

Ethnopharmacology of Five Flowers herbal Tea, a popular traditional beverage in South China

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Abstract

Background

It has been a long-standing tradition of using herbal tea for preventive and therapeutic healthcare in South China and Five Flowers Tea is one of the most popular herbal teas. Based on the principle of traditional Chinese medicine, the pharmacological functions are to clear heat and dispel dampness in the body. Heat and Dampness are thought to contribute to a range of health problems, especially during the hot and humid season in South China and Hong Kong. The most prevalent herbs in the formula contain bioactive compounds including flavonoids, alkaloids, and terpenoids, which have a wide range of pharmacological properties including anti-inflammation, antiviral, anti-diarrhoea, anti-bacteria and antioxidation. However, with the composition varies widely, the ethnopharmacological benefits described may not be delivered uniformly. This study is to provide a comprehensive analysis on the composition of the Five Flowers Tea sold in Hong Kong and investigate the rationale behind the selection of herbs used in the formula. This study also provides information on the variation and quality of the Five Flowers Tea in the market.

Methods:

Thirty-three Five Flowers Tea samples were collected from various locations in Hong Kong. The size, texture, colour, and organoleptic properties were documented. Macroscopic and molecular authentication methods were employed to identify the individual components.

Results

Macroscopic identification revealed there were 23 herbs belonging to 18 plant families. The most prevalent herb was *Bombax ceiba* L., followed by *Chrysanthemum morifolium*. Ten adulterants and the existing of insect *Lasioderma serricorne* were confirmed by DNA barcoding techniques.

Conclusion

This study employed a comprehensive approach to authenticate the herbs in Five Flowers Tea samples collected from various locations in Hong Kong. Macroscopic and molecular methods were used to identify the herbs and adulterants. The findings revealed the varied composition in Five Flowers Tea and the occurrence of adulterants in some samples. This shows that quality assurance of Five Flowers Tea is essential for the effective use of this popular folk medicine.

Introduction

Herbal tea, also called “cooling tea”, “herbal beverage”, or “liáng chá”, is widely used for preventive and/or therapeutic healthcare in Hong Kong and South China. Herbal tea is a decoction with a range of herbal ingredients prepared according to the ethnomedical experiences of indigenous and local people [1]. The use of herbal tea is also regarded as a popular primary health care system in many countries [2].

The history of drinking herbal tea in China can be traced back to the Tang dynasty (AD618-907), proposed in the ancient Chinese literature *Beiji Qian Jin Yao Fang* [3]. Southern China, especially in Lingnan region, has a rich tradition of drinking herbal tea [4]. Until now, herbal tea shops have been a fixture of Hong Kong landscape and still very much a part of daily life. Herbal tea has been inscribed in the national list of Intangible Cultural Heritage in 2006 [5].

Hong Kong has a subtropical climate with a wet-dry season [6]. Both written in Qing Dynasty (1644–1911), *Lingnan fengwu ji* (A record of the customs of Guangdong) and *Chronicle of Sun-On County* described the unfavorable climate and environmental conditions and frequent epidemic plagues happened in Hong Kong. From the perspective of Chinese Medicine, hot and wet weather are thought to contribute to a series of health problems. Exogenous heat and dampness evils attack the body, resulting in the abnormal consumption of qi (basic element that constitutes the cosmos) in the body and impairment of body fluids. In particular, the interstices and mysterious mansion on the skin remain open in summer and spring time to release the internal heat, and hence people

are susceptible to the illness. To prevent the above conditions and alleviate the symptoms, people gradually learnt to use local botanical resources for making herbal tea.

So far, over 400 plant species have been used in producing herbal teas in southern China [7, 8]. The formulae of herbal teas vary in different regions [4]. The most iconic and representative herbal tea is Five Flowers Tea. This decoction is mostly consumed during the spring and summer months. Five Flowers Tea can be found globally because it has developed a widespread consumer base. The growing Chinese populations in cities like Toronto, London, Los Angeles, and Sydney have boosted demand for traditional herbal remedies. These markets provide ingredients for expat communities and locals wanting to explore health traditions. The primary functions are to clear heat and expel dampness which helps to alleviate symptoms including stomachache, abdominal bloating, indigestion and diarrhea [1, 9]. There is no official formula of Five Flowers Tea in the Chinese Pharmacopoeia. On the other hand, some of the local publications suggested that the mainstream formula of Five Flowers Tea include the flower of *Lonicera japonica* (honeysuckle), *Plumeria rubra* (frangipani), *Pueraria lobata* (kudzu), *Bombax ceiba* (tree cotton) and *Sophora japonica* (Japanese pagoda tree) in equal weight ratio. Apart from the stated herbs, herbal tea shops and distributors may add some other herbs to reinforce the heat clearing and dampness expulsion function [9, 10]. The lack of standardization in Five Flowers Tea formula poses problems. Different producers may use substitutes or adulterated ingredients due to the availability issues or to cut cost. This inconsistency in formulation can undermine the therapeutic claims and safety. So far, information and analysis on the composition of Five Flowers Tea and the rationale of using the herbs are lacking. We therefore set forth to analyze the authenticity, diversity and quality of Five Flowers Tea found in the Hong Kong market.

Methods

2.1 Data collection

33 bags of Five Flowers Tea were collected from local grocery stores, herbs retailers, market stalls and Chinese medicine pharmacies. Purchase location, claims and instructions given by the suppliers, total weight and size of the parcel and species of herbs were recorded (Table S1).

2.2 Macroscopic examination

Macroscopic identification of the Five Flowers Tea samples includes shape, size, surface, texture, cross-section, and type of fracture. Organoleptic examination including colour, odour and taste were carried out by comparing with the herbs listed in the Chinese Pharmacopoeia [11].

2.3 Molecular authentication

Total DNA was extracted from 2 grams of each dried sample using a broad-spectrum plant rapid genomic DNA extraction kit (Biomed, China) according to the instructions of the kit manufacturer. DNA was visualized by electrophoresis in a 1.5% agarose gel and images were collected (Gel Doc EZ system, Bio-Rad, Hercules, CA). DNA concentration was measured by Nanodrop spectrophotometer (DeNovix, Wilmington, DE).

Universal primers (COI-F: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' and COI-R: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') were used for the amplification of the COI (Cytochrome Oxidase I) region. Another pair of universal primers (psbAF: 5'-GTT ATG CAT GAA CGT AAT GCT C-3' and trnHR: 5'- CGC GCA TGG TGG ATT CAC AAT CC-3') were used for amplification of the psbA-trnH region. A pair of universal primers (ITS2F: 5'- ATG CGA TAC TTG GTG TGA AT-3' and ITS3R: 5'-GAC GCT TCT CCA GAC TAC AAT-3') were used for amplification of the ITS2 region. The 30µl PCR reaction mixes included 21.4µl of ultrapure water, 3µl of DNA template, 1.2U of DNA polymerase, 3µl of 10X PCR buffer, 0.6µl of deoxynucleoside triphosphates (10nM), 0.3µl of each primer (10µM), and 1.2µl of 50mM MgSO₄. Amplifications were carried out using a thermal cycler (Veritipro 96W). The thermal regime consisted of an initial denaturation at 94°C for 5 minutes; 30 cycles of 94°C for 1 minute, 56°C for 1 minute and 72°C for 1.5 minutes; and a final extension at 72°C for 7 minutes. PCR products were visualised on 1.5% agarose gel. Amplicons of the correct sizes were selected and purified for subsequent sequencing by BGI, China. The obtained sequences were aligned by ClustalW multiple alignment in MEGA11. The identities of species were identified via BLASTN, NCBI in Genbank database at their highest similarity.

2.4 Data analysis

Data were statistically analyzed and organized using Microsoft Excel (version 16.68, 2022).

Results

3.1 Five Flowers Tea samples

We collected 33 Five Flowers Tea samples from different locations in Hong Kong. Most were packed by transparent plastic bags, with some of them having logos of the shops. A sample was wrapped by paper in the traditional way. Heat-seal was the major way to close the plastic bag. Other ways included tying by string and a knot directly on the bag (Fig. 1).

Among the 33 samples, 14 were labelled with description including the names of the herbal tea, herbs included or functions. Four were labelled with the brand name and 14 did not have label. One sample was labelled as Heat Dampness Clearing Tea instead of Five Flowers Tea.

3.2 Profiles of Five Flowers Tea samples

A total of 23 herbs in 18 families were identified by macroscopic identification (Table S2). Components of the herbs in each sample are listed in Table S1. Figure 2 shows that the most prevalent herb is *Bombax ceiba*, followed by *Chrysanthemum morifolium*. The third place is *Artemisia capillaris*, a herb collected in non-flowering period. It is popular for the shop owners to change some ingredients in the Five Flowers Tea formula to lower the cost or increase the potency.

3.4 Composition of parcel samples

Table S1 illustrates the composition of herbs in the different parcel samples. HK07, HK12, KL04, KL05, and NT04 contain *Bombax ceiba*, *Plumeria rubra*, *Chrysanthemum morifolium*, *Lonicera japonica* and *Sophora japonica*, which are herbs in the classical formula. However, the weights of *Lonicera japonica* and *Sophora japonica* in HK07 were exceptionally low compared to other samples. Two extra herbs, *Artemisia capillaris* and *Prunella vulgaris* were found. For HK12, the weight of *Bombax ceiba* was around 45g, triple to that other four ingredients *Plumeria rubra*, *Chrysanthemum morifolium*, *Lonicera japonica* and *Sophora japonica*, which were around 15g. For KL04, the weight of *Bombax ceiba* was over 40g. For sample KL05, *Bombax ceiba* outweighed all the herbs by 2 to 10 times, followed by an extra herb *Artemisia capillaris*. For NT04, the quantity of *Bombax ceiba* and *Plumeria rubra* were higher than the rest of the herbs. Non-traditional herbs prevailed in both HK06 and NT06. For HK06, 41.3% of the weight of the whole package was dried whole plant of *Polygonum chinense* L. For NT06, 55.9% was *Morus alba* L.

3.5 DNA barcoding for the authentication of herbs

We used DNA barcoding technique to identify the suspected adulterants. Barcoding region psbA-trnH was used followed by BLASTN search from GenBank database for the authentication of *Lonicera japonica* and *Artemisia capillaris*. The result suggested that four samples had *Lonicera macranthoides* instead of *Lonicera japonica*. For the suspected adulterant of *Artemisia capillaris*, BLASTN research indicated that one sample was *Potentilla chinensis*, while another was *Potentilla discolor*. ITS2 region was sequenced to examine four doubtful samples of *Lysimachia christinae*, and it was found that all were *Desmodium styracifolium*. Table 2 illustrates the ten found adulterants.

Table 1. Composition of Five Flowers Tea samples. Number in the Table denote weight in gram. MMH: *Bombax ceiba*, JDH: *Plumeria rubra*, JH: *Chrysanthemum morifolium*, JYH: *Lonicera japonica*, HH: *Sophora japonica*, GH: *Pueraria lobata*, YC: *Artemisia capillaris*, XKC: *Prunella vulgaris*, HTM: *Polygonum chinense*, SY: *Morus alba*, QCZ: *Oroxylum indicum*, GC: *Glycyrrhiza uralensis*, TC: *Tetrapanax papyrifer*, TFL: *Smilax glabra*, FL: *Poria cocos*, JYH: *Lysimachia christinae*, BX: *Dioscorea hypoglauca*, BMG: *Imperata cylindrical*, BZY: *Microcos paniculate*, PPY: *Eriobotrya japonica*.

	MMH	JDH	JH	JYH	HH	GH	YC	XKC	HTM	SY	QCZ	GC	TC	TFL	FL	JYH	BX	BMG	BZY	PPV
HK01	60	5	4	2			15	12												
HK02	62	3	2				13													
HK03	35	5	6	1			15													
HK04	47	9	15				12	23				7								
HK05	28		14	6			22	27			6				24					
HK06	45		6	1			19	19	74											
HK07	15	13	14	3	2		21	25												
HK08	51	23	16	5			29													
HK09	51	20	19	15			19													
HK10	107		20	7			42	9												
HK11	64	19	15	14		14	21													
HK12	46	15	14	14	16															
KL01	84		2	1		5	45	2												
KL02	35	12	13	5			5	19		6		7			12					
KL03	36	2	7	3			14	16	16	9	4	4								
KL04	41	13	27	2	7															
KL05	43	16	7	4	3	5	20													
KL06	62						14				5					6		8	10	
KL07	17		15				20	20	9			5								
KL08	27	15	20				43	26												21
KL09	28		13					24								14	11		34	
KL10	50	12	11			9	32													
NT01	31	5	7			4	25	15				10								
NT02	54	3	10	2			36	20												
NT03	34	10	9	6		6	32	28												
NT04	34	36	24	25	24															
NT05	37		6	2			19	10	16								25			
NT06	13		2				8	17		79			1		10					
NT07	35		11				55	32					1							
NT08	38	6	9	6		5	23	13												
NT09	42	3	14	4			45	26												
NT10	19		11	4		9	5													
NT11	56	18	19	7			36										17			

Table 2
Summary of identification of suspected adulterants using BLASTN search from GenBank

Voucher number	Species claimed	DNA barcoding region	Species identification using BLASTN	Max percent identity
HK09	<i>Lonicera japonica</i>	psbA-trnH	<i>Lonicera macranthoides</i>	100
HK10	<i>Artemisia capillaris</i>	ITS2	<i>Potentilla chinensis</i>	99.01
HK11	<i>Lonicera japonica</i>	psbA-trnH	<i>Lonicera macranthoides</i>	97.61
KL05	<i>Lonicera japonica</i>	psbA-trnH	<i>Lonicera macranthoides</i>	100
KL06	<i>Lysimachia Christinae</i>	ITS2	<i>Desmodium styracifolium</i>	100
KL09	<i>Lysimachia Christinae</i>	ITS2	<i>Desmodium styracifolium</i>	100
NT05	<i>Lysimachia Christinae</i>	ITS2	<i>Desmodium styracifolium</i>	100
NT07	<i>Artemisia capillaris</i>	ITS2	<i>Potentilla discolor</i>	99.71
NT11	<i>Lysimachia Christinae</i>	ITS2	<i>Desmodium styracifolium</i>	100
NT05	<i>Lonicera japonica</i>	ITS2	<i>Lonicera macranthoides</i>	97.79

3.6 Procedures for decoction generation

A total of 16 parcels were advised to bring up a boil, and all the parcels were advised to brew with different volumes of water, time length and level of heat. It was suggested that other ingredients can be added to increase the potency or flavour. Rock sugar and *Siraitia grosvenorii* (Lo Han Kuo) were the most prevalent optional additives to Five Flowers Tea, recommended by five store owners. For KL02, slab sugar but not rock sugar was suggested. Store owner of KL07 suggested to add *Lonicera japonica*. Table S1 summarizes the recommended procedures for preparing the decoctions.

3.7 Storage conditions and identity of insects

All 12 samples that contained insects were discovered to be moist. We found that all collected insect samples were *Lasioderma serricorne*, as revealed by sequencing the COI region and compared with the data in BOLD Identification System (BOLD-IDS) or BLAST search in GenBank.

Discussion

4.1 Variation of Five Flowers Tea formula

Etymologically, the term Wu Hua Cha can be directly translated into Tea with Five Flowers. The most used formula is based on the combination of five herbs, *Bombax ceiba*, *Plumeria rubra*, *Chrysanthemum morifolium*, *Lonicera japonica* and *Sophora japonica*. These five herbs possessed the function of heat clearing, dampness draining and detoxifying. The herbs would act on intestinal area and liver. In terms of pharmacological effect, most of the five herbs possess the function of anti-inflammatory, anti-bacterial, anti-viral, anti-diarrheal and diuretic effect, aligning with the ethnomedical claims.

In this study, we only found eight samples comprised of the five floral herbs. Some herbal shop owners mentioned that some herbs are cut to reduce the cost. Herbal tea is a grassroot remedy in respond to the expensive fee for consulting a clinician. Therefore, there is often a trade-off between the cost and potency of the herbal tea. *Prunella vulgaris*, for example, is a cheaper alternative to other expensive herbs like *Pueraria lobata* and *Sophora japonica*. Over half of the parcels (63.6%) contain it. *Prunella vulgaris* is renowned in the heat clearing function, and as a major ingredient in another popular herbal tea Xiasangju [12].

Artemisia capillaris was found in 28 out of 33 parcels (84.8%) (Fig. 2). This herb is not a component in the classic formula. Also, the herb is not in the flowering stage. The harvesting season of *A. capillaris* is between March and April [13]. It is believed that during this period, the medicinal properties of *A. capillaris* are at their strongest for clearing heat and draining dampness, surpassing its effectiveness when collected during the flowering period.

Artemisia capillaris was used to *Sophora japonica*. According to the principle of Chinese medicine, *A. capillaris* has a greater dampness draining ability, but *Sophora japonica* is better in heat clearing. Some studies suggested that *A. capillaris* may have hepatoprotective, anti-cancer, and anti-inflammatory properties [14–16]. Therefore, herbalists may preferentially select *A. capillaris* for its superior dampness dispelling capacity.

4.2 DNA authentication of herbs

Herbal remedies often face issues of adulteration due to the difficulty of visually identifying authentic herbs from fraudulent substitutes. The visual appearance of closely related herbal species can be highly similar, making organoleptic identification difficult for those without specialized knowledge of the subtle morphological differences. This confusion has led to many instances of adulteration, such as *Desmodium styracifolium* being used as *Lysimachia christinae*, *Potentilla* species substituting for *Artemisia capillaris*, and *Lonicera confusa* being used in place of *Lonicera japonica*.

DNA barcoding method was performed to identify the herbs used in Five Flowers Tea parcel. DNA barcoding is a molecular technique used to identify species based on species-specific differences in a short, standardized fragment of nucleus and organelle DNA from a specific gene or genome region. That DNA fragment is then compared to a reference DNA database to determine the identity [17].

This method can precisely and rapidly identify species from various raw materials. In plant identification, the common barcode regions used are ITS2 and psbA-trnH. The region ITS2 (Internal Transcribed Spacer 2) is located in the nuclear genome, between 5.8S and 28S rRNA genes [18]. The region psbA-trnH is in the non-coding region of the chloroplast genome. Due to their high variability, the region can serve as a distinctive marker for a particular species [19].

The COI region, which is located in the mitochondrial genome of eukaryotic organisms, was used to identify insects due to its significant level of intra-specifically similarity and inter-specifically variation [20].

Using DNA barcoding technique, we detected the substitution of *Lonicera japonica*, *Artemisia capillaris* and *Lysimachia christinae* by *Lonicera macranthoides*, *Potentilla* species and *Desmosium styracifolium* respectively. This may pose risks of having a reduced therapeutic efficacy or unintended side effects.

4.3 Reasons of using the adulterants and potential health benefits and hazards

The first herb in question was *Lonicera japonica*. While *L. japonica* and *L. macranthoides* show similar pharmacological efficacy as evidenced by their medicinal usage history, the Chinese Pharmacopeia (2005 Edition) lists them separately. Both *L. japonica* and *L. macranthoides* exhibit low toxicity [21]. There have been many clinical trials conducted on *L. japonica*. In contrast, clinical investigations on *L. macranthoides* are limited. Both *L. japonica* and *L. macranthoides* share similar properties, including being categorized as cold in nature, sweet in flavor, and having comparable therapeutic functions and applications. *L. macranthoides* is commonly cultivated and used as a substitute for *L. japonica* in China. Despite having similar chemical profiles and pharmacological effects, *L. macranthoides* offers a higher yield and lower production cost. This makes *L. macranthoides* an economical alternative to *L. japonica* [22].

The second herb in question was *Artemisia capillaris*. Studies have shown that *A. capillaris* has greatly benefited the treatment of many diseases such as liver inflammation, cirrhosis, and liver cancer, contributing to the wider development and advancement of traditional Chinese medicine [23]. *A. capillaris* and its active compounds have antioxidant, anti-inflammatory, antifibrotic, antiviral and anticancer effects. This also supports its traditional uses for treating liver and viral disorders as well as cancer [24–26]

We found that *Potentilla* species was used to replace *A. capillaris* in Five Flowers Tea. *Potentilla* species have been noted for their medicinal functions that are akin to those of *A. capillaris*, although there are variations between the herbs in terms of chemical profiles and effectiveness in treating certain conditions. *Potentilla* species have been used in traditional medicine, particularly in Chinese medicine, for a long time to treat ailments such as diarrhoea, hepatitis, rheuma, scabies, and for detoxification purposes [27]. *A. capillaris* was found to be adulterated by *Potentilla* species, in particular *Potentilla discolor* and *Potentilla chinensis*. The aerial and underground parts of *Potentilla discolor* have been used to treat inflammation, wound, cancer, infection caused by bacteria, fungi, and viruses, diarrhoea, and diabetes mellitus [28]. There have been no reports of toxic effects during the long history of using *Potentilla* species and their extracts in traditional medicine [27].

Substituting *A. capillaris* by *Potentilla discolor* and *Potentilla chinensis* may be appropriate due to the pharmacological properties of the two latter herbs. *A. capillaris* is indicated for dispelling dampness due to its bitter and pungent flavour. *P. discolor* and *P. chinensis*, with their stronger cooling properties, are more suitable for resolving excessive heat conditions compared to *A. capillaris*.

The third herb in question was *Lysimachia christinae*. *Lysimachia christinae* is a traditional herb that possesses heat-clearing, diuretic, detumescent, and detoxifying properties [29]. The adulterant *Desmosium styracifolium* is widely used in traditional Chinese medicine for its diuretic and heat-clearing properties and for the management of urinary calculi, cardiovascular and cerebrovascular disorder, and hepatitis [30].

From the Chinese medicine perspective, *L. christinae* and *D. styracifolium* have some similarities but also distinct differences in their indications and usage. Both herbs are valued for their diuretic and heat-clearing properties, which help resolve accumulated dampness-heat and dissipate pathogenic factors. However, *L. christinae* focuses on dispelling dampness-heat in liver and gallbladder. It is indicated for conditions that involve pathogenic factors affecting the hepatobiliary system. In contrast, *D. styracifolium* focuses more on dispelling dampness-heat in the urinary bladder and kidney for urological conditions.

L. christinae and *D. styracifolium* therefore should be used interchangeably as they are for treating different types of conditions.

4.4 Instructions on brewing and precautions

Instructions for brewing the tea differed among the different shops. The time, quantity of water, procedure of brewing and heat level vary (Table S1).

Furthermore, indications on the contradictions are simple and not mentioned in every parcel. For instance, sample HK10 stated that it is suitable for everyone regardless of their health condition. Sample NT02 suggested that people who are of “cold” constitution can only drink half of a bowl. G6PD deficient patients was suggested to avoid taking *Lonicera japonica* [31, 32]. Of the 33 samples surveyed, only one parcel provided notice that people with G6PD deficiency should avoid using the product.

Apart from the potential danger of G6PD, a retailer has mentioned that pregnant women should avoid taking the Five Flowers Tea. However, a survey has indicated that more than half of Chinese pregnant women living in Hong Kong have taken Chinese herbal medicine, including Five Flowers Tea and *L. japonica*, for improving their gastrointestinal and digestive health. The use of Chinese herbal medicine during pregnancy did not appear to affect the condition, incidence, or severity of jaundice in both normal and G6PD deficient infants at birth [33]. The herbs used in Five Flowers Tea are not included in the List of Restricted Chinese Herbal Medicines for Pregnant Women [34].

4.5 *Lasioderma serricorne* infestation and quality control

L. serricorne was first reported and identified on tobacco in France in 1848 [35]. It is currently globally distributed and exceptionally prevalent in tropical and subtropical areas and is the most destructive pest affecting a wide variety of materials derived from both plants and animals [36]. It has long been recognised as the dominant pest species that infests stored Chinese medicinal materials, resulting in significant economic loss [37]. In our study, 12 out of 33 Five Flowers Tea samples were contaminated by *L. serricorne*.

The larvae of *L. serricorne* can cause significant damage by feeding on herbal tea leaves, resulting in reduced quality and value. Some conditions give rise to the growth of *L. serricorne*. Firstly, since herbal tea parcels are stored in compacted form, they provide an ample food source for the beetle to feed on. Secondly, the beetles like to hide away from bright light and low humidity. The best temperature range and relative humidity for rapid development of *L. serricorne* are 29–35°C at 75% [38]. All our samples found with insects were moist. Hong Kong is warm and humid, not to mention the hot and humid storage conditions in warehouses and wet markets.

Thirdly, packaging material and styles would be another issue. The adult stage of the beetle is capable of biting through the packaging material [39]. In our study, different packaging styles are found (Fig. 1). Insect infestation is higher when the herbs are wrapped in a piece of paper or put in a transparent plastic bag with opening tied with a nylon. *L. serricorne* can easily penetrate the paper wrap, and paper wrapping cannot protect the herbs from the humidity. Nylon string tied on the bag opening is easily loosened, creating an entry point for the insect and moisture to enter.

The presence of contaminated and gnawed herbs, as well as the excrement, cast-off skin, and other products, can pose a menace to the health of consumers. *L. serricorne*, in particular, has been linked to cantharisis, a human enteric infection [40]. Cases of intestinal cantharasis have been reported in China and Malaysia [40, 41].

Effective pest management methods include phosphine fumigation, CO₂ treatment and radiation treatments and high temperature. In recent years, pest management has put more emphasis on friendly to the environment and cost-effectiveness [42]. For instance, the package can be treated with natural repellents that prevent *L. serricorne* from consuming the contents [43]. Examples of such repellents include crude leaf extracts of *Tecoma stans* and *Datura metel*, as well as various plant essential oils such as shiso oil and savory oil [44]. For storage, multilayer packaging is an alternative to applying pesticide or temperature control warehouse. *L. serricorne* was found to get through polyethylene, polypropylene, and polyester [45]. Thicker packaging material and multilayer packaging better deterred the spreading of the insect [39].

Conclusion

Five Flowers Tea has been consumed for prevention and therapeutic purposes in Southern China for centuries. This work provides a systematic analysis on the composition and quality of Five Flowers Tea parcels sold in Hong Kong. We have found the use of variety of herbs, adulterants, irregular instructions and insect infestation. A better quality control of this popular herbal tea is needed. Also, only with a standardized and authenticated formulation can consumers be confident that this culturally significant herbal remedy delivers its attributed effects.

Abbreviations

28S: 28S ribosomal RNA gene

5.8S: 5.8S ribosomal RNA gene

A. capillaris: *Artemisia capillaris*

BLAST: Basic Local Alignment Search Tool

BOLD: Barcode of Life Data System

CO₂: Carbon dioxide

COI: Mitochondrial cytochrome oxidase subunit 1 gene

D. styracifolium: *Desmodium styracifolium*

DNA: Deoxyribose nucleic acid

ITS2: Internal transcribed spacer 2

L. japonica: *Lonicera japonica*

L. christinae: *Lysimachia Christinae*

L. macranthoides: *Lonicera macranthoides*

L. serricorne: *Lasioderma serricorne*

P. chinensis: *Potentilla chinensis*

P. discolor: *Potentilla discolor*

PCR: Polymerase chain reactions

psbA-trnH: Intergenic spacer between Photosystem II protein D1 gene and tRNA-His gene

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

KTC: Investigation, Data Curation, Writing - Original Draft; HYW: Conceptualization; WYT: Data Curation; PPHB: Writing - Review & Editing; SCHC: Writing - Review & Editing; PCS: Supervision

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Figures

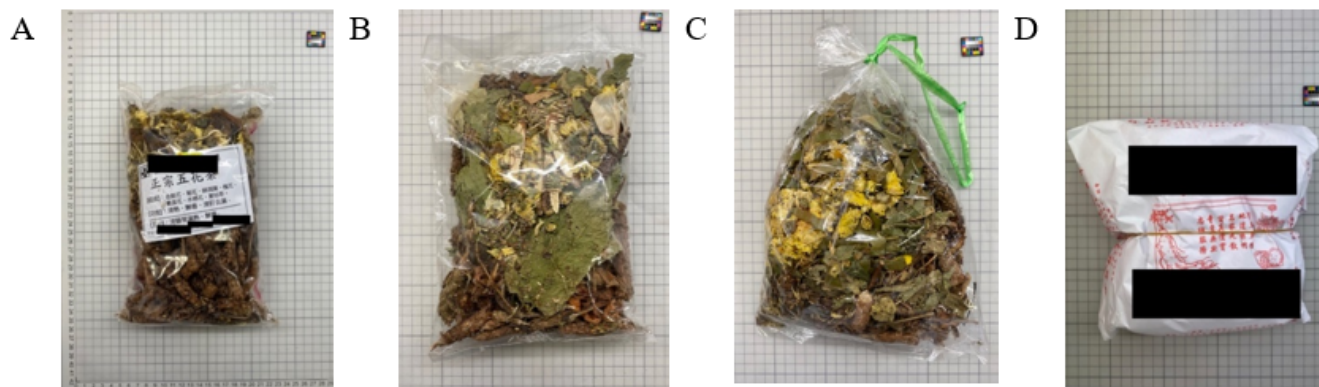


Figure 1

Packaging styles of Five Flowers Tea samples. (A) HK07: Transparent plastic bag with a piece of note stating their brand, name of the tea, ingredients, and functions. (B) KL03: Transparent plastic bag closing with heat-seal. (C) KL07: Transparent plastic bag tying by nylon string. (D) HK08: Wrapped by paper printed with company name and address.

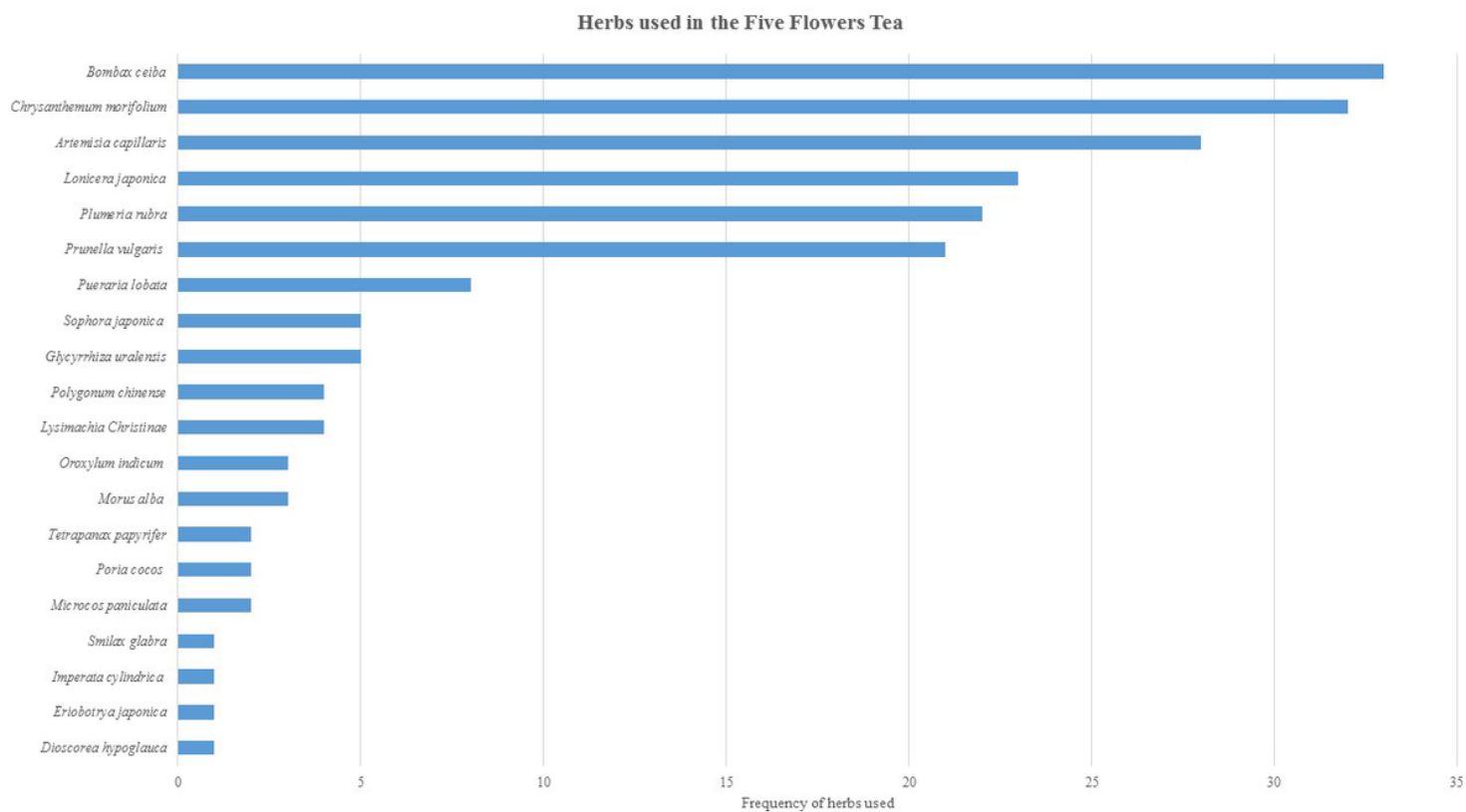


Figure 2

Herbs used in the Five Flowers Tea. Number denotes the frequency of herbs used.



Figure 3

Image of insects collected from sample HK08 as an example

Supplementary Files

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