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Special Issue: Research on Rare and Precious Medicinal Herbs
Guest Editors: Prof. Hong-Xi Xu Prof. Zhi-Xiu Lin

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Special Issue: Research on Rare and Precious Medicinal Herbs

Guest Editors-in-Chief

Hong-Xi Xu

Prof. Hong-Xi Xu has been actively involved in natural products research in various institutions in China, Japan, Singapore, and Canada. He has been appointed as a distinguished professor of Shanghai University of Traditional Chinese Medicine since 2011 and is currently the Honorable Dean of the School of Pharmacy. In recognition of his achievements, Prof. Xu was selected as a "Specially-Appointed Professor of Shanghai". He has published more than 350 SCI papers. His H index is 68 and i10-index is 257. His current research interests are focused on new drug discovery from natural resources, as well as the development of botanical dietary supplements from herbal medicines. Specifically, he is interested in finding natural lead compounds from medicinal plants and in developing new drug based on Chinese medicines against different disease targets such as cancer, HSV, and metabolic diseases. He applies multiple assay models targeting cell death (e.g. apoptosis, autophagy), metastasis, and quiescent cell recurrence to screen for anti-cancer natural compounds. With state-of-the-art technologies, he investigates the in vitro and in vivo mechanisms of action of the novel compounds from medicinal plants.

Zhi-Xiu Lin

Prof. Zhi-Xiu Lin graduated from Guangzhou University of Chinese Medicine in 1987 with a BSc in Chinese Medicine. After graduation, he worked as a Chinese medicine doctor at the Affiliated Hospital of Guangdong Provincial Research Institute of Chinese Medicine. In 1991 he moved to England to study English language, followed by a PhD degree study at the Department of Pharmacy, King College, University of London. He obtained his PhD degree in Pharmacognosy in 1999. From 1998 to 2002, he was employed as a Senior Lecturer and Programme Leader on the Chinese Medicine Programme, Middlesex University in London where he was involved with basic and clinical teachings of Chinese medicine. Prof. Lin joined Chinese University of Hong Kong in 2003, and is now Professor and Associate Director of the School of Chinese Medicine. He has published more than 200 SCI papers. His H index is 42 and i10-index is 113. His main research interests include: (1) Pharmacological studies on Chinese medicines for psoriasis, eczema, pancreatic cancer and Neuroprotection; (2) Clinical trials on effectiveness and safety of Chinese.

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Overview of Research Trends in Precious Chinese Medicines

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Introduction

Traditional Chinese medicine (TCM) has continued through centuries to this day since the ancient times and has contributed greatly to the well-being maintenance and disease treatment in China.[1-3] Before the ushering in of Western medicine into China in the 19th century, TCM had been the major healthcare and medical modality in Chinese communities.[4] This ancient art of healing has not diminished over time; conversely, it is now widely accepted by patients both in China and around the world owing to the shift of the disease spectrum and the rise of the "return to nature" paradigm. Moreover, TCM is attracting increasing attention from research circle worldwide.[5] At present, TCM is used in approximately 45% of the world's countries, and a large amount of Chinese medicine raw materials is exported from China, and some of these Chinese medicines are collected directly from the wild.[6,7] Owing to the popularity of Chinese medicine around the world, the natural resources required for Chinese medicine production can hardly meet the rapidly increasing demand. Wild herbal resources are decreasing by approximately 30% every year.[5,8,9]

For some "precious Chinese medicines," such as Ren Shen (人参 *Ginseng Radix et Rhizoma*), Ling Zhi (灵芝 *Ganoderma*), Xue Lian (雪莲 *Saussureae Involucratae Herba)*, Fan Hong Hua (番红花 *Stigma Croci*), Jin Xian Lian (金线莲 *Anoectochilus roxburghii*), Xiong Dan Fen (熊胆粉 *Ursi Fellis Pulvis*), Tie Pi Shi Hu (铁皮石斛 *Dendrobium officinale*), and Dong Chong Xia Cao (冬虫夏草 *Cordyceps*), the problem of the scarcity of wild resources is particularly serious, largely because of the destruction of natural habitats and overexploitation by humans. Some of these species are currently listed in the *Convention on International Trade in Endangered Species of Wild Fauna and Flora*.[10,11] These herbs are traded at extremely high market prices because of their scarcity, sometimes merely based on speculation. Although the emergence of cultivation and natural fostering techniques and the application of substitutes for some precious Chinese medicines in recent years have played an important role in resource conservation, the keen demand for precious Chinese medicines continues unabated.[11] Sustainable utilization of precious and rare Chinese medicine resources remains a great challenge.

The special issue published in *Chinese Medicine and Culture* provides a valuable summary on several precious Chinese medicines that have high market value and possess good clinical usage. The precious Chinese medicines discussed in this special issue include Zang Hong Hua (藏红花 *Stigma Croci*), Huang Jing (黄精 *Polygonati Rhizoma*), Huang Qi (黄芪 *Astragalus membranaceus*), Dong Chong Xia Cao (冬虫夏草 *Cordyceps*), Shan Ci Gu (山慈菇 *Pseudobulbus Cremastrae seu Pleiones*), Rou Gui (肉桂 *Cinnamomi Cortex*), Chen Xiang (沉香 *Aquilariae Lignum Resinatum*), and Jin Chai Shi Hu (金钗石斛 *Dendrobium nobile Lindl*). In this highlight, we provide brief overviews of their history, current usage, therapeutic efficacy, and pharmacological actions.

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Summary of the Precious Chinese Medicines Included in this Special Issue

Stigma Croci is an autumn-flowering perennial plant. The earliest use of *Stigma Croci* in TCM was recorded in the *Gang Mu Shi Yi* (《纲目拾遗》 *Supplement to the Compendium of Materia Medica*) during the Tang dynasty (741 A.D.). It has been globally recognized as one of the most expensive medicinal plants and the best dye substance since the ancient time. *Stigma Croci* was initially used as a precious spice for the royal family and noble officials and was later used as a valuable gynecological medicine in TCM.[12] Historically, China relied on imported *Stigma Croci* till the 20th century. In 1979, a Shanghai medicinal materials company imported 0.5 tons of *Stigma Croci* from Japan and successfully cultivated it in Ma Qiao town of Shanghai by combining the technology of cultivating corms in open fields but producing flowers indoors. Starting in 1987, China began to export *Stigma Croci*. After the 1990s, China made great progress in *Stigma Croci* cultivation and research, which resulted in expanding of the planting area. *Stigma Croci* is currently being cultivated in more than 20 provinces and cities in China, mainly in Tibet, Shanghai, and Zhejiang. Over 90% of China's total yield of *Stigma Croci* is harvested from Chongming District of Shanghai. Recent studies have identified the major bioactive compounds in *Stigma Croci* and various biological properties, including anti-cancer, anti-bacterial, anti-inflammatory, anti-nociceptive, hypnotic, anxiolytic, anesthetic, anti-depressant, and bronchodilatory effects.[13-16] In addition to serving as a Chinese medicinal herb, *Stigma Croci* is currently used in the industries of food, dye, and perfume.

Polygonati Rhizoma is one of the most popular Chinese medicines used in a dried form. It was first documented in the *Ming Yi Bie Lu* (《名医别录》*Miscellaneous Records of Famous Physicians*) in China.[17] In the *Zhong Guo Yao Dian* (《中国药典》*Chinese Pharmacopeia*) version 2020, *Polygonati Rhizoma* is stated to have the effects of replenishing qi, nourishing yin, invigorating the spleen, moistening the lungs, and strengthening the kidneys. *Polygonati Rhizoma* was originally used as a food for Taoist fasting (Bigu), which is regarded as a good strategy for resisting various chronic diseases such as diabetes and hyperlipidemia.[18] In Taoist medicine, *Polygonati Rhizoma* is regarded as an edible herb with little toxicity and few side effects. It is believed to improve one's appearance and prolong life. Pharmacologically, *Polygonati Rhizoma* has diverse therapeutic actions, including anti-oxidant, anti-diabetic, anti-osteoporosis, anti-cancer, anti-microbial, anti-hyperlipidemic, cardiomyocyte protection, immunomodulatory function, effects against infertility, and enhancement of sleep and memory.[17,19-21]

Astragalus membranaceus (*A. membranaceus*) is a major tonic used in TCM practice. The medicinal use of this herb was first recorded in the *Shen Nong Ben Cao Jing* (《神农本草经》*Shennong's Classic of Materia Medica*), which was written approximately 2000 years ago.[22] *A. membranaceus* is commonly used in TCM to treat anemia, wounds, fever, allergy, fatigue, loss of appetite, and abnormal menstrual bleeding.[23] At present, *A. membranaceus* is often prescribed with other herbs to treat a broad spectrum of diseases, such as diabetes, cirrhosis, leukemia, nephritis, viral infections, and cancer, without any side-effect trace of toxicity. In addition, *A. membranaceus* can enhance immunity, protect the liver, and possesses anti-aging, anti-stress, and anti-bacterial properties. More than 200 compounds have been identified and isolated from *A. membranaceus*. These compounds, which can be grouped into three main chemical classes, namely flavonoids, polysaccharides, and saponins,[24] are the major driving forces behind the diverse biological and pharmacological activities of *Astragalus* species.[25]

Most notably, *Cordyceps* refers to *Ophiocordyceps Sinensis* (*O. sinensis*). It a naturally occurring fungus-caterpillar complex that has been used in TCM and traditional Tibetan medicine since the 15th century. It is also known as "Dong Chong Xia Cao" (冬虫夏草) in Chinese, which means the mixture of "worm in the winter, herb in the summer."[26] It is used to strengthen the immune system, assist the lung yin, stop bleeding, transform phlegm, and cure low libido and impotence by correcting yang deficiency in TCM. *O. sinensis* is highly regarded as a precious Chinese medicinal tonic because it can only be harvested from remote locations, usually at altitudes exceeding 3800 m in Tibet, Qinghai, Yunnan, Sichuan, and Gansu provinces in China.[27] Owing to its limited quantity and high demand, *O. sinensis* is commercially cultivated for a short growing cycle and low cost. Both natural and cultivated *O. sinensis* contain bioactive compounds that are believed to confer therapeutic effects. Cultivated *O. sinensis* can be a good substitute for naturally occurring *Ophiocordyceps* because it contains higher amounts of cordycepin and a lower risk of contamination than its natural counterpart. However, the active compounds in cultivated *O. sinensis* responsible for its therapeutic effects require rigorous testing through randomized placebo-controlled trials to clarify their clinical efficacy.

Pseudobulbus Cremastrae seu Pleiones was first listed as a precious Chinese medicine in the *Ben Cao Shi Yi* (《本草拾遗》*Supplement to Materia Medica*) during the Tang dynasty.[28] According to the *Chinese Pharmacopoeia,* it is derived from the pseudobulbs of three orchidaceous plants, namely *Cremastra appendiculata* (D. Don) Makino, *Pleione bulbocodioides* (Franch.) Rolfe, and *P. yunnanensis* Rolfe. It has attracted the attention of researchers because it is commonly prescribed in TCM for the treatment of various

cancers, bacterial infections, burns, and frostbite.[29] However, most studies on the biological activities of *Pseudobulbus Cremastrae seu Pleiones* were based on *in vitro* bioassays. *In vivo* studies should be conducted in the future to discern the bioactivities and associated molecular mechanisms of this herb.

Cinnamomi Cortex is a famous Chinese herb, and the source of this herb is the bark. Its medicinal use has long been recorded in the *Chinese Pharmacopoeia*. The genus *Cinnamomum* of the family Lauraceae contains more than 250 species,[30] and each of them has specific morphological characteristics and a unique phytochemical composition.[31,32] Clinical studies that used *Cinnamomi Cortex* as an intervention revealed that supplementation at a dose as low as 1g/day could improve hemoglobin A1c levels in patients with poorly controlled diabetes. There is a dose-response relationship concerning postprandial blood glucose modulation, in which at least 5g of crude cinnamon appeared necessary for blood glucose control in healthy or prediabetic people; however, this dose-response relationship was not observed in the type 2 diabetes group. Prolonged supplementation with cinnamon might improve insulin sensitivity. Future studies on cinnamon should pay attention to such matters as the species and form (e.g., powdered, extract) of cinnamon.

Aquilariae Lignum Resinatum is a dark resinous substance found in the trunk of wounded *Aquilaria* plants. Known as "Chen Xiang" (沉香) in Chinese, it was first described in the *Miscellaneous Records of Famous Physicians* written in the Jin dynasty under different names such as Xun Lu Xiang (薰陆香) and Feng Xiang (枫香).[33] The character "Chen" means sink, whereas "Xiang" means fragrance or incense. The trunk has a strong fragrance, and it can be burnt as incense. *Aquilariae Lignum Resinatum* has many therapeutic functions, such as moving qi and relieving pain, directing the rebellious qi downward and stopping vomiting, and warming the kidneys to aid in qi absorption.[34] *Aquilariae Lignum Resinatum* is traditionally used to treat many ill conditions such as gastrointestinal disorders, asthma, and pain. A recent study on its phytochemistry and pharmacological effects revealed new bioactive compounds in *Aquilariae Lignum Resinatum*, and these compounds have various pharmacological effects such as anti-inflammatory, anti-tumor, anti-bacterial, and anti-fungal effects.[35] Although advances have been made in the discovery of new compounds and novel bioactivities, further studies are needed to evaluate their clinical efficacy.

Dendrobium nobile Lindl (*D. nobile*), a well-known precious plant, is also known as noble *Dendrobium*, as well as Jin Chai Shi Hu (金钗石斛) in Chinese. It is one of the main *Dendrobium* species recorded in the *Chinese Pharmacopoeia*, and it has both ornamental and medicinal value.[36,37] In China, the utilization of *Dendrobium* as a medicine and health food can be traced back to 1500 years ago. *D. nobile* has been used to treat diabetes, chronic atrophic gastritis, neurodegenerative diseases related to aging, and cardiovascular disease.[38,39] Various phytochemical compounds have been identified in *D. nobile*, such as alkaloids, bibenzyl, phenanthrene, phenylpropanoids, and polysaccharides. Pharmacological studies illustrated that these active ingredients of *D. nobile* have multiple health-promoting effects, and many of the pharmacological effects have significant ethno-medicinal values, such as anti-tumor, anti-inflammatory, immunomodulatory, anti-fatigue, anti-aging, and hypoglycemic.[40]

Future Perspectives

With its long history of practical uses, TCM is gaining popularity, and materials have been marketed as dietary supplements or been developed into herbal drugs. Because of increasing demand for medicinal plants, pressure is mounting on existing resources, threatening the depletion of some species in their natural habitats. The situation has spurred researchers to develop innovative methods for the cultivation of herbal plants to meet the market needs and encourage ecological conservation. Therefore, the sustainability of rare and precious Chinese medicine resources and Chinese medicine in general relies on a well-balanced combination of wild collection, cultivation, and natural fostering.

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This article does not contain any studies with human or animal subjects performed by either of the authors.

Author contributions

Hong-Xi Xu and Zhi-Xiu Lin wrote and reviewed the article.

Conflict of interest

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Chemistry Behind the Immunomodulatory Activity of *Astragalus membranaceus*

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Abstract

Huang Qi (黄芪 *Astragalus membranaceus*) is a well-known and widely used herb in traditional Chinese medicine (TCM) tonic preparations. It has been used for many ailments over the last 2000 years. Flavonoids, saponins, and polysaccharides have been shown to be the main compounds responsible for the biological and pharmacological activities, especially the immunomodulatory properties, of such tonic preparations. This review summarizes the published data on *Astragalus* extracts and fractions and the natural compounds responsible for the immunomodulatory activity with special reference to the modulation of nuclear factor-kappa B and related pathways (e.g., Nrf2). In addition, this review highlights the importance of *Astragalus membranaceus* in TCM for treating patients with diseases related to immunocompromised conditions, such as cancer and diabetes.

Keywords: Huang Qi (黄芪 *Astragalus membranaceus*), immunomodulatory, nuclear factor-kappa B, phytochemicals

Introduction to the Traditional Uses of Huang Qi (黄芪 *Astragalus membranaceus*)

Astragalus is one of the largest genera in the family Leguminosae and is widely distributed in temperate and arid regions as annual and perennial shrubs and subshrubs. It is estimated that the genus *Astragalus* contains more than 3000 species.[1-3] In traditional Chinese medicine (TCM), the roots of *Astragalus* are one of the major constituents of many herbal formulations. The *Astragali Radix* or "Huang Qi" is the dried roots of *Astragalus membranaceus* (*A. membranaceus*), which is a major component in tonic formulations used in TCM and is also popular worldwide.[1-4] The medicinal use of *Astragalus* boasts a history of over 2000 years and was first recorded in the *Shen Nong Ben Cao Jing* (《神农本草经》 *Shennong's Classic of Materia Medica*) in 200 A.D. This book was the first recorded document concerning herbal medicine in TCM.[5] In TCM, Huang Qi is commonly used to treat anemia, wounds, fever, allergies, fatigue, loss of appetite, and abnormal menstrual bleeding.[6-8] Nowadays, *Astragalus* is used to treat a broad spectrum of diseases, such as diabetes, hypertension, cirrhosis, leukemia, nephritis, viral infections, and cancer. Most importantly, it does not show any toxicity.[1-5] In addition, *Astragalus* enhances immunity, protects the liver, and possesses anti-aging, anti-stress, diuretic, anti-hypertensive, and antibacterial activity.[1-6] Owing to its medicinal importance and applications, *Astragalus* is included in the Grade-III National Protected Plant List in China.[7]

There have been many review articles published over the past few years and they highlight the importance of *Astragalus*

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species.[3,5,6] The current review focuses on two major *Astragalus* species, namely *A. membranaceus* (Fisch.) Bge. and *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao. which are categorized under the same species.[6]

Major Classes of Compounds Found in *Astragalus membranaceus*

More than 200 compounds have been isolated and identified from *Astragalus* species.[3] These compounds can be mainly grouped into three major classes of compounds, namely flavonoids, polysaccharides, and saponins [Figure 1]. These major compounds are responsible for the diverse pharmacological activities of *Astragalus* species.[3,5,6]

Astragalus polysaccharides are the most abundant substances[9] and they consist of dextran and heteropolysaccharides, *i.e.,* polysaccharides with multiple monosaccharide units such as glucose, rhamnose, arabinose, galactose, mannose, fructose, fucose, ribose, xylose, glucuronic acid, and galacturonic acid.[6,7,10] *Astragalus* polysaccharides are complicated macromolecules with molecular weights ranging from 8.7 to 4800 kDa and up to nine different monosaccharide units with different ratios.[10] Due to their complexity, to date, there have only been 24 polysaccharides isolated and identified from *A. membranaceus*.[10] Cycloartane- and oleanane-type saponins are important pharmacologically active substances in *Astragalus* species.[3,10] Astragalosides (AS) I (**1**), II (**2**), and IV (**3**) and isoastragalosides I (**4**) and II (**5**) [Figure 2] are the major saponin constituents (more than 80%) found in *A. membranaceus*.[11] Similar to other plants, *Astragalus* species are also rich in flavonoids, including abundant isoflavones and other flavanols, flavones, flavonones, isoflavans and pterocarpans.[12] More than 60 flavonoids have been identified from *A. membranaceus*.[3,11] Among them, calycosin-7-*O*-β-D-glycoside (**6**) [Figure 2] is a biomarker compound in the flavonoid fraction of *A. membranaceus*.[11]

Immunomodulatory Activity of *Astragalus membranaceus*

A. membranaceus has been extensively used in herbal formulations, especially in tonics, in order to strengthen the immune system. *A. membranaceus* is used for adults with weak immune system, for the patients suffering from chronic diseases (e.g., diabetes and cancer) with low-grade inflammation, and for those people under physiological stress.[13] Herbal formulations of *A. membranaceus* have been shown to regulate immunity by (i) exerting an effect on organs (e. g., thymus and spleen), lymphatic tissues, bursa of Fabricius (birds), and dendritic cells in bone marrow;[14,15] (ii)

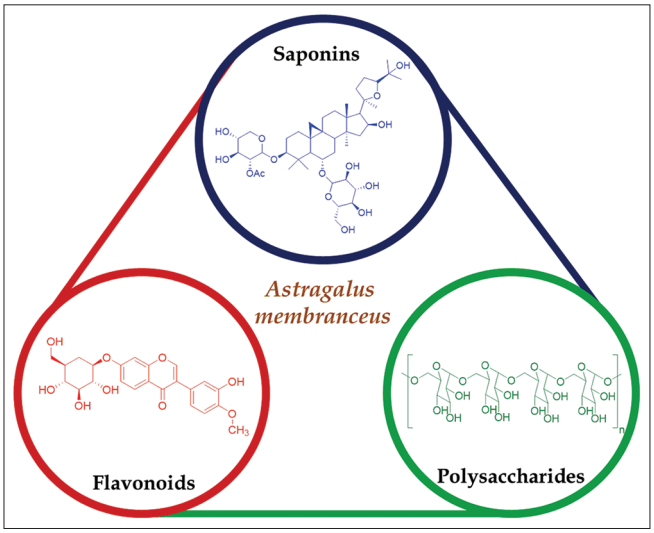

Figure 1 The three major classes of compounds present in *Astragalus membranaceus*

increasing the development of primary stem cells in lymph nodes (B and T lymphocytes) and regulating natural killer cells and macrophages;[16] (iii) influencing immunomodulatory compounds, which protect cells from increased levels of cytokines during an inflammatory response; (iv) affecting the secretion of immunoglobulins; and (v) influencing the immune signal transduction in immune signaling pathways [Figure 3].[17] To regulate immune signaling transduction, there is the nuclear factor-kappa B (NF-κB) transcription factors, which play an important role in the activation of the immune system are modulated.[18,19] NF- κB is a family of transcription factors that regulates gene expression as a result of immune and inflammatory responses in the body. This review will focus on the immunomodulatory activity of *A. membranaceus* with special reference to NF-κB and the chemical constituents and natural products responsible for the activity.

Innate or natural immunity and acquired or adaptive immunity are the two major immune responses in biological systems. Innate immunity provides the first line of immunity and is developed when an organism is born. When immunity is low, innate immunity is not always sufficient to protect the organism. In this situation, adaptive immunity plays a major role in protecting the organisms.[20] Cells in the adaptive immunity are capable of destroying foreign pathogens and dead cells, producing antigens, acting as messengers between the innate and adaptive immune systems, controlling and limiting the spread of microbial infections, damaging the cell walls of microorganisms, promoting immune activation, and controlling overactivation.[21-23] Macrophages, natural killer cells, dendritic cells, and erythrocytes are part of the innate

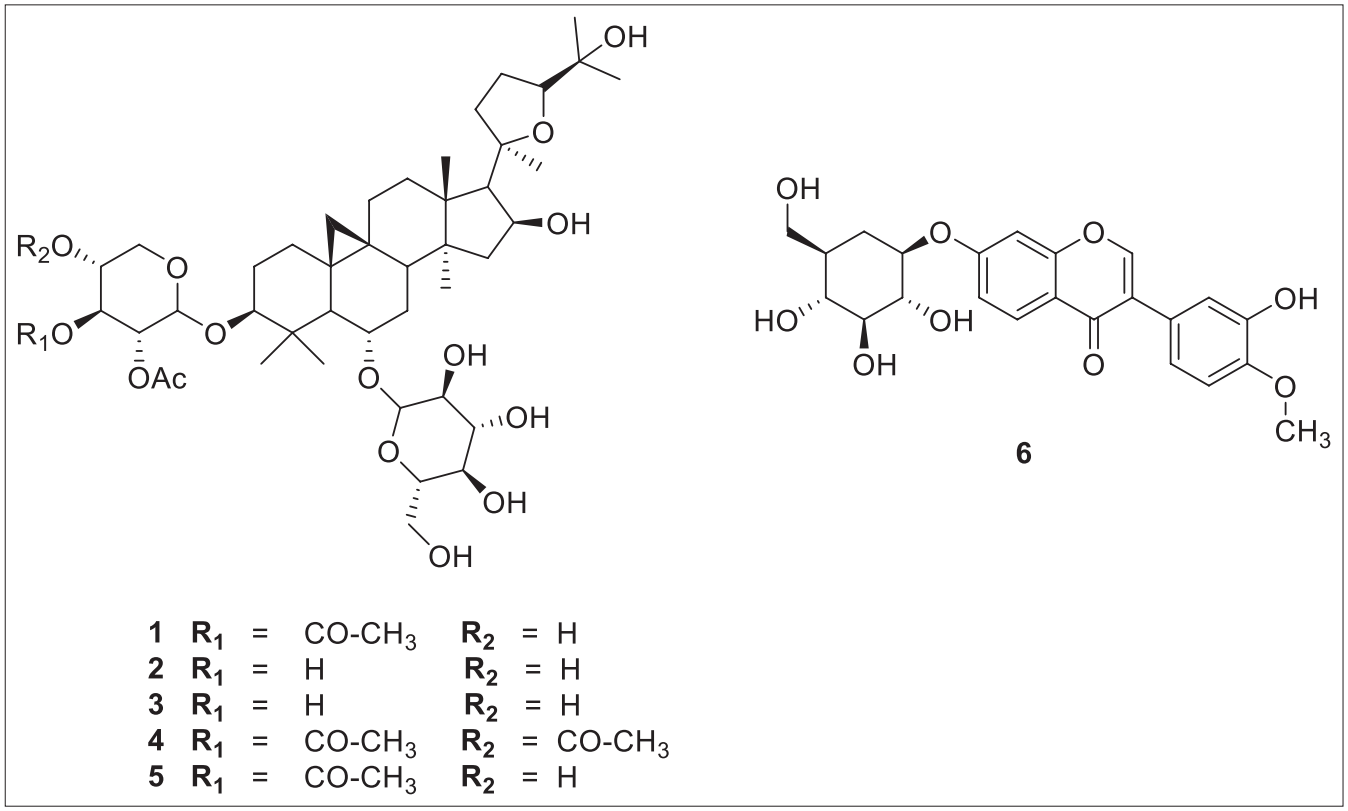

Figure 2 Biomarker compounds in *Astragalus membranaceus*

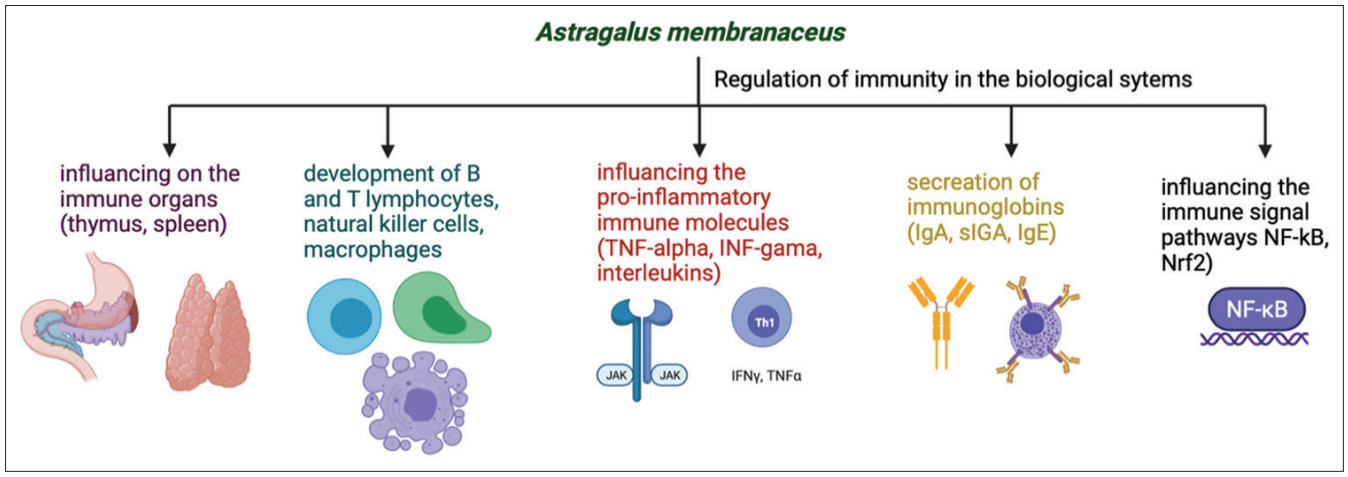

Figure 3 Immune enhancing effects of *Astragalus membranaceus* in biological systems

immune response, and T and B lymphocytes are part of the adaptive immune response. *A. membranaceus* extracts and fractions have been shown to have an ability to modulate the immune systems of biological organisms.[5,16]

Astragalus total extract

Administration of different parts (e.g., aerial parts and roots) of *A. membranaceus* in different forms (e.g., fine powder, decoction, or polysaccharide, saponin, or flavonoid fractions) in animal models has shown a significant development of immune system, especially in poultry.[24] Several studies have reported that administration of the total extract of *A. membranaceus* to 7-d-old chickens, in addition to a basic diet and water, significantly increased the development of immune organs before the chickens were 35 days old.[24,25]

Long-term administration of an *A. membranaceus* total extract has been shown to strengthen the immune organs of mice.[14,26] Several studies have concluded that the immunoregulation

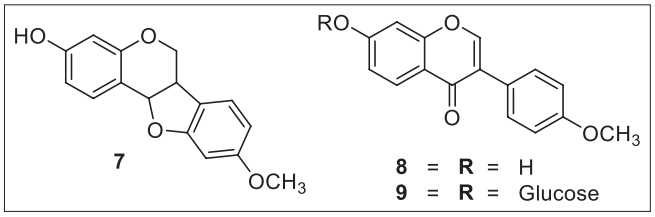

Figure 4 Bioactive flavonoids in *Astragalus membranaceus*

of *A. membranaceus* extracts or fractions was entirely dose dependent, and 300 mg/kg in mice was the optimum dose to enhance the immune function by improving cell proliferation in immune organs and balancing cytokine levels.[12,18,27] Demethylhomopterocarpin (**7**), formononetin (**8**), and formononetin-7-*O*-β-D-glucopyranoside (**9**) [Figure 4] in *A. membranaceus* extracts were found to be responsible for this immunoregulatory activity.[28] The ability of the total extract of *A. membranaceus* to cause neuroregeneration has been studied using a mouse model. The mice were dosed with 1.5 or 3.0 g/kg of extract daily for 4 weeks. The results showed that the neuronal function was significantly improved in the high-dose extract group. The levels of fibroblast growth factor, nerve growth factor, interleukin-1 (IL-1), and interferon (IFN) were decreased in the high-dose group but were increased in the low-dose group. Therefore, a low dose of the total extract of *A. membranaceus* could be helpful for the regeneration of nerves, especially in the case of nerve injuries.[28] Similarly, the total extract was administered to mice infected with chronic bronchitis. During the dosing, the inflammation in pulmonary tissues and the bronchus of infected mice was significantly reduced, and the proliferation of lymphocytes in the alveolar macrophages was increased.[29] These results indicated that an *A. membranaceus* crude extract could improve the immune system in the treatment of chronic bronchitis. During an infection, the migration of macrophages is an innate immune response. Heparanase (HPA) is a key regulator of migration and immune response mediators in macrophages. Qin *et al*. have shown that the total extract of *A. membranaceus* increased HPA activity, cell migration, and mRNA gene expression through the secretion of interleukin-1β (IL-1β and tumor necrosis factor [TNF])- α in macrophages. Therefore, the *A. membranaceus* crude extract can activate an immune response through HPA cell migration.[30] The effect of the total extract on the viability and apoptosis of different carcinoma cell lines has been studied. In one study, the human nasopharyngeal carcinoma CNE2 cell line was treated with the total extract of *A. membranaceus*. The results showed that the total extract was effective in inducing apoptosis in cancer cells. After treatment with the total extract, the apoptosis-related protein BCL-2 became underexpressed and caspase-3 and-8 and BAX proteins were overexpressed. The percentage of T-lymphocytes was also increased. This study suggested that *Astragalus* could alleviate the immunological effects of cancer.[31] The water and whole ingredient extracts of *A. membranaceus* have been investigated through the application different extraction methods. The *in vivo* immunomodulatory effect of the extracts was studied in cyclophosphamide-immunosuppressed mice. The results showed that polysaccharides, flavonoids, and saponins were abundant in the water extract. Oral administration of the water extract for 18 days significantly increased the immune responses in the tested mice, including effects on body weight, peripheral white blood cells, thymus and spleen indexes, splenocyte proliferation, natural killer cells activity, splenic lymphocytes, and serum levels of Immunoglobulin G and M (IgG and IgM).[4] The total extract of *Astragalus* was administrated both orally and intracolonically to Dawley rats with 2,4,6-trinitrobenzene sulfonic acid TNBS-induced colitis. In both cases, the extract protected the rats from the induced colitis. The extract decreased the colonic lesions and damage scores and ameliorated colonic myeloperoxidase activity. Moreover, oral administration of the extract was able to reduce the overexpression of *TNF-a*, *IL-1b*, and *IL-10* genes. Therefore, this study showed the applicability of *Astragalus* as a food supplement for immunocompromised patients.[32]

Astragalus polysaccharide fraction

Many research results have indicated that the polysaccharide fraction of *A. membranaceus* contains major bioactive constituents that are responsible for the diverse biological and pharmacological activities of *A. membranaceus*, including immunomodulatory, anticancer, antitumor, anti-inflammatory, neuroprotective, and antidiabetes effects.[10] Administration of a polysaccharide-rich fraction of *A. membranaceus* to ducklings infected with Muscovy duck reovirus (MDRV) showed thickened intestinal wall, inhibited reduction in lymphocytes and goblet cells because of the infection, and increased the levels of secretory immunoglobulin A (sIgA), cytokines (IL)-4, IL-6, and IL-15, and TNF-α[33] MDRV infections are a major problem among ducklings and are an economic trouble for the poultry industry.[33] Polysaccharide fractions have been administrated to mice inoculated with hepatitis B virus (HBV) to investigate the effect of immunomodulation on mice. Administration of the polysaccharide fraction increased the levels of HBV antibodies and T cells, which induced the production of IL-2 and IL-4, enhanced the activity of cytotoxic lymphocytes, and stimulated dendritic cells as an immune response.[34] Similarly, the polysaccharide fraction has been administered to chickens inoculated with avian infectious bronchitis virus (IBV) at

different doses over different time frames post inoculation. IBV-specific antibodies, lymphocyte proliferation, and expression of IL-1 β, IL-2, IL-8, TNF-α, and mRNA were found to be increased in the polysaccharide-administrated group.[35] Gao *et al*. have studied the immunological characteristics of *Astragalus* polysaccharides in the trinitrobenzene sulfonic acid (TNBS)-induced inflammatory bowel disease (colitis) in the mouse model. The rats were treated with the polysaccharide fraction at a dose of 0.5 g/kg daily for 14 days. Both macroscopic lesions and histological colonic damage induced by TNBS were found to be reduced, and the expression of T-beta and GATA-3 binding protein was enhanced. In addition, T helper cell-1 (Th-1)- and T helper cell-2 (Th-2)-specific transcription factors were overexpressed. These observations suggested that the polysaccharide fraction of *A. membranaceus* has therapeutic potential in colitis.[36] Li *et al*. have investigated the immunotherapeutic activity of *Astragalus* in diabetes using a mouse model. Diabetic mice treated with an *Astragalus* polysaccharide fraction showed decreased level of blood glucose, increased level of serum insulin, increased β-cell mass, and decreased level of apoptotic β-cells in the pancreas. The following were also observed:[37] downregulation of Th-1- and Th-2-specific transcription factors, a decrease in Th-1/Th-2 cytokine ratio, and upregulation of peroxisome proliferator activated receptor-γ gene expression in the spleen as immunomodulatory actions against diabetes (by increasing the insulin sensitivity).

Astragalus flavonoid fraction

Immunomodulatory actions associated with flavonoid fraction of *Astragalus* have been investigated using *in vivo* mice model and *in vitro* cell models. Histopathological studies on mice treated with the *Astragalus* flavonoid fraction showed that there was an increase in the macrophage index and decreased hypersensitivity. This suggests an enhanced nonspecific immunity by increasing the phagocytosis of macrophages, thus initiating immune reaction.[38] In addition, reduced ear and paw edema and vascular permeability as an example of the anti-inflammatory and immunomodulatory actions was observed.[38] In an *in vitro* cell model, the flavonoid fraction of *Astragalus* stimulated the expression of NO, IL-1β, IL-6, and TNF-α in RAW267.4 cells.[38] In a mouse model, the amount of food for the mice was restricted and the mice were forced to swim for 6 weeks to induce fatigue. The flavonoid fraction was given orally at different doses for 6 weeks. Immunohistopathological studies showed that mice administered with the flavonoid fraction showed increased cytokine production (high IL-2 and low IL-4 levels) and a high endurance capacity for swimming. The high capacity of the mice to endure these stressful conditions may be because of the immunomodulatory balance caused by the dominance of Th-1 over Th-2 cells and the secretion of specific cytokines.[27] Furthermore, flavonoid compounds, particularly formononetin (**8**), calycosin (**10**), and the saponin glycosides calycosin-7-*O*-β-D-glucopyranoside (**6**), and formononetin-7-*O*-β-D-glucopyranoside (**9**), which are present in *A. membranaceus* extracts enhanced the upregulation of phagocytic activity in macrophages.[27] Li *et al*. isolated 12 flavonoids from *A. membranaceus*, namely isoliquiritigenin (**11**), liquiritigenin (**12**), calycosin (**10**), calycosin 7-*O*-β-D-glucoside (**6**), formononetin (**8**), formononetin 7-*O*-β-D-glucoside (**9**), daidzein (**13**), daidzein 7-*O*-β-D-glucoside (**14**), methylnissolin (**15**), methylnissolin 3-*O*-β-D-glucoside (**16**), isomucronulatol (**17**), and isomucronulatol 7-*O*-β-D-glucoside (**18**) [Figure 5] and they investigated the effect of the compounds on cytokine production in bone marrow-derived dendritic cells. Isoliquiritigenin (**11**) demonstrated significant inhibition of the pro-inflammatory cytokines IL-6 and IL-12, as well as TNF-α.[39]

Astragalus saponin fraction

The mucosal immune system is made up of mucosa-bound lymphoid tissues that are widely distributed in the intestine, respiratory, and genitourinary tracts. This system regulates immunity by producing sIgA proteins and cytokines in the mucosa. Wu *et al*. have investigated the immune response in mucosal immunocompromised mice after administration of *A. membranaceus* saponin fractions for 14 days. IgA was found to be expressed in the intestinal and respiratory mucosal lymphoid tissues.[40] Administration of *A. membranaceus* saponin fractions significantly upregulated NO and TNF-α synthesis in macrophages and increased the phagocytic activity and the capacity in macrophages.[41-43] The antitumorigenic activity of *Astragalus* saponins has been investigated *in vitro* and *in vivo*. The proliferation, progression of cell cycle, and apoptosis of BGC-823 gastric cancer cells were inhibited by the saponin fraction acting as a tumor suppressor. In an *in vivo* study, BCG-823 cell xenografted tumors were induced, and the tumor volume was significantly reduced after injecting saponin fractions.[44] The saponin biomarker compound astragaloside IV (AS-IV) (**3**) has been investigated in human non-small cell lung cancer cell carcinoma (NSCLC). High doses of AS-IV (**3**) inhibited NSCLC cell growth, whereas low doses did not show any toxicity affecting the cell viability. In combination with the chemotherapy drug cisplatin, AS-IV (**3**) increased the chemosensitivity of cisplatin and inhibited the expression of mRNA and B7-H3 genes. The inhibition of the B7-H3 gene has an immunomodulatory action against NSCLC.[45] In addition, topical application of AS-IV (**3**)

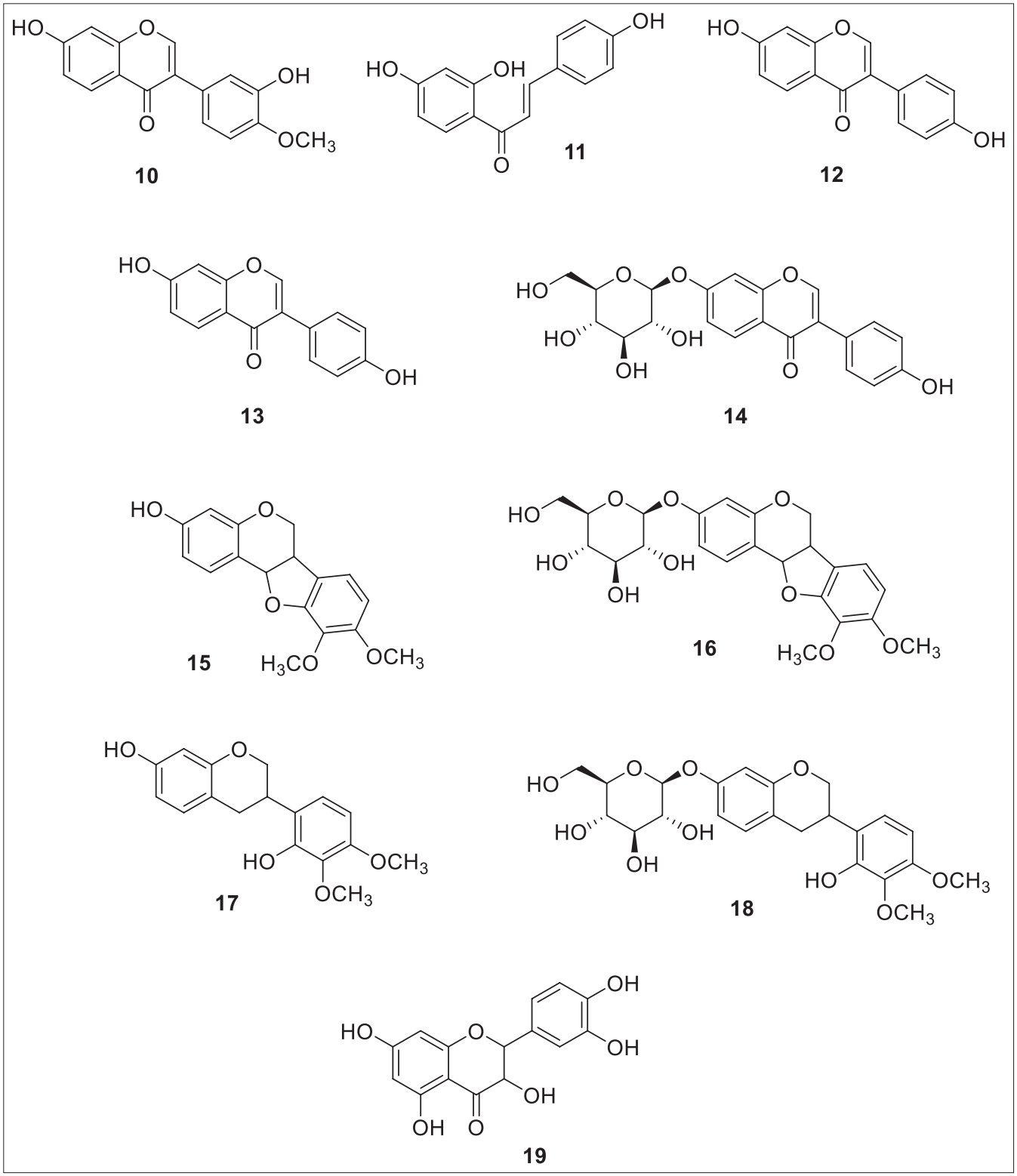

Figure 5 Bioactive flavonoids in *Astragalus membranaceus*

promoted the healing of wounds induced in diabetic mice by promoting re-epithelialization and collagen deposition. The expression of fibronectin and collagen IIIα genes was induced by AS-IV (**3**), which promoted the wound healing. In addition, AS-IV (**3**) promoted the formation of new blood vessels and endothelial cells through the expression of the responsible genes.[46] In addition, Du *et al*. have reported that oral administration of AS-IV (**3**) significantly

reduced eosinophilic airway inflammation induced in mice. AS-IV (**3**) also downregulated the IL-4 and IL-13 levels in the bronchoalveolar lavage fluid and immunoglobulin E (IgE) levels in the serum.[47] *A. membranaceus* extracts and fractions upregulated microphage stimulating and releasing factors and increased the Ca^{2+} ion concentrations in macrophages, and AS-IV (**3**) was determined to be responsible for these biological actions.[5] Astragaloside IV (**3**) is a biomarker in accessing the quality of Astragali Radix and showed widespread pharmacological activities, including anti-inflammatory, anticancer, antidiabetic, cardioprotective, and immunoregulatory effects.[5] A review of the properties of astragaloside IV (**3**) has been done by Zhang *et al*.[5]

Actions of the Nuclear Factor-Kappa B Pathway in Immune Regulation by *Astragalus membranaceus*

When an organism recognizes the presence of foreign pathogens, the organism tends to respond immediately to clear the pathogen. This process starts at the cellular level as a function of the immune system. NF-κB is inactive and available in the cytoplasm under normal circumstances. NF-κB is a family of inducible transcription factors that regulates a large array of genes involved in different processes of the immune and inflammatory responses. When stimulated by cytokines, inactive NF-κB is activated and translocated to the nucleus to induce the expression of the relevant genes.[48,49] The activation of NF-κB in an immune response mainly refers to a combination of two processes. The major pathway is classical (canonical) and is involved in the regulation of the immune response and the expression of cytokines, TNF receptors, and T cell and B cell receptors. The second pathway is alternative (noncanonical) and assists the classical pathway in the regulation of immune functions in the adaptive immune system.[50,51]

Innate immune cells, such as macrophages, dendritic cells, natural killer cells, and neutrophils are found in different tissues at different locations. These cells can be activated in an immune response against infections of microbes and changes in the microenvironment. As part of an immune response, these cells release cytokines.[52] Cytokines can be differentiated into pro-inflammatory cytokines, such as IL-1, IL-6, IL-12, and TNF-α that are necessary for the inflammatory process, T cells (Th-1 and Th-17) that mediate the inflammation, and anti-inflammatory cytokines (IL-10 and IL-13) that are important for the final healing stage.[53] Therefore, NF-κB is a key transcriptor that is important for inducing pro-inflammatory and anti-inflammatory genes to regulate the immune system.

In recent years, many studies have been carried out on *A. membranaceus* to investigate the mechanism of action of

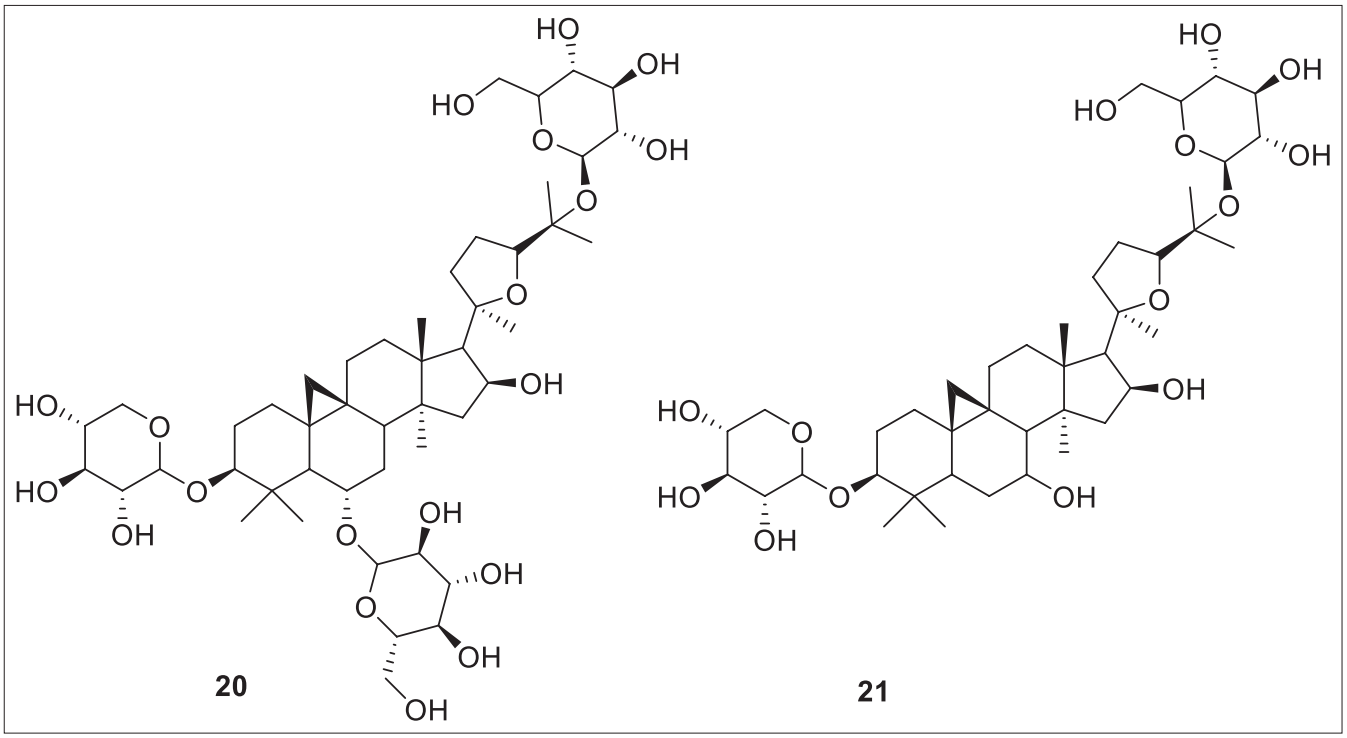

Figure 6 Saponins in *Astragalus membranaceus* responsible for the upregulation of pro-inflammatory cytokines

phytochemicals in *A. membranaceus* to understand how these phytochemicals regulate the immune systems of organisms via modulation of the NF-κB pathway. Qin *et al*. have studied the involvement of the NF-κB pathway in the pro-inflammatory response in Ana-1 macrophages treated with advanced glycation end products (AGE). The total extract of *A. membranaceus* inhibited the AGE-induced inflammatory cytokines, IL-1β and TNF-α, and mRNA expression, and these effects might have been mediated through the NF-κB signaling pathway.[54] Yang *et al*. have proven that *Astragalus* extract inhibited the translocation of NF-κB from cytoplasm to nucleus, abolished the expression of NF-κB in asthmatic mice, and consequently suppressed the pro-allergic cytokines IL-4 and IL-5.[55] Microglial cells treated with an *Astragalus* polysaccharide fraction showed downregulation of nitric oxide (NO), inhaled NO (iNO), and prostaglandin E genes. The downregulation of the pro-inflammatory cytokines IL-1β and TNF-α was observed in lipopolysaccharide-stimulated cells. Thus, *Astragalus* polysaccharides could inhibit the translocation of NF-κB induced by the inflammatory response in microglial cells.[56] Similarly, the *Astragalus* polysaccharide fraction has been shown to downregulate the pro-inflammatory cytokines IL-1 β, IL-6, IL-16, and TNF-α in dextran sulfate sodium-induced colitis mice model. This reduction in the levels of NF-κB and cytokines improved colitis in the *Astragalus*-treated group.[57] Lee and Jeon have demonstrated that *Astragalus* polysaccharides significantly stimulated macrophage expression of the NO synthase (iNOS) gene in mice. In addition, the polysaccharide fraction also induced iNOS and mRNA transcription in RAW 264.7 cells. Further, *in vivo* and *in vitro* investigations have indicated that nuclear translocation, activation of NF-κB, and DNA binding were strongly inhibited by the *Astragalus* polysaccharide fraction.[58]

In RAW264.7 cells, an *Astragalus* polysaccharide-rich fraction mixed with plant flavonoid quercetin (**19**) decreased NO production and iNOS gene expression, although quercetin alone had no effect. The combination of the polysaccharide fraction and quercetin inhibited cytokine production, cellular phosphorylation, and ultimately macrophage activation in the RAW264.7 cells,[59] and the polysaccharide fraction alone upregulated the production of NO, TNF-α, IL-6, and iNOS genes. More importantly, these findings indicated that the immunomodulatory effect of the polysaccharide fraction was mediated via the activation of the NF-κB p65/mitogen-activated protein kinase (MAPK) signaling pathway.[60] In a previous study, a combination of quercetin (**19**) and the polysaccharide fraction showed no effect on the NF-κB pathway to exert immunomodulatory activity.[59] Li *et al*. concluded that quercetin (**19**) might interfere with the immunomodulatory activity of the polysaccharide fraction of *A. membranaceus*.[59]

Several studies have investigated the involvement of active compounds in *Astragalus* extracts in immune-enhancing activity through the NF-κB pathway. The immune response activity of the saponin metabolites AS-VII (**20**) and macrophyllosaponin B (**21**) [Figure 6] has been studied by Nalbantsoy *et al*. in an albino mouse model. Both compounds strongly upregulated the inflammatory cytokines IL-2 and IFN-γ and downregulated Th-2 cytokine production (IL-4). Interestingly, the observed immune response did not affect the NF-κB pathway.[61] In streptozotocin-induced diabetic rats, the NF-κB activity and mRNA protein expression increased in the kidneys. These abnormalities were restored partially when the rats were treated with the saponin biomarker astragaloside IV (AS-IV) (**3**). Administration of AS-IV resulted in decreased levels of TNF-α and cytokine proteins MCP-1 and ICAM-1. AS-IV (**3**) inhibited the activation, translocation, and overexpression of NF-κB in diabetic nephropathy.[62] Thus, *Astragalus* total extracts, fractions, and phytochemicals can activate cell proliferation, increase cytokine production, and stimulate macrophages to express the iNOS gene through inhibiting the translocation and expression of NF-κB genes, thus deactivating the NF-κB pathway.

In a recent study, the active natural compound methylnissolin-3-*O*-β-D-glucopyranoside (**16**) was isolated from Jing liquor of which *A. membranaceus* is a major component. Wu *et al*. have studied the cytoprotective activity of methylnissolin-3-*O*-β-D-glucopyranoside (**16**). Methylnissolin-3-*O*-β-D-glucopyranoside (**16**) activated Nrf2 and antioxidant genes, such as HO-1 and NQO-1.[63,64] Adesso *et al*. have proven that the *Astragalus* polysaccharide fraction reduced the levels of pro-inflammatory cytokines IFN- γ, TNF- α, and iNOS in nontumorigenic intestinal epithelial cells and suppressed inflammation induced by lipopolysaccharides. The polysaccharide fraction induced the production of reactive oxygen species, thus activating Nrf2 genes.[65]

Conclusions and Future Perspectives

A. membranaceus has been used as a herbal medicine for more than 2000 years and has a broad spectrum of pharmacological and biological activities ranging from anti-inflammatory, anticancer, antidiarrhea, antiviral, and neuroprotective functions. In addition, *A. membranaceus* is also a common dietary supplement. There are many reports regarding the mechanism of action of *Astragalus* in

enhancing immunity in biological systems. However, there are few reports that summarize the immunomodulatory activity of *Astragalus* extracts and/or fractions and/or the responsible phytochemicals. This review focuses on the use of *Astragalus* for immune enhancement in diabetes, cancer, infections, colitis, chronic bronchitis, asthma, wound healing, viral infections, neuron regeneration, and fatigue, and summarizes the *in vitro* and *in vivo* investigations of the immunomodulatory activity of the total extract and the polysaccharide, flavonoid, and saponin fractions of *A. membranaceus*. Few studies have investigated the structure–activity relationship of the active compounds in *Astragalus,* which is worthy of further investigation. Supplementation of the daily diet with *Astragalus* may be beneficial for enhancing immunity. *Astragalus* is easily accessible from online shopping sites and is available in such forms as powders, capsules, drinks, injections, and granules.[7]

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Ethical approval

This article does not contain any studies with human or animal subjects performed by either of the authors.

Author contributions

Mallique Qader drafted and corrected the manuscript; Shugeng Cao conceived, guided, and revised this article. All the authors, Mallique Qader, Jian Xu, Yuejun Yang, Xiaohua Wu, Yuancai Liu, and Shugeng Cao, have read and agreed to the published version of the manuscript.

Conflict of interest

None.

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Rare and Precious Chinese Materia Medica: *Pseudobulbus Cremastrae seu Pleiones*

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Abstract

Shan Ci Gu (山慈菇 *Pseudobulbus Cremastrae seu Pleiones*), a rare and precious traditional Chinese medicine, has attracted attention for the treatment of various cancers and bacterial infections. According to the *Pharmacopoeia of the People's Republic of China*, Pseudobulbus Cremastrae seu Pleionesis sourced from the pseudobulbs of three plants in the Orchidaceae family: *Cremastra appendiculata* (D. Don) Makino, *Pleione bulbocodioides* (Franch.) Rolfe, and *Pleione yunnanensis* Rolfe. Extracts from *Pseudobulbus Cremastrae seu Pleiones* are used for the treatment of tumors, burns, and frostbite. The aims of this review are to provide information on the historical and herbological origins of *Pseudobulbus Cremastrae seu Pleiones*, to summarize research conducted on its phytochemical and biological activities over the last twenty years, and to detail planting efforts.

Keywords: Biological activity, *Cremastra appendiculata,* phytochemistry, planting, *Pleione bulbocodioides, Pleione yunnanensis,* Shan Ci Gu, *Pseudobulbus Cremastrae seu Pleiones*

Introduction

Traditional Chinese medicines (TCMs) have been widely used in China for thousands of years. Nowadays, the use of TCMs is rapidly increasing not only in tandem with the economic growth in China but also facilitated by national policies. In the last 10 years, huge growth has been seen in the TCMs market and their pharmaceutical development, including cultivation and processing.[1] Demand for rare TCMs such as *Panax ginseng*, *Ganoderma lucidum*, *Cordyceps sinensis*, *Fritillaria*, and Shan Ci Gu (山慈菇 *Pseudobulbus Cremastrae seu Pleiones*) for use in healthcare has increased. These materials typically command high prices.

Pseudobulbus Cremastrae seu Pleiones is a rare TCM that has attracted attention for the treatment of various cancers and bacterial infections. According to the *Zhong Guo Yao Dian* (《中国药典》*Chinese Pharmacopoeia*), *Pseudobulbus Cremastrae seu Pleiones* is extracted from pseudobulbs of *Cremastra appendiculata* (D. Don) Makino, *Pleione bulbocodioides* (Franch.) Rolfe, and *Pleione yunnanensis* Rolfe in the Orchidaceae family [Figure 1] and is prescribed for the treatment of tumors, burns, and frostbite.[2] In Chinese, *C. appendiculata* (D. Don) Makino is commonly referred to as Mao Ci Gu (Mao means hairy), and *P. bulbocodioides* (Franch.) Rolfe, and *P. yunnanensis* Rolfe are commonly referred to as Bing Qiu Zi (which means ice ball). Dried bulbs of *Tulipa edulis* (Miq.) Baker, which is commonly known in Chinese as Guang Ci Gu (Guang means smooth), are also sold in markets as *Pseudobulbus Cremastrae seu Pleiones* at a cheaper price than the above three species or mixed with authentic *Pseudobulbus Cremastrae seu Pleiones*. This plant is a perennial herb in the genus *Tulipa* and the family Liliaceae.

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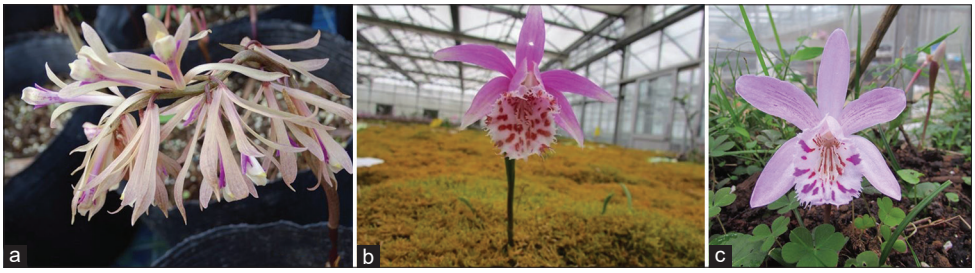

Figure 1 *Cremastra appendiculata* (D. Don) Makino (a) *Pleione bulbocodioides* (Franch.) Rolfe (b) and *P. yunnanensis* Rolfe (c) (by Feng Zhao)

Owing to its high ornamental and medicinal value, several orchid species that can be used to produce medicines, such as Shan Ci Gu (Pseudobulbus Cremastrae seu Pleiones) and Bai Ji (hyacinth orchid, *Bletilla striata* (Thunb.) Reichb. f.), have become rare and endangered. Therefore, artificial cultivation has been investigated actively, and a method for massive propagation from seeds and meristems has been successfully developed and applied to *C. appendiculata*, *P. yunnanensis*, and Bai Ji in Guizhou, China. Among them, the phytochemical and biological activities of *Bletilla striata* harvested from these plantations have been evaluated.[3]

The aims of this review are to provide information on the historical and herbological origins of *Pseudobulbus Cremastrae seu Pleiones*, its source plants, cultivation, phytochemical and biological activities.

History and Herbological Origin of *Pseudobulbus Cremastrae seu Pleiones*

Pseudobulbus Cremastrae seu Pleiones was first listed in the *Ben Cao Shi Yi* (《本草拾遗》 *Supplement to Materia Medica*) which was published in the Tang Dynasty and has been used as an antidote for the treatment of abscess (*i.e.*, carbuncles and furuncles), scrofulosis, snake bites, and worm bites. However, the origins of *Pseudobulbus Cremastrae seu Pleiones* are confused in the herb market because it is derived from many different plants and their records in ancient and modern literature with the same name. The earliest studies on the origin of Shan Ci Gu were performed in 1980s[4] and involved systematic investigation of the historical and herbological origins of *Pseudobulbus Cremastrae seu Pleiones* in China and Japan. It was concluded that the correct sources of *Pseudobulbus Cremastrae seu Pleiones* were *Cremastra. variabilis* (Bl.) Nakai (*C. appendiculata* (D. Don) Makino), and *P. bulbocodioides* (Franch.) Rolfe.

According to morphological descriptions in the *Zhen Lei Ben Cao* (《证类本草》 *Materia Medica Arranged According to Pattern*) which was published in the Song Dynasty, *Pseudobulbus Cremastrae seu Pleiones* used in the Tang Dynasty was sourced from *C. variabilis* (Bl.) Nakai and *P. bulbocodioides* (Franch.) Rolfe [Figure 2]. From the Song Dynasty to Ming Dynasty, *Pseudobulbus Cremastrae seu Pleiones* was sourced from several other plants in addition to *C. variabilis* (Bl.) Nakai and *P. bulbocodioides* (Franch.) Rolfe. For example, illustrations and/or morphological descriptions recorded in the *Ben Cao Gang Mu* (《本草纲目》 *Compendium of Materia Medica*), *Ben Cao Hui Yan* (《本草汇言》 *Treasury of Words on the Materia Medica*) and *Ben Cao Yuan Shi* (《本草原始》 *Origins of Materia Medica*) of the Ming Dynasty, as well as the *Zhi Wu Ming Shi Tu Kao* (《植物名实图考》*Illustrated Reference of Botanical Nomenclature*) of the Qing Dynasty indicate that *Pseudobulbus Cremastrae seu Pleiones* was sourced from *Lycoris* plants in the Amaryllidaceae family, *Dioscorea* plants in the Dioscoreaceae family, and *Amana edulis* (Miq.) Honda in the Liliaceae family, respectively. In addition, *Lycoris* plants in the Amaryllidaceae family and *Erythronium japonicum* Dence. were used in ancient Japan, which suggests that Japanese herbalists in the Edo period (1603–1867) did not have accurate information and/or knowledge about the origin of *Pseudobulbus Cremastrae seu Pleiones*.

The identification of the correct sources of *Pseudobulbus Cremastrae seu Pleiones* as *C. variabilis* (Bl.) Nakai (*C. appendiculata* (D. Don) Makino), and *P. bulbocodioides* (Franch.) Rolfe is supported by recent studies on botanical descriptions in Chinese ancient literature.[5] It should be noted that *Amana. edulis* (Miq.) Honda, formerly known as *Tulipa edulis* (Miq.) Baker, is a plant in the Liliaceae family. Dried bulbs of this plant are named Guang Ci Gu because the soft hairs on the surface are removed. On the basis of literature investigations, some researchers have argued that *T. edulis* should be listed as an authentic source of *Pseudobulbus Cremastrae seu Pleiones*.[6] Since 1995, *C. appendiculata*, *P. bulbocodioides*, and *P. yunnanensis* have been listed formally in the *Pharmacopoeia of the People's Republic of China* as the authentic sources of *Pseudobulbus Cremastrae seu Pleiones*.

Cremastra appendiculata (D. Don) Makino

Cremastra appendiculata is an orchid species in the genus *Cremastra*. This genus was established by J. Lindley (1833)

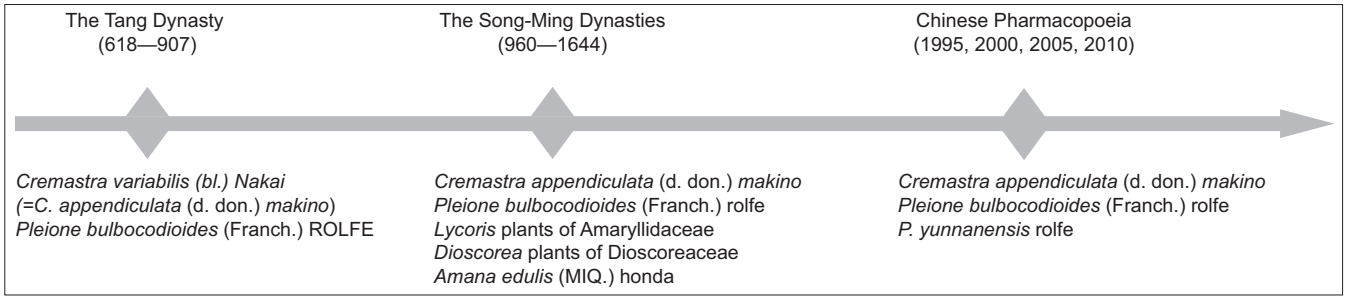

Figure 2 Historical and Herbological Origins of *Pseudobulbus Cremastrae seu Pleiones*

using a plant collected by N. Wallich in Nepal. It was previously described by D. Don (1825) as *Cymbidium appendiculatum*. However, Lindley apparently did not agree with the placement of this orchid in the *Cymbidium* genus and renamed the species *Cremastra wallichiana*. Nomenclature rules at the time were different and allowed for this change. Under current nomenclature rules, the correct name is *C. appendiculata* (D. Don) Makino.[7] The generic name for this species is derived from the Greek words kremannymi (hanging) and astron (star) because of its physical features.

Plant description and distribution

The *Cremastra* genus comprises seven species of plant.[8] Among them, *C. appendiculata* is the most common and widely distributed species. It is found in forests at an altitude of 300–2900 m in most regions south of the Yellow River, including Guizhou, Guangxi, Guangdong, and Yunnan provinces of China, Thailand, Vietnam, and Japan (Hokkaido, Honshu, Shikoku, and Kyushu).[8,9] This plant is a terrestrial herb with tuberous, clustered pseudobulbs, each of which bears a single, large elliptical leaf (of 20–30 leaves by 4–6 cm) that is plicated with three ribs and a long petiole. The floral scape arises from the side of the pseudobulb and carries a dozen floppy, scented, tubular flowers (up to 4 cm long) that look similar to lilies. These flowers do not open widely, and together looking rather like a stand. The flowers are yellow to orange with a white lip, and the lip and petals have bluish-violet spots.[9] Flowering occurs from May to June[5,9] and fruiting from September to December.[5] The common name of the plant in Chinese is Dujuan Lan and in Japanese is Saihai Ran.

This species has relatively wide green leaves that suggest high photosynthetic ability, but is usually found in the understory of humid and highly shaded forests. Therefore, it is unlikely to exert its photosynthetic ability to a sufficient level to support its own growth, which suggests that mycobionts may support the growth of this orchid in its natural habitat. Yagame *et al.*[10] found that saprobic Psathyrellaceae fungi in the Agaricales order induced seed germination of the photosynthetic orchid *C. appendiculata*. This was the first report of Psathyrellaceae fungi as mycobionts associated with photosynthetic orchids, and it suggested that *C. appendiculata* may depend on mycobionts to obtain sufficient nutrients for growth even in the adult stage. This ecological feature could contribute to the survival of this orchid under the highly shaded forest canopy.

Phytochemistry and biological activity

Numerous phytochemical studies have been conducted on *C. appendiculata* in recent decades by two research groups. A total of 108 natural chemical constituents, including 37 new compounds, have been isolated from *C. appendiculata*. Most of these compounds have been identified as phenanthrenes, biphenanthrenes, phenanthrene glucosides, bibenzyls, and terpenoids. Phenanthrenes are a relatively uncommon class of polycyclic aromatic metabolites that are thought to form by oxidative coupling of the aromatic rings of stilbene precursors.[11] Although phenanthrenes are considered to constitute a relatively small group of natural products, a fairly large numbers of phenanthrenes with promising biological activities have been isolated from vascular plants, mainly in the Orchidaceae family.[12]

From 2005 to 2008, Xue *et al*. isolated eight novel compounds and sixteen known compounds from an ethanolic extract of the tubers of *C. appendiculata*.[13-16] The new compounds identified were as follows: three monophenanthrenes, viz. 1-hydroxy-4,7-dimethoxy-1-(2-oxopropyl)-1H-phenanthren-2-one (1), 1,7-dihydroxy-4-methoxy-1-(2-oxopropyl)-1H-phenanthren-2-one (2), and 2-hydroxy-4,7-dimethoxyphenanthrene (3); two biphenanthrenes, viz. 2,7,2'-trihydroxy-4,4',7'-trimethoxy-1,1'-biphenanthrene (4),and 2,2'-dihydroxy-4,7,4',7'-tetramethoxy-1,1'-biphenanthrene (5); one triphenanthrene, viz. 2,7,2',7',2''-pentahydroxy-4,4',4'',7''-tetramethoxy-1,8,1',1''-triphenanthrene (6);[14] and two new terpenoids, viz. (−)-cadin-4,10 (15)-dien-11-oic acid (7) and (−)-ent-12β-hydroxykaur-16-en-19-oic acid, 19-*O*-β-D-xylopyranosyl-(1>6)-*O*-β-D-glucopyranoside (8).[14] The known compounds were identified as isohircinol (9), flavanthrinin (10), *p*-hydroxyphenylethyl alcohol (11), 3,4-dihydroxyphenylethyl alcohol (12), daucosterol (13), β-sitosterol (14),[15] cirrhopetalanthrin (15), 7-hydroxy-4-methoxyphenanthrene-2-*O*-β-D-glucoside (16), 4-(2-hydroxyethyl)-2-methoxyphenyl-1-*O*-β-D-glucopyranoside (17), tyrosol 8-*O*-β-D-glucopyranoside (18),

vanilloloside (19), *p*-hydroxybenzaldehyde (20), sucrose (21), adenosine (22), cirrhopetalanthin (23), and (+)-24,24-dimethyl-25,32-cyclo-5α-lanosta-9 (11)-en-3β -ol (24).[16] Among these compounds, 9–22 were isolatedfor the first time from this plant, and 9 was obtained from natural source for the first time.

The bioactivities of these compounds were evaluated against human colon cancer (HCT-8), hepatoma (Bel7402), stomach cancer (BGC-823), lung adenocarcinoma (A549), breast cancer (MCF-7), and ovarian cancer (A2780) cell lines.[13,14,16] Only two of the compounds (15 and 24) showed non-selective moderate cytotoxicity with half-maximal inhibitory concentration (IC_{50}) values of 8.4–13.3 μmol/L and selective cytotoxicity against human breast cancer cell lines with an IC_{50} value of 3.18 μmol/L.[14,16]

From 2013 to 2021, Liu *et al*. identified 15 new phenanthrenes in a high-polarity extract of *C. appendiculata* tubers. These compounds were 1-(3′-methoxy-4′-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol (25), 1-(3′-methoxy-4′-hydroxybenzyl)-7-methoxy-9,10-dihydrophenanthrene-2,4-diol (26), 1-(3′-methoxy-4′-hydroxybenzyl)-4-methox yphenanthrene-2,6,7-triol (27),[17] cremaphenanthrenes A–E (28–32),[18] cremaphenanthrene F–G (33 and 34),[19] and cremaphenanthrenes L–P (35–39).[20] This was the first report of the isolation of biphenanthrene atropisomers (33 and 34) from the plant kingdom.

Thirteen known compounds were obtained from petroleum ether and ethyl acetate extracts and they were identified as *p*-hydroxybenzaldehyde (20), 4,4'-dimethoxy-9,9',10,10'-tetrahydro-(1,1'-biphenant hrene)-2,2',7,7'-tetrol (40), 4,4',7,7'-tetrahydroxy-2,2'-dimethoxy-1,1'-biphenanthrene (41), 3,5-dihydroxy-2,4-dimethoxyphenanthrene (42), physcion (43), chrysophanol (44), emodin (45), genkwanin (46), quercetin (47), quercetin 3'-*O*-β-D-glucopyranoside (48), 3-methoxy-4-hydroxy phenylethanol (49), syringic acid(50), and vanillin (51).[21] Seven known phenanthrenes were isolated from an ethanolic extract and identified as 2,7,7′-trihydroxy-4,4′-dimethoxy-9′,10′-dihydro-1,2′-biphenanthreneether (blestrin C, 52), 2,7,7′-trihydroxy-4,5′-dimethoxy-9′,10′-dihydro-1,2′-biphenanthreneether (blestrin D, 53), 4,7,4′-trimethoxy-9′,10′-dihydro-(1,1′-biphenanthrene)-2,2′,7′-triol (54), phochinenin B (55), 2,7,2′-trihydroxy-4,4′,7′-tr imethoxy-1,1′-biphenanthrene (56), 2,2′-dihydroxy-4,4′,7,7′-tetramethoxy-1,1′-biphenanthrene (57),[18] and 1-(4′-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol (58).[17] Among these compounds, 40–42 and 44–51 were reported from this genus for the first time.

The bioactivities of these known compounds were investigated against colon (HCT-116), cervix (Hela), liver (HepG2), and breast (MDA-MB-231) human cancer cell lines and against A549 and MCF-7. Compounds 25–27 showed potent cytotoxicity against HCT-116 and MDA-MB-231 cell lines. The IC_{50} values against HCT-116 and MDA-MB-231 were 37.44 μmol/L and 10.42 μmol/L (25), 33.18 μmol/L and 11.92 μmol/L (26), and 14.22 μmol/L and 52.84 μmol/L (27), respectively. Compounds 28–32, 35, and 52–57 showed moderate or weak cytotoxicity toward HCT-116, MCF-7, MDA-MB-231, and Hela cell lines.[18,20] The new biphenanthrene atropisomers, cremaphenanthrene F (33) and G (34), showed butyrylcholinesterase inhibition with IC_{50} values of (14.62 ± 2.15) μ mol/L and (79.56 ± 0.78) μmol/L, respectively; however, they were inactive against acetylcholinesterase. These results suggest that compound 33 could act as a selective butyrylcholinesterase inhibitor for Alzheimer's disease prevention and treatment.[19]

Between 2004 and 2016, numerous other groups also investigated the phytochemistry and biological activity of *C. appendiculata* tubers, including Liu *et al.,*[22,23] Zhang *et al.*,[24] Wang *et al*.,[25] Ikeda *et al.,*[26] and Shim *et al*.[27]

Liu *et al*. isolated and identified two new phenanthrene glucosides named 2'-hydroxy-4,4',7'-trimethoxy-1, l'-biphenanthrene-2,7-di-*O*-β-D-glucoside (59) and 1-(4-hydroxybenzyl)-4-methoxy-2,7-dihydroxy-phenanthrene-8-*O*-β-D-glucoside (60). They have also identified the following 11 known compounds in ethyl acetate extracts of *C. appendiculata* tubers: cirrhopetalanthrin (15), 7-hydroxy-2,4-dimethoxy-phenanthrene (61), coelonin (62), shancigusin I (63), 4-*O*-β-D-glucopyranosyl cinnamate (64), bulbocodin D (65), blestriarene A (66), militarine (67), gastrodin (68), 3-hydroxyphenylpropionic acid (69), and cinnamic acid (70).[22,23] Among these compounds, 63–65, 69, and 70 were isolated from this plant for the first time.

Zhang *et al.* isolated the following seven known compounds from ethyl acetate extracts of *C. appendiculata* tubers for the first time: fumaric acid (71), dimethylhexyl phthalate (72), l-pyroglutamic acid (73), 2-furoic acid (74), vanillic acid (75), *p*-coumaric acid (76), and protocatechuic acid (77).[24]

Wang *et al.* obtained thirty four compounds, including eleven novel phenanthrenes, from high-polarity fractions (ethyl acetate and/or water) of *C. appendiculata*.[26] The chemical structures of the novel phenanthrenes were identified as 1-(4-β-D-glucopyranosyloxybenzyl) 4-methyl (2*R*)-2-isobutylmalate (78), 1-(4-β-D-glucopyranosyloxybenzyl) 4-ethyl (2*R*)-2-isobutylmalate (79), 1-(4-β-D-glucopyranosyloxybenzyl) 4-methyl (2*R*)-2-benzylmalate (80), 1,4-bis (4-β-D-glucopyranosyloxybenzyl) (2*R*)-2-benzylmalate (81), 7-hydroxy-4-methoxy-9,10-dihydrophenanthrene-2-*O*-β-D-glucopyranoside (82),

7-hydroxy-5-methoxy-9,10-dihydrophenanthrene-2-*O*-β-D-glucopyranoside (83), 4-methoxy-9,10-dihydrophenanthrene-2,7-di-*O*-β-D-glucopyranoside (84), 4,4'-dimethoxy-9,10-dyhydro-[6,1'-biphenanthrene]-2,2',7,7'-tetraol (85), (2,3-*trans*)-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-10-methoxy-2,3,4,5-tetrahydro-phenanthro[2,1-b] furan-7-ol (86), (2,3-*trans*)-3-[(2,7-dihydroxy-4-methoxy-phenanthren-1-yl) methyl]-2-(4-hydroxy-3-methoxyphenyl)-10-methoxy-2,3,4,5-tetrahydro-phenanthro[2,1-b] furan-7-ol (87), and (2,3-*trans*)-3-[2-hydroxy-6-(3-hydroxyphenethyl)-4-methoxybenzyl]-2-(4-hydroxy-3-methoxyphenyl)-10-methoxy-2,3,4,5-tetrahydro-phenanthro[2,1-b] furan-7-ol (88). Among these compounds, the structures of 87 and 88 were unusual as dimers because they possessed a phenanthrene or bibenzyl unit connected to C-3 of the 2,3,4,5-tetrahydro-phenanthrol [2,1-b] furan moiety. This was a novel discovery among the natural products isolated from plants in the Orchidaceae family.[25] The 23 known compounds were identified as flavanthrinin (10), adenosine (22), coelonin (62), blestriarene A (66), militarine (67), gastrodin (68), (−)-(2*R*,3*S*)-1-(4-β-D-glucopyranosyloxybenzyl)-4-methyl-2-isobutyltartrate (89), loroglossin (90), 7-hydroxy-2,4-dimethoxy-9,10-dihydrophenanthrene (91), 4,7-dihydroxy-1-*p*-hydroxybenzyl-2-methoxy-9,10-dihydrophenanthrene (92), 2-hydroxy-5,7-dimethoxyphenanthrene (93), 1-*p*-hydroxybenzyl-4-methoxyphenanthrene-2,7-diol (94), batatasin III (95), 3,3',5-trihydroxybibenzyl (96), 3,3'-dihydroxy-4-(*p*-hydroxybenzyl)-5-methoxybibenzyl (97),3,3'-dihydroxy-2-(*p*-hydroxybenzyl)-5-methoxybibenzyl (98), 3',5-dihydroxy-2-(*p*-hydroxybenzyl)-3-methoxybibenzyl (99), blestriarene B (100), blestriarene C (101), gymconopin C (102), blestrianol A (103), pleionesin C (104), and shanciol H (105). Among these compounds, 66, 89, 91, 92, 97, 100, and 102–105 were isolated from *C. appendiculata* tubers for the first time.[25] The cytotoxicity of all compounds was evaluated against A549 and Bel7402 cell lines. Compound 88 showed moderate cytotoxic activity (IC_{50} of 16.0 μM) against the A549 cell line, and all compounds were inactive towards Bel7402 cells (IC_{50} >50 μM).

A new pyrrolizidine alkaloid called cremastrine (106) was isolated by Ikeda *et al*.[26] and a homoisoflavanone called5, 7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)-6-methoxychroman-4-one (107) was isolated by Shim *et al*.[27] from *C. appendiculata* bulbs. Total synthesis of compound 106 was achieved in seven steps through construction of enantiopure idolizidines, pyrrolo[1,2-a] azepines, and pyrrolo[1,2-a] azocines for the synthesis of pyrrolizidine alkaloids from commercial materials with 25.2% overall yield.[28] Biological evaluation of compound 106 and the unnatural analog indicated that both were pan- muscarinic receptors (mAChRs) functional antagonists.[28] Compound 107 had inhibitory activities against angiogenesis,[27] retinal neovascularization,[29] UVB-induced skin inflammation,[30] mast cell activation and allergic responses.[31] To further explore the potential of homoisoflavanones as a treatment for neovascular eye disease, a novel isomer of compound 107 was synthesized (5,6-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)-7-methoxychroman-4-one, code: SH-11052). This compound had antiproliferative activity against human umbilical vein endothelial cells and ocular disease-relevant human retinal microvascular endothelial cells.[32]

Pleione bulbocodioides (Franch.) Rolfe

Pleione D. Don is a genus of mostly terrestrial Asian orchids that contains approximately 22 species, and is closely related to the *Coelogyne* and *Bletilla* genera.[33,34] This genus is named after Pleione who was the mother of the Pleiades (the seven daughters of Atlas) in Greek mythology. Species of this genus are mainly found in China, Vietnam, Myanmar, Bangladesh, and Northeast India at an elevation of 600–4200 m.[34] Most *Pleione* species are flowering in springat the beginning of their growth cycle. Because of their high ornamental and medicinal value, *Pleione* species have attracted much attention commercially. In China, the dry pseudobulbs from *P. bulbocodioides P. yunnanensis* and *C. appendiculata* are used as sources of the TCM *Pseudobulbus Cremastrae seu Pleiones*.[2]

Plant description and distribution

Pleione bulbocodioides is found in Yunnan, China[7,34] where it grows near shaded rocks or in raised beds. This species flowers during late spring (April–June)[5,10] and fruits in July.[5] The flower up to approximately 8cm high and 10cm wide, is bright pink with red spots, lamellae in the callus, and splashes within the lip.

Phytochemistry and biological activity

Early phytochemical research on *P. bulbocodioides* was conducted in the 1990s by Tagaki's group.[35-41] Twenty novel compounds were isolated from *P. bulbocodioides* tubers and their chemical structures were elucidated as follows: one dihydrophenanthropyran, namely shanciol (108);[35] one polyphenol, namely pleionol (109);[36] one bichroman, namely 6,6′-dihydroxy-4,4′-dimethoxy-3,3′-bichroman (110, pleionin A);[37] two flavan-3-ols, namely 4′-hydroxy-3′,5′,7-trimethoxy-5-(3′′-hydroxyphenethyl) flavan-3-ol (111, shanciol A) and 4′-hydroxy-3′,7-dimethoxy-5-(3′′-hydroxyphenethyl) flavan-3-ol (112, shanciol B);[38] four bibenzyls, namely bulbocodin (113), bulbocol (114), bulbocodins C (115) and D (65);[36,39] two bibenzyl glucosides, namely 3′-hydroxy-5-methoxybibenzyl-3-*O*-β-D-glucopyranoside (116) and 3′5-dimethoxybibenzyl-3-*O*-

β-D-glucopyranoside (117);[40] two dihydrophenanthropyrans, namely shanciol E (118) and F (119);[39] five stilbenoids, namely shancilin (120), shancidin (121), shanciguol (122),[41] shanciols C (123) and D (124);[38] and two lignans, namely sanjidins A (125) and B (126).[41] Ten known compounds were identified as coelonin (62),[41] batatasin III (95), 3′-*O*-methylbatatasin III (127),[40] 3,3′-dihydroxy-4-(*p*-hydroxybenzyl)-5-methoxybibenzyl (128), 3,3′-dihydroxy-2-(*p*-hydroxybenzyl)-5-methoxybibenzyl (129), 3′,5-dihydroxy-2-(*p*-hydroxybenzyl)-3-methoxybibenzyl (130),[36] lusianthridin (131),[41] bletilol A (132),[38] bletilol B (133),[35] and bletilol C (134).[38] The absolute configurations of bletilol B (133) were determined to be 11*S* and 12*S* using Horeau's partial resolution method and chemical correlations.[38]

From 2007 to 2012, several research groups worked on phytochemical studies of *P. bulbocodioides* tubers. Liu *et al*.[42-49] reported the isolation and identification of the following eight novel α, β-unsaturated butyrolactone derivatives: 4-(4″-hydroxybenzyl)-3-(3′-hydroxy-phenethyl) furan-2 (5H)-one (135), 3-(3′-hydroxyphenethyl) furan-2 (5H)-one (136),[42] (3-hydroxy-9-(4′-hydroxy-3′-methoxyphenyl)-11-methoxy-5,6,9,10-tetrahydrophenanthro [2,3-b] furan-10-yl) (137),[43] shanciol G (138), shanciol H (105),[44] 2-(4″-hydroxybenzyl)-3-(3′-hydroxyphenethyl)-5-methoxy-cyclohexa-2,5-diene-1,4-dione (139),[45] 9-(4′-hydroxy-3′-methoxyphenyl)-10-(hydroxymethyl)-11-methoxy-5,6,9,10-tetrahydrophenanthro[2,3-b] furan-3-ol (140), and 2-(4″-hydroxybenzyl)-3-(3′-hydroxyphenethyl)-5-methoxy-cyclohexa-2,5-diene-1,4-dione (141).[46] Twelve known compounds were also obtained and identified as gastrodin (68), 3-hydroxybenzic acid (142), *p*-dihydroxy benzene (143), gymconopin D (144), methyl-(4-OH)-phenylacetate (145), shanciol F (146), batatansin III (147), amentoflavone (148), kayaflavone (149), *p*-hydroxybenzaldehyde (20), *p*-hydroxybenzoic acid (150), and 4-oxopentanoic acid (151).[46,47]

From 2013 to 2020, elution–extrusion countercurrent chromatography was tested as a rapid and efficient method for preparative separation of the high-polarity compounds gastrodin (68) and benzyl ester glucosides from *P. bulbocodioides* tubers. Two new compounds, (*E*)-4-β-D-glucopyranosyloxycinnamic acid 9-(4-β-D-glucopyranosyloxybenzyl) ester (152) and (*Z*)-2-(2-methylpropyl) butenedioic acid bis (4-β-D-glucopyranosyloxybenzyl) ester (153), were isolated together with three known major components, militarine (67), gastrodin (68), and dactylorhin A (154).[48] Cui *et al*. also established a simple and quick high-performance liquid chromatography method to accurately determine militarine (67) and dactylorhin A (154) in *P. bulbocodioides*. Due to its good reproducibility, this method is used for quality control.[49]

Li *et al*.[50,51] isolated five novel compounds, including four pyrrolidone-substituted bibenzyls and a prenylated flavone, along with 29 known compounds from the pseudobulbs of *P. bulbocodioides*. The novel compounds were elucidated as dusuanlansins A–D (155–158)[50] and 3,5,7,3'-tetrahydroxy-8,4'-dimethoxy-6-(3-methylbut-2-enyl) flavone (159).[51] The former group of compounds are two pairs of epimers of pyrrolidone-substituted bibenzyls, and they were separated successfully using an LOD-RH C_{18} column. Their absolute configurations were elucidated by electronic circular dichroism. The known compounds were identified as 2,5,2′,5′-tetrahydroxy-3-methoxybibenzyl (160), batatasin III (95), 2,5,2′,3′-tetrahydroxy-3-methoxybibenzyl (161), bauhinol C (162), batatasin III-3-*O*-glucoside (163), arundinin (164), isoarundinin I (165), isoarundinin II (166), blestritin B (167), bulbocodin D (65), 5-*O*-methylshanciguol (168), blestriarene B (100), 4,4′,7,7′-tetrahydroxy-2,2′-dimethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene(169), phoyunnanin A (170), 1-(4-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol (171), 1-(4-hydroxybenzyl) 4,7-dimethoxy-9,10-dihydrophenanthrene-2-ol (172), 2,2′-dihydroxy-4,7,4′,7′-tetramethoxy-1,1′-biphenanthrene (173), militarine (67), bletillin A (174), 3,5,3′-trihydroxy-8,4′-dimethoxy-7-(3-methylbut-2-enyloxy) flavone (175), isorhamnetin-3,7-di-*O*-β-D-glucopyranoside (176), 3′-*O*-methylquercetin-3-*O*-β-D-glucopyranoside (177), 4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (178), 2,7-dihydroxy-4-methoxy-9,10-dihydrophenanthrene (179), lirioresinol B (180), sanjidin A (125), dactylorhin A (154), gastrodioside (181), and phenyl-β-D-glucopyranoside (182).[50,51]

Li's research group further isolated twenty eight novel compounds and thirty six known compounds from high polarity fractions of *P. bulbocodioides*.[52-55] The novel compounds and their absolute configurations were identified by NMR analysis and/or MS combined with experimental and theoretical electronic circular dichroism analyses. They were shown as follows: eight phenanthrenequinones (four pairs of enantiomers), namely bulbocodioidins A (9*R*, 9*S*) (183 and 184), B (9*R*, 9*S*) (185 and 186), C (9*R*, 9*S*) (187 and 188), and D (10*R*, 10*S*) (189 and 190);[52] four pairs of racemic bi (9,10-dihydro) phenanthrene and phenanthrene/bibenzyl atropisomers, namely *M*-bulbocodioidin E (191) and *P*-bulbocodioidin E (192), *M*-bulbocodioidin F (193) and *P*-bulbocodioidin F (194), *M*-bulbocodioidin G (195) and *P*-bulbocodioidin G (196), and *M*-bulbocodioidin H (197) and *P*-bulbocodioidin H (198);[53] ten glucosyloxybenzyl succinate derivatives, namely pleionosides A–J; (199–208);[54] and two phenylpropanoid

glycosidic compounds (a pair of epimers), namely pleionosides K (209) and L (210).[55] Compounds 183–190 possessed a 9 (10) H-phenanthren-10 (9)-one structure, which is rare in natural products. The thirty six known compounds identified were monbarbatain A (211), 2,7,2′-trihydroxy-4,4′,7′-trimethoxy-1,1′- biphenanthrene (212), blestriarene A (66), pleionesin B (213), shanciol H (105), 17-hydroxy-7′-(4′-hydroxy-3′-methoxyphenyl)-4-methoxy-9,10,7′,8′-tetrahydrophenanthro[2,3-b] furan-8'-yl methyl acetate (214), 1-*p*-hydroxybenzyl-4-methoxy phenanthrene-2, 7-diol (94), 1-*p*-hydroxybenzyl-4-methoxy-9,10-dihydrophenanthrene-2,7-diol (215), hircinol (216), coelonin (62), gigantol (217), batatasin II (218), syringaresinol (219), ergosta-4,6,8,22-tetraen-3-one (220),[56] asloroglossin (221), grammatophylloside A (222), cronupapine (223), (−)-(2*R*,3*S*)-1-(4-β-D-glucopyranosyloxybenzyl)-4-methyl-2-isobutyltartrate (224), vandateroside II (225), grammatophylloside B (226), bis[4-(β-D-glucopyranosyloxy)-benzyl] (*S*)-2-isopropylmalate (227), gymnoside I (228), militarine (67), dactylorhin A (154), gastrodin (68),[57] (−)-(2*R*,3*S*)-1-[(4-*O*-β-D-glucopyranosyloxy) benzyl]-4-methyl-2-isobutyltartrate (229), loroglossin (90), (−)-(2*S*)-1-[(4-*O*-β-D-glucopyranosyloxy) benzyl]-2-isopropyl-4-[(4-*O*-β-D-glucopyranosyloxy) benzyl] malate (230), syringaresinolmono-*O*-β-D-glucoside (231), (7*S*,8*R*)-dehydrodiconiferyl alcohol-9′-*O*-β-D-glucopyranoside (232), 5-methoxyl bibenzyl-3,3′-di-*O*-β-D-glucopyranoside (233), 3′-hydroxyl-5-methoxyl bibenzyl-3-*O*-β-D-glucopyranoside (234),[54] blestrianol A (103), pleionesin E (235), pleionesin D (236), and 3,3′-dihydroxy-2,6-bis *(p*-hydroxybenzyl)-5-methoxybibenzyl (237).[53] Among these compounds, 66, 211, 212, 216, 219, 220, and 221–227 were isolated from this genus for the first time.[56,57]

Biological investigations showed that compound 178 exhibited potent anti-inflammatory activity toward Lipopolysaccharide (LPS)-induced NO production in BV-2 microglial cells with an IC_{50} value of 5.44 μM.[50] The cytotoxic effects of the isolated new phenanthrenequinones were evaluated in several human cancer cell lines, and compounds 183 and 189 exhibited marked cytotoxic activity.[52]

Furthermore, three novel glucosyloxybenzyl succinate derivatives (201, 202, and 204) exhibited potent hepatoprotective activity against *N*-acetyl-*p*-aminophenol (APAP)-induced HepG2 cell damage in vitro, with cell survival rates of 31.89% (201), 31.52% (202), and 31.97% (204) at 10 μM (positive control: bicyclol, 31.90%).[54] Compound 198, a phenanthrene/bibenzyl atropisomer, displayed cytotoxic activity against colon cancer (HCT-116), liver cancer (HepG2), and breast cancer (MCF-7) cell lines with IC_{50} values of 7.6, 3.8, and 3.4 μM, respectively. Compound 235 showed cytotoxic activity against the MCF-7 breast cancer cell line with an IC_{50} value of 5.4 μM.[53]

The two phenylpropanoid glycosidic compounds 209 and 210 exhibited moderate hepatoprotective activity against APAP-induced HepG2 cell damage in vitro, with cell survival rates of 25.83% (209) and 28.82% (210) at 10 μM. They also displayed antioxidant activity against H_2O_2-induced toxicity in human SK-N-SH cells, with increases in viability at 10 μM of 24.9% (209) and 34.6% (210).[55]

Pleione yunnanensis Rolfe

Pleione yunnanensis (also known as the Yunnan Pleione) can be easily distinguished from other *Pleione* because it has rounded petals, a lip with broad rounded side lobes, and pale-colored flowers with five white lamellae.[34]

Plant description and distribution

Pleione yunnanensis is terrestrial or lithophytic and it grows in grassy meadows and open pine forests at elevations of 1100–3500 m in Tibet, Yunnan, Sichuan, and Guizhou province of China, as well as northern Myanmar.[5,34] The flowering period is between April and May, and the fruiting period is between September and October.[5]

Phytochemistry and biological activity

There are fewer phytochemical studies or biological investigations on *P. yunnanensis* before 2009 than on *C. appendiculata* and *P. bulbocodioides*. To date, two research groups have published several reports about *P. yunnanensis*.

Dong *et al*. and Cui *et al.* have isolated 29 novel compounds and 49 known compounds in 70%–95% ethanolic extracts of *P. yunnanensis* tubers.[58-61] The novel compounds have been identified are as follows: four new bibenzyl derivatives, *i.e.,* shancigusins A–D (238–241);[58] three new dihydrophenanthrofurans, i.e., pleionesins A–C (242–244);[59] five new glucosides, *i.e.*, shancigusins E–I (245–249);[60] and nine new glucosyloxybenzyl-2-hydroxy-2-isobutylsuccinates, *i.e.*, pleionosides M–U (250–258).[61]

The known compounds are identified as 2,6-bis (4-hydroxybenzyl)-3,3′,5-trihydroxybibenzyl (259), 3,3′-dihydroxy-2,6-bis (4-hydroxybenzyl)-5-methoxybibenzyl (260), 3′,5-dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl (261), 3,3′-dihydroxy-2-(4-hydroxybenzyl)-5-methoxybibenzyl (262), 3,3′-dihydroxy-4-(4-hydroxybenzyl)-5-methoxybibenzyl (263);[58] shanciol H (105), shanciol F (146),[59] 3′-hydroxy-5-methoxybibenzyl-3-*O*-β-D-glucopyranoside (264), 3′,5-dimethoxy-3-*O*-β-D-glucopyranoside (265), 3-hydroxy-3′,5-dimethoxybibenzyl (266), 3,3′-dihydroxy-5-methoxybibenzyl (267), 2,7-dihydroxy-1-(4-hydroxybenzyl)-

4-methoxy-9,10-dihydrophenanthrene (268), 4,7-dihydroxy-1-(4-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene (269), 2,7-dihydroxy-4-methoxy-9,10-dihydrophenanthrene (179), 4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (178), 2,7-dihydroxy-1-(4-hydroxybenzyl)-4-methoxyphenanthrene (270), 2,2′,7,7′-tetrahydroxy-4,4′-dimethoxy-1,1′-biphenanthrene (blestriarene C) (101), militarine (67), dactylorhin A (154), gymnoside I (228),β-sitosterol (14), daucosterol (13), (−)-syringaresinol (219), succinic acid (271), and adenosine (22),[60] 4,7-dihydroxy-1-(*p*-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene (272), (2,3-*trans*)-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-10-methoxy-2,3,4,5-tetrahydro-phenanthro[2,1-b] furan-7-ol (86), pleionesin B (213), blestriarene A (66), batatasin III (95), 3, 3'-dihydroxy-2-(*p*-hydroxybenzyl)-5-methoxybibenzyl (98), 3',5-dihydroxy-2-(*p*-hydroxybenzyl)-3-methoxybibenzyl (99), 3,3'-dihydroxy-2,6-bis (4-hydroxybenzyl)-5-methoxybibenzyl (273), triphyllol (274), pholidotin (275), (*E*)-*p*-hydroxycinnamic acid (276), (*E*)-ferulic acid (277), and (*E*)-ferulic acid hexacosyl ester (278),[62] Shancigusin H (248), dactylorhin A (158), gymnoside III (279), dactylorhin E (280), 1-[4-(β-D-glucopyranosyloxy) benzyl]-4-methyl-(*R*)-2-hydroxy-2-isobutylsuccinate (281), gymnoside I (228), loroglossin (90), bis (4-hydroxybenzyl) ether (282), 4-hydroxybenzyl alcohol (283), 4-hydroxybenzoic acid (284), and 4-hydroxybenzyl methylether (285).[61] Among these compounds, 66, 259–263, and 274–278 were isolated from this plant for the first time. Biological investigations showed that compounds 254, 255, 248, and 281 had significant *in vitro* hepatoprotective activity against d-galactosamine-induced toxicity in HL-7702 cells. The cell viability increased by 27%,

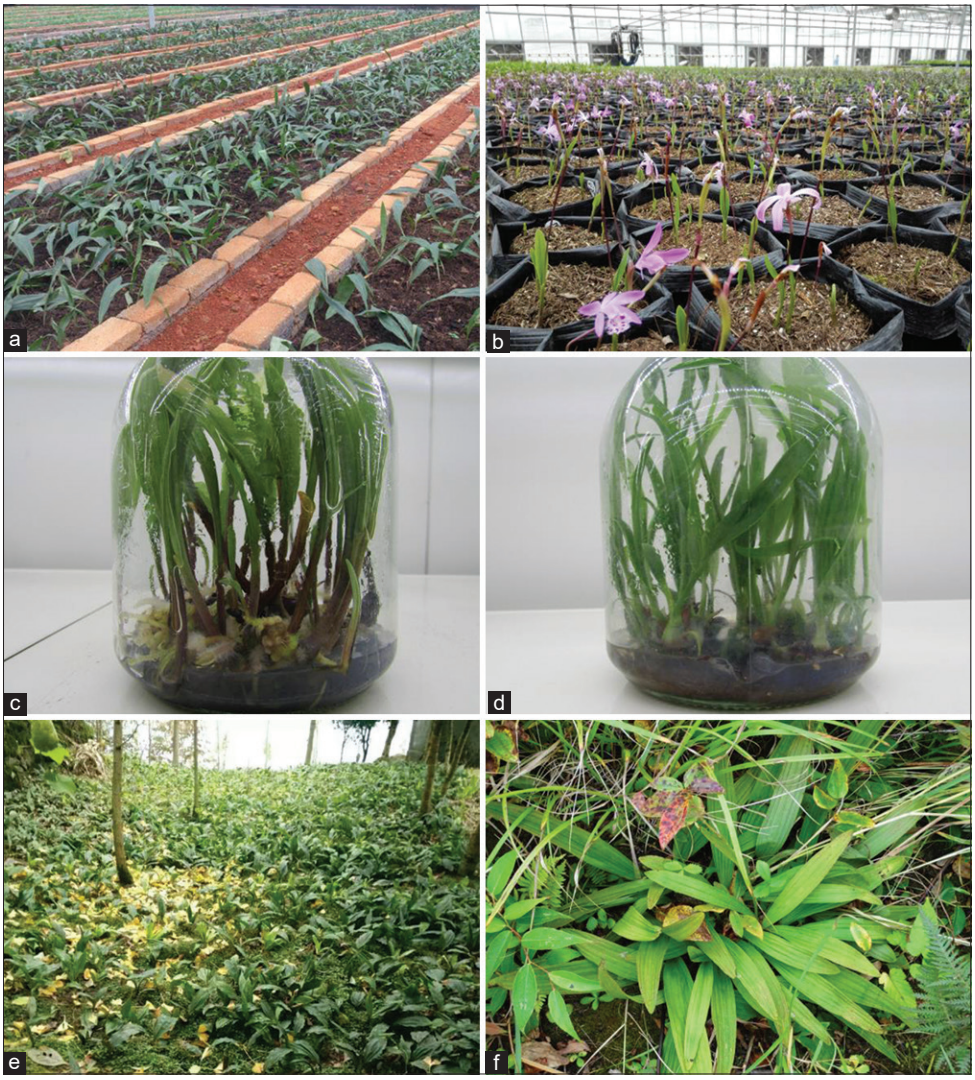

Figure 3 Plantations of *Cremastra appendiculata* (D. Don) Makino (a) and *Pleione yunnanensis* Rolfe (b) in greenhouse (seeds collected from wild) and their seeding from their meristems (c and d), and plantations under forests (e and f, planting with seeding from their meristems) (by Feng Zhao)

22%, 19%, and 31%, respectively, compared with the model group (bicyclol, 14%) at 10 μmol/L. Compounds 253, 258, and 158 exhibited moderate hepatoprotective activity against APAP-induced toxicity in HepG2 cells and increased cell viability by 9%, 16%, and 12%, respectively, compared with the model group (bicyclol, 9%) at 10 μmol/L.[61,62]

Cultivation and Production of *Pseudobulbus Cremastrae seu Pleiones*

Increase in global consumer demand for natural medicines and the rapid industrial development of TCMs have endangered wild Chinese herbal resources. Data analysis has shown that 1800–2100 medicinal species are facing extinction in China.[63] Currently, artificial cultivation of TCMs is used to ease pressure on natural reserves and increase wild medicinal resources. However, artificial cultivation requires a massive area of cultivable land and is not suitable for mountainous regions. Recently, another cultivation method, called natural fostering, has been suggested in China. This method involves planting herbs in forests to imitate their natural habitats. It resolves the land-area issue, decreases stress on the environment, and provides economic development in isolated mountainous areas. Natural fostering of *C. appendiculata* and *P. yunnanensis* has been investigated in Guizhou for many years and a successful method for mass propagation from their seeds and meristems has been developed [Figure 3]. Presently, seeding of TCMs such as *C. appendiculata* and *P. yunnanensis* is an area of rapid commercial growth in Guizhou.

Conclusions

Approximately 280 natural products, including 122 novel compounds, with potentially useful biological activity, have been isolated from the original source plants of *Pseudobulbus Cremastrae seu Pleiones*. Studies on the biological activities of these compounds conducted in recent years have improved understanding of the actions of *Pseudobulbus Cremastrae seu Pleiones* and its clinical application. Furthermore, these studies have identified various compounds that could be used as lead compounds or promising candidates for drug development. To date, most studies on the biological activities of these compounds have been conducted *in vitro*. Obviously, further research is required to systematically move these compounds to clinical trials. Planting of *C. appendiculata* and *P. yunnanensis* by mass propagation from their seeds and meristems could be an effective model to resolve supply issues, to meet market needs and to protect the environment.

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Ethical approval

This article does not contain any studies with human or animal subjects performed by either of the authors.

Author contributions

All authors participated in manuscript review and writing. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Conflict of interest

None.

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Cultivated *Cordyceps*: A Tale of Two Treasured Mushrooms

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Abstract

Ophiocordyceps sinensis and *Cordyceps militaris* both contain many bioactive compounds that confer potential therapeutic benefits. This review discusses the possible use of cultivated *C. militaris* as an effective substitute for native *O. sinensis* in the face of ever-increasing prices of *O. sinensis* because of its short supply. On the one hand, cultivated *C. militaris* contains higher levels of cordycepin when compared with that of wild-type *O. sinensis* and cultivation of *C. militaris* has been shown to be capable of reducing the risk of heavy metal contamination. On the other hand, there is a paucity of robust *in vivo* studies and randomized controlled tests comparing the pharmacology and use of *C. militaris* and *O. sinensis*. For extraction of cordycepin as western-style tablets, the use of cultivated *C. militaris* rather than *O. sinensis* represents the most appropriate future approach. For many other purposes, comparative pharmacology and clinical trials are in urgent needs.

Keywords: Cordycepin, cordyceps militaris, ophiocordyceps sinensis

Introduction

Dong Chong Xia Cao (冬虫夏草*Cordyceps*) is a genus of entomopathogenic ascomycetes, where entomopathogenic indicates that these species are parasites of insects, and ascomycetes indicate that this is a fungus that produces spores internally in sacs. The term "*Cordyceps*" has also been employed by the public to describe all of the commercial products that contain this fungus. Most notably, "*Cordyceps*" refers to *Ophiocordyceps sinensis* (*O. sinensis*), the most well-known and historically influential species, despite this fungus not falling into the *Cordyceps* subgenera according to a comprehensive phylogenetic analysis performed in 2007 to further categorize the *Cordyceps* genus into genera and subgenera through DNA sequencing.[1] In the previous studies, *O. sinensis* was named as *Cordyceps sinensis,* and the use of this term still prevails in some medical literature, for example, the latest edition of the *Zhong Guo Yao Dian* (《中国药典》*Chinese Pharmacopeia*) published at the end of 2020.

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Anti-cancer, anti-cirrhosis, disease-resisting, immunity-boosting, and life-prolonging are terms that have been associated with *Cordyceps*. As a medicinal mushroom that cures numerous diseases with few documented adverse effects,[2,3] *Cordyceps* has unsurprisingly garnered worldwide popularity in recent years. Shortages of the naturally existent fungi have resulted in *Cordyceps* being commercially cultivated to meet consumer demand.

History of *Ophiocordyceps sinensis*

Cordyceps as a health supplement has been used for many centuries. *O. sinensis* is a naturally existent fungus-caterpillar complex that has been used in Chinese and Tibetan traditional medicine since the 15th century.[3] *O. sinensis* is highly precious because it is harvested from remote locations of about 3800 m above sea level in Tibet, Qinghai, Yunnan, Sichuan, and Gansu provinces. Due to the limited quantity

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and high demand, *O. sinensis* was historically reserved for the most powerful and wealthiest people in society, such as members of the Emperor's Court in China.[4] In Tibet, this fungus was initially more known as a trade product than as a medicine. This is evident as the fungus has not been included in rGyud bzhi (《四部医典》*The Four Tantras*) and *Shel gong shel phreng* (《晶珠本草》*The Crystal Rosary*). The former is the fundamental treatise of Tibetan medicine dated between the 8th and the 12th century, while the latter is a highly comprehensive text devoted to Tibetan materia medica published in the 18th century.[5] Nonetheless, *O. sinensis* has long been included in Tibetan herbal preparations to improve energy levels and prolong lifespan. Since the 15th century, the fungus has been documented as a traditional Tibetan medicine to promote sexual virility.[5]

O. sinensis is "yartsu gambu" in Tibetan and "Dong Chong Xia Cao" in Chinese, which means "worm in the winter, herb in the summer."[6] This expression is in reference to how the fungus infects the caterpillar in late October but only grows out of the soil and matures in summer. As is shown in Figure 1, *O. sinensis* infects the underground larva of *Hepialus* Himalayan ghost moths and has the appearance of a silkworm. The exact mechanism that the fungus uses to plant its spores within the larvae is unknown.[3] Once inside the larvae, the fungus begins to consume the larvae and digests the inside of the larva, filling the larva with hyphae until only the exoskeleton remains. Hence, although the larva still appears intact in the caterpillar-fungus complex, its contents have already been entirely replaced by metabolites from the fungus. In spring, the fungus emerges from the fontanelle of the larva and grows out of the soil, this time with the ability to release ascospores that infect new larvae.[3] Thus, the fully mature form of *O. sinensis* can only be harvested from May to July.[6]

Current Usage of *Ophiocordyceps sinensis*

According to the *Chinese Pharmacopeia* (2020 Edition), *O. sinensis* is the sole legal source of *Cordyceps* for medicinal use. It is the main ingredient of the Bailing granule and Jinshuibao capsule sold in China. In traditional Chinese medicine (TCM) terminology, *O. sinensis* has the function to replenish the kidney, soothe the lung, stop blood bleeding and eliminate phlegm and is indicated for conditions with kidney deficiency, spinal and joint disorders, male sexual disorders, and respiratory diseases.[7] Consumed in its fermented or non-fermented state as a monotherapy or as part of a formulated therapy, *O. sinensis* is also used in clinical interventions for its immunomodulatory and apoptosis-modulating, anti-inflammatory, antioxidant, antitumor, as well as cardio-, reno-, osteo-protective and male sexual boosting effects in conventional medicine. These effects of *O. sinensis* have been reviewed by Xu *et al*.[8]

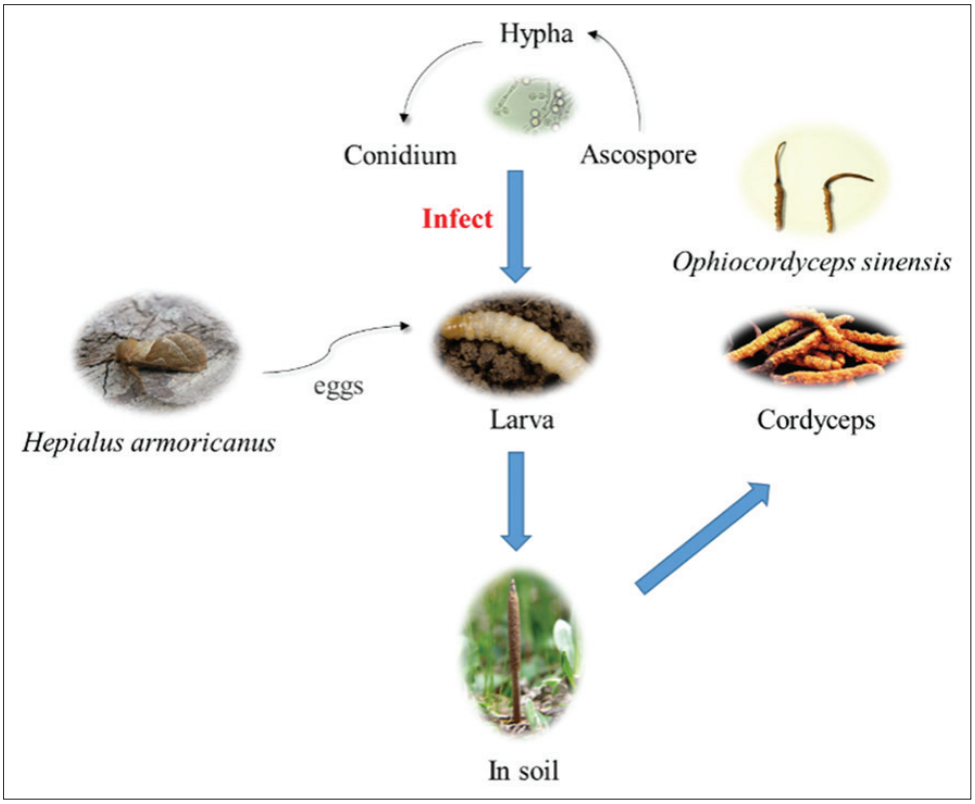

Figure 1 The life cycle of *Ophiocordyceps sinensis* in nature

For example, Jinshuibao capsule alleviated early diabetic nephropathy when used in conjunction with angiotensin receptor blockers, according to a meta-analysis performed on 26 studies.[9] *O. sinensis* extracts, including Bailing granule, and *O. sinensis* containing herbal formulae have been reported to ameliorate acute kidney injury (AKI) induced by ischemia-reperfusion and nephrotoxic agents in animal models.[10] In randomized controlled trials, Bailing granule mitigated AKI in intensive care unit patients; Bailing granule, Jinshuibao capsule and Chongcao Shenkang capsule, an *O. sinensis* containing herbal formula prevented AKI induced by contrast medium, severe brain injury or epidemic hemorrhagic fever.[10] All these studies were conducted with the Chinese people as the target and with relatively small sample sizes. Thus, a larger treatment group and more diverse population samples are required to reach more definitive conclusions.

Another major area of current use of *O. sinensis* is oncology. The scientific evidence on the antitumor effect of *O. sinensis* has been previously reviewed, involving multiple mechanisms, for example, inducing apoptosis of cancer cells, promoting immune responses against cancer, and regulating signal pathways mediating cancer invasion and migration.[8]

Side effects from treatment with *O. sinensis* have not been documented in detail. In immature (5-week-old) and mature (10-week-old) male mice, *O. sinensis* extracts increased plasma testosterone levels.[11] This supports the traditional use of *O. sinensis* to improve male sexual function but also necessitates caution for *O. sinensis* use among children and females. There were two cases of lead poisoning caused by contamination of *Cordyceps* powder, with a high lead concentration of 20,000 ppm. However, the side effects began to cease once people stopped taking the *Cordyceps* powder.[12] Otherwise, adverse effects and toxicity related to *O. sinensis* have not been reported in humans. In a preclinical study, rabbits were fed with 10 g/kg daily of *O. sinensis* for 3 months and the normal blood reports and function tests of the liver and the kidney suggest that *O. sinensis* is a rather nontoxic medicinal mushroom.[13]

The demand for *O. sinensis* has increased only because of the rising affluence of middle-class Chinese households who can now afford this fungus and the media coverage in the Western world. This growing demand, combined with the scarce supply, has resulted in the extremely high prices. In 2013, the annual harvest of *O. sinensis* was valued at 5–11 billion USD.[14] Harvesting *O. sinensis* has enabled rural Tibetan people to earn three times the average annual income of the region, leading to more collectors every year.[15] In a bid to harvest as many of the fungi as possible, *O. sinensis* are gathered even before they are sexually mature and have reproduced. The destruction of habitats caused by overharvesting has caused a decline in the quantity of *O. sinensis* collected. In a survey questionnaire given to harvesters in the Tibetan Autonomous Region, it was found that 95.1% believed the availability of *O. sinensis* in the pastures was declining, and 67.0% felt that the harvesting practices were not sustainable.[14]

Since the early 1980s, several scientific organizations have attempted to artificially culture the species to increase the availability and affordability of *O. sinensis*.[6] *O. sinensis* has both a sexual stage (teleomorph) and an asexual stage (anamorph). Commercially attempts to develop an efficient technology for the cultivation of fruiting bodies have yet to succeed. Anamorphic mycelia produced by fermentation thus was widely used as the alternative of natural *O. sinensis*. Industrial cultured mycelial products of *O. sinensis* claimed to have similar pharmacological activities to wild *O. sinensis,* although the cultured mycelial samples have higher contents of polysaccharides, adenine, and adenosine but much lower mannitol when compared with natural samples.[8] In China, research on cultivation of *O. sinensis* has made some progressions, including in the artificial feeding and reproduction of moth larvae of the genus *Hepialus*, the generation and isolation of *O. sinensis,* and the mechanism of fungal spores to attack the moth larvae.[16] Companies such as Aloha Medicinals based in the USA have filed patents for *O. sinensis* cultivation methods. However, there is conflicting literature on whether these companies have successfully cultivated *O. sinensis.* Some difficulties encountered during large-scale production attempts, for example, the cultivation of mycelia required a significant amount of energy as they needed a fixed temperature range for the incubation phase (15–20 °C) and larval phase (10–18 °C) of the host insect. Temperatures that were too high resulted in the death of the fungus. When the temperatures were too low, slow growth was observed. Humidity and soil moisture content had to be maintained over a narrow range. Furthermore, *O. sinensis* requires a lengthy production phase and is prone to contamination.[17] Nonetheless, the lack of data describing the production process may also be out of the reason that no official scientific report detailing the successes and specific cultivation methods has been published by any companies for fear that other institutions will capitalize on this lucrative industry.[6]

Advantages of Cultivated *Cordyceps militaris* as an Alternative

The economic production value and high content of bioactive compounds of *Cordyceps militaris* (*C. militaris*) make it the highest-selling cultivated species of *Cordyceps*, with an estimated annual revenue of 3 billion USD in China.[18] *C. militaris* is commercially cultivated because this fungus has a shorter life cycle than *O. sinensis,* and the production costs

are relatively less expensive. Unlike *O. sinensis,* there is no well-documented history recording the use of *C. militaris* as herbal medicine because it is comparatively scarcer in the wild than that of *O. sinensis*. While *O. sinensis* takes 1–2 years to fulfill its life cycle, *C. militaris* takes only 4–6 weeks as it does not require a host insect because it fruits readily in culture. Furthermore, the humidity and oxygen levels do not need to be controlled as strictly as the cultivated *O. sinensis*, making the production of this fungus economical. There are at least 36 approved supplements on the market made from *C. militaris.* This fungus can be consumed directly in its mushroom stage, in capsule form or as a mycelia powder, with all three forms approved by the Ministry of Health in China.[19] Official certification and improved availability of these products on the market should protect consumers from purchasing counterfeit and contaminated *C. militaris* products.

In contrast, counterfeit *O. sinensis* and related products remain a problem. To address this issue, a duplex polymerase chain reaction (PCR) method has recently been devised that uses primer pairs specific to naturally existent *O. sinensis* to successfully distinguish this fungus from artificial mimics and other *Cordyceps* species*,* including *C. militaris* and even its pure fermented mycelial form, which lacks the host species.[20] As this duplex PCR method can be quickly and inexpensively replicated in any molecular biology laboratory, authentication and certification by health authorities of *O. sinensis* should improve, which will quell the fear consumers have about unwittingly purchasing counterfeit *O. sinensis* products.

The richness of bioactive compounds in *C. militaris* and the associated pharmacological effects are substantial. For example, *C. militaris* was found to have an even higher cordycepin concentration than *O. sinensis.*[18,21] Cordycepin is a type of nucleoside, 3-deoxyadenosine, with a wide array of properties, such as anti-tumor activity [Table 1].[22-37] As shown in Figure 2, cordycepin has a similar structure to adenosine and thus functions as a nucleoside analog. This structural semblance enables cordycepin to be incorporated into DNA and RNA during the synthesis of nucleic acids instead of adenosine. However, the missing 3' hydroxyl group, which is present in adenosine, leads to premature transcription termination when cordycepin is added to the growing RNA chain because further bases cannot be added to the RNA chain.[38] This incorporation of cordycepin into nucleic acids can be helpful in cancer therapy, as it can stop the proliferation of tumor cells by terminating the cell cycle. In 1961, it was first shown that administering cordycepin over 7 days increased the survival time of mice with Ehrlich ascites carcinoma.[39]

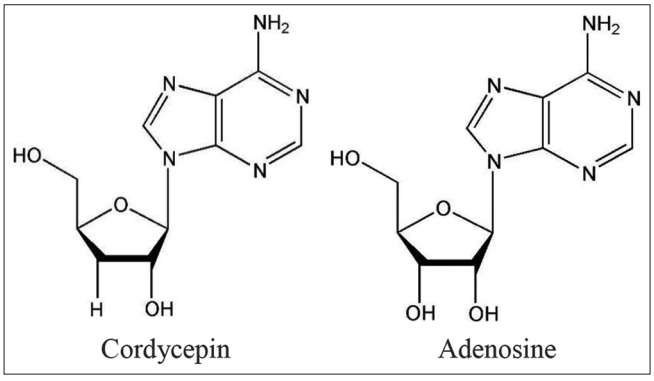

Figure 2 Chemical structures of bioactive compounds cordycepin (isolated from *Cordyceps militaris*) and adenosine

Cordycepin also demonstrates anti-tumor properties by causing tumor apoptosis via caspase-dependent pathways. This anti-tumor activity has been shown to be effective for human liver cancer cells (HepG2), human bladder carcinoma cells (T24 cells), human breast cancer cells (MCF-7 and MDA-MB-231), human renal cancer cells, and human non-small cell lung cancer.[40] Hence, the consumption of *C. militaris* should have a more potent anti-tumor effect than *O. sinensis* when administered at the same dose level.

In addition, the *C. militaris* protein (CMP), a cytotoxic antifungal protease, was identified in *C. militaris*. CMP was found to be effective against the fungus *Fusarium oxysporum in vitro*. Furthermore, CMP was cytotoxic against pure isolated human breast cancer MCF-7 and bladder cancer (5637) cell lines. These functions of CMP suggest that *C. militaris* might be an anti-fungal agent and could be used to treat cancer.[41] However, the experiment was not carried out *in vivo* and thus, potential interactions with other cells and substances within the body may affect CMP activity. In addition, CMP functions optimally at 37 °C and pH 7.0–9.0. Operating under nonoptimal conditions probably reduces the activity of CMP.

The anti-inflammatory activity of *Cordyceps* is also of interest. In lipopolysaccharides (LPS)-induced RAW264.7 cells, a *C. militaris* extract-mediated nano-emulsion exerted inhibitory effects on nitric oxide production and downregulated proinflammatory gene expression.[42] The biological activity of *C. militaris* was attributed to the saccharide and nucleoside contents. Chiu *et al*. isolated cerebrosides and nucleosides from the *C. militaris* extract and demonstrated the anti-inflammatory activity *in vitro*.[43] *C. militaris* contains β-(1R3)-D-glucan, an anti-inflammatory compound, which induces immune response by binding pattern recognition receptors (PRR) of cells similar to what pathogen-like molecules do and activating immune cells such as macrophages and dendritic cells. In an experiment, β-glucans were extracted from *C. militaris* and given to mice with peritonitis induced by LPS. The β-glucans inhibited the effects of IL-1β, TNF-α and COX-2 in a macrophage cell line *in vitro*, with β-(1R3)-D-glucan being the most potent. Furthermore, localized inflammatory

Table 1 Summary of the anti-tumor effects of cordycepin

Tumour type (cell line)	Mechanism	Molecular target	Reference
Human gastric cancer (SGC-7901 cells)	Inducing apoptosis	PI3K/AKT	[22,23]
Human gastric cancer (MGC-803 and HGC-27 cells)	Inhibiting proliferation and migration	CLEC2	[24]
Human non-small cell lung cancer cells	Inducing apoptosis and autophagy	c-FLIPL	[25]
Human liver cancer (HepG2 cells)	Inducing apoptosis	Caspase-8, Fas, FADD	[26]
Human liver cancer (HepG2 and Huh7 cells)	Inhibiting invasion and metastasis	CXCR4	[27]
Human bladder cancer (T-24 cells)	Inducing apoptosis	A3 adenosine receptors	[28]
Human renal cancer (TK-10 cells)	Inducing apoptosis	MKK7, JNK	[29]
Human breast cancer (MCF-7 and MDA-MB-231 cells)	Inducing apoptosis	Caspase-3,-8, -9, BCL-2	[30]
Human esophageal cancer (ECA109 and TE-1 cells)	Inducing apoptosis and G2/M phase arrest	Caspase-3, -9, BCL-2	[31]
Human uterine cervical cancer (ME180 and HeLa cells)	Inducing G2/M arrest	Cyclin A2	[32]
Human leukemia (NB-4 and U937 cells)	Inducting apoptosis and cell cycle arrest	Cyclin A2, cyclin E, CDK2, p53	[33]
Human prostate carcinoma (LNCaP cells)	Inhibiting migration and invasion	AKT	[34]
Human melanoma	Inhibiting invasion and metastasis	miR-33b, ZEB1, HMGA2, Twist1	[35]
Murine oral cancer (4NAOC-1 cells)	Inducing apoptosis, decreasing mitosis and EGFR signaling	Caspase-3, EGFR, IL-17RA	[36]
Murine testicular tumor (MA-10 cells)	Inducing apoptosis	AKT, MAPK	[37]

EGFR: epidermal growth factor receptor; FADD: fas-associated death domain; IL-17RA: interleukin 17 receptor A

pain both from peritonitis and induced by local tissue damage through injecting formalin into the paws of mice was reduced by β-glucans, suggesting that β-glucans from *C. militaris* could be used as a potential non-steroidal anti-inflammatory drug.[44] However, no consensus has been reached on what moiety of β-glucans binds to PRR, and the anti-inflammatory mechanism remains poorly understood. Additionally, although β-glucans found in *C. militaris* may demonstrate these effects, this does not guarantee that *C. militaris* would exert the same effects because of possible interactions with other substances.

There is no study that has demonstrated the anti-inflammatory effects of *C. militaris* in humans. A topical anti-inflammatory effect on mice with crouton-oil-induced ear edema has been examined. In this experiment, the mice had crouton oil injected into both ears. Acetonic solutions containing cultivated mycelia or fruiting bodies of *C. militaris* were applied to the right ear, whereas the left ear was left untreated. The difference in weight between the right and left ears was 51.8% for the cultivated mycelia and 58.7% for the fruiting bodies, thereby showing a strong inhibitory activity of edema and possibly inflammation.[45] In another study, the potential anti-inflammatory effect of an extract of *C. militaris* grown on germinated *Rhynchosia nulubilis* (GRC) fermented with *Pediococcus pentosaceus* ON89A (GRC-ON89A) was demonstrated in a mouse model of 1-fluoro-2,4-dinitrofluorobenzene-induced allergic contact dermatitis, in which GRC-ON89A reduced ear swelling and thickness.[46]

Cultivated *C. militaris* may be produced following good agricultural and manufacture practices, thus may be better positioned to avoid heavy metal poisoning, which can be hard to avoid from harvested native *O. sinensis*. In 2008, 13 out of 14 batches of native *O. sinensis* contained levels of arsenic, lead, mercury, cadmium, and copper that exceeded the green industry standard for medicinal plants, thereby posing a threat to humans if ingested. Under artificial cultivation, heavy metals can be monitored and kept at safe levels, hence avoiding poisoning.[47] However, mineral medicines containing mercury and arsenic, for example, An Gong Niu Huang Pill (安宫牛黄丸), is sometimes purposely added to preexisting formulations in TCM because of their sedative effects and they have been proven to have neuroprotective properties against cerebral ischemia-reperfusion injury with no hepatotoxic or nephrotoxic effects when administered over 7 days.[48]

Disadvantages of *Cordyceps militaris* as an Alternative

Native *O. sinensis* was found to have an inosine level higher than that of cultured *C. militaris* and *O. sinensis*. Inosine stimulates the growth of axons *in vitro* and the adult central nervous system.[49] Thus, native *O. sinensis* is a better source when using inosine as a marker of quality. Furthermore, for nerve damage treatment, native *O. sinensis* may represent the best option. In ddition, the level of D-mannitol, previously known as cordycepic acid before its identification in other species, was higher in native *O. sinensis* when compared with the levels in *C. militaris*. D-mannitol exhibits diuretic, cough relieving and anti-free radical activities. However, D-mannitol is found significantly higher in concentrations in fruits such as cranberries. Consequently, *Cordyceps* are not the best source of this compound.[50] Furthermore, consumers may be doubtful about whether cultivated and wild-type *Cordyceps* have uniform medical applications. A comparison of wild-type and cultivated *O. sinensis* indicated that the metabolites and protein compositions were similar, but the caterpillar bodies

differed from the stromata. Proteomic results also showed that artificial cultivation influenced the metabolism and amino acid synthesis pathways. The levels of four amino acids, lysine, threonine, serine and arginine, differed between wild-type and cultivated *O. sinensis*.[51] A study to identify a rapid and precise peptide marker to distinguish between native and cultivated *O. sinensis* through chemometrics and mass spectrometry found that native *O. sinensis* fruiting body, fermented *O. sinensis* mycelia powder and cultivated *O. sinensis* mycelia powder all had different marker peptides.[52] Hence, although the chemical components may be broadly similar, their pharmacological activities may vary because of differences in various peptides and cellular pathways. *C. militaris* has been artificially cultivated, but the development of the cultivation industry is slow, with the degradation of the fungus being the primary problem limiting the development of this industry. In addition, the unique climatic conditions of Xinjiang lead to unstable yields of fruiting bodies. The lack of effective scientific guidance can also lead to significant economic losses.

Conclusions

There is the evidence that novel compounds in cultivated *Cordyceps* can be used as potential medicinal or health products. These active compounds that may have therapeutic properties still require rigorous pharmacological and chemical testing and safety assessment through placebo-controlled trials. Furthermore, the pharmacological activities of cultivated *Cordyceps* have also not been compared in detail with their native species. Thus, it is difficult to confirm the therapeutic benefits of cultivated *Cordyceps*. Cultivation of *Cordyceps* should meet the current demand of this desirable resource, thereby ensuring a more sustainable harvesting practice and less environmental damage. However, the lack of quality control may be detrimental. Thus, a synchronized effort between the cultivated *Cordyceps* industry and scientific research communities is needed to future-proof this important industry.

Finally, although this paper primarily focuses on *O. sinensis* and *C. militaris*, other members of the megagenus *Cordyceps* may also have important roles to play in replacing *O. sinensis* in certain clinical applications. For example, in a recent report from China, ethyl acetate fractions of *O. xuefengensis* and *O. sinensis* both have *in vitro* anticancer activity. Eighty-two and 101 compounds were identified from the *O. xuefengensis* and *O. sinensis* extracts, respectively. Among these compounds, 68 existed in both *O. xuefengensis* and *O. sinensis*.[53] Thus, in addition to cultured *C. militaris* and mycelial products of *O. sinensis*, native and cultivated *O. xuefengensis* may also have its role to play in substituting *O. sinensis* in *Cordyceps*-based therapeutics.

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Ethical approval

This article does not contain any studies with human or animal subjects performed by either of the authors.

Author contributions

Anawinla Ta Anyu conceptualized this project, conducted initial literature retrieval and analysis, and wrote the first draft. Wen-Hui Zhang led the revision to the first draft, including amendments to the figures, as well as added more contents to the manuscript. Qi-He Xu supervised the whole process of this project and led the revision process before and after peer review. All authors agreed to the contents of the whole manuscript.

Conflict of interest

None.

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The History of Saffron in China: From Its Origin to Applications

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Abstract

Saffron (*Stigma Croci*) is an autumn-flowering perennial plant, and its use has a history of over 3500 years. Saffron has often been considered as the costliest medicinal plant, a premium spice, and the best dye with a golden yellowish color. Iran currently produces the finest quality saffron and dominates its global production (>90%). Other countries such as Australia, Canada, the USA, China, and some countries in Central Africa, produce saffron at a lower yield. In China, saffron is celebrated as "red gold" owing to the red stigmas of the flower and its price, which is comparable to the price of gold. Saffron has been one of the most attractive traditional Chinese medicine (TCM) herbs in the *Zhong Guo Yao Dian* (《中国药典》*Chinese Pharmacopoeia*) since its inclusion in the 2005 edition. The earliest use of saffron in TCM was recorded in the *Ben Cao Shi Yi* (《本草拾遗》*Supplement to Materia Medica*) written during the Tang dynasty (741 A.D.). However, saffron grown in inland China has been widely mistaken as originating from Tibet. This is because its Chinese name begins with "Xi" or "Zang," which sounds similar to its Tibetan name ("Xi Zang"). In this review, we clarify the origin of saffron and its introduction to China and summarize its various applications.

Keywords: Cosmetics industry, history, origin, perfumery, saffron, spice, traditional Chinese medicine

Introduction

Saffron (*Stigma Croci*) is the dried red stigmas of *Crocus sativus* L., a herbaceous flowering plant that belongs to the family *Iridaceae*, and the genus *Crocus* itself comprises approximately 80 species. Saffron is globally renowned as one of the most attractive spices owing to its unique properties that include a vivid crimson color, bitterness, and distinct aroma.[1] Saffron has been widely used in food, perfume, and cosmetics industries worldwide. In addition, recent studies have highlighted the therapeutic potentials of saffron including anticancer, antibacterial, and antioxidant activity.[2]

Saffron blooms in autumn, specifically in November, for only 15–20 days, and does not propagate by seeds. The cultivation and processing of saffron, including harvesting, drying, and screening, require delicate and manual handling. To produce 1 kg of saffron, approximately 70,000–200,000 flowers are required and each flower weighs approximately 0.3–1.0 g. This explains why saffron remains one of the world's most expensive medicinal plants. The price of top-grade saffron can reach 100 RMB (approximately 15–16 USD) per gram in China.[3] Globally, Iran produces saffron on the largest scale and its produce is of the highest quality. Other countries, such as Australia, Canada, the USA, China, and some countries in Central Africa, also produce and export saffron every year but at a lower yield.[4]

The applications of saffron have been extensively studied. There are contradictory reports in historical aspects, such as its origin and introduction to China, and there has been less emphasis on these topics. Hence, this review intends to clarify the origins of saffron and its introduction to China [Table 1], as well as to summarize its diverse applications in medicine, art, food, perfumery, and cosmetics industries in China and overseas [Figure 1].

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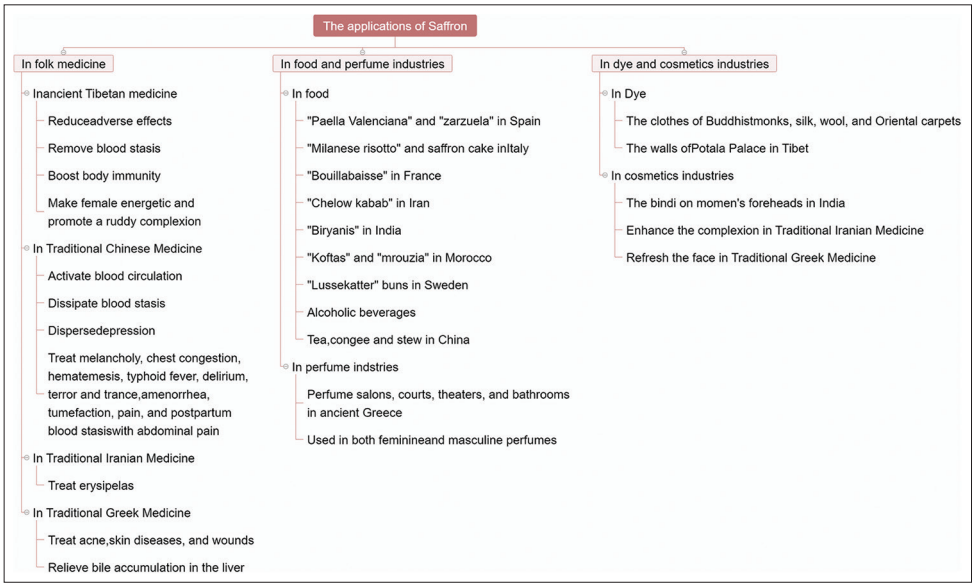

Figure 1 The application of saffron

Table 1 The history of saffron documented worldwide and in China

	Time period	Geographical area	Uses or properties of saffron	Related historical documentation
Worldwide	Around 2400 B.C.	Castilla-Mancha region of Spain	Fabric dye	[5]
	During the reign of Hammurabi (1800 B.C.–1700 B.C.)	Middle East	Condiment	[5]
	1550 B.C.	Frescoes in Minoic Palace of Knossos	Evidence of artificial cultivation	"Papyrus Ebers"[6]
	Late Bronze Age (1500 B.C. to 1100 B.C.)	Crete	Domestication of saffron	The Xeste 3 frescoes[7]
	12th century B.C.	Ancient Israel	Spice with unique aroma and bitterness	"Song of Songs" (Chapter 4, section 14) in the "Old Testament" of the *Bible*[8]
	10th century B.C.	Persia	Weave into carpets and shroud; Use in bath to relieve fatigue or cool off	[9]
	7th century B.C.	Assyria	Medicinal use	*A Botanical Dictionary of Assyrian*[10]
	Ancient Greek-Roman period (8th century B.C. – 3rd century B.C.)	Sicily	Cultivation of saffron	[11]
China	220 A.D. – 280 A.D.	China	Offered as part of prayer in temple; Drink with alcohol	*Nanzhou Records of Foreign Goods*[12]
	Tang dynasty (502 A.D. – 557 A.D.)		Offered as part of prayer in temple	*The Book of Liang*[13]
	Tang dynasty (741 A.D.)		TCM	*Supplement to Materia Medica*[14]
	Tang dynasty (742 A.D. – 805 A.D.)		Ornament	*Institutional History of Tang*[15]
	Yuan dynasty (1330 A.D.)		TCM	*Principles of Correct Diet*[16]
	Ming dynasty (1505 A.D.)		TCM	*Collected Essentials of Species of Materia Medica*[17]
	Qing dynasty (1745 A.D.)		Traditional Tibetan Medicine	*Jingzhu Materia Medica*[18]
	2005 A.D.		TCM	*Chinese Pharmacopoeia*[19]

TCM: Traditional Chinese Medicine

Methods

Electronic searches were performed on PubMed and China National Knowledge Infrastructure, from their inception to January 2021. The search terms used were saffron, 红花, *Stigma Croci*, and *C. sativus*. Articles were included if they covered the origin and application of saffron, and the full text

was retrievable. Articles were excluded if the used *Crocus* species were unspecified, or other species were used. Data from included articles were extracted and entered into a data extraction sheet.

The Origin of Saffron

The origin and history of saffron

The name "saffron" most probably originates from the Persian word "sahafaran."[20] Some researchers attribute the name "saffron" to the Arabic word "Zafaran," which means yellow.[1] Numerous legends have clouded the exact origin of saffron, while expert opinions on its origin are diversified as well. Vavilov suggested that saffron originated from the Middle East, while other researchers asserted Asia Minor or the South-Western Greek islands as its origin.[21,22] A recent study applied the genome-wide single-nucleotide polymorphisms method to examine the genetic make-up of saffron and affirmed that it evolved from *Crocus cartwrightianus* in Attica, Greece.[23] The earliest evidence of saffron as a fabric dye was unearthed in Castilla-Mancha, Spain, and has been dated to approximately 2400 B.C.[5] Then, during the reign of Hammurabi (1800 B.C.–1700 B.C.), saffron was used as a condiment.[5] The first evidence of saffron cultivation is of a much later date and can be traced to the Minoan civilization of 1550 years B. C., as inscribed in the Papyrus Eber.[6] This Egyptian medical document referred to the frescoes in the Palace of Knossos, which portrayed young girls picking saffron flowers while holding baskets.[24] Negbi *et al*. documented that saffron was probably selected for domestication in Crete during the latter years of Bronze Age (1500 B.C.–1100 B.C.).[7]

The unique aroma lends itself to be used as a spice. This was initially described in the ancient religious writings: The Old Testament of the Bible (12th century B.C.),[8] and the Hebrew Bible (known as the Tanakh) that was the first classic of Judaism (1000 B.C.).[25] During the reign of Ashurbanipal (7th century B.C.), the Assyrians pioneered the documentation of the therapeutic values of saffron in their botanical dictionary.[10] Manganaro *et al*. described their archeological findings of the ancient coins and inscriptions in Sicily, where saffron had been cultivated and used as medicine for gastrointestinal ailments and nephropathy since the Graeco–Roman era (8th century B.C. – 3rd century B.C.).[11] In Iran, which was historically known as Persia, saffron was woven into carpets and shrouds for the kings (10th century B.C.).[10] The Persians, later on, introduced saffron to the Kashmir region.[11] The saffron-bathing practice was initiated by the Persians to relieve fatigue or to cool off,[9] and this was expanded to Macedonia when Alexander the Great promoted it to the Greek soldiers (356 B.C.–323 B.C.).[26] Safranbolu, a city in northern Turkey, later developed a famous annual saffron harvest festival after saffron cultivation was introduced to the city. A similar "Saffron Rose Festival" has also been held annually on the last weekend of October in the town of Consuegra, Spain since the late 20th century.[27]

The introduction of saffron to China

Researchers hold contradictory opinions on when and how saffron was introduced to China. It is widely accepted that saffron was introduced to China through the Silk Road.[28] The Silk Road was initiated in the Western Han dynasty (202 B.C.–8 B.C.) when Emperor Wu tasked Zhang Qian (张骞) to open up a land passage from the capital, Chang'an (today's Xi'an), stretching through Central Asia and Western Asia (through Gansu and Xinjiang), and connecting to Mediterranean countries. Silk was exported from China westward toward India, Persia, Rome, and Egypt. In exchange, Western medicine and spices were imported to China. Saffron was probably introduced to China during that period.[29] Saffron was initially treated as a precious spice for the royal family and noble officials, and years later became a valuable gynecological medicine in Traditional Chinese Medicine (TCM).[30]

Some historians contended that the earliest introduction of saffron to China resulted from the Mongols' invasion of Persia, while others believed that saffron entered the capital of ancient China from Tibet during the Ming dynasty.[31] However, more researchers are convinced that saffron was introduced into China from India, along with the eastward dissemination of Buddhism during the Han and Jin dynasties (approximately 67 B.C.).[32] At that time, Indian Buddhists offered the whole saffron flower to the Buddha, and merchants were willing to sell the stigmas after the flower withered. Moreover, after the death of Shakyamuni, the Buddha, his corpse and clothes were also dyed with saffron, and the Buddhist disciples used saffron as the official color of dharma clothes. Buddhist scriptures also recorded the medicinal use of saffron for serious disease, in which patients were smeared with saffron, or bathed in water with saffron in it.[25]

"Saffron" was mentioned in many ancient works during medieval times, including *Tang Hui Yao* (《唐会要》*Institutional History of Tang*),[15] *Liang Shu* (《梁书》*The Book of Liang*),[13] and *Nan Zhou Yi Wu Zhi* (《南州异物志》*Nanzhou Record of Foreign Goods*).[12] "Saffron" was even cited in Chinese literature, specifically in the well-known poem *Ke Zhong Xing* (《客中行》*Writing as a Guest*) by Li Bai (李白) during the Tang dynasty. The word "saffron" quoted in these ancient works was misinterpreted as "Yu Jin Xiang" (郁金香) instead of "Xi Hong Hua" (西

红花).[32] In fact, "Yu Jin Xiang" is *Tulipa gesneriana* L., which is the national flower of the Netherlands. Criticisms made by Chinese scholars noted that the "Yu Jin Xiang" in those ancient works referred to saffron (*Stigma Croci*), rather than to *T. gesneriana* L. American sinologist Edward H. Schafer also noted that "Yu Jin Xiang" was quoted in the *Sa Ma Er Han de Jin Tao* (《撒马尔罕的金桃》*Golden Peach in Samarkand*), a catalog of exotic goods received in the capital city during the Tang dynasty.[33] He consistently specified that the "Yu Jin Xiang" presented by the state of Ghabia (today's Kashmir) to China was actually saffron, and interpreted all the references to "Yu Jin Xiang" in the poetry of the Tang dynasty as saffron.[32]

Historically, China relied on imported saffron for centuries until the 20th century. In 1965, a Chinese herbal medicine company attempted to plant a batch of saffron corms from West Germany in some open fields located in Shanghai, Hangzhou, Qingdao, Nanjing, and Sichuan.[34] Unfortunately, the corms degenerated and the effort to introduce saffron eventually failed. Later, in 1979, a Shanghai medicinal materials company imported 0.5 tons of saffron corms from Japan and successfully planted them in Ma Qiao town of Shanghai by combining the technology of cultivating corms in open fields with indoor flowering. From 1980, saffron corms were continuously imported from Japan, and 1.674 kg of saffron was harvested in China in that year and increased by 80% in 1981. Between 1981 and 1983, 36 tons of saffron corms were imported from Japan, and advancements in cultivation technology expanded the scale of production. China eventually began to export saffron from 1987.[35]

In 1990s, China made great progress in saffron cultivation and research, which contributed to the expansion of the planting area. At present, saffron has been successfully grown in more than 20 provinces and cities in China, especially Tibet, Shanghai, and Zhejiang. In Shanghai, saffron production is mainly distributed in Baoshan and Chongming districts. The saffron harvested from Chongming accounted for over 90% of China's total yield in recent years. Between 1981 and 1983, its sown area was 190 hectare and the cumulative yield of saffron exceeded 4300 kg.[35] Despite the advancement in saffron cultivation, its price remains high, primarily because of limited resources and production constraints.[35]

The Applications of Saffron

Saffron's use in folk medicine

As a part of TCM herbs, the earliest record of saffron in China was found in the *Ben Cao Shi Yi* (《本草拾遗》*Supplement to Materia Medica*) published during the Tang dynasty (741 A.D.).[14] During the Yuan dynasty, saffron was listed in the *Yin Shan Zheng Yao* (《饮膳正要》*Principles of Correct Diet*) by the name "Ji Fu Lan" (洎夫蓝).[16] During the Ming dynasty (1505 A.D.), the official revision of *Ben Cao Pin Hui Jing Yao* (《本草品汇精要》*Collected Essentials of Species of Materia Medica*) used "Sa Fu Lan" (撒馥兰) as the name of saffron, a synonym of "Fan Hong Hua" (番红花).[17] *Jing Zhu Ben Cao* (《晶珠本草》*Jingzhu Materia Medica*),[18] an ancient Tibetan medical book, recorded that "the appearance of saffron was similar to chrysanthemum, yellow and red, and with slightly fragrant aroma. Saffron was used to reduce adverse effects, remove blood stasis, and strengthen one's power." The medical book encouraged long-term intake of saffron to enhance beauty and boost body immunity. Females prone to qi and blood deficiency will find that taking saffron will make them energetic and promote a ruddy complexion.[18]

According to TCM, saffron stimulates blood circulation and nourishes blood. This effect was first included in the *Zhong Guo Yao Dian* (《中国药典》*Chinese Pharmacopoeia*) edition 2005 under the Chinese name "Fan Hong Hua" (番红花), "Xi Hong Hua" (西红花), or "Zang Hong Hua" (藏红花). In the latest 2020 edition, saffron is primarily indicated to activate blood circulation, dissipate blood stasis, and disperse depression. It is also used to treat melancholy, chest congestion, hematemesis, typhoid fever, delirium, terror and trance, amenorrhea, tumefaction, pain, and postpartum blood stasis with abdominal pain.[19]

Clinically, saffron has been used alone or in combination with other Chinese materia medica. A combination of saffron with Da Qing Ye (大青叶 *Folium Isatidis*) or Ban Lan Gen (板蓝根 *Radix Isatidis*) clears heat and removes toxins from the body.[36] When treating measles with fever and blood stagnation, saffron is usually applied together with Zi Cao (紫草 *Lithospermum erythrorhizon* Sieb. et Zucc.) and Chi Shao (赤芍 *Radix Paeoniae Rubra*), depending on the features of the rashes, such as whether they are dense or faint.[36] The efficacy of saffron on promoting blood circulation is further potentiated when combined with Yi Mu Cao (益母草 *Herba Leonuri*) and Dan Shen (丹参 *Radix Salviae Miltiorrhizae*).[37]

However, caution is needed when taking saffron as it may induce rhythmic uterine contractions, and large doses may trigger off spasmodic contractions and eventually cause miscarriage in pregnant women. During menstruation, the uterine contractions will consequently lead to abdominal pain, diarrhea, and even depletion of circulating blood volume. Saffron is, therefore, contraindicated for pregnancy and menstruation, particularly heavy bleeding.[38] Similarly, patients who have just undergone surgery or have obvious bleeding wounds should not take saffron as it is detrimental to wound healing and may even aggravate bleeding.[39]

Saffron is also used in other traditional medicine. For example, traditional Iranian medicine uses saffron to treat erysipelas. The Greeks use saffron to treat acne, skin diseases, and wounds, as well as to relieve bile accumulation in the liver.[26]

Saffron's use in food and perfume industries

Food industries all over the world use saffron to dye and season famous dishes, for example: "Paella Valenciana" and "zarzuela" in Spain, "Milanese risotto" and saffron cake in Italy, "Bouillabaisse" in France, "chelow kabab" in Iran, "biryanis" in India, "koftas" and "mrouzia" in Morocco, and "Lussekatter" buns in Sweden.[40] Saffron is also used in alcoholic beverages because of its aroma, coloring, and flavoring properties.[41] In China, people are accustomed to adding 5–10 saffron stigmas to tea and alcohol, and the stigmas are edible after the drinks are finished. A pinch of saffron is also added when making congee and stew, or as a seasoning when preparing assorted delicacies in China.[42] Chefs and saffron specialists describe the flavor similar to honey, but with a slight metallic note.[43] Some attribute the bitterness of saffron to picrocrocin, one of its key bioactive components, while its intense aroma is related to its volatile oil composition.[44]

The perfume industry also makes use of the unique aroma of saffron. In ancient Greece, saffron was initially used by the royal clan to perfume salons, courts, theaters, and bathrooms. Aristophanes noted that the pleasant aroma that was released from dried saffron was a "sensual smell".[45] A recent study has identified safranal, a cyclic terpenic aldehyde, as the principal component contributing to the aromatic property of saffron.[46] Today, saffron is often found in both feminine and masculine perfumes since it has a woody, sweet note, and pleasing scent.[47]

Saffron's use in dye and cosmetics industries

A natural dye, saffron contains pigments that impart its coloring power. The golden yellowish color of saffron is incorporated into painting and textiles, including the clothes of Buddhist monks, silk, wool, and Oriental carpets.[47] The walls of Potala Palace, a magnificent ancient building in Tibet, China, are repainted annually after the rainy season and before the winter season. The paint is mainly made of pure natural raw lime that is obtained from Yangbajing, Lhasa. Saffron, milk, sugar, and honey are added to thicken the paint for better protecting the building walls.[48] The advantage of saffron is its coloring property, high water solubility, and ability to reduce the oxidation of cellulose. Saffron is also stable in alkaline and acidic media because of its pKa (acid dissociation constant), dicarboxylic acids, esters, and nitrogen compounds.[49]

Saffron also makes a large contribution to the cosmetics industry. In India, women dab a yellow dot of saffron on their foreheads to make the bindi, symbolizing good fortune and conscience.[47] Traditional Iranian medicine applies saffron to enhance the complexion while traditional Greek medicine uses saffron to refresh the face.[9] Despite the beauty effects offered by saffron, only minute amounts are used in cosmetics because of its high price.

Current preclinical and clinical advancement of saffron

Recent studies have identified the major bioactive compounds of saffron as terpenoids, flavonoids, and anthraquinones.[50] Increasing evidence has demonstrated various biological properties of saffron-derived compounds, including anticancer, antibacterial, cosmetic, anti-inflammatory, antinociceptive, hypnotic, anxiolytic, anesthetic, antidepressant, and bronchodilatory effects. They also have therapeutic effects on the eye diseases, premenstrual symptoms, gastrointestinal tract disease, and liver disease.[51] For example, Al-Hrout *et al*. revealed that safranal, one of the primary bioactive components of saffron, inhibits the proliferation of HepG2 hepatocellular carcinoma cells by inducing endoplasmic reticulum (ER)-stress-mediated apoptosis and G2/M cell cycle arrest. More importantly, safranal promotes DNA damage through induction of DNA double-strand break and deregulation of DNA replication and transcription.[52] Safranal has recently been discovered to repress the recurrence of prostate cancer and suppress tumor growth *in vivo*.[53] The neuroprotective property of saffron is evident when the lipid peroxidation and levels of nitric oxide in the brains of saffron-treated mice were markedly reduced compared with those in the control group.[54]

In addition to its clinical use in TCM, saffron has been clinically tested in Iran for patients with metabolic syndrome, Alzheimer's disease, and depression. In an 8-week double-blind, randomized clinical trial, patients with metabolic syndrome who received 30 mg/d of saffron had improved lipid profiles compared with those receiving a placebo.[55] Another study randomized 46 patients with mild to moderate Alzheimer's disease and they received either 15 mg saffron, or a placebo, twice daily for 16 weeks. Compared with the placebo group, saffron improved both the cognitive functions and dementia symptoms of the treated patients.[56] Patients suffering major depressive disorder and anxious distress also benefitted from a 6-week daily treatment of 30 mg saffron. The beneficial effect of saffron in improving symptoms of depression and anxiety was comparable to citalopram, an antidepressant medication.[57] These clinical findings further corroborate the therapeutic potentials of saffron and saffron-derived components.

Discussion

Low yield, short flowering time, and high labor costs are the main challenges in lowering the price of saffron. Iran

and southern European countries yield more than 90% of global saffron production, while other countries produce saffron to a much less extent. Compared with the scale of other medicinal plants, the scale of saffron production is still limited, thus lowering the yield.[4] Moreover, saffron hardly grows in the wild because it does not propagate by seeds. Saffron is usually planted in April or May and it blooms in late October or November in Shanghai, China.[58] Because saffron is an autumn-flowering species, the month of planting may differ across regions, depending on geographical and climatic differences. It flowers for only 15–20 days,[59] and the quality of harvested saffron rapidly withered when exposed to sunlight, warranting speedy harvest before sunrise. Saffron production comprises many steps including harvesting, drying, stripping of stigmas, screening, and bundling. All these steps require delicate manual handling, thus raising the labor costs.[60] In addition, a massive amount of raw material only produces a small amount of saffron. Taking all these factors together, saffron remains the most expensive medicinal plant and premium spice that attracts the attention of various industries.[61]

Unfortunately, the prohibitive price of saffron leads to the phenomenon whereby illegal traders make substantial profits by selling fake or adulterated saffron. It is frequently adulterated with other parts of the *Crocus* flower, such as the styles, stamen, or strips of the corolla. Other common adulterants are safflower, calendula, poppy, arnica, onion skins, turmeric, annatto, capsicum, and stigmas of maize.[62] Adulteration of saffron dates back to the Middle Ages in Europe, where the penalty for adulterating merchants was execution. Identification of adulterations is of great importance in the contemporary saffron market. Recent research has focused on enhancing the yield and improving the quality of saffron stigmas to decrease its price and make it more affordable for common use.[63]

The long history, diverse uses, and growing evidence of its various therapeutic potentials mean that saffron deserves more in-depth mechanistic, phytochemical, and pharmacological studies to support its development as a therapeutic agent.

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Ethical approval

This article does not contain any studies with human or animal subjects performed by either of the authors.

Author Contributions

Rong-Chen Dai and Hong-Xi Xu contributed to the conceptualization and design; Rong-Chen Dai performed the literature search; Rong-Chen Dai and Wan Najbah Nik Nabil contributed to the data acquisition, data analysis, manuscript preparation and manuscript editing; Hong-Xi Xu edited and reviewed the manuscript.

Conflict of interest

Hong-Xi Xu is an editorial board member of Chinese Medicine and Culture. The article was subject to the journal's standard reviewing procedures, with peer review handled independently of this editorial board member and their research groups.

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Dendrobium nobile Lindl: A Review on Its Chemical Constituents and Pharmacological Effects

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Abstract

Dendrobium nobile Lindl (*D. nobile*), a well-known precious herb, has a long history of use as a medicine and health food in China. Phytochemically, *D. nobile* has been found to contain various bioactive compounds, such as alkaloids, bibenzyl, phenanthrene, phenylpropanoids, and polysaccharides. Its medicinal applications are closely correlated to its diverse pharmacological activities, including antitumor, anti-inflammatory, nervous system protective, antifatigue, hypoglycemic, and hypolipidemic actions. In this review, we provide a comprehensive summary of the main chemical constituents and pharmacological activities of *D. nobile*, as well as the underlying molecular mechanisms for its bioactivities. It is expected that this review will provide a helpful scientific reference for the development and use of *D. nobile*.

Keywords: Chemical constituents, *Dendrobium nobile* Lindl (*D. nobile*), pharmacological effect, Chinese medicine

Introduction

Dendrobium nobile Lindl (D. *nobile*) is also known as Noble Dendrobium or *Jin Chai Shi Hu* (金钗石斛) in Chinese. It is a perennial epiphytic herb of the genus Dendrobium in the Orchidaceae family.[1] *D. nobile* is one of the main Dendrobium species recorded in the Chinese Pharmacopoeia and it offers both ornamental and medicinal benefits.[2] There are about 1,500 species of Dendrobium around the world, and 50 of them have been found to be medicinally valuable in China. The history of the utilization of medicinal Dendrobium is one of 1,500 years in China and the first record of its use was found in the *Shen Nong Ben Cao Jing* (《神农本草经》*Shennong's Classic of Materia Medica*). Dendrobium together with Dong Chong Xiao Cao (冬虫夏草 *Cordyceps sinensis*), Ren Shen (人参 *Radix Ginseng*) and Ling Zhi (灵芝 *Ganoderma lucidum*) have been regarded as precious and top-grade traditional Chinese medicines (TCM) in China for thousands of years. Dendrobium is widely used as a traditional medicine for nourishing Yin, clearing heat, relieving coughs, brightening eyes, strengthening body constitution, and promoting longevity.

In recent decades, phytochemical investigations have revealed that the active chemical components of *D. nobile* mainly include alkaloids, polysaccharides, and phenols. Pharmacological studies have shown that the active ingredients of *D. nobile* have multiple health-promoting effects, and many of the pharmacological effects have ethmomedicinal values, including anti-tumor, anti-inflammatory, immune enhancing, anti-fatigue, anti-aging, and blood sugar reducing properties have ethnomedicinal values.[3] Furthermore, the application value of *D. nobile* is expanded from its medicinal property

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to ornamental plants in gardens. For instance, *D. nobile* is widely used for decoration purposes in houses or on the streets in Chinese cities because of its beautiful and elegant appearance. To date, to our knowledge, no authoritative systematic review has been published on *D. nobile*. Hence, the aim of this present summary is to provide an up-to-date and comprehensive literature review on *D. nobile,* with a particular focus on its botany, chemical constituents, and pharmacological effect, as well as on the potential molecular mechanisms underpinning the bioactivities of its components. It is expected that this review will provide new insights for further study and exploitation of *D. nobile* as medicinal agent or functional food in future.

Botany and Ethnopharmacology

D. nobile is known by other synonyms including "*Callista nobilis* (Lindl.) Kuntze; *D. coerulescens* Wall. ex Lindl., *D. formosanum* (H. G. Reichenbach) Masamune; *D. lindleyanum* Griff., *D. nobile var. alboluteum* Huyen and Aver., *D. nobile var. formosanum* H. G. Reichenbach and *D. nobile var. nobilius* (H. G. Reichenbach) M. Hiroe.[4] *D. nobile* is known to be distributed in several Asian countries, including China, Bhutan, India, Laos, Myanmar, Nepal, Thailand, and Vietnam. *D. nobile* is native to several provinces of China including Anhui, Guizhou, Taiwan, Hainan, Hubei, and Guangxi provinces. It usually epiphytizes on tree trunks in mountain forests, lithophytes on rocks in mountain valleys at 500–1,700 m altitude, and grows mainly in warm and humid environments.[5] Botanically, *D. nobile* is a small-sized plant with stems that are erect, fleshy and cylindric, reaching between 10 and 60 cm long and 1.3 cm in diameter with its upper part curving and dividing into sections,and turning to golden yellow when dry. The leaves are oblong, measuring (6-11) cm × (1-3) cm, leathery, apex obtuse and unequally bilobed, while its base is decorated with a cauline sheath.[6] The pseudobulb of *D. nobile* is the main source of dendrobium recorded in the Chinese Pharmacopoeia. According to the *Shennong's Classic of Materia Medica*, it possesses the therapeutic effects of "nourishing Yin and benefiting essence, thickening intestines and stomach, supplementing deficiency of internal organs, and relieving the body to prolong life".[2,7]

For thousands of years, *D. nobile* has been used as a traditional remedy for various diseases, such as diabetes, chronic atrophic gastritis, neurodegenerative conditions related to aging, and cardiovascular disease.[8,9] The pseudobulb is used to alleviate thirst, calm restlessness, accelerate convalescence, and reduce dryness of the mouth. The leaf extract of *D. nobile* is effective on freshly cut wounds and is used agaianst dermatologic disorders.[10] Overall, the fresh and dried pseudobulbs of this plant are well known as one of the most expensive tonics in TCM and may also be used as a dietary supplement. *D nobile* is worthy of been further studied and developed as a therapeutic agent or functional food in the management of human health. A photo of the entire *D. nobile* is shown in Figure 1.

Figure 1 *Dendrobium nobile* Lindl. (by Prof. Zhi-Li Zhao)

Phytochemical Constituents

To date, various phytochemical compounds have been isolated and identified from *D. nobile* and they are mainly alkaloids, bibenzyl, phenanthrene, phenylpropanoids, and polysaccharides, among other things. Some of them have been regarded as the biologically active components responsible for multiple bioactivities. Among these chemical compounds, alkaloids are high in content and widely used in research. The compounds isolated from *D. nobile* are listed in Table 1.

Alkaloids

Alkaloids are a huge group of naturally occurring organic compounds that contain at least one nitrogen atom or atoms (amino or amido in some cases) in their structures. These nitrogen atoms are responsible for the alkalinity of alkaloids compounds.[23] Alkaloids play an essential role in both human medicine and in an organism's natural defenses. Alkaloids make up approximately 20% of the known secondary metabolites found in plants. Alkaloids are the earliest chemical compounds isolated and purified from Dendrobium genus, and they are also important active components in *D. nobile*. The alkaloids from *D. nobile* have been found to own the potential of exhibit anti-cancer and neuroprotective effects.[24] Wang *et al*.[11] isolated nine sesquiterpene alkaloids from the extracts of the stem of *D. nobile*, including N-methyldendrobinium (1), dendrobine (2), mubironine B (3), nobilonine (4), dendramine (5), 6-hydroxy-nobiline (6), N-isopentenyldendrobinium (7), N-isopentenyl-6-hydroxydendroxinium (8), and N-isopentenyldendroxinium (9). Dendroxine (10) and

Table 1 Chemical compounds isolated from *Dendrobium nobile*

Classification	Number	Compound	Reference
Alkaloids	1-9	(1) N-methyldendrobinium; (2) dendrobine; (3) mubironine B; (4) nobilonine; (5) dendramine; (6) 6-hydroxynobiline; (7) N-isopentenyldendrobinium; (8) N-isopentenyl-6-hydroxydendroxinium; and (9) N-isopentenyldendroxinium	[11]
	10-11	(10) dendroxine; and (11) 6-hydroxydendroxine	[12]
Bibenzyls	12-15	(12) moscatilin; (13) crepidatin; (14) chrysotobibenzyl and (15) gigantol	[13]
	16-18	(16) nobilin A; (17) nobilin B and (18) nobilin C	[14]
Phenanthrene	19-30	(19) moscatin; (20) nudol; (21) bulbophyllanthrin; (22) fimbriol B; (23) plicatol A; (24) lhridinusiant; (25) coelonin; (26) erianthridin; (27) ephemeranthol A; (28) ephemeranthol C; (29), hircinol and (30) flavanthridin	[12]
	31-36	(31) fimbriatone; (32) flavanthrinin; (33) 4, 9-dimethoxyphenanthrene; (34) 2, 2, 6-diol; (35) 2, 5-diol, 5, 7-dimethoxyphenanthrene and (36) confusarin	[15]
	37-41	(37) 3,7-dihydroxy-2,4-dimethoxy-9,10-dihydrophenanthrene; (38) 3,7-dihydroxy-2,4-dimethoxyphenanthrene; (39) 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene; (40) 1,5,7-trimethoxyphenanthren-2-ol (TP) and (41) dehydroorchinol	[16,17]
Phenylpropanoids	42-46	(42) decumbic acids A; (43) decumbic acids B; (44) (−)-decumbic acid; (45) (−)-dendrolactone and (46) (+)-dendrolactone	[18]
Polysaccharides	47-51	(47) DNP-W1; (48) DNP-W2; (49) DNP-W3; (50) DNP-W4 and (51) DNP-W5	[19,20]
	52-55	(52) L-arabinose; (53) D-galactose; (54) D-glucose and (55) D-mannose	[21]
Others	56-68	(56) syringic acid; (57) 2-hydroxyphenylpropanol; (58) vanillin; (59) apocynin; (60) coniferyl aldehyde; (61) syringaldehyde; (62) syringylethanone; (63) p-hydroxybenzaldehyde; (64) 3-hydroxy-4-methoxyphenylethanol; (65) α-hydroxysyringylethanone; (66) dihydroxyconiferyl alcohol; (67) p-hydroxybenzoic acid and (68) p-hydroxyphenylpropionic acid	[14]
	69-72	(69) dendroflorin (A1); (70) denchrysan A (A1); (71) dengibsin (A1); (72) nobilone	[22]

6-hydroxy-dendroxine (11) have also been isolated as major alkaloids from *D. nobile*.[12] Among them, dendrobine (2) is the first identified active alkaloid of *D. nobile*[25] and is regarded as the standard agent for the qualitative and quantitative evaluation of *D. nobile*.[26]

Bibenzyls

Bibenzyl is a type of organic compound that is a derivative of ethane in which one phenyl group is bonded to each carbon atom. It is chemically constructed by two lunularin moieties with two diaryl-ether bonds, or two biaryl bonds, or one diaryl-ether and one biaryl bond.[27] Bibenzyls are found to be widespread in this plant. For instance, moscatilin (12), crepidatin (13), and chrysotobibenzyl (14) have been isolated from *D. nobile* by the screening of 31 extracts.[13] Moreover, moscatilin (12) and gigantol (15) were isolated from nearly 20 species of Dendrobium. Zhang *et al*. also identified three new bibenzyl compounds from an ethanol-water extract of *D. nobile*, namely nobilin A (16), nobilin B (17), and nobilin C (18), all of which were found to possess antioxidant properties.[14] In addition, seven bibenzyl compounds were isolated from the stem of *D. nobile* by using silica gel, MCI column chromatography, and preparative HPLC, among which 4,α-dihydroxy-3,5,3'-trimethoxybibenzyl was identified as a new compound and 4,5-dihydroxy-3,3'-dimethoxybibenzyl was isolated from the plant for the first time.[28]

Phenanthrenes

Phenanthrene is a polycyclic aromatic hydrocarbon consisting of three fused benzene rings.[29] The phenanthrenes isolated from the stem of *D. nobile* include moscatin (19), nudol (20), bulbophyllanthrin (21), fimbriol B (22), plicatol A (23), lhridinusiant (24), coelonin (25), erianthridin (26), ephemeranthol A (EA) (27), ephemeranthol C (28), hircinol (29) and flavanthridin (30).[12] Zhang *et al*. isolated 5 phenanthrene compounds including fimbriatone (31), flavanthrinin (32), 4, 9-dimethoxyphenanthrene (33), 2, 2, 6-diol (34), 2, 5-diol, 5, 7-dimethoxyphenanthrene (35) and confusarin (36) from stem extracts of *D. nobile*.[15] Another three phenanthrene derivatives were isolated from *D. nobile* and were structurally characterized as 3,7-dihydroxy-2,4-dimethoxy-9,10-dihydrophenanthrene (37), 3,7-dihydroxy-2,4-dimethoxyphenanthrene (38), and 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene (39).[16] In addition, Kim *et al*. isolated three phenanthrenes, namely EA (27), 1,5,7-trimethoxyphenanthren-2-ol (40), and dehydroorchinol (DO) (41) from *D. nobile*. These phenanthrenes were found to possess anti-inflammatory activities.[17]

Phenylpropanoids

Phenylpropanoids are a diverse family of organic compounds that are synthesized by plants from the amino acids phenylalanine and tyrosine.[30] Five new phenylpropanoids were first isolated from *D. nobile*, namely decumbic acids A (42), decumbic acids B (43), (−)-decumbic acid (44), (−)-dendrolactone (45) and (+)-dendrolactone (46), which have been reported to possess antifungal and antitumor activities.[18]

Polysaccharides

Polysaccharides are a type of biomacromolecule composed of ten or more monosaccharides for which the structure and sugar composition vary, and are one of the active

and important ingredients of TCM.[31] Several types of polysaccharides have been identified in Dendrobium plants. They are usually considered to be one of the main ingredients in *D. nobile* and have been shown to have obvious biological activity, such as immunomodulatory, anti-tumor, and antioxidant activities. Research findings have shown that five water-soluble polysaccharides, including Dendrobium Nobile polysaccharide (DNP)-W1 (47), DNP-W2 (48), DNP-W3 (49), DNP-W4 (50), and DNP-W5 (51) have been isolated from the stems of *D. nobile* and possess significant immune-modulatory activity.[19,20] Among these, two water-soluble polysaccharides, namely, DNP-W1 (47) and DNP-W3 (49) exhibited significant antitumor activities. One study reported the isolation of four polysaccharides from the dry stem of *D. nobile* including L-arabinose (52), D-galactose (53), D-glucose (54), and D-mannose (55) through subcritical water extraction.[21]

Fluorenone and phenolic acid

To date, apart from the above-mentioned chemical classes of components, other types of chemical constituents, such as phenolic acids and fluorenone, have also been purified from *D. nobile*. Briefly, compounds including syringic acid (56), 2-hydroxyphenylpropanol (57), vanillin (58), apocynin (59), coniferyl aldehyde (60), syringaldehyde (61), syringylethanone (62), p-hydroxybenzaldehyde (63), 3-hydroxy-4-methoxyphenylethanol (64), α-hydroxysyringylethanone (65), dihydroxyconiferyl alcohol (66), p-hydroxybenzoic acid (67), and p-hydroxyphenylpropionic acid (68) were identified as phenolic acids and were shown to exhibit antioxidant activity.[32] Dendroflorin (A1) (69), denchrysan A (A1) (70), dengibsin (A1) (71), and nobilone (72) were identified as derivatives of fluorenone.[22]

Pharmacological Effects of *D. nobile* and the Associated Molecular Mechanisms

D. nobile possesses multiple biological functions, including anti-tumor, anti-inflammatory, cardioprotective, neuroprotective, anti-aging, hypoglycemic, and hypolipidemic effects. The biological activities of *D. nobile* and the related molecular mechanisms are shown in Table 2 and Figure 2, respectively.

Anti-tumor effects

Multiple *in vitro* and *in vivo* studies have demonstrated the significant inhibitory effect of *D. nobile* against a variety of tumor cells. The possible mechanisms responsible for the antitumor activity of the isolated compounds from *D. nobile* are shown in Table 2. Two water-soluble polysaccharides of *D. nobile*, namely DNP-W1 and DNP-W3 exhibited potent antitumor activities against Sarcoma 180 *in vivo* and human promyelocytic leukemia HL-60 cells *in vitro*.[33]

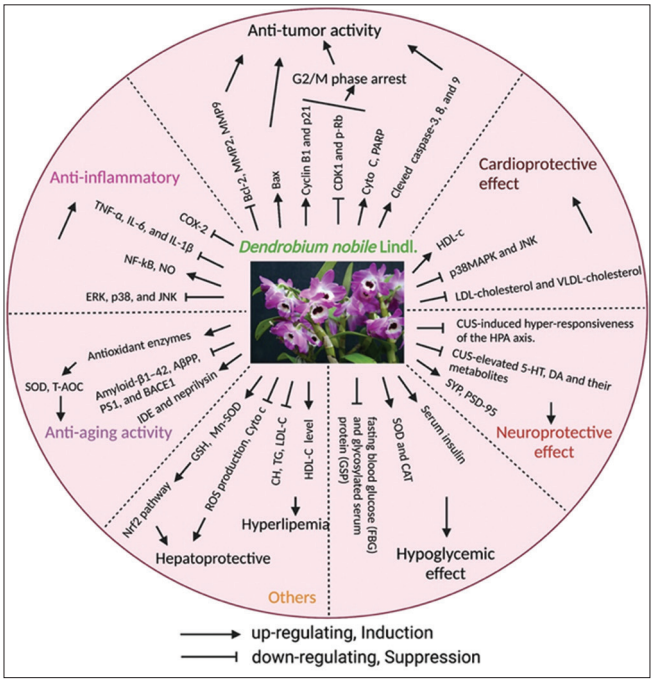

Figure 2 Schematic representation of the mechanisms underlying the bioactivities of the compounds isolated from *D. nobile*

Similarly, another study demonstrated that Nudol (20), a phenanthrene compound from *D. nobile*, induced cell cycle arrest (G2/M phase) and apoptosis and inhibited migration in osteosarcoma U2OS cells.[16] Dendrobine has been shown in an *in vivo* study to enhance the anticancer effect of cisplatin on non-small cell lung cancer cells via c-Jun N-terminal kinase (JNK)/p38 stress signaling, and could induce apoptosis via the pro-apoptotic proteins Bax and Bim.[34] In addition, denbinobin, another phenanthrene from *D. nobile,* was reported to induce apoptosis and inhibit cancer cell invasion in human gastric SNU-484 cells.[5] Moreover, two phenanthrenes compounds isolated from the aerial part of *D. nobile* viz, 4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene and denbinobin, were found to be cytotoxic against A549 (human lung carcinoma), SK-OV-3 (human ovary adenocarcinoma), and HL-60 (human promyelocytic leukemia) cell lines. The compounds also showed antitumor activity and enhanced lifespan on sarcoma 180 ICR mice.[35]

Anti-inflammatory effects

The anti-inflammatory effects of phenanthrenes EA (27), TP (40) and DO (41) were evaluated, and the results showed that EA, TP and DO at 50 μg/mL exhibited significant cytotoxic effects toward RAW 264.7 cells and attenuated inducible nitric oxide synthase protein level in the lipopolysaccharide (LPS) stimulated RAW 264.7 cells. EA significantly lowered the mRNA levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-1β in the LPS-activated cells. Notably, the mRNA levels of IL-6

Table 2 Chemical constituents of *Dendrobium nobile* and their pharmacological activities

Effects	Compound/extract	Type	Dosage/duration	Effect/mechanism	Reference
Anti-tumor	DNP-W1-6	*In vitro*	200 μg/mL	Exhibiting significantly higher antitumor activities against the growth of Sarcoma 180 tumor cells	[33]
	DNP-W1 and DNP-W3	*In vivo*	40 mg/kg (for 10 days)	Significantly inhibiting HL-60 leukemia cells growth	[33]
	Nudol	*In vitro*	20 μM	Inhibiting the viability of U2OS cells by enhancing the cell cycle arrest in the G2/M phase, induced cell apoptosis and inhibit cell migration	[16]
	Dendrobine	*In vitro/in vivo*	10 μg/mL; 50 mg/kg (for 10 days)	Enhancing chemotoxicity of cisplatin against A549 lung cancer cells *in vitro* and *in vivo*	[34]
	Denbinobin	*In vitro*	10 μM	Inducing apoptosis and inhibit cancer cell invasion in human gastric SNU-484 cell	[5]
	4,7-dihydroxy-2-methoxy-9, 10-dihydrophenanthrene and denbinobin	*In vivo*	20 mg/kg	Exhibiting a toxic effect on ICR mice	[35]
Anti-inflammatory	EA, (41) TP, (42) DO	*In vitro*	25 μg/mL	Exhibiting anti-inflammatory properties in activated macrophages by blockage of NF-κB activation and phosphorylation of MAP kinases in the macrophages	[17]
Cardioprotective	DNLA	*In vivo*	6 mg/kg	Alleviating the myocardial injury after ischemia-reperfusion	[36]
	SA	*In vitro*	10 μM	Attenuating renal I/R injury	[37]
	DNL	*In vivo*	80 mg/kg	Increased the potency in protecting the aged hearts against I/R injury and reduced the myocardial infarction, and improved myocardial function	[38]
Neuroprotective	DNLA	*In vitro*	0.25, 2.5 mg/L	Protecting cortical neurons against $A\beta_{25-35}$-mediated neurotoxicity and synaptic damage	[39]
	DNLA	*In vivo*	20 mg/kg	Attenuating anxiety/depression-like behavior and neuronal damage induced by CUS	[40]
Anti-aging	DNLA	*In vivo*	20, 40 mg/kg	Against aging-related cognitive deficits, neuron aging, damage, and loss	[41]
	DNLE	*In vitro*	50 μg/mL	Eliminating free radicals and suppresses cellular proteins expression disorder to protect cells from oxidant damage	[42]
Hypoglycemic	DN-powder	Clinic study	12 g/day	Hypoglycemic and lipid-lowering effects	[43]
	DNP	*In vivo*	200, 100, 50 mg/kg	Significant hypoglycemic activities	[44]
Others	DNLA	*In vivo*	10, 20 mg/kg	Improving CCl4-induced liver injury	[45]
	DNP	*In vivo*	40, 80, 160 mg/kg	Modulate fat extraordinary and abate liver fatty degeneration of the hyperlipemia rat model	[46]

I/R: Ischemia-reperfusion, EA: Ephemeranthol A, TP: 1,5,7-trimethoxyphenanthren-2-ol, DO: Dehydroorchinol, SA: Syringic acid, DNLA: Dendrobium nobile Lindl. Alkaloids, DNP: *Dendrobium nobile* polysaccharide, MAP: Mitogen-activated protein, ICR: Insitute of cancer research, CUS: Chronic unpredictable stress

and TNF-α decreased when compared with the level of the LPS-untreated control. DO also attenuated the mRNA levels of TNF-α, IL-6, and IL-1β in the LPS-activated cells, EA and DO downregulate the expression of LPS-stimulated COX-2 which is known as an inflammatory enzyme and a converting enzyme of prostaglandins. The inhibitory activities were associated with suppressing nuclear factor kappa-B (NF-κB) activation and phosphorylation of mitogen-activated protein kinases (MAP) kinases in macrophages.[17]

Cardioprotective effects

Cardiovascular diseases are the major cause of mortality in many parts of the world. Cardiomyopathy and high blood pressure are the most significant risk factors for cardiovascular diseases. Studies have suggested that *D. nobile* exerts its cardioprotective activity by ameliorating myocardial ischemia/reperfusion (I/R) injury. It has been shown that *Dendrobium nobile* Lindl. alkaloids (DNLA) may alleviate myocardial injuries after I/R through inhibiting the abnormal expression of FAT/CD36, decreasing the uptake of free fatty acids and reducing the abnormal accumulation of long-chain acyl-coenzyme A in the myocardium.[36] Studies have also laid bare that *D. nobile*-derived syringic acid attenuated renal I/R injury, and the related mechanisms were associated with downregulation of B-cell lymphoma 2 expression and

suppression of the expression of Bcl-2-like protein 4 (Bax) and cleaved caspase-3 in H9c2 cardiomyocytes induced by hypoxia/reoxygenation. SA also alleviated H/R-induced phosphorylation of p38 MAP kinase and JNK in H9c2 cardiomyocytes.[37] Moreover, *D. nobile* Lindl increased the potency of SA in protecting aged hearts against I/R injury, and the protective mechanism may be related to the reduction in the activity of the TLR4/MyD88/NF-κB signaling pathway and subsequent modulation of inflammatory cytokines and endogenous antioxidants.[38]

Neuroprotective effects

Alzheimer, characterized by the progressive deterioration of learning, memory and cognition, is the most common form of neurodegenerative condition. *D. nobile* has been subjected to many studies for its neuroprotective activity. It was revealed that *D. nobile*-derived DNLA could reduce the cytotoxicity induced by Aβ25-35 in cultured rat primary neurons. The protective mechanism that DNLA confers on the synaptic integrity of cultured neurons might be mediated, at least in part, via the upregulation of neurogenesis related proteins synaptophysin and postsynaptic density-95.[39] Another study showed that DNLA attenuated anxiety/depression-like behavior and neuronal damage in a chronic unpredictable stress rat model. The mechanisms may be related to the modulation of CUS-induced aberrant hippocampal gene expression, such as the decreasing of adrenocorticotropic hormones and expression of corticotropin-releasing hormone receptor-1, increasing expression of glucocorticoid receptor in the brain.[40] Additionally, DNLA significantly improved learning and memory functions in APP/PS1 transgenic mice owing to its ability to promote intracellular Aβ degradation by increasing the protein level of v-ATPase A1 and improving autolysosomal acidification and proteolysis.[47] Overall, DNLA displayed excellent neuroprotective properties and it could be a promising compound for development as a pharmaceutical drug for neurodegenerative diseases.

Anti-aging

Aging is a natural phenomenon and is the primary risk factor for the functional decline observed in most human body organs.[41] Aging is regulated by genes and the living environment. The human lifespan is partially determined by genetics (20%–30%) and partially by environmental factors such as lifestyle, diet, and intake of poisons and drugs. As alluded to the above aspects, DNLA is the active ingredient in *D. nobile* to improve learning and memory declines. Another study also reported the anti-aging effects out of long-term administration of DNLA during the aging process in the senescence-accelerated mouse-prone 8 (SAMP8) mice. The results showed that DNLA could protect against aging-related cognitive deficits, neuron aging, damage, and loss in SAMP8 mice and aging cells. The underlying mechanisms were associated with increased Aβ clearance, activation of autophagic activity, and upregulation of Klotho.[41] Many lines of evidence suggest that oxidative stress is linked to human diseases and aging. The research results showed that pretreatment with *D. nobile* liquor extract (DNLE) could attenuate the oxidative damage to cells caused by H_2O_2 and suppress the aberrant protein expressions caused by oxidative stress. Moreover, DNLE was also capable of mitigating the unfolded protein response and cell cycle disorder caused by oxidative stress.[42]

Hypoglycemic effect

Metabolic syndrome is a common chronic disease characterized by obesity, dyslipidemia, raised blood pressure, and high glucose levels. MS significantly augments the risk of type 2 diabetes and adversely affects human health. Several studies have extensively explored the antidiabetic potential of *D. nobile* using various experimental models. The findings revealed that *D. nobile* exhibited an outstanding hypoglycemic effect. *D. nobile* has been proven for its hypoglycemic and lipid-lowering effects without obvious side effects.[43] A number of experimental studies have shown that *D. nobile* plays an important role in glucose and lipid metabolism. *D. nobile* could reduce the serum levels of aspartate aminotransferase and alanine aminotransferase, attenuate the production of malondialdehyde (MDA), and improve ultrastructural morphology in hepatocytes to reduce the CCl_4- induced liver damage. *D. nobile* is also beneficial to the expression of genes involved in glucose and lipid metabolism due to its ability to enhance the expression of genes related to the Nrf2-antioxidant pathway.[48,49] Oral administration of polysaccharides from *D. nobile* (DNP) confers significant hypoglycemic activities evidenced by the decreased levels of fasting blood glucose and glycosylated serum protein, as well as by the increased level of serum insulin in alloxan-induced diabetic mice.[44]

Other pharmacological effects

It was found that *D. nobile* also has hepatoprotective and hypolipidemic effects. One study showed that DNLA, a *D. nobile*-derived alkaloid, improved CCl_4-induced liver injury, and the mechanism was related to the improvement of mitochondrial oxidative stress and mitochondrial dysfunction, evidenced by a decrease in mitochondrial H_2O_2 content and MDA production, as well as a marked increase in glutathione level and Mn-superoxide dismutase activity. The effect of DNLA-modulated hepatoprotection is dependent on the activation of the Nrf2 signaling pathway.[45] Furthermore, DNLA has also been shown to have glucose-lowering and antihyperlipidemic effects. Repeated administration of DNLA for 8 days modulated liver metabolism genes for glucose (Glut2, Glut4, FoxO1, PGC1α) and lipid (Acox1, Cpt1a, Srebp1, PPARα and ATGL/Pnpla2). In addition, DNLA could also induce hepatic antioxidant components (MT-1 and Nqo1) and decrease mRNA transcription from the Srebp1

gene in the liver. DNLA treatment was able to 'program' the liver under normal physiological conditions and constitutes a pharmacological basis for DNLA use in hyperglycemia and hyperlipidemia treatment.[49] In addition, DNP could not only extraordinarily modulate fat but also abate liver fatty degeneration in a hyperlipemia rat model. The mechanisms of the effects of DNP on hyperlipemia and liver fatty degeneration were possible through decreasing the levels of cholesterol, triglyceride, and low-density lipoprotein cholesterol, as well as increasing the level of high-density lipoprotein cholesterol.[46]

Conclusions and Future Perspectives

D. nobile, is one of the most popular species of *Dendrobium* and it has long been considered as a precious herb and health food in TCM and folk medicine. This review has so far summarized the botany, phytochemical constituents, and pharmacological activities of *D. nobile*. The health and pharmaceutical industries have been increasingly interested in *D. nobile* due to its multiple biological properties, as well as its various health and nutritional benefits. Phytochemically, *D. nobile* mainly contains alkaloids, bibenzyl, phenanthrene, phenylpropanoids, polysaccharides, fluorenone, and phenolic acid [Table 1]. Some of these representative compounds are the biologically active constituents of *D. nobile* with extensive pharmacological properties which include anti-tumor, anti-inflammatory, cardioprotective, neuroprotective, anti-aging, hypoglycemic, hypolipidemic and hepatoprotective activities. The relevant research published so far indicates that *D. nobile* is a promising candidate to treat diabetes and cardiovascular diseases. Furthermore, *D. nobile* can be applied in the prevention and treatment of oxidative stress and aging-related diseases.

A number of problems exist in the research that aims to bridge the gap between biological activities and the bioactive constituents of *D. nobile,* and these need to be elucidated further. First, although many studies have so far concentrated on the crude extracts of *D. nobile,* the compounds from these crude extracts responsible for the observed bioactivities still remain largely elusive. Hence, in-depth investigations to clarify the biological actions and mechanisms of the active constituents found in *D. nobile* should be conducted further. Secondly, information related to the toxicity of *D. nobile* is still lacking. Further study on toxicity and safety studies of *D. nobile* should be carried out. Finally, efforts need making to clarify the pharmacokinetics of *D. nobile* involving absorption, distribution, metabolism, and excretion studies, and to identify the metabolites of the bioactive phytochemicals using *in vivo* experimental models.

In conclusion, *D. nobile* as one of the common medicinal herbs in TCM appears to have great potentials as a functional food and candidate for further development into pharmaceutical agents. Future investigations should focus on in-depth explorations of on the biological functions of this commonly prescribed Chinese herb to maximize its health-related benefits.

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Ethical approval

This study does not contain any studies with human or animal subjects performed by any of the authors.

Author contributions

Zhi-Xiu Lin, Hong-Xi Xu and Yan-Fang Xian conceived and designed the study. Juan Zhang drafted the manuscript. Zhi-Li Zhao provided the photo for the manuscript. Yan-Fang Xian, Hong-Xi Xu and Zhi-Xiu Lin revised the manuscript.

Conflict of interest

None.

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Clinical Research on *Cinnamomi Cortex*: A Scoping Review

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Abstract

There are over 250 species of cinnamon. Each has its distinct morphology and phytochemical composition, which may result in varied therapeutic effects. However, clinical studies have rarely put emphasis on the species of cinnamon being investigated. This scoping review summarized the clinical evidence of *Cinnamomum cassia* (also known as *Cinnamomum aromaticum*), which is the species of cinnamon used in traditional Chinese medicine. Electronic searches were conducted on PubMed from its inception till August 2021. Clinical studies that were published in English, stating monotherapy with *Cinnamomum cassia*, *Cinnamomum aromaticum*, or "Rou Gui" were included. The 15 included clinical studies investigated the effects of *Cinnamomum cassia* on type-2 diabetes patients (n=7), and healthy adults (n=8). In the type 2 diabetes population, *Cinnamomum cassia* supplementation of as low as 1 g/d seemed to improve HbA1c in only poorly controlled diabetes. In the healthy population, *Cinnamomum cassia* supplementation appeared to influence blood glucose response in a dose-dependent manner, with current studies indicating a minimum of 5 g/d to achieve significant improvement. Studies also showed potential improvement in insulin sensitivity with prolonged *Cinnamomum cassia* supplementation. However, there were apparent heterogeneity among studies and uncertainties regarding the accuracy of reported cinnamon species. Therefore, the therapeutic effects of *Cinnamomum cassia* remain inconclusive. Future larger scale and more rigorous clinical studies, with clear identification of *Cinnamomum* species used, are needed for more conclusive evidence of the clinical effects of *Cinnamomum cassia*.

Keywords: *Cinnamomum cassia*, *Cinnamo*, Rou Gui, review, clinical trial

Introduction

Cinnamomi Cortex is part of the Lauracae family, and its genus *Cinnamomum* contains more than 250 species.[1] Each of the species has specific morphological characteristics and phytochemical compositions.[1,2] However, there is often a lack of clear indications of the *Cinnamomum* species being investigated within studies,[3-7] and systematic reviews often evaluate "cinnamon supplementation" without differentiating the species.[8-19]

The Chinese herb known as "Rou Gui" has been recorded in the Chinese Pharmacopoeia as the bark of *Cinnamomum cassia* (syn. *Cinnamomum aromaticum*).[2,20,21] This scoping review aims to summarize the currently available clinical evidence on this specific species of cinnamon used in traditional Chinese medicine.

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Methods

Data sources and search strategies

Electronic searches were conducted on PubMed from its inception to August 2021. The search strategy used here was as follows:

a. "rou gui"
b. "rougui"
c. "cinnamon"
d. "cassia"
e. "cinnamomi cortex" [Supplementary Concept]
f. "cinnamomum" [Mesh]
g. "cinnamomum aromaticum" [Mesh]
h. "cinnamomum cassia"

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i. "Chinese cinnamon"
j. "Chinese cassia"
k. "cinnamon bark"
l. #a or #b or #c or d or #e or #f or #g or #h or #i or #j or #k
m. "Randomized Controlled Trials as Topic" [Mesh]
n. "Randomized Controlled Trial" [Publication Type]
o. "Clinical Trial" [Publication Type]
p. "Clinical Trials as Topic" [Mesh]
q. "Controlled Clinical Trial" [Publication Type]
r. "Clinical Study" [Publication Type]
s. "Clinical Studies as Topic" [Mesh]
t. "Observational Study" [Publication Type]
u. #m or #n or #o or #p or #q or #r or #s or #t
v. #l AND #u.

Study selection criteria

Studies were included if they were published in English; if they were clinical studies (i.e., randomized controlled trials, controlled trials, observational studies) that evaluated therapeutic effects in humans; if they specifically mentioned monotherapy with *Cinnamomum cassia*, *Cinnamomum aromaticum*, "Rou Gui," or Chinese Cassia, and if the full article were retrievable. Studies were excluded if they did not specify the *Cinnamomum* species used or mentioned the use of species other than *Cinnamomum cassia*, *Cinnamomum aromaticum*, "Rou Gui," or Chinese Cassia. Studies that mentioned the use of "Gui Zhi" or cinnamon twigs were also excluded because that refers to a different herb used in traditional Chinese medicine. Studies were also excluded if the intervention involved a formula or a mixture of herbs or if the intervention involved specific phytochemical compounds of cinnamon.

Study screening and data extraction

A data extraction sheet was created by using Microsoft Excel based on the data items listed on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis extension for Scoping Reviews.[22]

All hits from the electronic database searches were exported to EndNote X9,[23] and duplicates were removed by using the "Find Duplicates" function. The initial screening of titles and abstracts, subsequent full article screening, and data extraction were conducted by one author and checked by another author. Any discrepancies were resolved through discussion. The final extracted data were tabulated into summary tables for ease of reference.

Results

Description of studies

In total, 206 hits were obtained from the electronic searches [Figure 1]. After initial screening, 165 were excluded for not meeting the inclusion criteria. Forty-one full articles were sought, but one full article could not be obtained. Among the 40 full articles for evaluation, 19 articles did not specify the *Cinnamomum* species used, four used species other than *Cinnamomum cassia*, while one did not use monotherapy because it used *Cinnamomum cassia* mixed with zinc gluconate and tricalcium phosphate, and one was an editorial. A total of 15 studies were included in this review.

The examined studies included five from the United States, three from the United Kingdom, two from Sweden, and one each from China, Germany, the Netherlands, Pakistan, and Switzerland. Eleven studies used capsules containing freshly ground or extracts of *Cinnamomum cassia* or *Cinnamomum aromaticum*, three studies added cinnamon to a meal, and one study used cinnamon tablets. The dosages of cinnamon ranged from 0.5 g to 14.4 g/d with varying administration protocols. Eleven studies compared the intervention to placebo, one study compared the intervention to usual care, and three studies that added cinnamon to meals compared the intervention to the same meal without cinnamon.

Seven studies involved patients with type-2 diabetes,[24-30] with one study specifically focusing on type-2 diabetes in postmenopausal patients.[30] Another eight studies enrolled healthy subjects,[31-38] including one study involving both healthy and obese adults,[35] one study involving overweight or obese adults with impaired fasting glucose,[36] one study involving obese women,[37] and one study involving healthy adults with impaired glucose tolerance.[38] It is interesting to note that all the included studies investigated physiological parameters relating to blood glucose.

Type-2 diabetes population

Among the seven studies involving patients with type 2 diabetes, six of them were placebo-controlled studies, and one study compared cinnamon to usual care [Table 1].[26] The latter study only evaluated hemoglobin A1c (HbA1c) and found that supplementation with 1 g/d *Cinnamomum cassia* for 90 days significantly lowered HbA1c and that the treatment was effective as an adjunct to usual care. The author attributed the positive outcome to the study population of patients with "poorly controlled" type 2 diabetes.

Among the six placebo-controlled studies, the outcomes were conflicting without an obvious correlation with the cinnamon dosage or treatment duration. The study by Khan *et al.*[27] was one of the first clinical studies using *Cinnamomum cassia*, and it has been a common reference and comparator for newer studies. Their study found that daily supplementation with 1 g, 3 g, or 6 g of *Cinnamomum cassia* for 40 days significantly improved fasting blood glucose, low-density lipoprotein (LDL), triglyceride (TG), and total cholesterol levels. However, later studies indicated that Khan *et al.*'s study may have obtained positive outcomes because the study

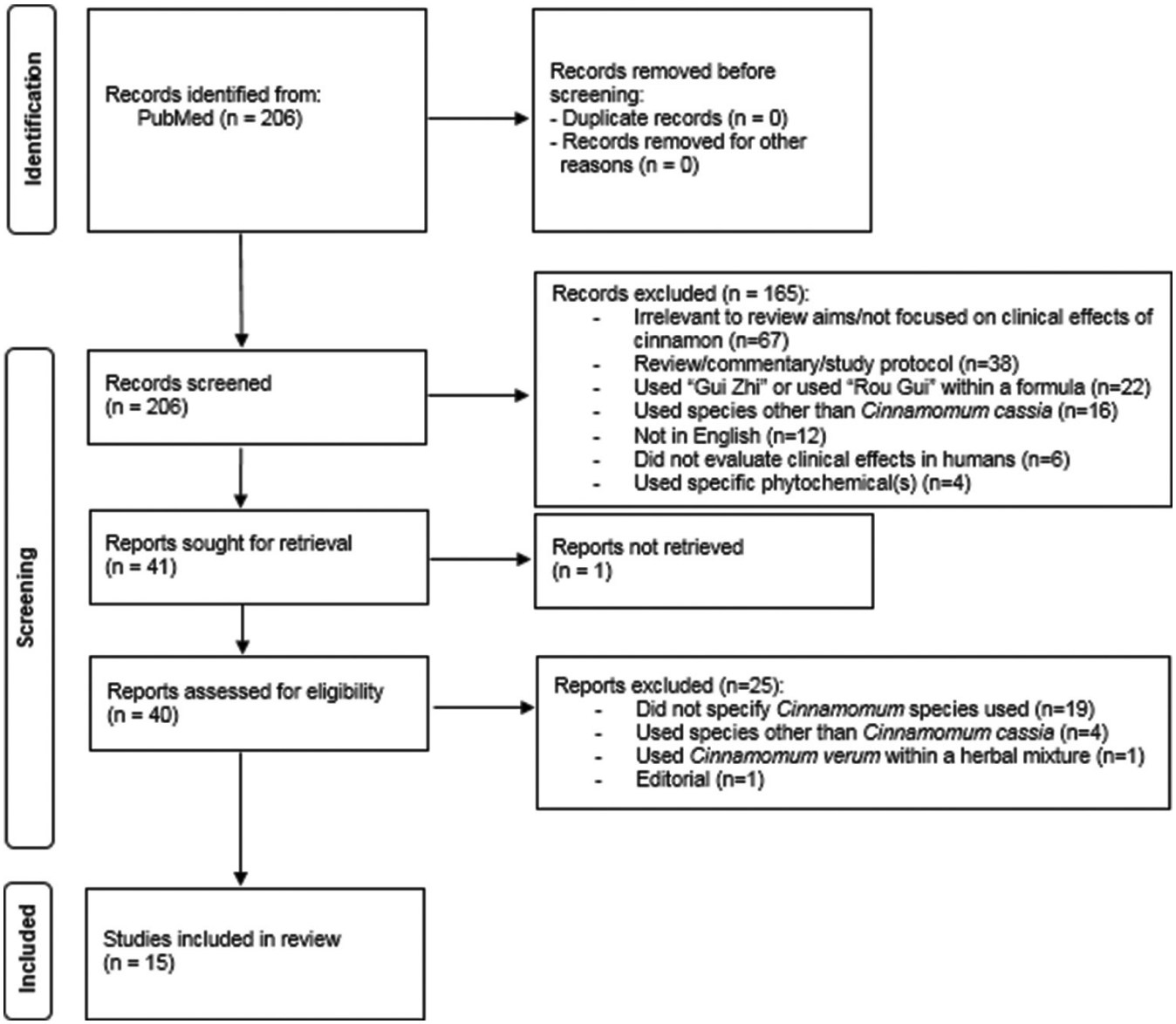

Figure 1 Study flow diagram

population had poorly controlled diabetes, citing the high fasting glucose level at baseline.[25,29,30] Although Khan *et al.* did not evaluate HbA1c, Mang *et al.* stated that the mean fasting blood glucose in the former study corresponded to HbA1c levels of 8.0%–10.5%.[29] Lu *et al.*[28] highlighted that *Cinnamomum cassia* supplementation significantly improved HbA1c levels in studies of patients with higher baseline HbA1c levels, as observed in three clinical studies of patients with a baseline level of HbA1c >8%,[24,26,28] in addition to Khan *et al.*'s study.

While, most studies employed cinnamon powder in capsules, it is noteworthy that two studies applied *Cinnamomum cassia* extracts, which may be a confounding factor when comparing interventions. One study indicated that each capsule contained 60 mg of aqueous extract isolated from 2.4 g of cinnamon, and the daily dose of cinnamon was two or six capsules.[28] This may be interpreted as 4.8 g of cinnamon every two capsules and 14.4 g of cinnamon every six capsules, and the latter dose was significantly higher than other studies, which may have impacted study outcomes. Another study used an aqueous extract in which each capsule contained 112 mg of extract, equivalent to 1 g of cinnamon.[29] Although the daily dose of cinnamon in this study was comparable to those in other studies, the different forms of the extract could be a potential explanation for the significant improvement in fasting blood glucose even though the baseline HbA1c levels were <8%. This extract form was selected as a safer option because it contained <0.1% coumarin and <0.1% essential oil to avoid a potential influence on blood coagulation and reduce allergic potential.[29] Moreover, the aqueous fraction of the extracts

Table 1 Summary of studies of type 2 diabetes populations

Author, origin, year	Sample size, study method	Treatment duration	Cinnamon treatment regime	Control intervention	Outcome improved in the cinnamon group	
					Significantly	Insignificantly
Crawford, US, 2009[26]	109 (55 cinnamon, 54 control), RCT	90 days	Cinnamon capsules 1 g daily ± oral antidiabetics/ insulin	Oral antidiabetics/ insulin	HbA1c	-
Akilen *et al*., UK, 2010[24]	58 (30 cinnamon, 28 control), RCT	84 days	Cinnamon (powder-filled) capsules 2 g daily + oral antidiabetics	Starch-filled capsules 2 g daily + oral antidiabetics	HbA1c, systolic and diastolic blood pressure	FBG, HDL, LDL, TG, total cholesterol, body weight, waist circumference, BMI
Blevins *et al*., US, 2007[25]	57 (29 cinnamon, 28 control), RCT	90 days	Cinnamon capsules 0.5 g twice daily ± oral antidiabetics	Wheat flour-filled capsules 0.5 g twice daily ± oral antidiabetics	None	HbA1c, FBG, HDL, LDL, TG, total cholesterol, blood insulin
Khan *et al*., Pakistan, 2003[27]	60 (10 subjects/ group), RCT	40 days, then 20 days washout period	Cinnamon (finely ground-filled) capsules 0.5 g (1 g, 3 g or 6 g daily) + oral antidiabetics	Wheat flour-filled capsules 0.5 g (1 g, 3 g, or 6 g daily) + oral antidiabetics	FBG, HDL (3 g/d group only), LDL, TG, total cholesterol	HDL (1 and 6 g/d groups)
Lu *et al*., China, 2012[28]	66 (23 cinnamon 0.12 g/d, 23 cinnamon 0.36 g/d, 20 control), RCT	90 days	Cinnamon tablets (from aqueous extract) 0.12 g or 0.36 g daily + oral antidiabetics	2 placebo (unspecified ingredients) tablets daily + oral antidiabetics	−0.12 g and 0.36 g daily: HbA1c, FBG −0.12 g daily: TG	HDL, LDL, Total cholesterol, AST, ALT
Mang *et al*., Germany, 2006[29]	65 (33 cinnamon, 32 control), RCT	120 days	Cinnamon capsule (from aqueous extract) 1 g thrice daily + oral antidiabetics or diet	Cellulose-filled capsule thrice daily + oral antidiabetics or diet	FBG	HbA1c, HDL, LDL, TG, total cholesterol
Vanschoonbeek *et al*., the Netherlands, 2006[30]	25 (12 cinnamon, 13 control), RCT	42 days	Cinnamon (powder-filled) capsules 0.5 g thrice daily + oral antidiabetics	Wheat flour-filled capsules 0.5 g thrice daily + oral antidiabetics	None	HbA1c, FBG, blood insulin, HDL, LDL, TG, total cholesterol, insulin sensitivity and resistance

ALT: alanine transaminase; AST: aspartate aminotransferase; BMI: body mass index; FBG: fasting blood glucose; HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; LDL: low-density lipoprotein; RCT: randomized controlled trial; TG: triglyceride

contains the vital constituents of *Cinnamomum cassia* that enhance the effects of insulin.

Healthy population

The remaining eight included studies involved healthy populations, including a subgroup of healthy adults with impaired glucose tolerance and a subgroup of healthy but overweight or obese people with impaired fasting glucose or impaired glucose tolerance [Table 2]. One study involved two study populations: normal healthy young adults (aged 18–30) and obese (body mass index [BMI]≥30) young adults (aged 18–30).[35] However, the authors found no difference in the glycemic response between the two populations, attributing the lack of abnormalities in glucose metabolism in this obese population to the subjects being otherwise young and healthy. The authors subsequently incorporated the two populations into one group to compare the effects of the two interventions. Accordingly, this review evaluated the results of the paper under the "healthy population." Meanwhile, another study that included obese but otherwise healthy but otherwise healthy women, stated that, of their 10 participants, four may have had impaired fasting glucose, and another two met the criteria for impaired glucose tolerance.[37] Therefore, this study was evaluated under the subgroup of healthy but overweight or obese with impaired fasting glucose or impaired glucose tolerance.

Four studies evaluated the acute effects of cinnamon on glycemic response when it was taken 12 h before or together with a meal,[31-33,35] whereas one study evaluated the effects of cinnamon when taken daily for 14 days as well as the persistence of the effects after stopping cinnamon.[34] Hlebowicz *et al*.[31] found that 3 g of cinnamon taken with a meal significantly reduced postprandial insulin levels and increased glucagon-like peptide 1 levels. Although 1 g of cinnamon also showed a decrease in postprandial insulin levels, the difference was insignificant, plausibly because of the small sample size. Hlebowicz *et al*. also postulated that a higher dose of cinnamon might be required to influence the gastric emptying rate and postprandial blood glucose concentrations. This notion is apparently supported by the significant reduction of postprandial blood glucose levels in other studies that applied 5 g-6 g of cinnamon.[33,35] Interestingly, one study that employed 4 g of cinnamon found no significant changes in postprandial blood glucose levels.[32] Aside from the dose of cinnamon, the authors also considered the difference

Table 2 Summary of studies of healthy populations

Author, origin, year	Study population	Sample size, study method	Treatment duration	Cinnamon intervention	Control intervention	Outcome improved in cinnamon group	
						Significantly	Insignificantly
Hlebowicz *et al*., Sweden, 2009[31]	Healthy adults	15, crossover study	3 sessions (1 week interval)	300 g of rice pudding mixed with 1 or 3 g of *C. cassia* in different sessions	300 g of rice pudding without *C. cassia* in the other session	Postprandial: insulin, GLP-1	GER, satiety Postprandial: blood glucose, GIP, ghrelin
Mettler *et al*., Switzerland, 2009[32]	Healthy adults, non-smokers	27, crossover study	4 sessions (completed within 4 weeks, ≥2 days interval)	Milk rice meal + 4 g *C. cassia*, 28 mmol acetic acid, or cinnamon-acetic acid combination	Milk rice meal	-	Satiety, postprandial blood glucose
Magistrelli and Chezem, US, 2012[35]	Normal (BMI=18.5-24.9) or obese (BMI ≥30), aged 18-30 years	30, crossover design	2 sessions with ≥7 days interval	74 g of cream of wheat instant farina cereal with 6 g of ground cinnamon in 1 session	74 g of cream of wheat instant farina cereal in another session	Postprandial blood glucose	-
Solomon and Blannin, UK, 2007[33]	Healthy, lean, sedentary males	7, crossover design	3 sessions with ≥5 days interval	Wheat flour-filled capsules 5 g at 12 h and *C. cassia* capsule 5 g just before OGTT Cinnamon capsule 5 g at 12 h and just before OGTT	Wheat flour-filled capsule 5 g at 12 h and just before OGTT	Plasma glucose response, insulin sensitivity during OGTT	Serum insulin response, fasting insulin sensitivity and insulin secretion index
Solomon and Blannin, UK, 2009[34]	Healthy, lean, sedentary males	8, single-blind crossover study	Days 0-14: cinnamon or control; days 15-19 control; 2 weeks washout period prior to starting another arm	Cinnamon (powdered) capsule 3 g daily	Wheat flour-filled capsule 3 g daily	Plasma glucose response, plasma insulin response, insulin sensitivity	-
Wickenberg *et al*., Sweden, 2014[38]	Adults with impaired glucose tolerance	17 (9 cinnamon, 8 control), RCT	84 days	Cinnamon capsules 6 g twice daily	Cellulose-filled capsule 700 mg twice daily	-	HbA1c, FBG, fasting insulin, insulin sensitivity, HDL, LDL, TG, total cholesterol, AST, ALT, bilirubin, ALP, GT, PK
Gutierre *et al*., US, 2016[37]	Young, sedentary, obese (BMI unspecified) women	10, crossover study	2 sessions (1 week interval)	Take cinnamon capsule 5 g in 1 session	Take cellulose-filled capsule 5 g in another session	Plasma glucose response (at 30 min post-OGTT), serum insulin	Insulin resistance and sensitivity
Roussel *et al*., US, 2009[36]	Overweight or obese (BMI=25-45) adults, IFG (FBG=100-125 mg/dL	22 (11 cinnamon, 11 control), RCT	84 days	Cinnamon (from dried aqueous extract) capsule 250 mg twice daily	Placebo (ingredient unspecified) twice daily	FRAP, plasma MDA, plasma SH groups, FBG	RBC antioxidant enzymes, fasting insulin

ALT: alanine transaminase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BMI: body mass index; FBG: fasting blood glucose; FRAP: ferric reducing activity of plasma; GER: gastric emptying rate; GIP: glucose-dependent insulinotropic polypeptide; GLP-1: glucagon-like peptide 1; GT: gamma-glutamyl transferase; HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; IFG: Impaired fasting glucose; LDL: low-density lipoprotein; OGTT: oral glucose tolerance test; PK: pyruvate kinase; Plasma MDA: plasma malondialdehyde; Plasma SH groups: plasma thiol groups; RBC: red blood cells; RCT: randomized controlled trial; TG: triglyceride; *C. cassia*: *Cinnamomum cassia*

in the carbohydrate/cinnamon ratio and suggested that a carbohydrate/cinnamon ratio of approximately 15 or lower, as recorded in other studies,[33,35] may be required to achieve a dose-response effect on postprandial blood glucose.

In the study on prolonged cinnamon supplementation, daily supplementation with 3 g of cinnamon for 14 days significantly reduced plasma glucose levels and insulin response starting from Day 1, as well as improved insulin sensitivity on Day 14, indicating that prolonged cinnamon supplementation may be a factor in improving insulin sensitivity.[34] In relation to the aforementioned dose-response requirement, this study, which employed 3 g of cinnamon, detected significantly reduced plasma glucose levels on Day 1, but the change did not achieve significance on Day 14. The authors speculated that the study was underpowered to detect significance, which would have been achievable with a larger sample size.

Healthy adults with impaired glucose tolerance

There was only one study in this subgroup; however, the baseline BMI of the study population was notably above 25, indicating that participants were likely overweight.[38] This study found no significant changes in fasting blood glucose, fasting insulin, HbA1c, insulin sensitivity, lipid profiles, and liver enzymes in the experiment group. It should be noted that the dosage of cinnamon used in the study was unclear because the dosage reported in the abstract, discussion, and conclusion was 12 g/d, but the methods section reported the use of two capsules a day, with each capsule containing 0.5 g of cinnamon and 0.2 g of cellulose. Cinnamon extracts may have been used so that each capsule contained extracts equivalent to 6 g of cinnamon, but this was not clarified. As such, it is difficult to compare the outcomes of this study with those of other studies. Furthermore, this study reported a significant decrease of fasting insulin levels and a significant increase of insulin sensitivity in the placebo group. Cellulose might have been a sufficient additional source of fiber that affected the insulin response. Another study that used microcrystalline cellulose observed the same effects.[29]

Healthy but overweight/obese population with impaired fasting glucose or impaired glucose tolerance

In this population, one study evaluated the acute effects of cinnamon, which was taken by participants 3 h before an oral glucose tolerance test,[37] whereas another study evaluated supplementation with cinnamon over 12 weeks.[36] The former reported significant improvements in plasma glucose response and serum insulin levels with 5 g of cinnamon,[37] which supported the aforementioned dose-response theory. In the latter study, the authors wanted to investigate the effects of cinnamon in improving oxidative stress in overweight or obese people, subsequently reducing the risk of further health complications.[36] They found that the cinnamon extract (Cinnulin PF) at 0.5 g/d for 12 weeks resulted in significant improvements in fasting blood glucose levels and oxidative stress markers such as plasma malondialdehyde, plasma thiol group oxidation, and ferric reducing activity of plasma. This study, however, did not report the amount of crude cinnamon that is equivalent to the dose of cinnamon extract that was used. The extract was reported to be sourced from *Cinnamomum cassia*, but further investigations revealed that Cinnulin PF is a registered trademark product that has been reported to be sourced from *Cinnamomum burmannii*.[40] An extract dose of 0.5 g was said to be equivalent to approximately 10 g of whole cinnamon powder (extract ratio of 20:1).[39]

While previous reviews discussed the clinical effects of cinnamon at length, this is the first review that focused on one specific species, namely *Cinnamomum cassia*. One of the main challenges in conducting this review was to identify studies that accurately reported the use of *Cinnamomum cassia*. Although all authors of the 15 studies included in this review mentioned the use of *Cinnamomum cassia*, we discovered potential inaccuracies in species identification, such as the use of Cinnulin PF, as discussed previously. Another example is a study that cited the use of *Cinnamomum cassia* from the commercially available brand Puritan's Pride;[26] however, on the brand's official website, the source of cinnamon quoted was *Cinnamomum burmannii*.[41]

The main bioactive component of *Cinnamomum cassia* is cinnamaldehyde (75%–90%),[42] where its content, as is outlined in the Pharmacopeia of the People's Republic of China, determines the quality of the plant.[43] Preclinical findings of cinnamaldehyde include antitumor activity by inducing cancer cells to apoptosis or by inhibiting tyrosinase activity in cancer cells. Cinnamaldehyde also demonstrates anti-inflammatory action through the suppression of lipopolysaccharide-stimulated inflammatory cytokines, nitric oxide production, and elevation of antioxidants such as catalase, superoxide dismutase, and glutathione peroxidase.[43] Coumarin, another bioactive compound found in the barks and twigs of *Cinnamomum cassia*, possesses analgesic effects. In the case of rats injected with chemotherapy oxaliplatin, coumarin blocked oxaliplatin-induced neuropathic cold allodynia by suppressing the activation of glial cells in the sacral spinal cord.[43] Other compounds of *Cinnamomum cassia* include polyphenols, benzaldehyde, cinnamic acid, eugenol, and cinnamyl alcohol, which contribute to its pharmacological effects such as hypoglycemic activity, antibacterial, antifungal, and cardioprotective actions.[43,44]

The names "cinnamon" and "cassia" have been used commonly and synonymously in the US.[45] Furthermore, many people

are unaware of or confused about the difference in species among the commercially available cinnamon, possibly because of labeling errors.[46] The importance of identifying the specific species used in clinical trials has been reported by many researchers,[30,45] especially in consideration of the difference in phytochemical compounds among different *Cinnamomum* species, which may result in different clinical effects. For example, Wickenberg reported a study that investigated the effects of *Cinnamomum cassia* on postprandial blood glucose and obtained positive outcomes, but another study using *Cinnamomum zeylanicum* did not replicate these results.[38] Furthermore, correctly identifying the species will allow the identification and management of potential health risks. For example, high coumarin levels, which were detected in *Cinnamomum cassia* but not in *Cinnamomum verum*,[20] may lead to adverse effects such as hepatoxicity[20] or blood coagulation problems,[32] especially in clinical trials where higher than usual doses may be used. With this information, researchers would be able to take appropriate precautions, monitor relevant parameters, and/or employ safer methodologies. For example, one study included the monitoring of liver enzymes,[38] whereas another study used an aqueous extract of *Cinnamomum cassia* that is nearly free of lipophilic compounds, including coumarin.[29] Chen *et al*.[45] further emphasized the importance of identifying the species of cinnamon and the importance of characterizing the composition of cinnamon samples before administration to facilitate comparisons between studies and elucidate potential differences in outcomes.

Conclusions

This scoping review included 15 clinical studies that used *Cinnamomum cassia* as an intervention, with all studies investigating outcomes related to the glycemic response. A summary of the findings is as follows: (1) As little as 1 g/d *Cinnamomum cassia* may improve HbA1c in patients with poorly controlled diabetes (HbA1c >8%); (2) There may be a dose-response relationship between *Cinnamomum cassia* and postprandial blood glucose levels, as a dose of at least 5 g of crude cinnamon appeared necessary in healthy or pre-diabetic people. This dose-response relationship was not observed in the type 2 diabetes population; (3) Prolonged cinnamon supplementation might improve insulin sensitivity; (4) Future studies on cinnamon should clearly identify the species of cinnamon used, as well as the form of cinnamon (i.e., powdered, extract, etc.), and enroll larger sample sizes for stronger evidence.

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Ethical approval

This study does not contain any studies with human or animal subjects performed by any of the authors.

Author contributions

Hsiewe Ying Tan and Wan Najbah Nik Nabil participated in conceptualization, research design and literature search; Hsiewe Ying Tan participated in data acquisition and data analysis; Hsiewe Ying Tan and Wan Najbah Nik Nabil prepared and edited the manuscript; Hong-Xi Xu edited and reviewed the manuscript.

Conflict of interest

None.

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Botany, Traditional Uses, and Pharmacology of *Polygonati Rhizoma*

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Abstract

Huang Jing (黄精 *Polygonati Rhizoma,* PR) was first documented as a herbal medicine in *Ming Yi Bie Lu* (《名医别录》 *Miscellaneous Records of Famous Physicians*) in China. However, there was no comprehensive review on the botany, traditional uses, and pharmacological effects of PR till now. In this study, the botany, traditional uses including Taoist medicine, and pharmacological effects of PR were reviewed and summarized to provide insights on drug development of PR. In Taoist medicine, PR maintains agerasia and helps prolong human life-span, and is used for fasting (Bigu). In the *Zhong Guo Yao Dian* (《中国药典》 *Chinese Pharmacopeia*) version 2020, PR exerts replenishing qi and nourishing yin, invigorating the spleen, moistening the lung, and strengthening the kidney. Pharmacological studies show that PR has effects of anti-oxidation, anti-diabetes, anti-osteoporosis, anti-cancer, anti-hyperlipidemia, cardiomyocyte protection, immunomodulatory, and thus can be used for treatment of infertility, anti-microorganisms, and improving sleep and memory. In conclusion, PR may play a potential role for chronic disease management and health preservation and this very role deserves a more in-depth research.

Keywords: Botany, pharmacology, *Polygonati Rhizoma,* materia medica

Introduction

Huang Jing (黄精 *Polygonati Rhizoma*, PR) is the dried rhizomes from *Polygonatum kingianum* Coll. et Hemsl. (滇黄精), *Polygonatum sibiricum* (PS) Red. (黄精), and *P. cyrtonema* Hua (多花黄精). It is one of the most popular traditional Chinese medicine items and Taoist medicine items. PR has been used as complementary food for Taoist fasting.[1,2] The Chinese name of PR, Huang Jing, refers to "essence of immortals," which origins from Taoist theory.[3] It was firstly documented in *Ming Yi Bie Lu* (《名医别录》 *Miscellaneous Records of Famous Physicians*) in China during the Northern and Southern Dynasties, in which its properties was described as "sweet, neutral and no toxic", and functions as "tonifying the spleen and stomach (middle jiao) and replenishing qi, expelling wind and damp, and calming Five-zang."[4] However, there was no comprehensive review on PR especially its Taoist medicine folk use till now. In this study, the botany, traditional uses, and pharmacological effects of PR are to be reviewed and summarized to provide insights in drug development of PR.

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Botany

In the *Chinese Pharmacopeia* (Version 2020), the resource of drug PR is dried rhizomes from three species of Genus

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Polygonatum, Family *Liliaceae*, including *Polygonatum kingianum* Coll. et Hemsl. (滇黄精), PS Red. (黄精), and *Polygonatum cyrtonema* Hua (多花黄精). *P. kingianum* is characterized by pink or white flowers with leaves in whorls, linear to lanceolate, and apex strongly cirrose or curved; while PS as white and slight yellow flowers, and leaves in whorls, abaxially glaucous, linear-lanceolate; and *P. cyrtonema* as yellowish green flowers, and leaves in alternate, petiole short; leaf blade elliptic to oblong-lanceolate, occasionally falcate, apex usually acuminate [Table 1 and Figure 1].

Traditional Uses

The properties of PR are sweet and neutral. In fact, there are a variety of descriptions in meridian tropism and traditional uses in the classical works and the *Chinese Pharmacopeia*. In *Miscellaneous Records of Famous Physicians*, PR exerts tonify the middle jiao and replenish qi, expel wind and damp, and calm the Five-zang.

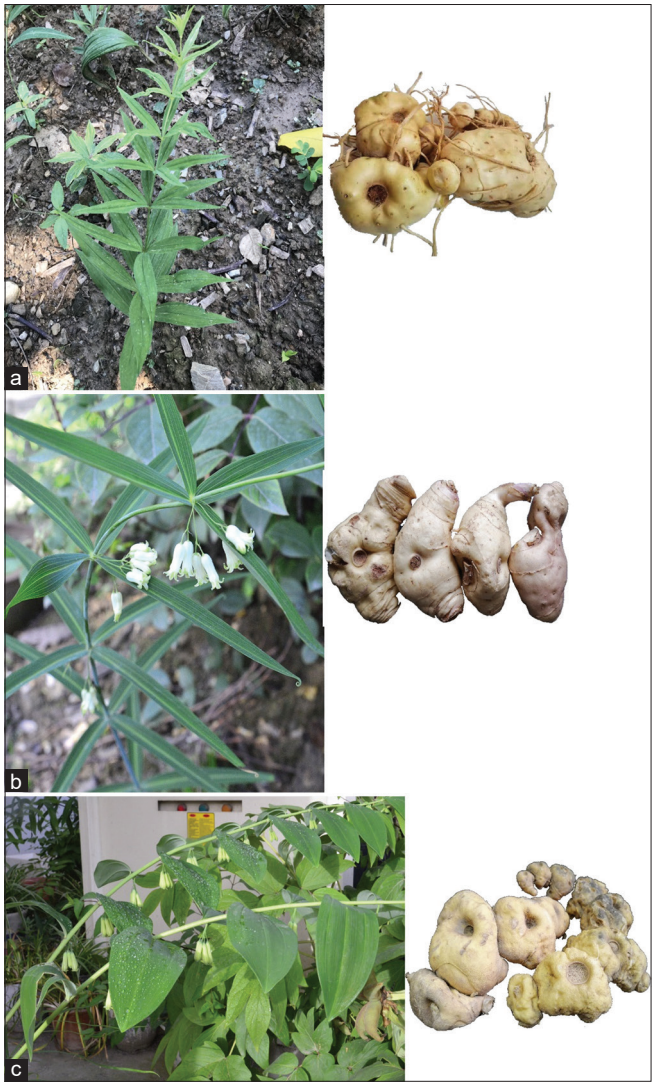

Figure 1 Three sources of *Polygonati Rhizoma* (a) *Polygonatum kingianum* Coll. et Hemsl. and its rhizomes (b) *Polygonatum sibiricum* Red. and its rhizomes (c) *Polygonatum cyrtonema* Hua and its rhizomes

Taoist medicine emphasizes that PR-contained formulae maintain agerasia (助颜), e.g., Wang Mu Niang Niang Zhu Yan Pill (王母娘娘驻颜仙丹), Guan Yin Li Fu Pill (观音丽肤丹),[8] and promote longevity (益寿), e.g., Wang Mu Si Tong Powder (王母四童散).[9] The Taoists have been preparing the PR-contained formulae, Er Jing Pill (二精丸), Huang Jing Pill (黄精丸), Huang Jing Wine (黄精酒), Huang Jing Di Huang Pill (黄精地黄丸), Bai Zhu Pill (白术丸),[2] and Bi Gu Formula (辟谷药饵)[10] for Taoist health preservation or fasting (辟谷 Bigu) for centuries.[8] These herbal formulae were made into pills or powder.[2,9] Furthermore, PR was documented in some classics of Chinese medicine by Taoist medical practitioners, e.g., *Dian Nan Ben Cao* (《滇南本草》 *Materia Medica in South Yunnan*), and *Zheng Tong Dao Cang* (《正统道藏》 *Collected Taoist Scriptures*) [Table 2].[3,11]

Now the authorized traditional usage is documented in the *Chinese Pharmacopeia* (Version 2020). PR is said to invade spleen, lung and kidney meridians, and exerts the effects of replenishing qi, nourishing yin, invigorating the spleen, moistening the lung, and strengthening the kidney [Table 3].[4]

Pharmacological Actions

Anti-oxidation

The anti-oxidative components of PR include PR water extracts, polysaccharides, galactoses and polyphenols. Polysaccharides in PS (PSP) inhibit the production of free radicals[19] and lipid peroxidation via increasing the activation of superoxide dismutase (SOD) and decreasing the level of malondialdehyde (MDA).[20] Thus PR water extracts and PSP protect liver from the oxidative stress.[21] The anti-oxidative capacity of PSP is more than that of the polyphenols.[22]

Anti-diabetes

The anti-diabetic components of PR include glucosides (saponins) and polysaccharides.[23] The saponins in PS regulate the gut microbiota, improve the state of insulin resistance), and increase glucose consumption, intracellular glycogen level and the activity of hexokinase and pyruvate kinase. It can also alleviate the symptoms of polyphagia and polydipsia in diabetic mice.[24] PSP decrease the levels of fasting blood glucose and glycated hemoglobin in blood and elevate the levels of insulin and C-peptide in plasma of diabetic rats.[25] Syringaresinol-di-O-β-d-glucoside, a phenolic glucoside in PS exerts an anti-diabetic effect on streptozocin-induced diabetic mice and the underlying mechanism may be associated with its anti-oxidative activity.[26]

Anti-osteoporosis

Osteoporosis is a common condition among aged people with osteopenia and decrease of bone strength. The main

Table 1 Botanical character of the three resources of *Polygonati Rhizoma*

Species	Chinese name	Main productive provinces	Botanical character
Polygonatum kingianum Coll. et Hemsl[5]	滇黄精/大黄精	Guangxi, Guizhou, Sichuan, Yunnan	Rhizome subterete or submoniliform, 1-3 cm thick. Stem erect, 1-3 m, glabrous, apex subscandent. Leaves in whorls of 3-10, sessile, linear to lanceolate, 6-20 (or 25) cm length× 0.3-3.0 cm width, herbaceous or leathery, apex cirrose. Inflorescences 2-4 (or 1-6)-flowered; peduncle 1-2 cm, pendulous; bracts borne usually on proximal part of pedicel, small, membranous. Pedicel 0.5-1.5 cm. Perianth pink or white, cylindric-campanulate, 1.8-2.5 cm; lobes 3-5 mm. Filaments filiform or compressed, 1.7-5.0 mm, glabrous or slightly papillose; anthers 4-6 mm. Ovary 4-6 mm. Style 1 (or 0.8)-1.4 cm. Berries red, 1.0-1.5 cm in diameter, 7-12-seeded. Flowering: March to May; fruiting: September to October
Polygonatum sibiricum Red[6]	黄精/鸡头黄精	Anhui, Gansu, Hebei, Heilongjiang, Henan, Jilin, Liaoning, Nei Mongol, Ningxia, Shaanxi, Shandong, Shanxi, Zhejiang	Rhizome usually shortly branched, subterete or tuberous terete, 1-2 cm thick. Stem erect or sometimes subscandent, 50-90 (or 140) cm, glabrous. Leaves in whorls of 4-6, sessile, abaxially glaucous, linear-lanceolate, 8-15 cm×4-16 mm, glabrous, apex strongly cirrose or curved. Inflorescences umbel-like, usually 2-4-flowered; peduncle 1-2 cm; bracts borne at base of pedicel, subulate to linear-lanceolate, 3-5 mm, membranous, 1-veined, persistent. Flowers pendulous; pedicel 4 (or 2.5) -10 mm. Perianth milky white to pale yellow, cylindric, slightly constricted in middle, 0.9-1.2 cm; lobes ca. 4 mm. Filaments 0.5-1.0 mm; anthers 2-3 mm. Ovary ca. 3 mm. Style 5-7 mm. Berries black, 7-10 mm in diameter, 4-7-seeded. Flowering: May to June, fruiting: August to September
Polygonatum cyrtonema Hua[7]	多花黄精/姜形黄精	Anhui, Fujian, Guangdong, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi (Qin Ling Mountain Area), Sichuan, Zhejiang	Rhizome usually moniliform or tuberous moniliform, rarely subterete, 1-2 cm thick. Stem erect, 50-100 cm, glabrous. Leaves 10-15, alternate; petiole short; leaf blade elliptic to oblong-lanceolate, occasionally falcate, 10-18 × 27 cm, apex usually acuminate. Inflorescences umbel-like, 2 (or 1) -7 (or 14)-flowered; peduncle 1-4 (or 6) cm; bracts borne on proximal part of pedicel, small, or absent. Flowers pendulous; pedicel 0.5-1.5 (or 3) cm. Perianth yellowish green, campanulate-cylindric, 1.8-2.5 cm; lobes ca. 3 mm. Filaments slightly compressed, 3-4 mm, papillose or shortly cottony, apically slightly dilated or saccate-convex; anthers 3.5-4 mm. Ovary 3-6 mm. Style 1.2-1.5 cm. Berries black, ca. 1 cm in diameter, 3-9-seeded. Flowering: May to June, fruiting: August to October

anti-osteoporosis components are polysaccharides in PR. On the one hand, PSP induce the differentiation from bone marrow mesenchymal stem cells to osteoblasts.[27] On the other hand, PSP inhibit bone turnover and reverse bone loss in ovariectomized rats.[28]

Anti-cancer

A vast amount of studies has been reported that polysaccharides and steroidal glycosides can inhibit cancer through arresting cell cycle, inducing apoptosis, blocking invasion and migration, and improving immunofunction as well as inhibiting inflammation-cancer interactions. PSP arrest G0/G1 phase of H22 in mice,[29] and induce cancer cell apoptosis,[30] whereas methyl protodioscin in PS arrests G2/M phase and induces apoptosis in human cervical carcinoma HeLa cells.[31] In the case of cancer metastasis, PSP can block invasion and migration in human esophageal carcinoma cell line Eca109.[32] Regarding inflammation-cancer interaction, PSP enhance the activity of Natural Killer cells, inhibit the secretion of inflammatory cytokines to inhibit the proliferation of gastric cancer.[33]

Anti-hyperlipidemia

The active components for anti-hyperlipidemia are PSP and kaempferol. PSP regulate lipid metabolism-associated factors,[34] inhibit lipid oxidation,[35] and regulate gut microbiota.[36] Kaempferol reduces adipogenesis and balances lipid homeostasis.[37] Jiu Zhuan Huang Jing Pill (九转黄精丸), with PR as its main ingredient, relieves mitochondrial dysfunction and attenuates high-fat diet-induced metabolic dysfunction-associated fatty liver disease.[38]

Cardiomyocyte protection

The active components for anti-cardiomyocyte injury are polysaccharides. PSP decrease the level of interleukin-6, tumor necrosis factor-alpha and MDA, activate glutathione peroxidase, and reduce apoptosis of cardiomyocytes.[39]

Immunomodulation

The active component for immunomodulatory is PSP. PSP, which regulate immune function by promoting the development of immune organs, lymphocyte proliferation, and macrophage phagocytosis, increasing the spleen index and thymus index, as well as regulating serum cytokine levels.[40,41]

Treatment of infertility

PR decoction and water extracts treat infertility by regulating sperm abnormalities, and restoring damaged testicular tissue.[42]

Anti-microorganisms

The active anti-bacterial and anti-fungi components of PR are extracts of PR and polysaccharides. The effects of crude PR

Table 2 Traditional usages of *Polygonati Rhizoma*

Literature	Property	Meridian	Traditional usage
Ming Yi Bie Lu (《名医别录》 *Miscellaneous Records of Famous Physicians*)[4]	Sweet, neutral and not toxic	NA	Tonifying the middle jiao and replenishing qi, expelling wind and damp, and calming Five-zang
Si Sheng Ben Cao (《四声本草》 *Materia Medica of Si Sheng*)[12]	Cold	Spleen, lung and kidney	NA
Ri Hua Zi Ben Cao (《日华子本草》 *Materia Medica of Ri Hua Zi*)[13]	Sweet and neutral	NA	Relieving fatigues and dysfunctions, assisting sinew and bone, stopping hunger, resisting cold and heat, tonifying the spleen and stomach, and nourishing the heart and lung, improving looks and prolonging life
Kai Bao Ben Cao (《开宝本草》 *Materia Medica of the Kaibao Reign*)[14]	Sweet, neutral and not toxic	NA	Tonifying the center and replenishing qi, expelling wind and damp, and calming Five-zang, and prolonging life by a long-time administration
Lei Gong Pao Zhi Yao Xing Jie (《雷公炮制药性解》 *Master Lei's Discourse on Medical Property During Processing*)[15]	Sweet, neutral and not toxic	Spleen and lung	Tonifying the center and replenishing qi, expelling wind and damp, and calming Five-zang, improving looks and prolonging life by a long-time administration
Dian Nan Ben Cao (《滇南本草》 *Materia Medica in South Yunnan*)[11]	NA	NA	Supplementing the deficiency and strengthening essence
Ben Cao Gang Mu (《本草纲目》 *Compendium of Materia Medica*)[16]	Sweet, neutral and not toxic	NA	Supplementing various deficiencies, resisting cold and heat, strengthening essence, and dispelling the evil and parasites
Zheng Tong Dao Cang (《正统道藏》)[3]	NA	NA	Tonifying the middle jiao and replenishing qi, calming Five-zang, strengthening sinew and bone, improving looks and blackening hair, prolonging life, and dispelling the evil and parasites
Ben Cao Qiu Zhen (《本草求真》 *Seeking Accuracy in Materia Medica*)[17]	Sweet	Entering spleen dominantly, and lung and kidney	Tonifying the center and Five-zang, replenishing the spleen and stomach, nourishing the heart and lung, supplementing essence, strengthening sinew and bone, expelling wind and damp, relieving hungriness
Chinese Pharmacopeia (V.2020)[18]	Sweet and neutral	Spleen, lung and kidney	Replenishing qi and nourishing yin, invigorating the spleen, moistening the lung, and strengthening the kidney

NA: Not available

Table 3 Formulae containing *Polygonati Rhizoma* in the *Chinese Pharmacopeia* (edition 2020)

Number	Formula name	Components	Traditional usages	Dosage
1	Yi Gan Yang Yin Huo Xue Granules (乙肝养阴活血颗粒)	Rehmanniae Radix 66.67 g, Glehniae Radix 83.33 g, Ophiopogonis Radix 66.67 g, Ligustri Lucidi Fructus (with wine) 83.33 g, Schisandrae Chinensis Fructus 55.56 g, Astragali Radix 111.11 g, Angelicae Sinensis Radix 66.67 g, Polygoni Multiflori Radix Praeparata 83.33 g, paeoniae Radix Alba 83.33 g, Asini Corii Colla 83.33 g, Lycopi Herba 83.33 g, Ostreae Concha 111.11 g, Citri Exocarpium Rubrum 55.56 g, Saviae Miltiorrhizae Radix Rhizoma 111.11 g, Toosendan Fructus 55.56 g, PR (steamed) 83.33 g	Tonifying the liver and kidney, activating blood and dissipating stasis	10-20 g, p.o., t.i.d
2	Tian Ma Shou Wu Tablet (天麻首乌片)	Gastrodiae Rhizoma 33.75 g, Angelicae Dahuricae Radix 26.25 g, Polygoni Multiflori Radix Praeparata 56.25 g, Rehmanniae Radix Praeparata Praeparata 56.25 g, Saviae Miltiorrhizae Radix Rhizoma 56.25 g, Chuanxiong Rhizoma 22.5 g, Angelicae Sinensis Radix 75 g, Tribuli Fructus Praeparata 37.5 g, Mori Folium 37.5 g, Ecliptae Herba 75 g, Ligustri Lucidi Fructus (with wine) 75 g, paeoniae Radix Alba 75 g, PR (steamed) 75 g, Glycyrrhizae Radix et Rhizoma 11.25 g	Tonifying the liver and kidney, activating blood and arresting wind	6 tablets, p.o., t.i.d
3	Gu Han Yang Sheng Jing Liquid (古汉养生精口服液)#	Ginseng Radix et Rhizoma, Astragali Radix (Praeparata), Rosae Laevigatae Fructus, Lycii Fructus, Ligustri Lucidi Fructus (Praeparata), Cuscutae Semen, Epimedii Folium, Paeoniae Radix Alba, Glycyrrhizae Radix et Rhizoma Praparata Cum Melle, Hordei Fructus Germinatus (fried), PR (Praeparata)	Tonifying qi, nourishing the kidney, and essence	10-20 ml, p.o., b.i.d or t.i.d
4	Gu Han Yang Sheng Jing Tablet (古汉养生精片)#	Ginseng Radix et Rhizoma, Astragali Radix (Praeparata), Rosae Laevigatae Fructus, Lycii Fructus, Ligustri Lucidi Fructus (Praeparata), Cuscutae Semen, Epimedii Folium, Paeoniae Radix Alba, Glycyrrhizae Radix et Rhizoma Praparata Cum Melle, Hordei Fructus Germinatus (fried), PR (Praeparata)	Tonifying qi, nourishing the kidney, and essence.	10-20 g, p.o., b.i.d
5	Gu Han Yang Sheng Jing Granules (古汉养生精颗粒)#	Ginseng Radix et Rhizoma, Astragali Radix (Praeparata), Rosae Laevigatae Fructus, Lycii Fructus, Ligustri Lucidi Fructus (Praeparata), Cuscutae Semen, Epimedii Folium, Paeoniae Radix Alba, Glycyrrhizae Radix et Rhizoma Praparata Cum Melle, Hordei Fructus Germinatus (fried); PR (Praeparata)	Tonifying qi, nourishing the kidney, and essence	10-20 g, p.o., b.i.d

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Table 3 Contd...

Number	Formula name	Components	Traditional usage	Dosage
6	Feng Liao Xing Feng Shi Die Da Medical Wine (冯了性风湿跌打药酒)	Erycibes Caulis 2500 g, Platycodomis Radix 75 g, Ephedrae Herba 93.8 g, Notopterygii Rhizoma et Radix 7.5 g, Angelicae Sinensis Radix 7.5 g, Chuanxiong Rhizoma 7.5 g, Angelicae Dahuricae Radix 7.5 g, Psoraleae Fructus 7.5 g, Olibanum 7.5 g, Gleditsiae Fructus Abnormalis 7.5 g, Citri Reticulatae Pericarpium 33.1 g, Atractylodis Rhizoma 7.5 g, Magnoliae Officinalis Cortex 7.5 g, Cyperi Rhizoma 7.5 g, Aucklandiae Radix 7.5 g, Aurantii Fructus 50 g, Atractylodis Macrocephalae Rhizoma 7.5 g, Dioscoreae Rhizoma 7.5 g, PR 20 g, Cuscutae Semen 7.5 g, Foeniculi Fructus 7.5 g, Armeniacae Semen Amarum 7.5 g, Alismatis Rhizoma 7.5 g, Trogopterus Faeces 7.5 g, Bombyx Mori Faeces 16.2 g, Moutan Cortex 7.5 g, Myrrha 7.5 g	Dispelling wind, relieving damp, activating the blood and arresting pain	10-15 ml, p.o., b.i.d or t.i.d
7	Lao Nian Ke Chuan Tablet (老年咳喘片)	Astragali Radix 110 g, Atractylodis Macrocephalae Rhizoma 66 g, Saposhnikoviae Radix 66 g, Glycyrrhizae Radix et Rhizoma 44 g, PR 66 g, Epimedii Folium 66 g, Psoraleae Fructus 66 g	Supplementing qi and Yang, strengthening and restoring	4-6 tablets, t.i.d
8	Zai Zao Sheng Xue Tablet (再造生血片)	Cuscutae Semen (with wine) 85 g, Ginseng Radix et Rhizoma Rubra 25.5 g, Spatholobi Caulis 59.5 g, Asini Corii Colla 25.5 g, Angelicae Sinensis Radix 42.5 g, Ligustri Lucidi Fructus 25.5 g, Astragali Radix 42.5 g, Leonuri Herba 25.5 g, Rehmanniae Radix Praeparata 42.5 g, Paeoniae Radix Alba 25.5 g, Polygoni Multiflori Radix Praeparata 42.5 g, Epimedii Folium 25.5 g, PR (with wine) 34 g, Cervi Cornu Pantotrichum (without fur) 25.5 g, Codonopsis Radix 34 g, Ophiopogonis Radix 25.5 g, Agrimoniae Herba 34 g, Atractylodis Macrocephalae Rhizoma (Praeparata) 25.5 g, Psoraleae Fructus (with salt) 25.5 g, Lycii Fructus 34 g, Ecliptae Herba 25.5 g	Tonifying the liver and kidney, nourishing qi and blood	5 tablets, p.o., t.i.d
9	Zai Zao Sheng Xue Capsule (再造生血胶囊)	Cuscutae Semen (with wine) 85 g, Ginseng Radix et Rhizoma Rubra 25.5 g, Spatholobi Caulis 59.5 g, Asini Corii Colla 25.5 g, Angelicae Sinensis Radix 42.5 g, Ligustri Lucidi Fructus 25.5 g, Astragali Radix 42.5 g, Leonuri Herba 25.5 g, Rehmanniae Radix Praeparata 42.5 g, Paeoniae Radix Alba 25.5 g, Polygoni Multiflori Radix Praeparata 42.5 g, Epimedii Folium 25.5 g, PR (with wine) 34 g, Cervi Cornu Pantotrichum (without fur) 25.5 g, Codonopsis Radix 34 g, Ophiopogonis Radix 25.5 g, Agrimoniae Herba 34 g, Atractylodis Macrocephalae Rhizoma (Praeparata) 25.5 g, Psoraleae Fructus (with salt) 25.5 g, Lycii Fructus 34 g, Ecliptae Herba 25.5 g	Tonifying the liver and kidney, nourishing qi and blood	5 capsules, p.o., t.i.d
10	Zhuang Yao Jian Shen Pill (壮腰健身丸)	Ligustri Lucidi Fructus (with wine) 24 g, PR 24 g, Rehmanniae Radix Praeparata 36 g, Rosae Laevigatae Fructus 24 g, Cibotii Rhizoma 24 g, Polygoni Multiflori Radix Praeparata 15 g, Flemingia Prostrata Radix 30 g	Strengthening the waist and kidney	9 g, p.o., b.i.d
11	Qi Zhi Jiang Tang Tablet (芪蛭降糖片)	Astragali Radix 1000 g, Rehmanniae Radix 830 g, PR 830 g, Hirudo 670 g	Tonifying qi and yin, activating blood and dissipating stasis	5 tablets, p.o., t.i.d
12	Qi Zhi Jiang Tang Capsule (芪蛭降糖胶囊)	Astragali Radix 1000 g, Rehmanniae Radix 830 g, PR 830 g, Hirudo 670 g	Tonifying qi and yin, activating blood and dissipating stasis	5 capsules, p.o., t.i.d
13	Shen Yan Shu Tablet (肾炎舒片)	Atractylodis Rhizoma 125 g, Poria 150 g, Imperatae Rhizoma 125 g, Stephaniae Tetrandrae Radix 75 g, Ginseng Radix 50 g, PR 75 g, Cuscutae Semen 75 g, Lycii Fructus 75 g, Lonicerae Japonicae Flos 125 g, Taraxaci Herba 150 g	Tonifying the kidney and spleen, inducing urination and dispersing swelling	6 tablets, p.o., t.i.d
14	Jin Hua Ming Mu Pill (金花明目丸)	Rehmanniae Radix Praeparata 210 g, Cuscutae Semen (with salt) 140 g, Lycii Fructus 140 g, Schisandrae Chinensis Fructus 21 g, Paeoniae Radix Alba 70 g, PR 210 g, Astragali Radix 140 g, Codonopsis Radix 70 g, Chuanxiong Rhizoma 63 g, Chrysanthemi Flos 42 g, Cassiae Semen (fried) 70 g, Plantaginis Semen (fried) 70 g, Buddleja officinalis Flos 42 g, Galli Gigerii Endothelium Corneum (fried) 70 g, Fagopyri Dibotryis Rhizoma 70 g, Crataegi Fructus 70 g, Cimicifugae Rhizoma 42 g	Tonifying the liver and kidney, and improving eyesight	4 g, p.o., t.i.d
15	Jiang Zhi Ling Tablet (降脂灵片)	Polygoni Multiflori Radix Praeparata 222 g, Lycii Fructus 222 g, PR 296 g, Crataegi Fructus 148 g, Cassiae Semen 44 g	Tonifying the liver and kidney, activating blood and improving eyesight	5 tablets, p.o., t.i.d
16	Jiang Zhi Ling Granules (降脂灵颗粒)	Polygoni Multiflori Radix Praeparata 369.8 g, Lycii Fructus 369.8 g, PR 493.1 g, Crataegi Fructus 246.6 g, Cassiae Semen 73.3 g	Tonifying the liver and kidney, activating blood and improving eyesight	3 g, p.o., t.i.d

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Table 3 Contd...

Number	Formula name	Components	Traditional usage	Dosage
17	Jiang Tang Jia Tablet (降糖甲片)	Astragali Radix 428.4 g, PR (with wine) 428.4 g, Rehmanniae Radix 428.4 g, Pseudostellariae Radix 428.4 g, Trichosanthis Radix 428.4 g	Tonifying qi of the middle jiao, nourishing yin and producing body fluid	
18	Shen Jing Zhi Ke Pill (参精止渴丸)	Ginseng Radix et Rhizoma Rubra 135 g, Astragali Radix 135 g, PR 270 g, Poria 135 g, Atractylodis Macrocephalae Rhizoma 135 g, Puerariae Lobatae Radix 135 g, Schisandrae Chinensis Fructus 27 g, Coptidis Rhizoma 27 g, Rhei Radix et Rhizoma 27 g, Glycyrrhizae Radix et Rhizoma 27 g	Tonifying qi of the middle jiao, producing body fluid and quenching thirsty	10 g, p.o., b.i.d or t.i.d
19	Wei An Capsule (胃安胶囊)	Dendrobii Caulis 50 g, Phellodendri Chinensis Cortex 50 g, Adenophorae Radix 100 g, Crataegi Fructus 100 g, Aurantii Fructus (fried) 100 g, PR 100 g, Glycyrrhizae Radix et Rhizoma 50 g, Paeoniae Radix Alba 50 g	Nourishing Yin and the stomach, softening the liver and arresting pain	2 g, p.o., t.i.d
20	Huo Li Su Liquid (活力苏口服液)	Polygoni Multiflori Radix Praeparata 1000 g, Epimedii Folium 300 g, PR (Praeparata) 440 g, Lycii Fructus 300 g, Astragali Radix 440 g, Saviae Miltiorrhizae Radix Rhizoma 220 g	Tonifying qi and blood, Nourishing the liver and kidney	10 ml, p.o., q.d
21	Huo Xue Tong Mai Tablet (活血通脉片)	Carthami Flos 36 g, Dalbergiae Odoriferae Lignum 36 g, Curcumae Radix 45 g, Notoginseng Radix et Rhizoma 91 g, Chuanxiong Rhizoma 27 g, Citri Reticulatae Pericarpium 91 g, Aucklandiae Radix 36 g, Acori Tatarinowii Rhizoma 45 g, Lycii Fructus 91 g, PR (with wine) 182 g, Ginseng Radix et Rhizoma 45 g, Ophiopogonis Radix 91 g, Borneolum Syntheticum 9 g	Promoting flow of qi and blood circulation, freeing arteries and veins and arresting pain	5-8 tablets, p.o., t.i.d or q.i.d
22	Jin Li Da Granules (津力达颗粒)	Ginseng Radix et Rhizoma 184.5 g, PR 244.5 g, Atractylodis Rhizoma (fried with wheat bran) 122.2 g, Sophorae Flavescentis Radix 100 g, Ophiopogonis Radix 244.5 g, Rehmanniae Radix 184.5 g, Polygoni Multiflori Radix Praeparata 149 g, Corni Fructus 244.5 g, Poria 149 g, Eupatorii Herba 100 g, Coptidis Rhizoma 100 g, Anemarrhenae Rhizoma 122.2 g, Epimedii Folium (Praeparata) 100 g, Saviae Miltiorrhizae Radix Rhizoma 160 g, Puerariae Thomsonii Radix 244.5 g, Lichi Semen 244.5 g, Lycii Cortex 149 g	Tonifying qi and yin, invigorating the spleen and inducing fluid	9 g, p.o., t.i.d
23	Guan Mai Ning Capsule (冠脉宁胶囊)	Saviae Miltiorrhizae Radix Rhizoma 112.5 g, Myrrha (fried) 25.5 g, Spatholobi Caulis 112.5 g, Draconis Sanguis 25.5 g, Corydalis Rhizoma (with vinegar) 45 g, Angelicae Sinensis Radix 45 g, Curcumae Radix 45 g, Polygoni Multiflori Radix Praeparata 75 g, Persicae Semen (fried) 30 g, 酒PR 75 g, Carthami Flos 30 g, Puerariae Lobatae Radix 112.5 g, Olibanum (fried) 25.5 g, Borneolum Syntheticum 4.5 g	Activating blood and dissipating stasis, promoting flow of qi and arresting pain	4-5 capusules, p.o., t.i.d
24	Jian Nao An Shen Tablet (健脑安神片)	PR (with wine) 47 g, Epimedii Folium 39 g, Lycii Fructus 16 g, Cervi Cornu Pantotrichum 0.8 g, Cervi Cornus Colla 2 g, Cervi Cornu Degelatinatum 5 g, Ginseng Radix et Rhizoma Rubra 2 g, Jujubae Fructus (without pit) 16 g, Poria 8 g, Ophiopogonis Radix 8 g, Testudinis Carapax et Plastrum 4 g, Ziziphi Spinosae Semen (fried) 8 g, Schisandrae sphenantherae Fructus 31 g, Polygalae Radix (Praeparata) 16 g, Rehmanniae Radix Praeparata 8 g, Xanthii Fructus 31 g	Tonifying and strengthening body, and tranquilizing the mind	5 tablets, p.o., b.i.d
25	Zhi Mai Kang Capsule (脂脉康胶囊)	Pu'er Tea 100 g, Acanthopanacis Senticosi Radix et Rhizoma Seu Caulis 100 g, Crataegi Fructus 100 g, Raphami Semen 50 g, Nelumbinis Folium 50 g, Puerariae Lobatae Radix 50 g, Chrysanthemi Flos 50 g, Astragali Radix 50 g, PR 50 g, Polygoni Multiflori Radix 100 g, Leonuri Fructus 50 g, Eucommiae Cortex 50 g, Rhei Radix et Rhizoma (with wine) 30 g, Notoginseng Radix et Rhizoma 50 g, Sophorae Flos 100 g, Taxilli Herba 50 g	Promoting digestion, lowering lipid, promoting blood circulation, and tonifying qi and blood	5 capsules, p.o., t.i.d
26	Tian Meng Liquid (甜梦口服液)	Acanthopanacis Senticosi Radix et Rhizoma Seu Caulis 53 g, PR 67 g, silkworm moth 13 g, Mori Fructus 33 g, Codonopsis Radix 40 g, Astragali Radix 40 g, Amomi Fructus 5 g, Lycii Fructus 40 g, Crataegi Fructus 160 g, Rehmanniae Radix Praeparata 27 g, Epimedii Folium (Praeparata) 27 g, Citri Reticulatae Pericarpium 27 g, Poria 27 g, Strychni Semen Praeparata 1.3 g, Pinelliae Rhizoma Praeparata 27 g, Alismatis Rhizoma 40 g, Dioscoreae Rhizoma 27 g.	Tonifying qi and the kidney, strengthening the spleen and stomach, and nourishing the heart and quieting the spirit	10-20 ml, p.o., b.i.d
27	Tian Meng Capsule (甜梦胶囊)	Acanthopanacis Senticosi Radix et Rhizoma Seu Caulis 178 g, PR 222 g, silkworm moth 44 g, Mori Fructus 111 g, Codonopsis Radix 133 g, Astragali Radix 133 g, Amomi Fructus 18 g, Lycii Fructus 133 g, Crataegi Fructus 533 g, Rehmanniae Radix Praeparata 89 g, Epimedii Folium (Praeparata) 89 g, Citri Reticulatae Pericarpium 89 g, Poria 89 g, Strychni Semen Praeparata 4.4 g, Pinelliae Rhizoma Praeparata 89 g, Alismatis Rhizoma 133 g, Dioscoreae Rhizoma 89 g	Tonifying qi and the kidney, strengthening the spleen and stomach, and nourishing the heart and quieting the spirit	3 capsules, p.o., b.i.d

Contd...

Table 3 Contd...

Number	Formula name	Components	Traditional usage	Dosage
28	Ke Le Ning Capsule (可乐宁胶囊)	Astragali Radix 312.5 g, PR (with wine) 312.5 g, Rehmanniae Radix 312.5 g, Pseudostellariae Radix 312.5 g, Trichosanthis Radix 312.5 g	Tonifying qi and yin, producing body fluid and quenching thirsty	4 capsules, p.o., t.i.d
29	Zhang Yan Ming Tablet (障眼明片)	Acori Tatarinowii Rhizoma 30 g, Cassiae Semen 30 g, Cistanches Herba 37 g, Puerariae Lobatae Radix 37 g, Celosiae Semen 30 g, Codonopsis Radix 48 g, Viticis Fructus 30 g, Lycii Fructus 48 g, Plantaginis Semen 37 g, Paeoniae Radix Alba 45 g, Corni Fructus 24 g, Glycyrrhizae Radix et Rhizoma 22 g, Cuscutae Semen 61 g, Cimicifugae Rhizoma 7 g, Prinsepiae Nux (without peel) 37 g, Chrysanthemi Flos 37 g, Buddleja officinalis Flos 37 g, Chuanxiong Rhizoma 30 g, PR (with wine) 37 g, Rehmanniae Radix Praeparata 61 g, Phellodendri Amurensis Cortex 30 g, Astragali Radix 48 g.	Tonifying the liver and kidney, and improving cataract and benefiting	0.8 g, p.o., t.i.d
30	Wen Xin Tablet (稳心片)	Codonopsis Radix 675 g, PR 900 g, Notoginseng Radix et Rhizoma 135 g, Amber 90 g, Nardostrachyos Radix et Rhizoma 450 g	Tonifying qi and yin, activating blood and dissipating stasis	4 tablets, p.o., t.i.d
31	Wen Xin Capsule (稳心胶囊)	Codonopsis Radix 675 g, PR 900 g, Notoginseng Radix et Rhizoma 135 g, Amber 90 g, Nardostrachyos Radix et Rhizoma 450 g	Tonifying qi and yin, activating blood and dissipating stasis	4 capsules, p.o., t.i.d
32	Wen Xin qi of the middle jiao (稳心颗粒)	Codonopsis Radix 300 g, PR 400 g, Notoginseng Radix et Rhizoma 60 g, Amber 40 g, Nardostrachyos Radix et Rhizoma 200 g	Tonifying qi and yin, activating blood and dissipating stasis	9 g or 5 g (without sugar), p.o., t.i.d
33	Ao Tai Le qi of the middle jiao (澳泰乐颗粒)	Asteris Radix et Rhizoma 1000 g, Curcumae Radix 50 g, PR 50 g, Paeoniae Radix Alba 15 g, Hordei Fructus Gerjminatus 100 g	Soothing the liver and regulating the normal flow of qi, clearing heat and detoxifying	5-15 g, p.o., t.i.d
34	Tang Mai Kang Tablet (糖脉康片)	Astragali Radix 240 g, Rehmanniae Radix 260 g, Paeoniae Radix Rubra 260 g, Saviae Miltiorrhizae Radix Rhizoma 240 g, Achyranthis Bidentatae Radix 150 g, Ophiopogonis Radix 150 g, Puerariae Lobatae Radix 150 g, Mori Folium 150 g, Coptidis Rhizoma 50 g, PR 150 g, Epimedii Folium 200 g	Norishing yin and clearing heat, activating blood and dissipating stasis, tonifying qi and kidney	
35	Tang Mai Kang Capsule (糖脉康胶囊)	Astragali Radix 200 g, Rehmanniae Radix 216.7 g, Paeoniae Radix Rubra 216.7 g, Saviae Miltiorrhizae Radix Rhizoma 200 g, Achyranthis Bidentatae Radix 125 g, Ophiopogonis Radix 125 g, Puerariae Lobatae Radix 125 g, Mori Folium 125 g, Coptidis Rhizoma 41.7 g, PR 125 g, Epimedii Folium 166.7 g	Nourishing yin and clearing heat, activating blood and dissipating stasis, tonifying qi and kidney	6 capsules, p.o., t.i.d
36	Tang Mai Kang qi of the middle jiao (糖脉康颗粒)	Astragali Radix 240 g, Rehmanniae Radixg, 260 g, Paeoniae Radix Rubra 260 g, Saviae Miltiorrhizae Radix Rhizoma 240 g, Achyranthis Bidentatae Radix 150 g, Ophiopogonis Radix 150 g, Puerariae Lobatae Radix 150 g, Mori Folium 150 g, Coptidis Rhizoma 50 g, PR 150 g, Epimedii Folium 200 g	Nourishing yin and clearing heat, activating blood and dissipating stasis, tonifying qi and kidney	5 g, p.o., t.i.d

#Content of the components is not given. p.o.: Oral administration; b.i.d: Twice a day; t.i.d: Triple a day; q.d: Once a day; PR: *Polygonati Rhizoma*

extracts are better than that of processed PR. The anti-bacterial effect of PR extracts, from high to low order is: N-butanol > ethyl acetate > water > petroleum ether. The order of anti-mold effect of PR extracts is: N-butanol > petroleum ether > ethyl acetate.[43] PSP inhibits *Escherichia coli*, *Salmonella enterica*, *Staphylococcus albus* and *Staphylococcus aureus*. PR is also used for anti-dermatophytes.[44]

Improving sleep

PS regulates nonrapid eye movement and increases the length of sleep. The active components extracted from PS contain oleamide, glyceryl monolinoleate, and γ-aminobutyric acid.[45-47]

Improving memory

The active components of PR that can improve memory competence include PS polysaccharides, total glucosides, and PS decoction and ethanol extracts. PS ethanol extracts and PSP relieve cerebral ischemia and oxidative stress.[48,49] PS decoction reduces the injury of neurons after global cerebral ischemia-reperfusion.[50] PSP also improve the learning and memory abilities of D-galactose-induced aging rats.[51] Moreover, PS-containing oral liquid (whose formulation includes PS, *Ginseng Radix et Rhizoma, Lycii Fructus*, and *Polygoni Multiflori Radix*) promotes synaptic remodeling and improves learning and memory in rats with vascular dementia.[52]

The progress made in both phytochemistry and pharmacology would facilitate the study of the potential roles in oxidation, diabetes, osteoporosis, cancer, hyperlipidemia, cardiovascular system, immunomodulatory, infertility, microorganisms, and mental and nervous system.

Future Perspectives and Conclusions

Chronic diseases including cardiovascular diseases, cancer, diabetes, cognitive disorder and memory loss, as well as aging are dominant factors that affect human life span.[53] Drugs and functional food for preventing and/or treating such diseases may prolong human life span. PR is an edible herbal medicine exerting replenishing qi, nourishing yin, invigorating the spleen, moistening the lung, and strengthening the kidney.[4] The Taoists have made formulae with PR into pills or powder for health preservation or fasting.[3,8,9] Fasting is a good strategy for preventing various chronic diseases, e.g., diabetes and hyperlipidemia.[54,55] Furthermore, PR is an edible herb which has little toxicity or side-effect indication. Thus, it is suggested that PR should be thoroughly studied about its effects on fasting, anti-oxidation, anti-diabetes, anti-cancer, anti-hyperlipidemia, as well as improving mental and nervous system. This may be a good way to reach the full potential of PR for either diseases treatment or health preservation.

In conclusion, PR may play a potential role for chronic disease management and health preservation. Thus, PR deserves more in-depth research.

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Ethical approval

This article does not contain any studies with human or animal subjects.

Author contributions

Xuan-Bin Wang, Hong-Liang Li, and Bei Li designed the study; Xiao-Jing Chen, Ju-Feng Duan, Kai-Qi Liu, Ying-Ying Guo, Ming Liu, Dong-Peng Wang, and Dan Zhao collected the data; Xiao-Jing Chen, Ju-Feng Duan and Kai-Qi Liu wrote the manuscript; Xuan-Bin Wang, Hong-Liang Li and Bei Li revised the manuscript. This manuscript has been read and approved by all the authors.

Conflicts of interest

None.

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Medicinal Uses of Agarwood

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Abstract

Agarwood from *Aquilaria* plants, also known as Chen Xiang (沉香), is traditionally used for the treatment of abdominal pain and as a sedative. Because of the great demand and the rareness of agarwood, extensive harvesting of *Aquilaria* plants has nearly led to the extinction of the species. To fully utilize this resource, the use of different parts of *Aquilaria* needs to be investigated. This article will focus on the pharmacological properties and the mechanism of action of different parts of *Aquilaria* plants.

Keywords: Agarwood, *Aquilaria*, Chen Xiang (沉香), Chinese medicine

Introduction

Agarwood is a dark resinous material found in the trunk of wounded *Aquilaria* plants. It is also known as Chen Xiang (沉香) in Chinese, *Kritsana* in Thai, and *Agar* in India. The word *Chen* (沉) means sink, while Xiang (香) means fragrance or incense. As the Chinese name implies, this wood has a strong fragrance and is burnt as incense. The aromatic oils present in agarwood mean agarwood to be heavier than water; thus, agarwood sinks rather than floats when placed in water. Currently, there are 17 species of *Aquilaria* known to produce agarwood. Among these species, *Aquilaria malaccensis* and *Aquilaria crassna* are the most well-known species and are commonly found in the Southeast Asian countries such as Thailand, Indonesia, Cambodia, Laos, Vietnam, and Malaysia. Agarwood is formed unevenly in the stem of the plant. Naturally grown *Aquilaria* generally does not contain a large amount of agarwood, and it is estimated that only 10% of natural *Aquilaria* species are potent to produce agarwood.[1] Generally, agarwood can only be obtained from injured *Aquilaria* plants. When injured, the plant responds to the stimulation by activating secondary biosynthetic pathways to produce a resinous compound. This response is initiated by injuries, such as wounds and bacterial[2] and fungal infections.[3] The main markets for agarwood are in the Middle East, South Asia, and East Asia. As the use of agarwood is increasing, there is a growing demand for agarwood. Because of both the difficulties in the production of agarwood and the high demand for the product, the price of agarwood ranges from 20-6000 USD/ kg per kilogram. In addition, the price of essential oils extracted from agarwood can be as high as 30, 000 USD/kg, depending on the grade and quality of the agarwood. The quality of agarwood was traditionally assessed by the resin content, density, color, scent/aroma, the agarwood-inducing method, formation time, and place of origin. Now, the quality assessment of agarwood is performed by chemical analysis which identifies the constituents in the agarwood.[4]

Agarwood is widely used in many areas of religion, literature, art, and medicine. In many religions, incense is burnt as a symbolic form of worship or offering to deities and spirits. This tradition can be traced back to ancient Egypt, where frankincense and myrrh resins were used to produce incense.[5] In China, the tradition of burning incense was widespread during the Song Dynasty. During that period, incense burning was considered one of the four arts of scholars, along with tea sipping, picture, and flower-branches arranging.[6] The ingredients for making incense mainly come from agarwood. Agarwood was a symbol of nobility. Incense can also be used

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for expelling insects that transmit diseases, such as mosquitoes and flies, which is a practice for disease prevention. The fragrance from incense can also be used for aromatherapy. The essential oil extracted from agarwood is added to cosmetics to enhance the scent of the products. The use of other parts of *Aquilaria* is often neglected when harvesting, as the wood from *Aquilaria* is soft and not suitable for making furniture. However, in some religions, *Aquilaria* wood is crafted into sculptures or ornaments.

Because of the great demand on agarwood, the extensive harvesting of *Aquilaria* has disrupted the natural growing cycle. In the past, agarwood was harvested from forests and the entire *Aquilaria* trees were cut down to obtain the dark resin inside the infected trunk. The resulting deforestation has led to the near extinction of the species. In 2005, *Aquilaria* species were listed as the endangered under the Convention on International Trade in Endangered Species Appendix II as endangered.[7] The governments of many countries are undertaking legal action to control the trade in *Aquilaria* species. More sustainable ways of producing agarwood have been investigated to increase the production rate and stop the destruction of natural forests. In addition, methods for agarwood induction have been investigated to increase the production of agarwood and prevent the extinction of *Aquilaria*. One of the methods used to harvest agarwood more sustainably is the infection and wounding of the trees. Farmers in Southeast Asia are starting to cultivate *Aquilaria* trees, and simple wounding techniques, such as cutting with an axe, nailing, or bark removal, are used to induce agarwood production. Because these techniques do not always produce the same amount of agarwood, therefore the production is not stable and can only produce a small amount of resin, and the products are considered to be low-grade agarwood.[8] Recently, scientific methods, such as fungal infection and chemical induction, have been investigated to increase the production of agarwood.[9] The following of this article will cover the medicinal use of agarwood in traditional Chinese medicine (TCM) and the pharmacological properties of agarwood.

In the TCM theory, agarwood has a spicy flavor, a warm property, and enters to the spleen, stomach, and kidney meridians. Agarwood was first described by Tao Hongjing (陶弘景) in the *Ming Yi Bie Lu* (《名医别录》 *Miscellaneous Records of Famous Physicians*) and has many different names, such as Xun Lu Xiang (薰陆香), Ji She Xiang (鸡舌香), and Feng Xiang (枫香).[10] Agarwood was described in the *Ming Yi Bie Lu* as being effective for the treatment of cholera and heart troubles. In a another book, the *Hai Yao Ben Cao* (《海药本草》 *Overseas Materia Medica*) described the use of agarwood for the treatment of psychological and neurological symptoms, such as mental problems and coma. The *Overseas Materia Medica* also mentioned the use of agarwood in ointments for treating swollen wounds.[11] The *Ri Hua Zi Ben Cao* (《日华子本草》 *Materia Medica of Ri Hua Zi*) included the use of agarwood to treat cold and dampness related to pains in the joints.[12] The *Yao Xing Fu* (《药性赋》 *Medicine Properties in Verse*) added alleviating nausea and asthma to the uses of agarwood.[13] The *Ben Cao Jing Shu* (《本草经疏》 *Commentary on the Materia Medica*) described the effect of agarwood in regulating the ascending or descending of qi.[14] It also mentioned the property of agarwood to alleviate edema; the flavor of agarwood is spicy; thus, agarwood can dry dampness in the spleen and reduce the edema. The contraindications of agarwood are also mentioned in the *Commentary on the Materia Medica*. Agarwood is not suitable for patients with qi weakness of middle jiao affecting the flow of qi back to its origin and patients with excess pathogens in the heart meridian. The warming kidney property of agarwood is mentioned in the *Yao Xing Jie* (《药性解》 *Exploration on Medicine Properties*). Li Zhongzi (李中梓) observed that agarwood sinks when placed in water, and pointed out the properties of agarwood to direct qi downward and nourish yin.[15] Thus, agarwood is said to have an effect on the lower parts of the human body, such as the life gate (命门) and the kidneys. Because the use of aromatic and spicy herbs will lead to dryness, agarwood is suitable for slows down deficiency cold pattern but not in the case of excessive ministerial fire. The actions of agarwood are summarized in the Chinese Pharmacopoeia as moves qi and relieves pain, directs rebellious qi downward and stops vomiting, and warms kidneys to aid in qi absorption.[16] The following section will describe the use of agarwood in TCM and the pharmacological properties and mechanisms of action of the constituents of agarwood.

Use of Agarwood in Traditional Chinese Medicine

Moving qi and relieving pain

When exogenous pathogenic cold attacks the body, it slows the flow of qi, and if the cold congeals in the stomach, it will cause stagnation of qi and lead to pain. Agarwood, with its warming property, can be used for pain related to stagnation of qi. Therefore, agarwood is suitable for use in qi stagnation caused by cold congealment. Additionally, dampness may affect the spleen's function in transportation and transformation, but agarwood, with its aroma, can be used to awaken the spleen. Thus, agarwood can be effective to treat cold dampness encumbering the spleen.

Directing inverse qi downward and stopping vomiting

Nausea and vomiting often occur with abdominal pain, mainly because of the counter flow of stomach qi. As mentioned, agarwood is heavier than water; this promotes a descending movement of qi. Therefore, the ability of agarwood to reduce nausea and vomiting will be more effective when combined with medicines that alleviate vomiting.

Warming kidneys to aid in qi absorption

One of the functions of the kidneys in TCM theory is absorbing qi. The life gate of the kidney requires yang to keep ministerial fire (相火) functional. If the kidneys lack yang, normal functions such as absorbing qi are not maintained. One of the common outcomes of this dysfunction is that the kidneys fail to receive qi in the lung. This failure leads to many symptoms, such as difficulty in inhalation, shortness of breath, and asthma. Many TCM practitioners have suggested that agarwood is warm in property and spice in flavor. It was recorded in the *Ben Cao Qiu Zhen* (《本草求真》 *Seeking Accuracy in Materia Medica*) that agarwood has the function of tonifying yang. And it enters the kidney meridian. Thus, agarwood can treat failure of the kidneys in absorbing qi pattern. Furthermore, agarwood can also be used for treating weakness and coldness in the lower back and knee joint pains caused by the weakness of kidney fire that is unable to warm the lower back and the knees.

Pharmacological Properties and Mechanism of Action of Agarwood

As mentioned, agarwood has been traditionally used for treating many abnormal physical conditions, including gastrointestinal disorders, asthma, and pain. Recent pharmacological research has revealed new bioactive compounds as possible drug candidates and the mechanism of action of compounds from agarwood. Traditionally, only the resin of *Aquilaria* has been used for medicinal purposes and the other parts of *Aquilaria* are often wasted during the harvesting process. This waste will not be sustainable in the long term, neither is it economically nor environmentally viable. Thus, it is important that the use of different parts of *Aquilaria*, such as the leaves and trunk, is to be considered. With the support from new technologies, the chemical compounds in agarwood can be isolated and identified. Recently, more than 300 types of compounds have been isolated from *Aquilaria*,[17] including potentially new active compounds. The main constituents of agarwood are 2-(2-phenylethyl)-4H-chromen-4-one derivatives, terpenoids, and flavonoids, including sesquiterpenes and diterpenes. The pharmacological properties and the mechanisms of action of these compounds can be grouped as follows.

Effects on the neurological system

Agarwood has been used as a sedative in many traditional medicines by inhalation of the burnt fumes or oral ingestion. Much research has focused on the effect of agarwood in neural activity. A study conducted by Okugawa *et al*. compared the sedative effects of petroleum ether, benzene, chloroform, and water extracts from agarwood. The results showed that only the benzene extract had a sedative effect. In particular, the benzene agarwood extract reduced spontaneous motility, increased the sleeping time when administered with a barbiturate, reduced the rectal temperature, and had the potential to provide pain relief from acetic acid-induced pain.[18] The extract demonstrated similar effects after both peritoneal and intracerebroventricular administration.[19] Further studies by this group have suggested that agarospirol from agarwood can reduce the incidence of writhing in mice from acetic acid-induced pain.[20] Agarwood oil contains benzylacetone, calarene, and alpha-gurjunene. Takemoto *et al*. have reported that inhalation of these compounds produced a sedative effect in mice.[21] One of the components released from burning agarwood is benzylacetone. Miyoshi *et al*. investigated 17 benzylacetone derivatives from agarwood and found that the most effective derivatives that have sedative effects were (S)-4-phenyl-2-butanol and (R)-4-phenyl-2-butanol.[22] Agarwood essential oil administered at 60 mg/kg had a sedative-hypnotic effect in pentobarbital-induced sleeping mice. The sedative-hypnotic effect of agarwood essential oil is mediated through regulating the gene expression of gamma-aminobutyric acid ($GABA_A$) receptors and potentiating $GABA_A$ receptor function.[23] Agarwood may also be used for treating anxiety. For example, a study has shown that alpha-agarofuran and the derivative 4-butyl-alpha-agarofuran can regulate serotonin and dopamine in rats with serotonin-induced anxiety.[24] Agarwood essential oil had anxiolytic and antidepressant effects in rats, and the mechanism of action was via inhibition of corticotropin-releasing factor and hyperactivity of the hypothalamic-pituitary-adrenal axis.[25] Chronic inflammation in physically ill patients and metabolic syndromes can lead to many neurological disorders such as depression. Studies showed glutamate[26] and corticosterone[27] often associated with depression. A 2-(2-phenylethyl) chromone derivative isolated from the ethanolic extract of agarwood showed a significant neuroprotective effect in glutamate and corticosterone-induced neurotoxicity.[28] Inhibitors of serotonin and norepinephrine reuptake may be potential antidepressants. Two sesquiterpene derivatives (+)-8β-hydroxy-longicamphenylone and 11β-hydroxy-13-isopropyl-dihydro-dehydrocostus lactone have been isolated from agarwood petroleum ether extract. An *in vitro* study of these compounds showed that both compounds have potent antidepressant activity by inhibiting serotonin reuptake in rat brain synaptosomes.[29] In another *in vitro* study, antidepressant activity of 11 agarwood compounds was preliminarily evaluated using rat brain synaptosomes and the compounds displayed antidepressant activity by inhibiting both serotonin and norepinephrine reuptake. Among the 11 compounds investigated, aquilarabietic acid A, aquilarabietic acid H, and aquilarabietic acid I showed antidepressant activity *in vitro* by inhibiting norepinephrine reuptake in rat brain synaptosomes.[30]

Effect on digestive system

Traditionally, agarwood has been used for pain relief, particularly for abdominal pain. A methanol extract of *Aquilaria agallocha* had a protective effect on the intestinal mucosa after 5-fluorouracil-induced intestinal mucositis. Administration of the *A. agallocha* methanol extract also improved food intake and the injury to the intestinal mucosa, and alleviated the weight loss and severe diarrhea. The mechanism of action occurred through an increase in the expression of proliferating cell nuclear antigen and inhibition of cyclooxygenase-2 (COX-2) and tumor necrosis factor-α.[31] An *A. agallocha* ethanol extract had a spasmolytic effect on gastrointestinal motility by decreasing gastric emptying and small intestinal transit. They observed that a decrease in the contractions induced by acetylcholine *in vitro* was probably mediated through inhibition of muscarinic receptors and blockade of calcium influx and NO release.[32] One study has indicated that ethanol extract produced by the whole-tree, agarwood-inducing technique can alleviate intestinal damage, suggesting that this extract could be used as an intestinal protective adjuvant therapy drug for intestinal injury induced by chemical drugs. This effect may be mediated by reducing pro-inflammatory mediators, such as NO, interleukin-17 (IL-17), and IL-13, and increasing glutathione and superoxide dismutase levels. The possible mechanism of this effect may be realized via the regulations of the nuclear factor-E2-related factor 2-antioxidant response element and nuclear factor-κ B pathways. Apart from pain relief, agarwood also has a laxative effect. Kakino *et al*. found that an ethanol extract of *Aquilaria sinensis* and *A. crassna* leaves had laxative effect in a mouse constipation model and did not cause diarrhea. A possible mechanism for this effect is realized via acetylcholine receptors.[33,34] The main constituent of acetone extract of *A. sinensis*, genkwanin 5-*O*-β-primeveroside, has been shown to increase bowel movement. The mechanism of action of this effect may involve stimulation of intestinal motility via acetylcholine receptors.[35] Another study has shown that mangiferin and iriflophenone 2-*O*-α-L-rhamnopyranoside were the most abundant compounds in the extracts of *Aquilaria* leaf tea. These compounds may possess laxative effect.[36] Another less well-known property of agarwood is the regulation of blood sugar. Pranakhon *et al*. have reported that a methanolic extract from *A. sinensis,* containing iriflophenone 3-*C*-β-glucoside as the main constituent, reduced blood glucose levels and enhanced glucose uptake in rat adipocytes.[37] Methanol and water extracts of agarwood leaf enhanced glucose uptake activity in rat adipocytes, and the effect was comparable to insulin.[38]

Anti-inflammatory effect

Inflammation is a defensive process in which the immune system reacts to antigens. However, an overreaction of the immune system or chronic inflammation can lead to a variety of diseases. 1-(12-*O*-(2′E,4′E)-6-oxohexa-2′,4′-dienoylphorbol-13-acetate), 12-*O*-deoxyphorbol 13-decanoate, and 1,3-dioleoyl glyceride isolated from *A. malaccensis* seeds showed potent inhibitory activity against formyl-methionyl-leucyl-phenylalanine/cytochalasin B -induced elastase released by human neutrophils.[39] Pilloin, a flavonoid compound from *A. sinensis*, significantly suppressed the production of pro-inflammatory molecules, such as tumor necrosis factor-α, IL-6, COX-2, and inducible nitric oxide synthase, in lipopolysaccharide-treated RAW 264.7 macrophages. A possible mechanism for this effect is through the inhibition of inflammatory-related mitogen-activated protein kinase pathways.[40] Agarwood oil significantly reduced the skin thickness, ear weight, oxidative stress, and production of pro-inflammatory cytokines in a 12-O-tetradecanoylphorbol -13-acetate-induced mouse ear inflammation model, which contributed toward validation of the traditional use of agarwood in the treatment of inflammation-related ailments.[41] Three compounds from the whole-tree, agarwood-inducing technique also exhibited anti-inflammatory activity in lipopolysaccharide-induced inflammation in RAW264.7 cells.[42] Microglial hyperactivation and neuroinflammation are known to induce neuronal death, which can lead to many neurodegenerative disorders, including Alzheimer's disease. Lee *et al*. found that a dichloromethane fraction of *A. lignum* reduced the levels of NO, COX2, prostaglandin E2, and IL-1β in lipopolysaccharide-stimulated BV2 microglial cells and the mechanism may involve the regulation of Nod-like receptor family pyrin domain-containing 3.[43]

Anti-asthmatic effect

Agarwood has been used to treat shortness of breath, which is an asthma-like symptom. Aquimavitalin extracted from the seeds of *A. malaccensis* has inhibitory activity against antigen-induced degranulation; therefore, aquimavitalin is a potential drug to treat allergic reaction-related diseases.[44] Although traditional use of agarwood to treat asthma has been recorded in ancient scripts, there are not many studies regarding the use of agarwood as an anti-asthma and anti-allergic agent; the mechanisms of action are not clear and need to be further investigated.

Effect on constraining bacteria and fungi

Although agarwood is not well favored for treating infectious diseases, many studies have reported antibacterial and antifungal activity from extracts of different parts of the *Aquilaria* plants. Kamonwannasit *et al*. have reported that *A. crassna* leaf extract was active against *Staphylococcus epidermidis*. The extract triggered swelling and distortion of the bacterial cells and inhibited bacterial biofilm formation. Rupture of the bacterial cell wall occurred after treatment with this extract for 24 h.[45] Oxidoagarochromone A and

oxidoagarochromone B from *A. sinensis* ethanolic extract showed inhibitory effects against *Staphylococcus aureus* and *Ralstonia solanacearum*.[46] β-caryophyllene from the essential oil of *A. crassna* demonstrated selective antibacterial activity against *S. aureus*.[47] Four compounds from agarwood oil exhibited antibacterial activity against both *S. aureus* and *R. solanacearum* and one compound had inhibitory activity against *S. aureus* only.[48] An inhibitory effect of agarwood oil against the growth of *Bacillus subtilis* has been demonstrated.[49] There are some concerns regarding artificially inducing the formation of agarwood. One study has shown that artificial agarwood obtained by a comprehensively stimulated method had an antibacterial effect on the bacterial strains, *S. aureus* and anti-methicillin-resistant *S. aureus*, and an antifungal effect on seven fungal strains: *Penicillium melinii*, *Penicillium adametzii*, *Penicillium urticae, Penicillium notatum*, *Paecilomyces variotii*, *Mucor saturninus* Hagem, and *Aspergillus niger*.[50]

Antitumor effect

Many compounds derived from Chinese herbs have shown promising cytotoxic effects, and some of these compounds are candidates for new antitumor drugs. Agarwood essential oil generated a reduction in the cell numbers in both cell viability and attachment assays, suggesting a cumulative effect on cell death, inhibition of cell attachment, and/or causing cells to detach.[51] 4′,7-dimethoxy-6-hydroxy chromone isolated from methanol extract of agarwood from *Aquilaria filaria* had antitumor activity against multidrug-resistant tumor cell lines, including lung carcinoma, epidermoid carcinoma of the nasopharynx, and breast cancer cell lines.[52] Seven compounds from ethanol extract of agarwood from *A. sinensis* inhibited the growth of SMMC-7721, MGC-803, and OV-90 cell lines; however, these compounds showed only weak cytotoxic activity.[53]

Conclusions

The pharmacological properties of compounds from different parts of *Aquilaria* plants have many properties that have not been recorded in ancient scripts. Although new uses and new compounds have been discovered, data from clinical experiments and knowledge of the mechanisms of action are lacking and need to be further investigated, especially the network pharmacology mechanism of multicomponent and multitarget of *Aquilaria* plants. With the help of data from the chemical analyses in this article, the quality control of agarwood can be quantified and standardized. These data could be used for the control of the quality and price of agarwood according to standard set by committees.

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Ethical approval

This article does not contain any studies with human or animal subjects performed by either of the authors.

Author contributions

Lertnimitphun Peeraphong wrote and reviewed the article.

Conflict of interest

None.

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Chinese Medicine and Culture

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References should be numbered consecutively in the order in which they are first mentioned in the text (not in alphabetic order). Identify references in text, tables, and legends by Arabic numerals in superscript with square bracket after the punctuation marks.

Papers in Journals

1. Standard journal papers (for up to six authors):

Parija S C, Ravinder PT, Shariff M. Detection of hydatid antigen in the fluid samples from hydatid cysts by co-agglutination. Trans. R.Soc. Trop. Med. Hyg.1996; 90:255–256.

2. Standard journal papers (for more than six authors): List the first six contributors followed by *et al*.

Roddy P, Goiri J, Flevaud L, Palma PP, Morote S, Lima N. *et al*., Field Evaluation of a Rapid Immunochromatographic Assay for Detection of *Trypanosoma cruzi* Infection by Use of Whole Blood. J. Clin. Microbiol. 2008; 46: 2022-2027.

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