000 years ago. The founder of Chinese medicine, Shen Nung, is regarded as the Father of medicine [11]. Unlike many breakthroughs in drug discovery that came through serendipity, such as the discovery of penicillin and digoxin, it is believed that the discovery of the medicinal value of Chinese medicinal herbs was not by chance. History records that Shen Nung sampled plants in the hundreds to examine their effects, both good and bad. With the accumulation of medical experiences, the theories and concepts of TCM emerged. The "Classic of

Shen Nung's Materia Medica" is said to be the first book that summarised the properties and functions of over 300 herbs [9]. As communication links improved, cultural exchanges between neighbouring nations, Korea, Japan and India, brought about more plants with nutritional value and more ways to treat diseases.

The concepts of Qi, Yin and $Yang$, and the Five Elements in Chinese medicine, subsequently evolved to comprise ideas that climates (Wind, Cold, Summer, Heat, Dampness, Dryness and Fire) and emotions (Joy, Anger, Grief, Anxiety, Melancholy, Terror and Fright) influence bodily disorientations. In later years, the concept that, "if there were no pathogens, there would be no diseases"

Table 1.   Summary of Phytochemicals and their Effects on Immune Cells and Microbes

No	Species	Plant part	Chemical compound	Chemical identity	Biological activity
1	*Aloe vera*	Leaves	CARN750	polysaccharide	Selectively stimulates cytokines, activates lymphocytes [18]
2	*Angelica dahurica*	Roots	5,8-di (2,3-dihydroxy-3-methylbutoxy)-psoralen	furocoumarins	Toxic to *Aspergillus candidus* [24]
			(R)-heraclenol	furocoumarins	Toxic to *Bacillus subtilis* [24]
			Ferulic acid, Byakangelicin	furocoumarins	Toxic to *Cladosporium herbarum* [24]
	Angelica gigas	Roots	Angelan	polysaccharide	Selectively modulates cytokines [30]
3	*Astragalus membranaceus*	Roots		polysaccharide	Increase macrophage count [33]
				soluble extracts	Selectively stimulates cytokines [31]
4	*Ganoderma lucidum*	Fruiting bodies	water soluble extracts	glycoproteins	Selectively stimulate cytokine [35]
			GLIS	proteoglycan	Stimulate lymphocytes [39]
			water- and ethanol-soluble extracts	polysaccharide	Increase natural killer cell count [40]
5	*Panax notoginseng*	Roots	Pananotin	peptide	Toxic to *Coprinus comatus, Physalospora piricola, Botrytis cinerea, Fusarium oxysporum* [52]
			Panaxagin	peptide	Fungal ribosome inactivating protein [53]
	Panax ginseng	Roots	Panaxadiol, Panaxatriols	saponins	Stimulate lymphocytes and cytokines [46]
			Ginsan	acidic polysaccharide	Stimulate IL-1, IL-12, TNF-α and IFN-γ [48]
			Rhamnogalacturonan II	saponins	IL-6 stimulant [47]
	Panax ginseng	Leaves	Rhamnogalacturonan II	saponins	Enhance macrophage actions, stimulate IL-6 activity [74,75]
6	*Scutellaria barbata*	Roots	Apigenin	flavonoid	Toxic to MRSA [55]
	Scutellaria baicalensis		Wogonin	flavonoid	Stimulates TNF-α, activates iNOS [61]
	Scutellaria albida		Linalool	flavonoid	Toxic to *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* [62]
7	*Zingiber officinale*	Rhizome	Citral		Antimicrobial [65]
			Curcumin	sesquiterpene	Toxic to *Rhizoctonia solani* [65]
			Dehydrozingerone		Antifungal [65]
			1,7-bis(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one	shogasulfonic acid	Toxic to *Pyricularia oryzae* [70]
				ethanol soluble extracts	IL-1, IL-6 stimulant [72]

emerged. Such was the foundation for the established fact that disease enters through openings in the body and that treatment of diseases should be targeted at combating the pathogens [9].

A search of the literature revealed seven TCM herbs have been studied, in some detail, for their immunomodulatory and antimicrobial effects. These herbs are also commonly used by TCM practitioners here in Singapore. Table **1** presents an overview of the chemicals present in the plants and their respective biological activities. This review also examines in greater detail the studies done with specific phytochemicals and their effects on some biochemical parameters of the immune system.

ALOE VERA ("LU HUI")

"Four vegetables are indispensable for the well being of man: Wheat, the Grape, the Olive and the Aloe. The first nourishes him, the second raises his spirit, the third brings him harmony and the fourth cures him." – Christopher Columbus

The *Aloe vera,* originated from Africa, was introduced to China though the Silk Road. The plant became a treasured herb by the 7th century. Containing major constituents such as aloin, ρ-coumaric acid, aldopentose, calcium oxalate and polysaccharides [12], *Aloe vera* is a common element in cosmetic products. While raw leaf juice was traditionally used as laxatives, its mucilaginous gel is casually used to treat burns and cuts [13].

The antimicrobial activity of aloe extracts was postulated as early as 1939 [14]. Isolates from *Aloe vera* were shown to inhibit microbes like *Staphylococcus spp* [15] and *Candida spp* [16]. Clinical studies have revealed several immunomodulatory properties. Stronger leukocyte infiltration was seen in injured areas where aloe extracts were used in treatment [17], and surface wound recovery was stimulated by polyuronic acids [13]. Acemannan, the major fraction of aloe polysaccharides, has been extensively studied for immunomodulatory effects. Reports showed that these $\beta(1,4)$-linked acetylated mannans are able to increase phagocytic activities [18,19,20]. CARN 750, an acemannan, stimulated leukocytes and lymphocytes in a dose-dependant manner, as well as triggered the release of IL-1, IL-6 and TNF-α. Administrations of CARN 750 also showed a positive influence on lymphocyte proliferation in the spleen and bone marrow [18], both of which are essential lymphoid organs that produce and differentiate lymphocytes. In fact, earlier reports mentioned the ability of acemannans to stimulate T_H2 cells [21]. It has been postulated that the actions of acemannan may be attributed to the residual presence of aloerides [13]. In accord with this postulate, polysaccharides from crude extracts have been shown to enhance transcription of cytokines. High concentrations of aloeride also seemed to enhance macrophage activities [13], and may be a contributing factor for the increased phagocyte stimulation by acemannan.

ANGELICA SPECIES

Traditionally, *Angelica sinensis,* because of its high phytoestrogenic content, is reputed to have a stabilising effect on the female hormonal system, making it useful in treating menstrual problems. In China, it is often referred to as 'female ginseng'. It is also one of the more commonly prescribed herbs, used to nourish blood. Its roots and leaves are commonly used for its medicinal purposes. Constituents of *A. sinensis* include ligustilide, butylidene phthalide and β-sitosterol [12].

Essential oil extracts from *Angelica* were shown to inhibit selected pathogens [22], and polysaccharides were shown to induce activation of both specific and non-specific immune components [23]. One study tested 8 constituents of *Angelica dahurica* root for antifungal and antibacterial activity. 5,8-di (2,3-dihydroxy-3-methylbutoxy)-psoralen was found to be highly toxic to *Aspergillus candidus* [24]. This aromatic compound intercalates between DNAs, cross-links bases when exposed to light [25], and produces a genotoxic effect. Apart from its effects on fungi, psoralen was later shown to cause cell cycle delays in *Saccharomyces cerevisiae* [26], and had bactericidal effects on *S. aureus* and *E. coli* as well [27]. Ferulic acid and byakangelicin from *A. dahurica* inhibited *Cladosporium herbarum* growth, while (R)-heraclenol showed potent antibacterial activity against *Bacillus subtilis* [24]. These furocoumarins induce toxicity, mainly by causing abnormal DNA-protein interactions, or DNA inter-strand interactions [28]. The resistance of some microbes to these furocoumarins may be due to the presence of feruloyl catabolising enzymes [29]. In a later study, a polysaccharide, angelan, isolated from roots of *Angelica gigas*, was shown to trigger the release of cytokines IL-2, -4, -6 and INF-γ from macrophages. Cytokine-release was found to occur in a sequential manner, with IL-6 presenting an almost immediate increase, followed by IL-4, with IL-2 having the slowest rate of increase [30]. The increase in IL-2 may be attributed to the preceding increase in IL-6. In accord with the type of cytokines released, it can be postulated that with the initial rapid rise in mediators that activate T_H2 cells, the primary effect of angelan is the enhancement of T cell-dependent antibody production.

ASTRAGALUS MEMBRANACEUS ("HUANG QI")

Native to Northern China, Shen Nung regarded *Astragalus membranaceus* as one of the most important herbs. Containing active constituents such as glucuronic acid, β-sitosterol, astragalosides, isoflavone and asparagines [12], it is usually taken to manage conditions of fatigue, loss of appetite and diarrhoea. *Astragalus* has also been traditionally used to strengthen the immune system, and as a treatment for respiratory infections.

Song *et al*. [31] had reported an increase in activated B cells, together with a suppression of IFN-γ levels, in response to *Astragali* extracts. However, IL-6 mRNA expression was found to be suppressed. More recently, Young *et al*. [32] have shown that water-soluble extracts of *Astragalus* radix were able to stimulate the proliferation of splenic lymphocytes, as well as increase the mRNA expression of the cytokines (IL-1, IL-6 and TNF). The extracts also increased IL-1 and IL-2 as well as macrophage activity in the immunosuppressed mouse [31]. The latter finding is in accord with earlier studies of Wang *et al*. [33] that *Astragali* polysaccharides increased macrophage count,

and promoted opsonization, via the C3 complement component.

GANODERMA LUCIDUM ("LING ZHI")

The virtues of Ganoderma have resulted in the documentation of this herbal fungus as one of the superior grade herbs, and as having the most effective healing powers, in some instances, even exceeding the reputation of ginseng. The anti-ageing effects of this medicinal fungus have also been clearly demonstrated [34]. Its traditionally accepted benefits include removal of toxins, healing of stomach diseases and combating mushroom poisoning. Also known as the 'King of Herbs', *Ganoderma* was among those herbs that were used to strengthen the immune system.

A glycoprotein from the water-soluble fractions of the crude extracts was found to activate IL-1, IL-2 and IFN-γ. Fucose residues on this glycoprotein were found to be indispensable for this activity [35]. Polysaccharides from fruiting bodies were also shown to increase levels of IL-1, IL-6, IFN-γ and TNF-α [36]. Specifically, 3 polysaccharide isolates were able to stimulate activation and proliferation of B and T cells [37]. All 3 glucans possess β-D-glucopyranosyl residues. Earlier studies performed on spores of *G. lucidum* had confirmed that glucans with glucopyranosyl residues had immunostimulating properties [38]. Another proteoglycan isolated from fruiting bodies, termed GLIS, specifically induces B cells to express activation and proliferation markers on the cell membrane. Consequently, increases in plasma B cell count and circulating antibodies were noted [39]. The finding parallels the reported increases in T_H2 and B cell-activating cytokines, IL-1 and IL-6 [35]. Both water-soluble and ethanol-soluble extracts from fruiting bodies were also shown to increase natural killer cell count [40], validating earlier reports of enhanced IFN-γ production by polysaccharide derivatives [35]. IFN-γ may also be responsible for the increase in differentiation and activation of dendritic cells (DC). Zi and Li [41] reported that APCs, particularly DCs, were found to be stimulated in the presence of polysaccharides. DC, which are major antigen processors, present the processed antigens to T cells, which in turn are activated. Hence, an indirect activation of T cells may be achieved through increased activation of DC. The increased activation of T cells reported by Sahar *et al.* [42] may, therefore, be attributed to stimulation of DC.

GINSENG SPECIES

Ginseng is one of the most popular herbs in the world. The medical benefits of ginseng have their roots in Chinese folklore. An anecdotal report of a farmer's discovery that an injured snake could recover after eating this plant provided the impetus that led to the discovery of its manifold benefits [43]. The Materia Medica, by Shen Nung, records ginseng as one of the highest quality herbs. The term 'panax' means 'all healing' in Greek. Ginseng has been used for centuries to reduce stress and boost energy. Containing active ingredients, such as panax-type ginsenosides, mono- and polysaccharides [12], ginseng is believed to stimulate every aspect of the immune system in vertebrates.

Indeed this is true. Ginsenosides are one of the major constituents of ginseng, with over 28 types identified so far [44]. These steroidal saponins were shown to enhance both B and T cell-mediated immune responses [45]. Many constituents of *ginseng* stimulate the immune system: protopanaxadiol and protopanaxatriol induce proliferation of lymphocytes and cytokines [46]; rhamnogalacturonan II ginsenosides [47] stimulate IL-6 activity; and, ginsan (an acidic polysaccharide) induces activities of IL-1, IL-12, TNF-α and IFN-γ [48]. Several of these constituents have been deemed responsible for the antimicrobial property of ginseng. Acidic polysaccharides isolated from *Panax ginseng* have been shown to inhibit *Helicobacter pylori* induced heamagglutination, possibly due to the presence of uronic acids, a major component of the acidic polysaccharide [49]. Another polysaccharide isolated from *P. ginseng* also decreased concentration of *S. aureus* in the plasma in a dose-dependant manner [50]. This effect may in part be due to the increased activation of macrophages [51], as well as intra-macrophage levels of nitric oxide [50], leading to significant bacterial cytotoxicity. Proteins have recently been found in ginseng. Pananotin, from the roots of *Panax notoginseng* was found to be toxic to *Coprinus comatus, Physalospora piricola* and the phytopathogens, *Botrytis cinerea* and *Fusarium oxysporum* [52]. Other proteins isolated from the *Panax* family (panaxagin and quinqueginsin) also exhibit antifungal activity [52]. Panaxagin seems to have some conserved residues in its N-terminal sequence, which have been deemed important for fungal ribosome inactivation. Hence, it has been postulated that panaxagin may exert its antifungal activity as a ribosome inactivating protein [53].

SCUTELLARIA SPECIES

Scutellaria has been used in TCM for centuries to cleanse the body of toxins. In China, *Scutellaria baicalensis* is referred to as one of the '3 cold brothers'. Traditionally, it is prescribed for fever, cold, and vomiting of blood. Its roots are among those that contain high concentrations of flavonoids ($\approx$35%) [54]. Its active compounds include baicalin, baicalein, wogonin derivatives and β-sitosterol [12].

Antibacterial properties have also been demonstrated in extracts of *Scutellaria barbata*, but they were specific to *S. aureus*. Two ethanol-soluble extracts with NMR peaks corresponding to luteolin and apigenin were examined [55]. Luteolin has been shown to have anti-allergic properties that are mediated through the inhibition of IgE [56]. The apigenin analogue had MICs for MRSA strains that were lower than those of the antibiotics, methicillin and oxacillin. Also, there seemed to be no significant activity against ten other bacterial strains tested [55], suggesting that the flavonoids in *Scutellaria barbata* extracts may be toxic only to *Staphylococcus*. *Scutellaria baicalensis,* however, was also active against a wide range of bacteria, including *cholera, typhoid, Streptococcus, E. coli, Pneumococcus* [57, 58], *Klebsiella pneumoniae, Proteus vulgaris, Mycobacterium tuberculosis, Pseudomonas aeruginosa* and *Corynebacterium diphtheria* [59]. *S. baicalensis* has also been shown to induce TNF-α [60], possibly due to the effects of the flavonoid, wogonin [61]. Since TNF-α acts to increase nitric oxide production, the effect of wogonin on

nitric oxide levels was examined. Results showed that wogonin increased nitric oxide production at lower concentrations and reduced its production at higher concentrations [61]. Hence, dose-dependant induction of microbial cytotoxicity may be a possibility. Linalool, a chemical isolated from *Scutellaria albida*, showed highly toxic effects against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa.* Except for *E. coli*, the other 3 bacteria proved to be more sensitive to linalool than to streptomycin [62]. In addition to its antibacterial activity, diterpenoids from *Scutellaria* have also shown activity against pathogenic plant fungi [63].

ZINGIBER OFFICINALE ("SHENG JIANG")

Ginger is indigenous to Southeast Asia; a city with its Sanskrit name, Shunti, was already in existence in 200 B.C. In Ayurvedic medicine, ginger is called "The Great Medicament" and is generally used to treat a variety of ailments. This holds true in Chinese culture too. Shen Nung was one of the first to mention dried ginger as a medicament. Known to be a classic gastric tonic, this tropical rhizome is recommended for gastric pain, vomiting, diarrhoea, coughs and colds [9]. Little wonder then that Chinese sailors chewed ginger to prevent and treat sea-sickness. It contains more than 20 active compounds, including curcumene and gingerol derivatives [12].

Ginger is a domestic remedy also known for its anti-infectant effects. Essential oil constituents from rhizomes of *Z. officinale* were found to decrease growth rate of a variety of bacteria and fungi, including *Staphylococcus* and *Candida* [64]. The most effective antimicrobial constituent was found to be citral. Curcumene, a sesquiterpene, from ginger oil was found to inhibit *Rhizoctonia solani* [65]. In another advance, it was shown that ethanol extracts of *Z. officinale* were able to inhibit growth of both gram-negative and gram-positive bacteria [66], although the inhibitory effect was more pronounced for gram-positive bacteria [67]. Bactericidal activity against the highly resistant gram-negative bacteria *Pseudomonas aeruginosa* was notable [68]. One of the constituents described for its antifungal activity is dehydrozingerone [65]. Another structurally characterised compound, 1,7-bis(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one also showed inhibitory effects on *Pyricularia oryzae* [69]. This structure seems to be the skeletal structure of a shogasulfonic acid isolated by Yumiko *et al.* [70]. Ethanol-soluble extracts from the rhizomes of *Z. officinale* were tested for their action on cytokines and found to promote the secretion of IL-1 and IL-6 in a time- and dose-dependant manner [71]. Early isolation experiments showed the presence of highly cytotoxic compounds (diacetylafzelin, diferuloylmethane, feruloyl-p-coumaroylmethane and di-p-coumaroylmethane) from the species of the family *Zingiberaceae* [73]. Pure *Zingiberis* isolates have been structurally characterised, but their anti-microbial effects were not studied [69, 70].

DISCUSSION

The seven medicinal herbs reviewed here are commonly used in many prescriptions of local TCM practitioners. In addition to their easy availability, the many benefits of these herbs are also internationally recognised. In TCM practice, prescriptions are made for various combinations of the herbs, each in different amounts. For this reason, it is not possible to conclude whether a single herb from a prescription, or a single compound from a particular herb, is solely responsible for the effect(s) on the immune system. Nonetheless, it is useful to recognise the molecular and biochemical effects of constituents, isolated from individual herbs, as they may explain the basis for the purported immunomodulatory and anti-microbial effects of the herbs.

This review shows that herbs exert immunostimulating effects in many ways. Immunostimulatory agents do not directly affect immune memory cells, as activation and differentiation of memory cells require precise cell-cell and MHC-antigen interactions. However, they are specific in that immunostimulants enhance particular immune responses to combat specific pathogens. Immunostimulating activities may be divided into those that (1) enhance phagocytic activities, and (2) effect cell-mediated and humoral immunity. Constituents that stimulate phagocytic activities include ginsan (polysaccharide from *ginseng*) and *Astragali* radix extracts. In this review, it was found that virtually all the herbs have stimulatory effects on humoral immunity. Herb constituents such as aloeride (polysaccharide from *Aloe vera*), angelan (polysaccharide from *Angelica gigas*), glucopyranosyl-containing polysaccharides (from *Ganoderma lucidum*), ginsenosides (from *Ginseng*) and gingerols (from *Zingiber officinale*) induce the activity of IL-6, a potent B cell stimulant. As many specific immune responses require T helper cells, it is not unexpected to find many of these constituents being able to stimulate cell-mediated immunity as well.

The extracts were also shown to selectively stimulate cytokine release, confirming that specific herbs are used to combat bacteria-, fungi- and viral-induced infections. In most cases, polysaccharides, proteoglycans, and flavonoids play a major role in preventing or controlling infectious microbes. Some of these constituents directly disable the pathogens, by disengaging their virulence factors, as well as inhibiting their growth rates. Furocoumarins from *Angelica* species present a hazard to microbes, possibly by inducing genotoxic effects. It has been hypothesized that panaxagin from *ginseng* works as a fungal ribosome inactivating protein, thus exerting its antifungal effects. Some ginsenosides may be able to interact with phospholipid derivatives on the cell membrane of microbes, causing toxicity by destabilising cell structure. This effect may also prevent microbial attachment to host cells. Such actions can limit the damage done to the host by pathogenic bacteria. Thus, in addition to enhancing the immune response, the antimicrobial effects of these herbs are beneficial to the host.

MULTI-INGREDIENT HERBAL MIXTURES

In many cases, the effects of a single herb result from the overall activities of its constituents. For example, as shown in the case of *A. membranaceus*, the general increase in B cells is due to the balanced effects between the decreases in IL-6 and IFN-γ. Various factors influence the concentration of bioactive compounds in the herb. The proportion of constituents may vary in different parts of the plant and the

age of the plant; the timing of its harvest and soil conditions can all affect the efficacy of the constituents. For example, where ginseng is concerned, it is generally believed that the older the root, the greater the concentration of ginsenosides and the more potent its activity. The relative levels of each cytokine may finally be affected by the presence of other herbs as well.

Although there have been relatively few reports validating the benefits of herb combinations, research into this area is increasing. Investigation into the effects of herbal formulas would prove to be rather useful since multi-ingredient prescriptions are a norm in Chinese medicine. The need to test the effects of prescribed formulas is prudent as the activity of a particular herb in a mixture might differ from its activity as a single entity. For example, the effects of four herbs (*Scutellaria barbata, Oldenlandia diffusa, Ligustrum lucidum and Astragalus membranaceus*) on aflatoxin-B mediated hepatotoxicity were examined. At the lowest dose tested (1.5mg/plate), *S. barbata* and *O. diffusa* exhibited moderate levels of protection. However, when used in combination at the same dose, the hepatoprotective effects were potentiated to optimal levels. The study also showed that *L. lucidum* was more effective when used singly than when used in combination with *A. membranaceus* [76]. "Xiao Chai Hu Tang" (XCHT) is a 7-herb mixture that includes *Scutellaria baicalensis*, *Panax ginseng* and *Zingiber officinale*. Experiments have shown that XCHT is able to act as a free-radical scavenging herbal product and is especially useful in preventing CCl₄-induced liver damage. Its anti-oxidant activity is largely attributed to a major constituent in the mixture, *bupleurum* [77], from *S. baicalensis*. However, reports have shown that *S. baicalensis* is also able to exert anti-oxidant activities primarily by increasing production of H_2O_2 [78]. In another example, the 10-ingredient mixture "Bu Zhong Yi Qi Tang" (which include *Astragalus membranaceus, Panax ginseng, Angelica sinensis* and *Zingiber officinale*) inhibited proliferation of hepatoma cells more significantly, compared to the inhibitory effects of individual herbs [79]. Hence the different mechanisms of action of the herbs in the combination appear to synergise to achieve optimal effects.

HERBAL TOXICOLOGY

In contrast to chemically synthesized medicinal drugs, herbs are generally thought to be harmless. However, herbal poisoning is not as uncommon as previously thought because of numerous herb-drug interactions, and high concentrations of metabolites that are toxic, when taken improperly. For example, selenium (Se) toxicity associated with high concentrations of Se is believed to be due to the continuous generation of free radicals, such as superoxide and their derivatives. *Astragalus* is an example of a plant that is able to grow in selenium rich areas, and is able to store large amounts of Se. At extremely high concentration, its metabolite, hydrogen selenide, is formed, which appears to be a major factor causing hepatotoxicity. Animal toxicity studies determined that while L-seleno derivatives were not toxic to animals at low doses, high concentrations of either isomer were noxious [80].

Pyrrolizidine alkaloids (PA) are highly toxic compounds found mostly in the leaves of plants such as *Heliotropium*

spp. and *Crotolaria spp.*. Roder [81] provides explicit information on the presence of these alkaloids in Chinese medicinal plants, while Denham [82] evaluated their hepatotoxicity. More recently, Deng [83] has highlighted the hepatotoxicity of other herbs like Jin Bu Huan (*Lycopocium serratum)*. The herbs reviewed here, however, have not been shown to be hepatotoxic when taken according to prescription. They are also not known to contain significant amounts of PAs.

Adriane [84] and Ikegami [85] have written two informative reviews on the effects of herb-drug interactions. It should be noted that these interactions do occur, and are a major cause of health complications in herb consumers. In this regard, pharmaco-vigilance is of critical importance to minimise or avoid the occurrence of such deleterious effects. As a safeguard, it is also crucial that the consumer, especially if he is taking Western drugs, have access to information on potential herb-drug interactions from reliable sources before starting on herbs.

In summary, a review of the existing literature has illuminated the existence of specific chemical entities in seven TCM herbs with immunomodulating and antimicrobial activities. It is, however, not possible to conclude with any great certainty that these compounds are solely responsible for a particular herb's effects, as several of these herbs are given in combination in TCM formulae. It appears that they act in concert to produce the desired outcome. It also seems possible that as yet undiscovered compounds from such herbs may exert differing and possibly synergistic effects on host immunity as well as against microbes. The identification and evaluation of new bioactive compounds from herbs can help in the development of novel drugs, leading the way to discovering interesting, possibly less harmful, and also clinically useful combinations, to support the immune system as well as inhibit or kill microbes.

ABBREVIATIONS

APC = Antigen presenting cell

DNA = Deoxyribonucleic acid

IFN = Interferon (chemokine)

IL = Interleukin (chemokine)

iNOS = Inducible nitric oxide synthase

MIC = Minimum inhibitory concentration

MRSA = Methicillin-resistant *Staphylococcus aureus*

NK = Natural killer cell

NMR = Nuclear magnetic resonance

TCM = Traditional Chinese medicine

TNF = Tumour necrosis factor (chemokine)

XCHT = Xiao Chai Hu Tang

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