Advances in Propolis Research and Propolis Industry in China

Chen Lihong[1, 2], Romanee Sanguandeekul[3], Zhang Fuxing[2]
Sureerat Deowanish[4], Ratna Thapa[5], Siriwat Wongsiri [5,*]

[1] Agricultural Technology Programme, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand
[2] Apiculture Research Institute of Chinese Academy of Agriculture Science, Xiangshan,100093, Beijing , China
[3] Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok,10330, Thailand
[4] Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand
[5] School of Science, Mae Fah Luang University, Chiang Rai, Thailand

Abstract

China has the world's largest apiculture products as well as the highest consumption of bee products. Since the 1980s, propolis has become one of the most important honeybee products in China. Currently, propolis is one of the fastest growing honeybee products, with demand for outstripping supply.

With increasing consumption of propolis products, propolis research has also seen significant expansion. We present here an overview of different methods of extracting raw propolis, along with contemporary research on the functions of propolis, such as its anti-bacterial, anti-inflammatory, anti-oxidant, anti-tumour and anti-cell toxin, activities softening blood vessels, improvement in blood microcirculation, enhanced immunity, anaesthetization and promotion of tissue regeneration. The past decade has witnessed a tremendous boom in the use of propolis in health care products and folk medicine for treating diabetes, cardiovascular diseases, neurodegenerative diseases, and some cancers, in China. It is also widely applied in animal husbandry, plant protection, and food industry. The new Chinese Pharmacology has included propolis in its descriptions of Apitherapy medical applications.

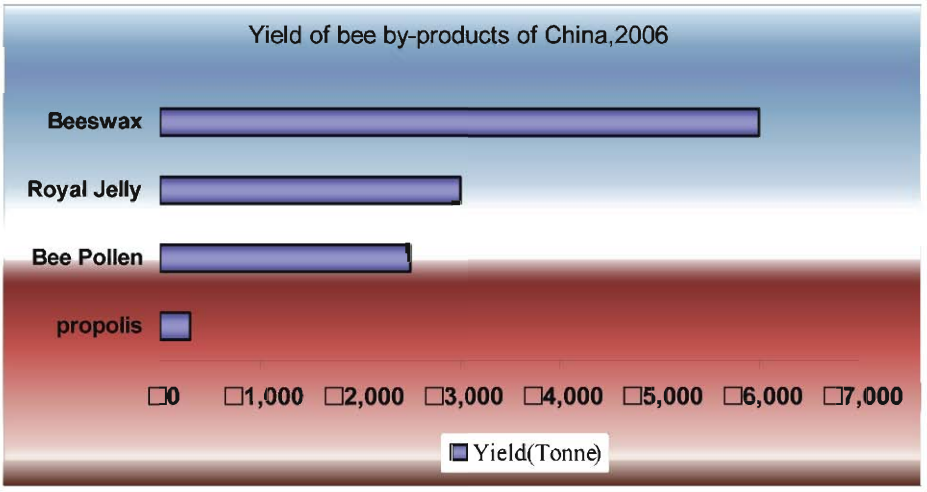
A number of propolis products in Chinese markets are also reviewed, such as propolis tablets, propolis oral membrane, propolis honey, propolis tincture, propolis dental liquid and toothpaste, propolis spray and ointment. We also review propolis cosmetics, such as soap, body cream, and skin care products.

Key words: propolis, bee products, apitherapy

Introduction

China has the largest apiculture industry in the world, with approximately 7,000,000 honeybee hives (*Apis. mellifera* 5,000,000 hives, and *Apis cerena* 2,000,000 hives). As such, it also has the highest output of bee-products. Honey is the main product, with output averaging 250,000 tonnes per year. There are also several significant bee by-products on the market. Although the yield of the by-products is lower than honey, their output is still ranked as the highest in the world. Royal jelly production is approximately 3,000 tonnes per year and more than 90% of the global supply comes from China. Bee pollen output is approximately 2,500 tonnes, beeswax 6,000 tonnes, propolis 300 tonnes, along with further output of drone pupae and larvae products (Fig 1). The majority of these byproducts are exported, with the exception of propolis: more than 80% of propolis is used domestically (ASAC, 2006). Today we introduce the limited yield but popular bee by-product: China propolis.

Fig 1 Yield of Bee By-products in China 2006 (Except Honey, 250,000 tonnes)



The word "propolis" originated in Greece, first used by Aristotle: "Pro" means "before" and "polis" means "city"—"Defender of the City." As research has shown, the name, "Defender of the City", is a very appropriate term to describe the role of propolis in the beehive. In his writings, Aristotle showed a remarkably accurate and detailed knowledge of bee propolis.

Propolis is a sticky resinous substance collected by European honeybees (*Apis mellifera*) from tree buds, tree bark, tree gum, or other botanical sources. Honeybees pack it into the orbiculae (pollen baskets) on their hind legs, carry it home and mix it

with wax secreted from their special glands. Honeybees use propolis as a building material to seal up any cracks or gaps where microorganisms could flourish, to decrease the size of nest entrances and make them smooth for passing bee traffic. Honeybees also use propolis as a thin layer to varnish inside brood cells before the queen lays eggs into them; presumably this provides a strong, waterproof and hygienic unit for developing larvae; and to wrap the bodies of intruding insects and other enemies (such as mice and small house geckoes, too large to remove from the nest). The volatile oils in propolis appear to serve as a kind of antiseptic air-freshener, keeping the bee (hives) dry, cozy and hygienic, ensuring a clean environment for the rearing of the brood (Chen Yaochun, 1993)

Plant Sources of Propolis in China

The main sources of propolis in China are poplar, pines, cypress, willow and sumacs (Fig 2), located mainly in north and central China, and to a lesser extent in several southern provinces. There are also some minor sources distributed all over the country such as peach, plum, almond, eucalyptus, rubber plant and helianthus, etc. (Chen Yaochun, 1993). It is also widely agreed that propolis production is best suited to the climate in the north zone of China.

Fig 2 The main sources (plants) of Propolis of China

Populus(poplar)

Cypress

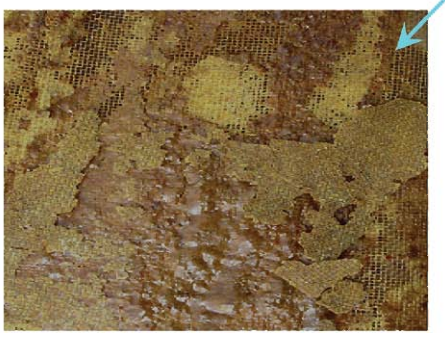
Willow

Methods of Producing Natural (Raw) Propolis

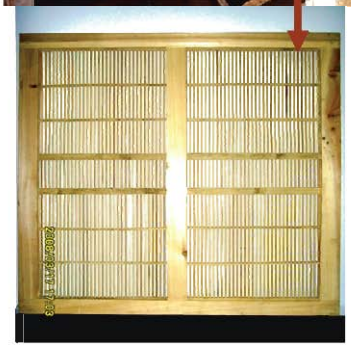
There are three main methods of producing natural propolis in China. Firstly, propolis is collected directly from the bar frames in the hives. Secondly, propolis is collected from the cloth net slipcover that covers the top of the hive inside the coping (Fig 3). Thirdly, propolis is collected from the crown board. In order to encourage honeybees to produce more propolis, beekeepers place a perforated, plastic or bamboo grid on top of the hive. The bees will seal up the slots with propolis. When it is full, they

take out the grid and put it in a freezer. By flexing the cold sheet, propolis pieces will drop out. This has proved to be an effective way to harvest propolis (Chen Lihong 2002; Zhang Zhongying, 2007). The last two methods are special in accordance with the "Production Bases of Safety and Standardization of Bee Product (Propolis)" of ASAC in China (Zhang Fuxing, 2004). The Production Bases of Propolis of ASAC are usually set up in jungle areas in order to collect natural propolis. (Fig 3)

Fig 3 Production Bases of Bee Product (Propolis) of ASAC



Cloth net slip cover



Bamboo grid on the top of the bee hive

Methods of Extracting Propolis

Raw propolis cannot be used directly. IMPURITES MUST BE REMOVED after harvesting it. The procedure involves freezing, grinding and extracting with alcohol, ether, or other organic solvents, and then refining. There are also several new extraction methods, which can be varied by using different solvents, aimed at increasing the efficiency of the extraction process. The selection of the method and solvent depends on the final use of the extract and on technical feasibilities:

Ethanol extracted propolis (EEP)

EEP is the most common method for extracting propolis in China. Extraction with ethanol is particularly suitable in order to obtain dewaxed propolis extracts rich in polyphenolic components. Before 2000, 70% ethanol was most commonly used to extract propolis (Chen Weixian et al. 2006). Subsequent research showed that 95% ethanol was better. Some researchers used various concentrations of ethanol and measured the absorption spectra of the different extracts. The 95% ethanol extraction yielded the highest concentration of flavonoids and the lowest amount of beeswax (Wang Chunling et al, 2004).

Preparation of raw propolis begins by freezing propolis in order to make a fine powder. This is dissolved in ethanol (the most frequently used proportion is 1:10, w/v), and left for 24h at room temperature. After filtering, this procedure is repeated several times. Alternatively, the raw propolis powder is dissolved by shaking at 70°C for 30 min (National Standard of Propolis, 2007).

Soxhlet extraction method (SE)

In the Soxhlet Extraction Method, the raw propolis powder is dissolved in the Soxhlet extractor with 100ml ether. Heating and distillation turn it transparent (Wang Xiaoping et al, 2007).

Ultrasonic wave extraction (UE)

For the UE method, the raw propolis powder is dissolved in 50ml ether with an ultrasonic device for 10min and filtered; the residues are then put through the same procedure two more times. The UE method can extract flavonoids at a higher rate than the SE method (Guizhou Univ.). UE was shown to be the most efficient method based on yield and selectivity, and requires a shorter time (Li Yaping et al, 2007).

Aqueous extracted propolis (AEP) or Steam-distilled extraction

The AEP method actually utilizes a lower concentration of ethanol, eg., 40% ethanol, at 80°C for 10 min. Then it is mixed with hot water. Some scientists use water and a small quantity of natural surfactant. Although not all the ingredients are water soluble, aqueous extracts have been shown to exhibit bactericidal and fungicidal effects.

Supercritical extracted propolis (SEP)

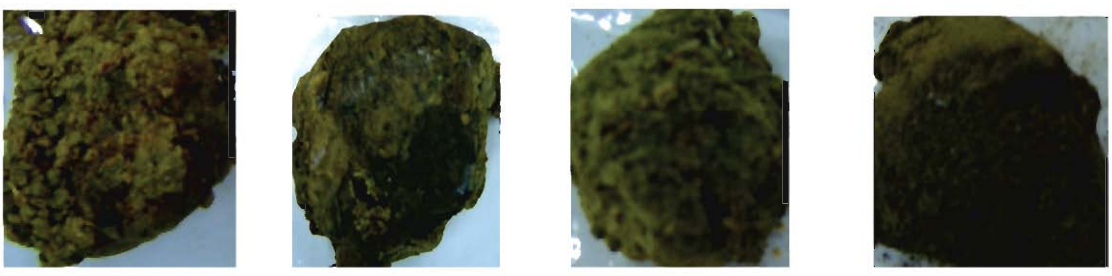
Supercritical Extraction is a new method currently used in China. SEP was developed for the fractionation of propolis tincture to obtain flavonoids, terpenes and essential oil fractions. It removes high molecular mass components. Flavonoids are practically insoluble in pure CO_2, but sufficiently soluble in CO_2+ ethanol to be separated from high molecular mass components. Researchers at Jiangsu University developed the method using CO_2 or dimethylmethane as a solvent to extract propolis, and also by using microwave to assist in extracting the rest of the propolis material (Gao Yinyu et al, 2000).

Character and Composition

Physical character

Raw propolis is very complex. It varies according to the sources (including plants, hives, districts, seasons, etc.). Natural propolis is an opaque solid with either a rough or a smooth surface. Normally, the colour varies between golden brown, grayish brown, grayish green and dark green with lustre, depending on its botanical sources and regions (Fig 4). Generally, natural propolis smells fragrantly, with a milky fragrance when being burned. It is sticky and pliant to the touch, with a slightly bitter and pungent taste. Below 15°C , it turns hard, crisp and fragile. Heated to 36°C, it becomes soft, pliable, plastic and sticky. When the temperature rises to 60-70°C, it melts into a sticky liquid from which bee's wax is extracted. It is difficult to dissolve in water, slightly dissolved in turpentine, and easily dissolved in ether, chloroform and ethanol. When dissolved in 95% ethanol it turns transparent and maroon in colour, with sedimentation of granules.

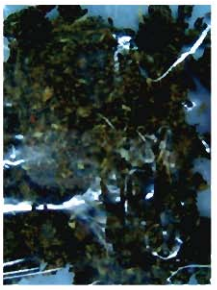
Fig 4 Nature (raw) propolis samples in 2007 (from different botanic source and regions of China)



Poplar (Jilin) Poplar & willow (Jilin) Heterophylla & Acacia(Beijing) Coniferea (Hubei)


Poplar (Hubei)


Poplar (Hunan)


Herbaceous(Xinjiang)


Heterophylla(Xiangxi)


Lichi & Longan(Guangxi)

Chemical composition

Natural propolis has an intricate composition, which is closely related to its botanic sources. Some ingredients come from the honeybees themselves. Normally, Chinese propolis composition is approximately as follows (Chen Yaochun 1993, Fang Zhu,1998 and Hu Fuliang, 2005):

- Resinous compounds and balsam (aromatic oils): 55%
- Beeswax: 30%
- Bee Pollen: 10%
- Others and Impurities: 5%

The main chemical component found in propolis extracts is as follows (Cao Wei, 2007; Li Yaping, 2007; Li Yi 2007 and Yu Shifeng, 2007):

- Flavonoids are the main components of propolis, and 36 kinds of flavonoids have been identified in the propolis of China, including: rutin myricetin, quercetin, apigenin, galangin, kaempferol, pinocembrin, pinostrobin, pinobanksin, chrysin, luteolin, etc.
- Terpenes are another component of propolis extract, with 17 varieties identified in China (LI Yaping et al, 2007).
- Amino acids: 18 kinds of amino acid, such as arginine, etc.
- Minerals: 32 kinds of mineral have been identified.
- Other components: polyphenol and polysaccharide, alcoholic aldehyde, other organic acids (including cinnamic acid, vanillin, caffeic acid, ferulic acid etc.), enzymes, vitamins, etc. (Zhang Zhongyin, 2007).

Propolis Action on Health

In recent years, the growing interest in the health functions of food has led to increased research on the functions and efficacy of propolis. Research shows that propolis possesses extensive pharmacological properties and functions in anti-bacterial, anti-

inflammatory, anti-oxidative, anti-tumour and anti-cell toxin, softening blood vessel, improving blood microcirculation, enhancing immunity, anaesthetization and promoting tissue regeneration, etc. Chinese Pharmacopoeia (2005) now includes propolis for medical applications. However, the claim of beneficial properties need to be fully evaluated.

Antibacterial and anti-pathogenic microbial activity

Many of the tests suggest that propolis has widespread antibacterial and anti-pathogenic microbial activity when used in various extracts and concentrations (Lan Taofeng, 2006; Yang Yanfeng, 1999; Zhang Wei, 1998 and Hu Fuliang, 1998)

The research also suggests that efficacy is different depending upon the different extraction methods and their concentration: methylene dichloride extract > chloroform extract > ethanol extract (Yang Yanfeng, 1999 and Yangyanbing, 1999).

Ethanol extract, for example, has an inhibitory effect on different food pathogenic bacteria (Zhang Wei, 1998). Ethanol extract also has a positive inhibitory action on tooth decay pathogenesis (Wang Yinlong, 1996).

For its anti-pathogenic microbial activity, propolis is used to treat clavus, tympanitis, oral erosions and ulcers. Dermatological direct external application of ethanol extracts or concentrated ointments (with up to 33% propolis) has given good results in veterinary use for healing wounds and sores. Plastic surgery employs extracts for improving wound healing and reducing scar tissue development. It is also effective in treating burn wounds.

Anti-inflammatory activity

Studies of propolis in mice have demonstrated anti-inflammatory and anti-pathogenic microbial activity. The studies show that propolis plays a positive role in assisting the treatment of arthritis, pleurisy, pneumonia and bronchitis (Li Yinhua, et al., 2002; Hu Fulang, et al., 2003). It also appears to be very effective in treating gastric and/or duodenal ulcers.

Anti-oxidative activity

Recently, the marketing and consumption of antioxidant products have increased in light of evidence regarding the important role of antioxidants in human health (Wollgast, 2002; Madhavi, 1996). Flavonoids, the main component of propolis, are

powerful antioxidants. Coffee acid, caffeic acid, and ester of propolis are also key antioxidants. The other components of propolis such as xanthine and quercetinic acid have certain anti-oxidative properties. Other four furfuran lignans were isolated from Chinese propolis by column chromatography. They were identified by spectroscopic methods as sesamin, yangambin, (+)-pinoresinol and (+)-syringaresinol. Among them, (+)-pinoresinol and (+)-syringaresinol were isolated for the first time from propolis. The antioxidant activity was evaluated by measuring the inhibition of lipid peroxidation in rat liver microsomes (Cui Guiyou, et al 2002).

In China, numerous experiments have been carried out on the anti-oxidative functions of propolis in aged mice. The results show that propolis indeed possesses good anti-oxidative properties that play a protective role for human health. Furthermore, these studies also demonstrated that the EEP extraction method demonstrates higher anti-oxidation (Wang Chunling, et al 2004; Hu Chunsheng, et al 2005; Lang Liuyun, et al 2006; Xuan Hongzhuan, et al 2007 and Peng Zengqi, et al 2005). Therefore, some studies suggest that propolis might be a kind of high quality food ingredient that could produce antioxidative functional food (Sheng Haitao, Cao Wei ,2001).

Indeed, EEP was studied with lard oil, rapeseed oil, peanut oil, soybean oil and colza oil to determine their peroxide value (POV value). The results showed that EEP not only could inhibit oil oxidation, but also the antioxidation effect of 0.5% EEP and better than butylated hydroxyanisole (BHA). It is also reported that propolis was a natural and safe antioxidant for food (Xu Ying; et al 2007; Guo Zhifang, et al 2007 and Cao Wei, et al 2002).

Anti-cancer and anti-radiation activity

Since 2000, there has been increasing research on the inhibitive properties of flavonoids, caffeic acid, and terpenes of propolis on cancer. The studies showed that in certain cases propolis possessed an inhibitory effect on cancer cell growth (Wu Ming, 2002) and was also found to actually kill human hepatocellular carcinoma cells (Zhenyi 2002). The propolis also has anti-mutational and anti-radiational effects, inhibiting and preventing production of carcinogens and potential carcinogens (Hang Liantang, 2001). In addition, the anti-tumor mechanism of propolis was analyzed in China. It increasingly appears that there is a bright and positive future for the use of propolis in health care (Long Suzheng, et al 2000; Chen Donghai, et al 2003 and Li Yinghua, et al 2006).

Regulation on blood lipid and blood sugar activity

The water extract (WEP) and ethanol extract (EEP) of propolis were also studied for the inhibitory effects on activities of α-glucosidase, which is responsible for the breakdown of carbohydrate to monosaccharides in the process of intestinal absorption. When comparing WEP and EEP, it was found that both of them acted as significant inhibitors of α-glucosidase. Scientists observed the effect of propolis on blood glucose in mice. Studies show that propolis has both hypoglycemic and blood lipid regulatory effects (Wu Chuiwen, et al 1998).

The function of propolis extract (EEP) on reducing blood lipids effect was also observed. SD rats with high levels of blood lipids were divided into four groups and received orally administered propolis in different concentrations for four weeks. The blood lipids level was then measured. The results show that propolis has a regulating effect on blood lipids (Qian Ronghua 2003). Studies on the effects of WEP and EEP on blood lipids show that propolis extracted by either method had significant inhibitory effects on the level of triglyceride (TG), total cholesterol (TC), low-density lip cholesterol (LDL-C) in serum, and TC and TG in liver. EEP also can regulate lipid metabolism to reduce TC, TG, LDL-C (Zeng Zhijiang, et al 2006; Zheng Hongyan, et al 2004; Hu Fuliang, et al 2004; Zhang Jie, et al 2005, Hu Fuliang, et al 2003 and Zhao Wen, et al 2005).

Immunization activity

Researchers found that propolis has strong immunity adjustment functions. It can increase the lymphocyte proliferation in mice by 3.7 to 6 times (Tang Zhongyen, et al 2006; Shen Zhiqiang, et al 1989), restrain red blood cell dissolution (Zhou Jinjin, et al 2003), enhance macrophage vigour and strengthen phagocytosis (Zhao Yan, et al 2005; Zhang Xinjun, et al 2005). Besides, propolis was also found to enhance the killing action of NK cells (Xie Ni , et al 2004).

Others

Propolis is also widely used in animal husbandry, plant protection, food processing, and cosmetics. Because of positive studies on its effects on tissue regeneration and renovation, the most popular use of propolis has been in cosmetic applications. Together with its bactericidal and fungicidal characteristics it provides many benefits in various applications in cosmetics.

Propolis Products on the Market

As noted above, the research and development of propolis products have made rapid progress during the past 20 years (Tables 1 and 2). Propolis research has promoted commercial applications in food, health care and medicine. Propolis preparations have also seen rapid development, from propolis liquid and ointment in the 1980s, to propolis spray and capsules in the 1990s. After the year 2000, there hasbeen development of propolis soft capsule products, and cosmetic products have seen dramatic growth.

In the honeybee consumer market, demand for propolis continues to grow, as it reaches mass consumption and wider retail outlets. Currently, a wide variety of propolis products can be found in supermarkets, shopping malls, and specialty shops, including propolis capsule, propolis tablets, propolis liquid, propolis oral membrane, propolis honey, propolis tincture, propolis dental liquid, propolis sprayer and ointment, etc.

Table 1 Preparation of Propolis Products for Medical Condition

Preparation	**Application**
Propolis Ointment	Dermatology, Surgery, Gynecology (e.g.: Uses to treat scalds proliferation scars Eczema, dermatitis, belt-shaped blister measles, etc.)
Propolis Tincture	
Propolis Ulcer spirit	Stomatology (Oral cavity ulcer, mucous membrane floccosoids)
Propolis Ulcer membrane	
Propolis Sprayers	Stomatology, Otorhinolaryngology (rhinitis, pharyngitis, laryngitis, sinusitis)
Propolis Drops	

Table 2 Preparation of Propolis Health Food Products

Preparation	**Application**
Propolis Soft Capsule	● Reduce blood Lipid and blood Sugar ● a diabetes ● Other cardiac, cerebral and vascular disease ● Inhibition of cancer ● Enhance immuno competence ● Protect from colds
Propolis Tablet	
Propolis Extract Liquid	
Hard Capsule	

With continued research and development, the range of propolis cosmetic pro ducts has increased: propolis skin cream, propolis jelling cream, propolis astringent, propolis facial cleanser, propolis moisturizers, propolis body lotion, propolis hand wash, propolis body wash, propolis shampoo etc.

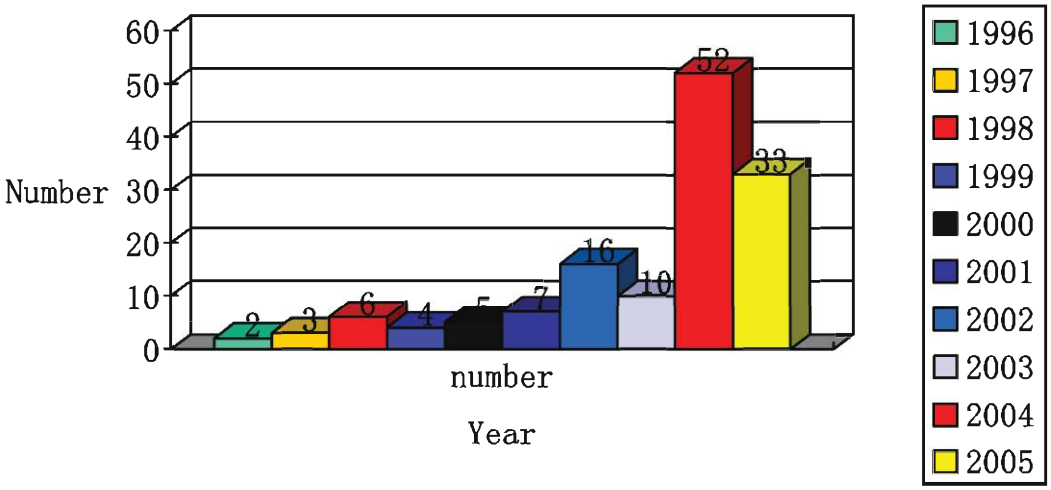
There are also some international propolis products to fit different market segments in China, particularly the soft capsule used for glycemia. It is clear that the Chinese propolis market has huge potential for both domestic and foreign products.

Government Interest in the Propolis Industry

The Chinese government has shown strong interest in the technological development of propolis. Over the past decade, the Ministry of Health of China approved 138 kinds of propolis food products and five medical products MOH (Fig 6). Propolis food products have also enjoyed steady growth, especially in 2004 and 2005. These pro ducts take the following forms: 72% capsules, 17% liquid propolis preparations, 9% tablet, 1.5% ointment, and 0.5% membrane Bulletin 1996-2005 (Fig 7).

After 2000, the National Science Committee has elevated propolis research into the Key Science and Technology Attack Project of Ministry of Agriculture (MOA). MOA also gives large amounts of funds to support propolis research and development.

Fig 5 The number of propolis health care, food and medicines approved by the government (1996-2005)



Propolis also gained entrance to the "Chinese Pharmacopoeia 2005". The new national standard was also published in 2008 (Fang Zhu and Wongsiri Siriwat, 2008).

The authoritative Apicultural Science Association of China (ASAC) plays a key role in the developing propolis industry, particularly in quality control. Setting up "Production Bases of Safety and Standardization of Propolis Products" has become one of ASAC's most important projects.

Conclusions

Overall, a growing number of studies shows that propolis is a natural bee product with important and powerful benefits for human health. It is becoming clearer that propolis products promoting health protection and disease prevention effects are extremely valuable and face growing demand.

Increased funding will allow scientists to continue their research into the details of propolis benefits for human health and other applications. The market for propolis will prosper (not only) in China but throughout the world.

The Properties of Propolis

The beneficial properties of propolis products are cited claims from Chinese literature. Further substantiations derived from proper clinical studies are recommended.

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