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Xinyi Huang April 11, 2016

Investigation on effectiveness of traditional Chinese topical antimicrobial uses of *Ginkgo Biloba*

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
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Bachelor of of Sciences with Honors

Biology Department

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Abstract

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Ginkgo biloba, originated from China, has been spread as an ornamental tree around the world. Its seeds have been regarded as snacks and food materials in Asia since pre-modern time, while its leaf extracts became a source of rising pharmaceutical commerce related to brain health in the last century. Besides studies about the neuro-protective effects of Ginkgo, its antibacterial activities have gained more attention from researchers in the past decades, but its leaves were the main focus. However, 500 years ago, the traditional *Chinese Material Medica*, *Ben cao gang mu*, had already recorded prescriptions involving Ginkgo seeds to treat infectious skin diseases. Therefore, we are interested in whether different Ginkgo tree parts, leaves, seeds and branches, have antibacterial activities, especially against skin pathogens. In this study, Ginkgo plant materials were separated by part into leaves, branches, seed kernel, seedcoats and immature seeds. Extraction methods included ethanol (80%) maceration, water decoction and sonication, and oil infusion. Those extraction methods were selected based on the prescriptions of Ginkgo's topical uses in traditional Chinese remedies. Skin pathogenic bacteria studied included *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Propionibacterium acnes* and *Acinetobacter baumannii*. Known chemicals present in Ginkgo plant (terpene lactones, flavonoids and ginkgotoxin) were also tested individually against the pathogens. Out of the 27 extracts, 17 demonstrated growth inhibition to at least one skin pathogen. Ginkgo extracts were more effective against Gram-positive

bacteria. The flavonoids tested demonstrated activity against *S. aureus*; however, the other chosen chemical standards did not demonstrate any activity. Ethanolic extracts were most effective compared to extracts produced by other methods. In summary, we demonstrate here that other Ginkgo parts, especially seeds, may have some antibacterial activity meriting further study. Importantly, this study demonstrated efficacy of this TCM use of *Ginkgo biloba*.

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TABLE OF CONTENTS

List of tables

List of figures

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to my advisor, Dr. Quave, for making all of those happen, guiding me and encouraging me. I would also like to thank my committee members, Dr.Reinders, for his expert help related to traditional Chinese culture; and Dr.Cole, for her extraordinary advices in the thesis process. I am fortunate to be their student.

I would like to thank Dr. Lyles and Kate from the Quave lab for teaching me and helping me all the time. I appreciate their friendly guidance and patience.

I am grateful for my parents. Even though they cannot understand the language or the science of my thesis, they always cheer for me.

Finally, my appreciation to everyone who suffered form the special smell of Ginkgo seeds during my collection period yet still being supportive to my investigation.

Chapter 1 Introduction

When it comes to Traditional Chinese Medicine (TCM), the first thought in people's minds is most likely to be "*yin* and *yang*". Ancient Chinese people believed that everything in nature was composed of two opposite qualities, and the balance between those two extremes was the basis of our bodies (Liu 1988). Possibly because the origin of understanding of health was the inner balance, Chinese physicians pursue the eventual cure by reset the original inner balance, even for skin diseases. Nonetheless, there were still topical uses for herbal products. Ginkgo is one of the plants that were recognized in Traditional Chinese medicine for both internal and external treatments. In the *Traditional Chinese Materia Medica*, *Ben cao gang mu* (本草纲目), Ginkgo (*Ginkgo biloba*) was recorded in prescriptions for lung diseases, urine circulation diseases, as well as several kinds of skin diseases (Li 1518-1593). From the perspective of modern medicine, it can be surprising that those diseases belonging to different pathological groups can be included in prescriptions using one plant.

In TCM system, the lung can represent a single organ, or a system, the lung channel (肺经), which includes *qi* (the flow of energy) and circulation. In this case, human skin is not only the body surface, but also a site that regulates *qi*; for example, pores were called "gates of *qi*" by some physicians (Liu 1988). Skin diseases are usually regarded as manifestations of internal diseases. For instance, eczema is thought to be caused by dampness which disrupts the dispersing of *qi*; thus, the dampness inside the body must be removed in order to cure the skin. In short, the lung, the skin, and urination belongs to the lung channel, which is susceptible to pathogen like dampness. Ginkgo has characteristics against those pathogens and thus it can treat diseases mentioned above.

Compared to drugs for oral administration, which are composed of specific processed and precisely weighted herbs, the compositions of topical medicines are simpler, even down to a sole herb. Directly applying the raw Ginkgo seed kernel is one of the methods more often recorded in *Ben cao gang mu*. However, most of time, the balance of different characteristics from multiple herbs, or the synergetic effects of them, are more commonly seen in TCM prescriptions. There are two ways to view why a single herb can be prescribed for skin diseases. One is that there was no sophisticated system, such as the balance of *yin* and *yang,* developed for skin diseases; two is that the physicians had confidence on the effectiveness of one plant. At the time before antibiotics, pre-modern Chinese physicians seemed to be somewhat superstitious when it came to topical medicines and skin diseases. According to *Ben cao gang mu*: Ginkgo seeds have the ability to expel parasites and the noxiousness, because they are "*yin* and sinister." Thus, Chinese people get that conclusion of its character because Ginkgo tree flowers blossom in the evening, the *yin* hours of a day (Li 1518-1593).[1] However, without acknowledgement of the existence of bacteria, pre-modern Chinese understood that threatful things are a double-edged sword—Ginkgo seeds are "sinister" but they can also kill bad things on people.

Nowadays, *Ginkgo biloba* is an ornamental plant, with eye-catching bright yellow leaves in autumn; it is also known as a complementary and alternative medicine (CAM) for brain health maintenance. Before the plant's introduction in Europe in the 1730s (Singh, Kaur et al. 2008) and its leaf CAM discovery last century, its seed plays an key role rather similar to antibiotics nowadays: both save people from infectious skin diseases.

Were the original texts wrong? Do they need to be updated? It is reasonable to stop using something if it is not efficient enough to work against bacteria. However, there is a chance that

[1] Translated by the author. More information about this text and its translation will be discussed in Chapter 3, section of "Historical text review".

we neglect that Ginkgo seeds could be a cure for infectious skin diseases, simply because the pieces of evidence are before the time of antibiotics. Researchers have been working hard to discover new chemicals in order to fight bacteria. But what if there were already solutions in old remedies?

In this study, we address whether Ginkgo has antibiotic activities. Ginkgo tree parts are extracted using methods originated from traditional *Chinese Materia Medica*. Seed kernels, which is recorded in the historical text, leaf, which is more frequently studied nowadays, are included, as well as seedcoats, immature fruits and branches. Four bacteria strains that are common causes of skin diseases are chosen: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Propionibacterium acnes* and *Acinetobacter baumannii*. Each Ginkgo extracts are used to treat those skin pathogens. We determine whether a Ginkgo extract is active against a bacteria strain by testing if the bacteria growth is inhibited after treated with the extract.

Our hypothesis is that Ginkgo extracts inhibit growth of skin pathogenic bacteria. Since the Ginkgo extracts in this study were made based on the traditional Chinese remedies, we also examine the efficacy TCM use of *Ginkgo biloba*. Because the remedy of the same plant includes certain prescription for a particular skin disease, we have the reason to believe that Ginkgo extracts prepared differently can have different level of effectiveness to each specie of bacteria. Therefore, we aim to find the method that can produce the most effective Ginkgo extracts, looking at several factors that may contribute: the plant part, the extraction solvent, the gender of the Ginkgo tree and the storage method.

Chapter 2 Background

2.1 Antibiotic resistance: the need of new solutions

Potencies of many antibiotics to the bacteria are questioned nowadays, because it has been decades since they were used and the treatment outcomes have become lower than expected. Therefore, we cannot underestimate the antibiotic resistance that the pathogens carried out. Here I will introduce the four skin pathogens chosen, along with their capability of resistance to antibiotics.

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium. It is a major cause of hospital-acquired infection worldwide and one of the leading causes of skin and soft tissue infections (SSTIs) (Dragulescu and Codita 2015, Lacey, Geoghegan et al. 2016). Treatment of *S.aureus* infections becomes more obstructed because of the antibiotic resistance of the pathogen. In particular, methicillin resistant *S. aureus* (MRSA) increasingly causes skin infections and has become resistant to even last resort antibiotics.

Propionibacterium acnes (*P. acnes*), is also a Gram-positive bacterium; it is also an anaerobic organism, thus it has slow growing rate comparing to other strains we chosen. One of the most common disease it causes is acne vulgaris, a chronic inflammatory disorder of the sebaceous follicles that affected up to about 80% of people in their adolescence (Grange, Chéreau et al. 2009). A large scale survey in China recently shown that macrolide–lincosamide–streptogramin (MLS) has been a main group of antibiotics prescribed for acne vulgaris patients, and over half of *P. acnes* strain are resistant to MSL(Fan, Hao et al. 2015). To be more specific, erythromycin was an effective topical treatment of acne vulgaris since 1950s, but its efficiency

has decreased to 33 –50% of the previous level (Simonart and Dramaix 2005). Also, application history of tetracycline is indicated to be a cause of reduction in its susceptibility to *P. acnes*.

Acinetobacter baumannii, a Gram-negative bacterium, is also a prevalent nosocomial pathogen. Multidrug-resistant *Acinetobacter baumannii* (MDRAB) is a frequent cause of difficult-to-treat nosocomial infections since some of its isolates are resistant to all clinically relevant antibiotics (Weber, Ly et al. 2015). Beside its drug resistance, vigilance for SSTIs caused by *A. baumannii* is necessary, since it becomes more common as *A. baumannii* associated infections have been increasing in health care settings (Guerrero, Perez et al. 2010). More specifically for patients who experience trauma, *A. baumannii*-associated SSTIs are emerging infections (Sebeny, Riddle et al. 2008).

Klebsiella pneumonia is also a Gram-negative bacterium. It is an opportunistic pathogens causing life-threatening nosocomial infections, such as pneumonia, bloodstream infections, wound or surgical site infections, and meningitis (Hackstein, Kranz et al. 2013). However, it is not a very common cause of skin diseases. Extended-spectrum β-lactamases (ESBLs) producing *K. pneumoniae*, which is resistance to all penicillins and cephalosporins, is the most common drug resistant *K. pneumonia*. Like *A. baumannii*, it is also a pathogen that rises more attentions nowadays when it comes to infections on wounds (Sensenig, Murray et al. 2012).

2.2 *Ginkgo biloba*

Ginkgo biloba (Ginkgoaeae), or maidenhair tree, is also called "duck flippers" in China(Li 1518-1593) due to its unique fan-shaped leaves. Its Chinese names *Bai guo*, or *yin xing*, all refer to its seeds with white inner integument. Seeds can only be produced by the female ginkgo tree, and the mature seeds have a distinguishable "stinky" smell due to butyric acid produced by their seed coats. A ginkgo tree has an equal chance of being male or female, and its gender can be validated after it reaches its fruiting age, which is usually 20-40 years (Isah 2015); thus ginkgo tree is also called "grandpa-grandson tree", because people can wait for two generations to get a female tree producing seeds. Fruiting season for the female trees vary with location and weather, but usually in the fall when leaves are still green; ginkgo leaves then start to turn to bright yellow and fall.

Figure 1 Pictures of *Ginkgo biloba*. Both captured on Emory University campus, September 30th, 2015. Left: Female Ginkgo tree, height about 20 meters. Right: Male Ginkgo tree, height about 12 meters.

Ginkgo is known as “living fossil” because its existence can be traced back to 200 million years ago; its origin is believed to be in the Zhejiang province of China(Singh, Kaur et al. 2008). It does not have any close relatives in the plant kingdom, since other members of the order Ginkgoales are extinct(Isah 2015). In order to thrive for this long, *Ginkgo biloba* must have evolved good pathogen defense mechanisms (Major 1967). There are previous studies that isolated antifungal chitin binding protein(Huang, Xie et al. 2000).

Ginkgo biloba was introduced in Europe in around 1730s(Singh, Kaur et al. 2008), and extraction of *Ginkgo biloba* leaves, or EGb761, started a rising commerce in Europe last century. Commercial leaf extracts with brand names Rökan, Tanakan, and Tebonin (2003), are used for treating neural system disease, such as dementia(Ahlemeyer and Krieglstein 2003), as well as keeping senior brain health. The standard extraction method results in a composition of 24% flavonol glycosides, primarily quercetin and kaempferol, and isorhamnetin, and 6% terpene of which 2.9% is bilobalide and 3.1% ginkgolides.(Ross 2015) in which higher concentration than found naturally in ginkgo leaves, also limit ginkgolic acid composition to less than 5ppm (Gauthier and Schlaefke 2014). Studies have shown that EGb 761 has a neuroprotective effect, as well as increases peripheral blood flow and reduce viscosity by inhibit platelet clotting (Shen and Cui 1998, Ahlemeyer and Krieglstein 2003). Besides the widely-used EGb 761, there were more than 3000 patents filed on Ginkgo and its constituents before 2010(van Beek and Montoro 2009). *Ginkgo biloba* extract has been in the top ten frequently used herbal dietary supplement in the United States since last century(Chan, Xia et al. 2007).

In China, Ginkgo seed has been playing a more important role than Ginkgo leaf. Eating ginkgo seed in fall has been advocated until nowadays, because fall has been regarded as a “dry season” and ginkgo can “moisturize the lung”(Li 1518-1593). Boiling, cooking with other food

material as soup or with rice as porridge, are common food remedies. In Chinese *Materia medica*(Li 1518-1593), records about ginkgo indicated only the seed, as the plant was included in the fruit chapter. Ginkgo seed was recorded as drug to treat respiratory system diseases, such as asthma, as well as urinary system diseases. However, topical use was also recorded as an important application of ginkgo seed. Infectious skin diseases were treated with raw ginkgo seed; the remedies included cutting the shell open and scrubbing on skin, pounding to paste or pounding with drinking alcohol and apply on infected areas, and immersing in oil. Usage of leaves involved is only included in Chinese herbal remedy in rather recent medical books. Despite of the variety of ginkgo seed applications, the seed is always consumed in small doses. Neurotoxin 4'-O-methylpyridoxine, or ginkgotoxin, can cause convulsion even death if more than several dozen are ingested at once, especially lethal for children(Wada, Ishigaki et al. 1988). However, there was no such toxin caution for topical use of ginkgo seed, and the absorbance through skin has not been investigated, therefore, the risk of intoxication for topical application is not clear. Ginkgotoxin is also find in a trivial amount in leaves, but the amount is too small to have any detrimental effect (Arenz, Klein et al. 1996).

2.3 Compendium of Materia Medica, "*Ben cao gang mu*"

Chinese herbal medicine system is said to be founded by *Shennong*(神农), who tasted all the herbs he could find, record their tastes as well as any effects they had on him (Hinrichs 2013). Thanks to this brave man in legendary, Chinese physicians were able to add more useful herbs or usage on known ones, and make corrections. Li shizhen(1518 -1593), the author of *Compendium of Materia Medica*, *Ben cao gang mu*(本草纲目), was one of the physicians who contribute the most. Li summarized over 40 earlier pharmacopoeia and more than 300 medical texts into his book; moreover, he traveled across China to interview local people in order to validate names and usage of recorded medicines and find new ones (Hinrichs 2013). Like *Shennong*, Li tasted many of the medicines and made valuable annotations. Therefore, *Ben cao gang mu* is not merely an herbal medicine record, but a historical guide to medicine (not limited to plants), including culture, past annotations from famous physicians, remedies to diseases, and prescriptions.

One of the first things mentioned when introducing any herbal medicine is its characters (性) and tastes(味). Character and taste can be regarded as two further categorizations to a medicine, besides its nature, which is *yin* or *yang*. An herb's character can be cold or hot, or something in between: cool or warm. Tastes includes: sweet, sour, bland, salty, bitter, astringent and spicy. On one hand, based on the legendary of *Shennong*, tasting seems to be a proof of credibility of the record; on the other hand, it is believed that the medicine's taste and character indicates its medicinal effect. In general, herbs with sweet taste helps tonifying and moistening; sour consolidates; astringent constricts, which is similar to sour taste herbs; bland promote body fluid absorption; salty softens; spicy helps circulation of *qi* and blood (Gao 1997).

In *Ben cao gang mu*, the first sentence that leads into the medicinal record of Ginkgo is about its character and taste. (see Figure 2)

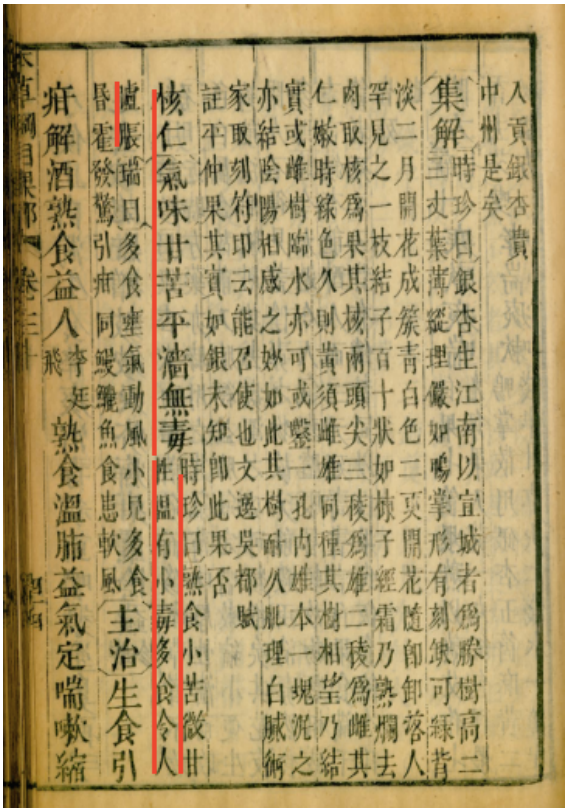

Figure 2 Picture of *Ben cao gang mu* text. A block-printed copy made in China, 1826, stored in Emory University Pitts Theology Library. The page shown here is the second page of Ginkgo record.

“核仁 【气味】甘、苦，平，涩，无毒。
时珍曰:熟食，小苦微甘，性温有小毒。多食令人胪胀。”

“Kernel [smell and taste]: sweet, bitter, bland, astringent, not poisonous. Shizhen commented: After being cooked, [Ginkgo] tastes a little bitter and sweet; its character is bland and a little poisonous. Eating too many makes people’s bellies bloated.” The major effect of Ginkgo seeds are tonifying the lung, stopping coughs, reducing urine and helping clear urine; reducing sputum, cleansing and expelling parasite; curing skin disease. If we look across the taste and effect of Ginkgo, many of its medicinal use could be indicated by its taste according to the empirical summarization of taste and function.

2.4.1 What are known: antimicrobial activities

Standardized extract EGb761, organic solvent extract of leaves and seedcoat, as well as isolated proteins and lipids that unique to ginkgo were found to show various bioactivity towards microorganisms. For example, ginkbilobin, extract from ginkgo leaves showed inhibitory activity towards variety of fungi, including *Botrytis cinerea*, *Mycosphaerella arachidicola*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Coprinus comatus* (Wang and Ng 2000). Ginkgo seedcoat extract exerted antifungal activity against *Rhizoctonia solani*, or rice sheath blight (Oh, Koo et al. 2015). Ginkgo leaf extracts can also be used in oil fields, where its activity is against bacteria growing in oil pipes (Chen, Zhang et al. 2013). Due to ginkgo extracts' effectiveness against plant pathogenic fungi and other microorganisms, they can be applied to various fields such as food preservation. Nonetheless, researchers invested most attention on ginkgo's effect on human health.

There are previous studies to claim that, aqueous Ginkgo leaf extract and extracts of Ginkgo fruits [seeds] exhibited antimicrobial activity against Gram-positive bacteria, but no activity against Gram-negative bacteria (Brantner and Grein 1994, Boonkaew and Camper 2005). For example of bactericidal activity against Gram-positive bacteria, ethyl acetate partition product of methanolic leaves extract was against *Staphylococcus sanguis* and *Enterococcus faecalis*, *Staphylococcus aureus, Staphylococcus epidermidis;* noticeably, this extract also inhibited growth of yeast strains *Candida parapsilosis, C. krusei*, *C. tropicalis* and *C. albicans* (Mazzanti, Mascellino et al. 2000). However, more recent studies provide counterevidence to the activity limitations towards Gram-negative bacteria. Methanolic extract of leaves were proved to be effective against both Gram-positive and Gram-negative phytopathogenic bacteria:

Agrobacterium tumefaciens-, *Bacillus subtilis*, *Escherichia coli*, *Erwinia chrysanthemi*, and *Xanthomonas phaseoli* (Sati and Joshi 2011)

Focusing more on infectious disease treatment, other than applications for plant pathogen, researchers found Ginkgo extracts also to be effective. Salicylic acid from extracted from Ginkgo sarcotesta (seedcoat), showed antibacterial activity against vancomycin-resistant Enterococcus (VRE), *E. faecalis* (Choi, Jeong et al. 2009) Ginkgolic acids extracted from Ginkgo leaves exhibited high antibiofilm formation activity against Enterohaemorrhagic *Escherichia coli* (EHEC) and multi-drug resistant *Staphylococococus aureus* without inhibiting planktonic cell growth (Lee, Kim et al. 2014). *S. aureus* growth was also inhibited by the lipid extract from Ginkgo leaves, nerolidol, and synergistic mixture of Ginkgo biloba polyprenols (GBP) with isophytol, which also inhibited *Salmonella enterica* and *Aspergillus niger* (Wang and Ng 2000). Ginkgolide A, ginkgolide B, bilobalide and the standardized Ginkgo leaf extracts (Tanakans) exhibited antimicrobial activity against *Streptococcus pyogenes* (Boonkaew and Camper 2005), which is a common cause of pharyngitis and impetigo. Growth of *Staphylococcus epidermidis*, a member of common skin flora, was also inhibited by Bilobalide and Tanakans (Boonkaew and Camper 2005). Ginkbilobin from Ginkgo leaves also exhibited a moderate antibacterial action against *S. aureus*, and also *Pseudomonas aeruginosa*, which can cause dermatitis and soft tissue infections (Wang and Ng 2000).

As we can see from the previous studies, there were investigations on Ginkgo specific to infectious skin disease, but Ginkgo seeds were not focused on. Nonetheless, traditional Chinese medicine records from hundreds years ago claimed ginkgo seed to be effective to treat infectious skin diseases. Several diseases were listed out: genital infectious diseases such as crab louse, genital sores; rosacea, which is likely caused by *Helicobacter pylori*; breast carbuncle, a possible

consequence of *S.aureus* infection; general shingles, sores on skin. Therefore, the effectiveness of traditional ethno-use is worth to be validated or exanimated, exploring potential usages of Ginkgo that might be neglected recently.

2.4.2 What are known: chemical composition

Plant parts	General categories	Examples of constituent
Leaves	Terpene lactones	Ginkgolides, bilobalide
	Flavonoids	Quercetin, kaemferphol, isorhamentin
	Others	Ginkgolic acid
Seeds	Terpene lactones	Ginkgolides, bilobalide
	Flavone glycosides	Quercetin, kaemferphol, isorhamentin
	Proteins	Ginkgobilobin
	Others	Ginkgolic acid, ginkgotoxin, polysaccharides
Seedcoats	Others	Ginkgolic acid, polysaccharides
Branches	Terpene lactones	Ginkgolides, bilobalide
	Flavone glycosides	Quercetin, kaemferphol, isorhamentin

Table 1 Summarization of chemical composition of Ginkgo tree parts.

Since Ginkgo leaf extract EGb761 brought the plant in scientific interest, Ginkgo leaf's chemical composition was more well-studied compared to other parts of the tree. Terpenoids, flavonoids, and ginkgolic acid are the compounds found in all parts of the plant, though the amounts contained vary. Among the terpenoids, there are several unique terpene lactones that present only in Ginkgo, ginkgolides and bilobalide. Those constituents are thought to be the major factors that contribute to neutron-protective effect of Ginkgo extracts (Ahlemeyer and Krieglstein 2003). Flavonoids in the Ginkgo have highest antioxidant activities (Stefanovits-Bányai, Szentmihályi et al. 2006), quercetin, kaemferphol and isorhamentin are the flavonol glycosides that were studied most frequently. Ginkgolic acid is the component which contain is restricted in standard extract to below 5ppm (10^{-6} g/g of extract), because it can causes damage to the brain(Ahlemeyer, Selke et al. 2001). However, it still keep researcher in interest because it

shows antimicrobial activities in previous researches: it is against certain bacteria, mite, and fungi (Yang, Chen et al. 2002, Pan, Luo et al. 2006, Lee, Kim et al. 2014). Ginkgotoxin is a neurotoxin that can causes seizure even death, since it competitively inhibits Vitamin B6 and thus cause improper neural impulse; It is mainly found in the seed kernel. Ginkbilobin is a protein found in the seed that have shown antifungal activates (Sawano, Miyakawa et al. 2007). Polysaccharides are found in Ginkgo seedcoats and seeds (also in leaves, but not much mentioned); they have shown activities to be antiviral drug (Lee, Park et al. 2015), or immune enhancer (Chen, Meng et al. 2015). Ginkgo branches is not as intensely researched as the other tree parts; as other tree parts, it has flavones as well as terpene lactones, which is about twice as leaf contains (Liu, Zhao et al. 2015).

Chapter 3 Materials and Methods

3.1 Historical text review

There are two sources of historical texts that I refer to: one is *Ben cao gang mu*, and the other one, which is minor, is *Summary of Chinese Herbal Medicine*, *quan guo zhong cao yao hui bian* (全国中草药汇编). The records about Ginkgo is recorded in the Book of Fruits, volume 30, section 2: Ginkgo, in *Ben cao gang mu*. The whole record is translated in English by the author. Since there is only one sentence in *Summary of Chinese Herbal Medicine* that is relative to this study, the whole text is not included in the translation.

Because *Ben cao gang mu* is a collection of previous annotations, the references of each prescription related to skin diseases were checked. Two original references are found: *Simple remedies for emergencies, jiu ji yi fang* (救急易方) and *General remedies, pu ji fang*(普济方), which each included prescriptions for "carbuncles on breast festering" and "furuncles full with fluid or with dark colors." However, according to those two texts, there are several mistakes in *Ben cao gang mu*, either a missing character or a wrong usage. More detail will be discussed in the translation portion of this section. The effect of those information can be huge, since this version of *Ben cao gang mu* was block printed in the Qing Dynasty, uncountable copies was generated by then, and the mistakes can be carried along the time.

Below is the translation of the section of Ginkgo in *Ben cao gang mu*:

"**Ginkgo (*yin xing*)** (daily use)

Explanation of its name

[alternate name] **White Fruit (*Bai guo*)** (daily use) [other name] **Duck Foot**

Shizhen commented: [Ginkgo] originated in Jiangnan (region south of Yangtze River). Its leaves look like duck flipper, so it got its nickname: the Duck Foot. Starting from Song Dynasty, Ginkgo was a tribute to royal families and was re-named the silver apricot (*yin xing*), because it looks like small apricot and its inner nutshell is white. Nowadays, it is called the white fruit (*bai guo*). Mei Raochen wrote a poem: "The Duck Flipper looks like a green (immature) plum. It is famous because of its interesting-shaped leaves." Ouyang Xiu wrote a poem: "The beautiful Ginkgo fruit became a Royal tribute, it became a treasure in central China."

Annotations

Shizhen commented: Ginkgo trees grow in Jiangnan, the best producing area is Xuan. The tree is usually two to three *zhang* (about 6~10 meters). The leaves are thin and the veins align vertically. They look just like duck flippers as the leaves have cleavages. [The leaves][2] are green and the backside of the leaves are lighter. In February [usually March in the Gregorian calendar], the tree flowers in clusters; the blossoms are in between light green and white. They bloom in the second watch of the night [9~11pm] and immediately fall, so people can hardly see them. One branch has about a hundred fruits, shape like *lian zi* (chinaberry); they mature and become rotten in the Fall, after frost. After peeling the outer seedcoats and keeping the inner nuts, the two tips of its shell are pointed; triple-edged ones are male fruits, double-edged ones are female fruits. Their kernels are green when they are tender, and will become yellow later. In order to bear fruit, the male and female have to be planted together and the trees must grow near to each other; or the female tree has to be next to water or a stream; or if one cuts the female tree and seals a piece of male tree trunk in it, it can also bear fruit. The interaction between Yin and Yang is indeed fascinating. The Ginkgo tree can survive very long, and its texture is white and smooth; therefore, magicians believe that it can summon a [spirit] messenger, so they use Ginkgo wood to make amulets. There is an annotation in *Literary Selection of Wu*:" there is kind of fruit in Pingzhong, the fruits look like silver; could it be Ginkgo?"

Kernel [smell and taste]: sweet, bitter, bland, astringent, not poisonous

Shizhen commented: After being cooked, [Ginkgo] tastes a little bitter and sweet; its character is bland and a little poisonous. Eating too many makes people's bellies bloated.

Rui commented: Consumption of too many [Ginkgo] blocks *qi* and causes dizziness. Children who eat too many Ginkgo will faint, have panic attacks, and have digestive diseases. Consuming Ginkgo and eel together can lead to paralysis.

[2] The character Ke, 可, is used here, but is clearly incorrect. From the context, I interpret the meaning as "The leaves"

Major uses:

Raw administration causes digestive diseases and can sober up drunk people; Cooked Ginkgo are good for people. (Li Yanfei)

Cooked [Ginkgo can] warm the lung, benefit the circulation of *qi*, stop coughing, constrict urine, cure turbid micturition. Raw [Ginkgo are] sputum reducing, detoxifying and insecticidal. By chewing it for its juice and applying the paste on face and limbs, chapped skin and freckles, scabs, digestive diseases, and crab louse can be treated. (Shizhen)

Discovery

Shizhen commented: People started to know Ginkgo in the Song Dynasty, but the physicians did not include it in medicinal herb records. More recent remedies sometimes include it. It does not have a strong smell but have strong tastes; it is astringent and constrictive, its color is white and it belongs to Metal [of the five phases system]. Thus, it can be used to treat lung diseases, such as promotion of *qi* circulation, cure of coughing, and urine constriction. The pestle paste of raw Ginkgo fruit can wash away oil stains, thus we can see its effectiveness to get rid of sputum and turbidity. Because Ginkgo flowers bloom in the night and cannot be seen by people, Ginkgo is yin and sinister, so it can kill insects and eliminate noxiousness. However, eating too many Ginkgo leads to too much constriction, blocking the *qi*, belly bloating and fainting. Thus *The Book of Folk Encyclopedia* recorded: "Ginkgo can intoxicate people". But *The Book of Sanyuan for longevity*[3] mentioned: "People who eat a thousand Ginkgo will die". It also mentioned that once there were hungry men, they ate Ginkgo as meals, and they all died the day after.

Appendix: Prescriptions, a new list of 17 items

Cough and sputum due to cold: seven Ginkgo. Stew the Ginkgo, stuff each into cooked mugwort, wrap with paper and stew until they smell, peel the mugwort and eat the Ginkgo. (*Remedy from Mi yun, the book of secret prescriptions*)

Cough and sputum because of asthma: "Duck flipper powder". Five Ginkgo, two and half *qian* of ephedra, two *qian* of licorice (roasted). [Add] one and half *zhong* of water, decoct to seven-tenth of the volume, drink at bed time. Another prescription of asthma from a drug store in Jinling city, the "Ginkgo asthma-cure broth", was effective to every patient; the owner was able to start his business because of this prescription. The prescription is: twenty-one Ginkgo fry to yellow, three qian of ephedra, two qian of

[3] Abbreviation of 三元延寿参赞书.

perilla seed, two qian of tussilago, standardized processed pinellia and bark of white mulberry honey roasted, one *qian* of peeled almond, [dried root of] *Scutellaria baicalensis* and ?[4], one qian of half licorice; [add] three *zhong* of water, decoct until two *zhong* [of the liquid left], divide [the drug] into two serving to drink , no use of ginger. （combine *Prescriptions of health preservation*）

Frequent micturition: fourteen Ginkgo, eat seven raw and seven stewed, stop the remedy after it works.

White and turbid urine: ten raw Ginkgo kernel, pestle with water, drink once per day, stop the remedy after it works.

Leucorrhea contains blood, infirmity of kidney: five *qian* (about 16 grams) of Ginkgo, lotus seeds, and sticky rice, one and half (about 5 grams) black pepper. Pestle all in powder. Stuff in a silkie chicken, stew with crockery. Eat on an empty stomach.

Hematofecia/ hemorrhoidal hemorrhage: Stew Ginkgo in order to remove its hot *qi*, eat and deliver with rice water.

Hematofecia/ dysentery: fourty nine Ginkgo, crack open the shell and pestle the raw kernel. Decoct with *bai yao* (gallnut, *wu bei zi*, fermented with tea leaves) to powder and make into a drug ball. Eat one or two each time, eat on an empty stomach and chew thoroughly, deliver with rice water. (*Essence treatments* by Dai Yuanli)

Tooth decayed by worms: Chew one or two raw Ginkgo after meals. (*Illustrated remedies*)

Chapped hands and foot: Raw Ginkgo, chew up and apply to the affected part every night.

Nose like distillery draff (pustule/rosacea): Chew up Ginkgo and distillery draff, apply in the evening and wash away in the morning. (*Collection of medicinal reports*)

Tinea and skin ulcer on head and face: cut open Ginkgo kernel, rub on affected parts. (*Remedies out of experience*, by Shao)

Sores on genital parts: pestle raw Ginkgo and apply on affected parts.(by Zhao Yuanyang)

Crab louse induced itchiness: itchiness cause by insects like louse, white or red, grows in skin under pubic hairs. Kernel of Ginkgo, chew up and rub on affected area.

Skin ulcer caused by dog bite: Kernel of Ginkgo, chew up and rub on affected area.

Carbuncles on breast festering: half *jin* (about 250 grams) of Ginkgo, half pestle with wine and drink, the other half pestle [with water][5] and apply on affected parts. (*Simple remedies for emergencies, jiu ji yi fang*)

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[4] Fraction of two Chinese characters, thus unreadable. See the Appendix for an copy of the texts.
[5] The character for water, shui, 水, was missing, but was recorder in the original reference *jiu ji yi fang*.

Furuncles full with fluid or with dark colors: furuncles with fluid are yellow, and cause numbness but not pain; dark color furuncles are protruding and red, and cause confusion and madness. Pierce their edge then [apply][6] the paste of Ginkgo that was de-shelled and immersed in oil for a long time. (*General remedies, pu ji fang*)"

[6] The character Yin, which refers to the other character Yin, 饮, is a misreport according to the original text of *General remedies*.

3.2.1 Collection

Ginkgo tree samples were collected from a female and a male tree on Emory University Campus in September and October, 2015. The trees were green throughout the collecting period; leaves that were damaged or had yellow color on any part were not included in further extraction, in order to retain active constituents, such as flavonoids and terpene trilactones, in highest level(Ellnain-Wojtaszek, Kruczynski et al. 2001, Ellnain-Wojtaszek, Kruczyński et al. 2002, Goto and Usuki 2012). Voucher specimens (XH-01 and XH-03, see figure 3.1 and 3.2) were deposited at the Emory University Herbarium (GEO) in Atlanta, GA, USA.

Figure 3. Pictures of *Ginkgo biloba* vouchers. Vouchers were deposited in Emory University herbarium. Left: figure 3.1, the voucher specimen of female Ginkgo tree, XH-01. Right: figure 3.2, the voucher specimen of male Ginkgo tree, XH-03.

The gender of the Ginkgo trees was determined by the following criteria: 1. Fruiting. Female Ginkgo tress can bear fruits while male trees cannot (McKenna, Jones et al. 2001). 2.

Shape of the leaves. Li (2013) summarized Chinese empirical methods to distinguish gender of Ginkgo tree before fruiting age (which is usually more than 20 years.) Leaves from male trees have large and deep notch in the middle while leaves from female trees have not.

Whole mature ginkgo seeds that were newly fallen were collected; immature ginkgo seeds were collected directly from branches. Additional fresh seeds were bought from local farmer's market; their seedcoats were removed and the nutshell were cleaned before sale.

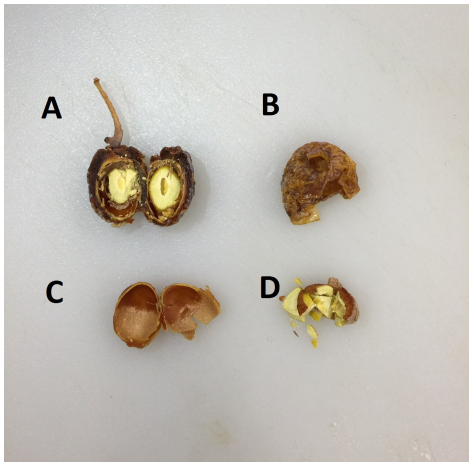


Figure 4 Dissection of Ginkgo seed. (a) Cross section of whole ginkgo seed. (b)Testa, or seedcoat. (c) Nutshell, not included in assay. (d) Seed, or endosperm.

Samples were separated by gender, then by different parts: leaves, branches, seeds and immature whole seeds. Mature seeds were separated into seedcoat (testa/integument) and seed(endosperm) (figure 4). Two types of samples were prepared from the plant materials: dry samples and fresh-frozen samples. Dry samples were prepared by dehydrating the plant materials in drying cabinet for at least 5 days, under 35°C and continuous removal of humidity; after dehydration, samples were grinded into fine powder with a Thomas Wiley Mill at a 2 mm mesh size (Thomas Scientific). Fresh-frozen were prepared by freezing the plant materials forthwith collecting under -20°C and being stored until extraction.

3.2.2 Extraction

Pre-modern Chinese valued Ginkgo differently than the Western countries. However, it is also not unusual that people's understanding to Ginkgo varied in time. Li shizhen, the author of *Ben Cao Gang Mu*, used the comment "invention" under introduction of the plant Ginkgo: "Ginkgo was not famous until the Song Dynasty (10-13 century)." (Li 1518-1593)

According to *Ben Cao Gang Mu*, the topical usage of Ginkgo seed involved delivery media of water, alcohol, and oil. Thus, extractions of Ginkgo seeds were processed with those solvents. Despite that methanol was used in majority of previous studies, for example, Sati et al. (Sati and Joshi 2011) claimed that methanol leaf extract was more active than ethanol leaf extract, 80% ethanol was chosen. In the traditional remedy, whether the alcohol mentioned was for consumption (e.g. wine) or for medication (e.g. tincture), ethanol was the form of alcohol available and commonly used. 80% was an adjust to enhance efficiency of extraction. The type of oil was not specified in the ancient remedy, but rape seed oil was a major source of oil in 16th century China, and still available nowadays. Decoction was performed to Ginkgo leaves, since in *Quan guo zhong cao yao hui bian*, the *Summary of Chinese Herbal Medicine*, Ginkgo leaf boiled water was a tropical treatment for children enteritis.

Samples were extracted with solvent in ratio of 1g: 10mL, regardless sample types or solvent type. For maceration, plant materials were mixed with EtOH or type II dH_2O, set in hood with daily agitation, for at least 72 hrs. After filtration, plant marcs were macerate in same manner for successive 72 hrs. For decoction, plant materials were heated with dH_2O on hot plate with a stir bar for 25min, at 100°C; the decoction products were then filtered after cooled to room temperature.

Vacuum filtrations were done in coarse (Fisherbrand P8) then fine (Fisherbrand P2) steps for ethanol extracts. For aqueous extracts, materials were filtered through cheese cloth, then centrifuged for 10 min under "speed 7"(~2500rpm). Supernatant were then filtrated through fine (Ahlstrom) filter paper. Filtered first maceration and second maceration were combined, and solvent was evaporated at <58 atm pressure at 38°C with rotary evaporators. Extracts were re-suspended with dH_2O and lyophilized. Extracts were stored in -20°C until being tested.

For MIC assays, ethanol extracts were dissolved in DMSO and aqueous extracts were dissolved in 50% DMSO to 10 mg/mL. For aqueous extracts, dH_2O was added and followed by DMSO, otherwise the extracts cannot be fully dissolved. DMSO was added to prevent microbial growth in dissolved aqueous extracts, because the aqueous extracts were susceptible to microorganism growth since the plant material were not sterile. Sterile filtered sterile filter (pore size=0.2 μm) was not suitable for processing the water dissolved extracts either, because extract particles were filtered out of the result solution.

3. 3 Microbiological assay: minimum inhibitory concentration (MIC)

Tests were performed based on guidelines of Clinical Laboratory Standards Institute (CLSI) M100-S23. The processes for different bacteria strains are the same, with variations in broth media, growth conditions, incubation lengths and antibiotic controls, details are listed in Table 2. The following description of MIC assay is in instance of *A. baumannii*. A single colony of *A. baumannii* is randomly selected from it agar plate, which is stored in -4°C until tested, and incubated in TSB broth media for 22hrs. Concentration of the overnight culture is determined at Cytation3 multimode plate reader (Biotek) at OD 560nm, then were standardized to 0.0006 under OD_{560} read ($5*10^5$ CFU/mL) with CAMHB media. Tests were performed in 96 wells plates (Cat No.655-185 Grenier-Bio). Vehicles (DMSO or 50% DMSO) and antibiotics, Gentamycin and Tetracycline, were tested as controls for each test performed. The concentrations of extract/control tested were from 512 μg/mL to 4 μg/mL in serial dilution, and for antibiotics 64–0.5 μg/mL were tested (4-0.03125 μg/mL for Cln and Ery only). All concentrations were tested in triplicates.

Plates were read by Cytation3 at OD 600nm at 0 hour and after 22 hours of static incubation with humidity at 37°C (see Figure 5). To determine the effectiveness of extracts, percentage inhibition of bacteria growth was calculated using Equation 1 below. MIC50 and MIC90 were determined as the concentration in which 50% and 90% of bacteria growth were inhibited, comparing to bacteria growth in media with vehicle added.

$$\% \text{ inhibition} = \left(1 - \left(\frac{\Delta OD_{extract}}{\Delta OD_{vehicle}}\right)\right) \times 100$$

Equation 1. Equation for percentage inhibition of an extract. ΔODs are the difference in OD_{600nm} read before and after incubation. Vehicle stands for wells containing vehicle and bacteria culture; extract stands for wells containing extract and bacteria culture.

MIC	strain	Incubation time (hours)	media			antibiotics
			plate	Overnight culture	incubation	
EU27	*A. baumannii*	22	TSA	TSB	CAMHB	Gent, Tet
EU32	*K. pneumoniae*	18	TSA	TSB	CAMHB	Gent, Tet
UAMS1	*S.aureus*	18	TSA	TSB	CAMHB	Amp, Van
AH1263	*S. aureus*	18	TSA	TSB	CAMHB	Amp, Van
AH430	*S. aureus*	18	TSA	TSB	CAMHB	Amp, Van
ATCC6919	*P. acnes*	72	TSA+10%SB	BHI+1%dextrose	BHI+1%dextrose	Cln, Ery

Table 2. Variations of growth condition for different bacteria strains. The abbreviations of the chemicals included in this chart are explained in the end of this section.

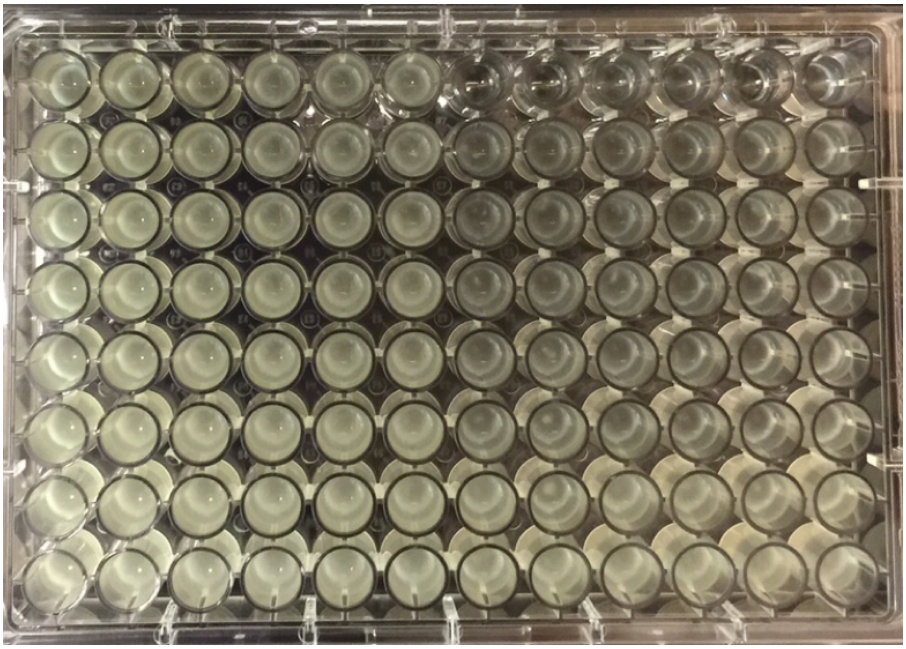


Figure 5. A plate after incubation in MIC assay. Wells with darker color contain more cells, indicating more bacteria growth. Wells with clearer color contain less cell, indicating bacteria growth was inhibited more.

3. 4 Cytotoxicity assay

The extracts' toxicity to human keratinocyte, a predominant type of cell in the outermost layer of the skin, were tested according to cytotoxicity assays by Quave et al. (Quave, Lyles et al. 2015). Human immortalized keratinocytes (HaCaT cell line) were maintained in DMEM medium with L-glutamine and glucose (4.5 g/L), supplemented with 10% heat-inactivated FBS, Penicillin (100 IU) and Streptomycin (100 μg/mL) at 37°C, 5% CO_2, in 75 cm^2 flasks (Greiner Bio-One). After the cell line growth reached 90–95% confluence, HaCaT cells were detached from the flask bottom by adding 0.25% typsin-0.1% EDTA in HBSS (without Ca^{2+}, Mg^{2+} and $NaHCO_3$) and gentle rocking. Before exposure to exracts, the cell culture was standardized to 4 x 10^4 cells/mL using a hemocytometer and 200 μL was added to each well in a 96 well tissue culture treated Microtiter plate (Falcon 35–3075); plates were incubated for 48 hours to allow for seeding to the bottom of flasks. Media were aspirated and fresh media containing either extracts or vehicle were serially diluted 2-fold (8–512 μg mL^{-1}) was added. All concentrations were tested in triplicates.

Toxicity of extracts were evaluated with the LDH Cytotoxicity assay (G-Biosciences) after 24 hours of incubation, following manufacturer's protocol for chemical induced cytotoxicity. Plates were read by Cytation3 at OD 490nm (Figure 6). To determine the cytotoxicity of extracts, percentage cytotoxicity was calculated using Equation 2 below.

$$\% \text{ inhibition} = \left(1 - \left(\frac{\text{OD}_{extract} - \text{OD}_{\text{spontaneous}}}{\text{OD}_{max}}\right)\right) \times 100$$

Equation 2. Equation for percentage cytotoxicity of an extract. OD_{max} is the OD_{490nm} read for wells treated with lysis buffer after incubation to achieve maximum of cell lysis. $OD_{extract}$ is the OD_{490nm} read for wells containing extracts or vhicle. $OD_{spontaneous}$ is the OD_{490nm} read for wells without treatment.

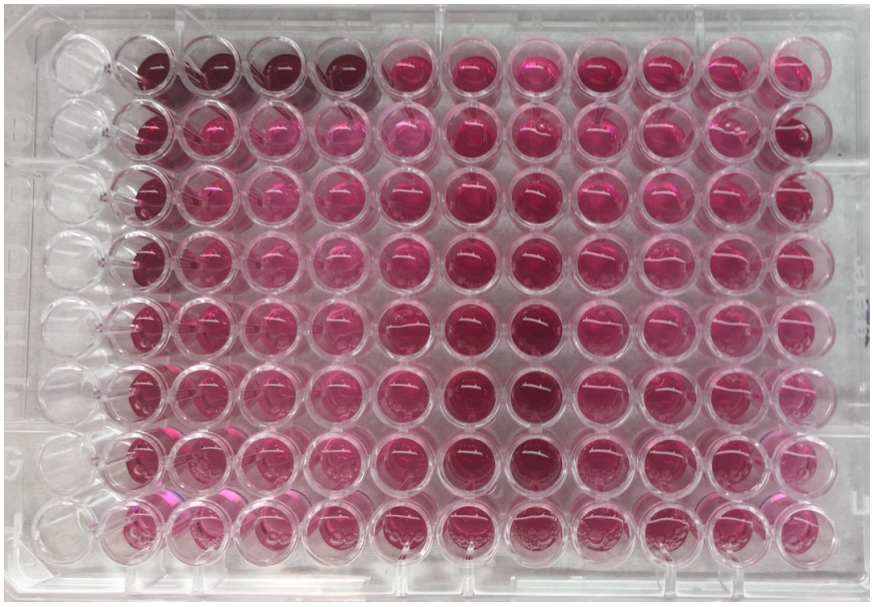


Figure 6. A plate to be read for cytotoxicity calculation. Darker color indicates more LDH presence, which represent cell damage, thus the toxicity of the treatment.

Abbreviations:

TSA: trypticase soy agar
TSB: trypticase soy broth
SB: sheep blood
BHI: Brain-heart infusion
CAMHB: Cation-Adjusted Mueller-Hinton Broth
Gent: Gentamicin
Tet: Tetracycline
Amp: Ampicillin
Van: Vancomycin
Ery: Erythromycin
Cln: Clindamycin
DMEM: Dulbecco's modified Eagle's medium
FBS: fetal bovine serum
EDTA: ethylenediaminetetraacetic acid
HBSS: Hanks' balanced salt solution

Chapter 4 Results

4.1 Extraction outcomes

In this chapter, I discuss the results generated in the progress of extraction, as well as the MIC assays for both extracts and chemical standards.

Plant parts	**Dry**			**Fresh-frozen**			
	Extraction method		**Percentage yield (%)**	**Extraction method**		**Percentage yield (%)**	**Percentage yield excluding water (%)**
Leaves	female	80% EtOH	27.62	female	80% EtOH	10.57	14.78
		decoction	21.82		decoction	7.06	9.86
		water	22.81		water	10.22	14.28
	male	80% EtOH	26.76	male	80% EtOH	12.22	17.66
		decoction	15.72		decoction	7.48	10.81
		water	23.90		water	63.74*	92.12
Branches	female	80% EtOH	8.19	female	80% EtOH	—	
					water	5.64	11.82
	male	80% EtOH	9.51	male	80% EtOH	—	
					water	4.62	9.68
Seeds (endosperm)	80% EtOH		8.34	80% EtOH		4.60	8.12
	water		10.28	water		11.02‡	9.11
				Oil infusion		—	
Seedcoat (Testa/ integument)	80% EtOH		74.01	80% EtOH		31.65	50.37
Immature seeds				80% EtOH		29.37	

Table 3 Percentage yield of Ginkgo extracts. Without specification, maceration is the method applied. —, percentage yield not applicable. *, final extract not fully dried. ‡, result for extract 689, not 675.

Yields were calculated using Equation3 below, dividing weight of plant material used by weight of lyophilized extract. In general, seedcoats generate highest yield of extract, followed by immature seeds, then leaves, then seeds, and branches are the lowest (see Table3). We have the reason to believe that there is still certain amount of water in the two seedcoat extracts, contributing to the high final weight of the seedcoat extracts, because the two extracts are sticky semi-fluid instead of power, like other extracts. The immature seeds extracts showed similar character, so water can be a reason of its high yield, too.

$$\%\ \text{yield} = \left(\frac{\text{weight of plant material}}{\text{weight of extract}}\right) \times 100$$

Equation 3. Equation used to calculate percentage yield of Ginkgo extracts.

The factor that contribute to the difference of yield between same plant part the most is the storage method, dry and fresh-frozen. The percentage yield of fresh-frozen plant materials had lower yield than the dried ones. The water content when calculating the yield is not what causing the difference, since the percentage yield of fresh-frozen plant materials excluding weight of water is still lower than that of dried ones. Percentage yield exclude the water content is calculated still with Equation 3, but the weight of plant material excludes the weight of water. See Table4 for the water content for each tree parts. Yield loss of fresh-frozen extracts could be generated in the step of centrifugation (while dry samples did not have this step), due to remaining filtrate in centrifuge pellets and plant marcs.

Plant part	gender	Percentage water loss(%)
leaves	female	28.45
	male	30.81
branches	female	52.31
	male	52.28
seedcoats		37.18
seeds		43.35

Table 4. Percentage water loss of fresh Ginkgo tree parts. Water contain was determined by drying the fresh plant materials in drying cabinet, under 35°C and continuous removal of humidity. The plants parts were dried for 24hr and weighted every 3~4hr, the final dry weights were determined when the weight loss was less than 1% than previous weighing. The percentage water loss was calculated by dividing the fresh weight by the final extract weight.

4.2 Antibacterial activities—MIC results

For the MIC assays, if an extracts fail to inhibit bacterial growth by 50% at the highest concentration tested, which is 512 μg/mL (or 64 μg/mL for the chemical standards), it is regarded as inactive against that bacteria strain.

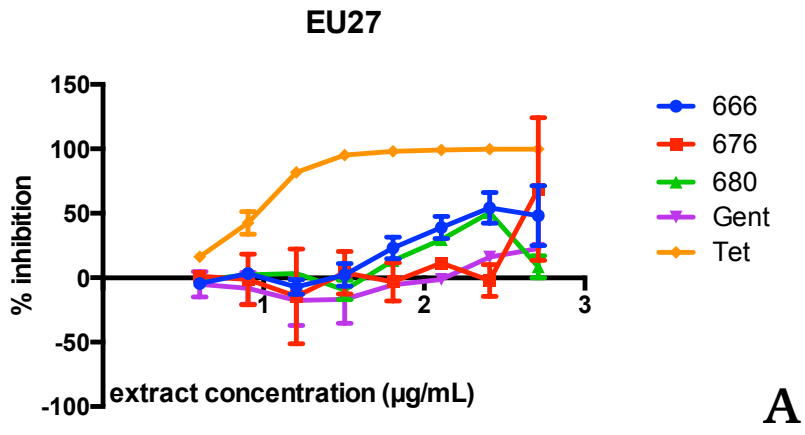
For the extract MIC assays that are active, beside a summary chart (Table 5), scatter plots of their dose responses, which is the percentage inhibitions under different extract concentration are also included here (Figure 7).

	A. baumannii	*K. pneumoniae*	P. acnes	S. aureus		
	EU27	EU32	ATCC6919	UAMS1	AH1263	AH430
Ginkgolide A	-	-	-	-	-	-
Ginkgolide B	-	-	-	-	-	-
Bilobalide	-	-	-	-	-	-
Ginkgotoxin	-	-	-	-	-	-
Quercetin	-	-	-	32	8	16
Isohamentin	-	-	-	-	64	-
Kaemferphol	-	-	-	64	16	-

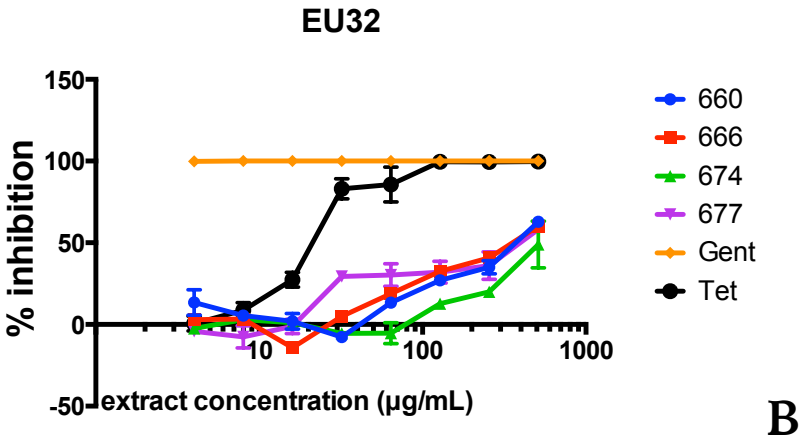
Table 5. Summary chart of MIC result for the chemical standards.

					A. baumannii	*K. pneumoniae*	*P. acnes*		*S. aureus*					
					EU27	**EU32**	**ATCC6919**		**UAMS1**		**AH1263**		**AH430**	
					MIC50	MIC50	MIC50	MIC90	MIC50/90	MIC90	MIC50/90	MIC90	MIC50/90	MIC90
extract#	part	solvent	gender	storage										
660	leaves	EtOH	female	dry	-	512	256	256	128	-	256	-	512	512
661	leaves	decoction	female	dry	-	-	-	-	-	-	-	-	-	-
662	leaves	water	female	dry	-	-	-	-	-	-	-	-	-	-
663	seeds	EtOH	female	dry	-	-	-	-	-	-	-	-	512	-
664	seeds	water	female	dry	-	-	-	-	-	-	-	-	-	-
665	seedcoats	EtOH	female	dry	-	-	64	64	32	-	128	-	128	-
666	branches	EtOH	female	dry	256	512	128	256	64	-	512	-	-	-
667	leaves	EtOH	male	dry	-	-	128	256	-	-	512	-	256	-
668	leaves	decoction	male	dry	-	-	-	-	512	-	512	-	-	-
669	leaves	water	male	dry	-	-	-	-	-	-	-	-	-	-
670	branches	EtOH	male	dry	-	-	64	64	-	-	256	-	128	-
671	leaves	EtOH	female	fresh-frozen	-	-	256	512	256	512	512	-	512	-
672	leaves	decoction	female	fresh-frozen	-	-	-	-	-	-	-	-	-	-
673	leaves	water	female	fresh-frozen	-	-	-	-	-	-	-	-	-	-
674	seeds	EtOH	female	fresh-frozen	-	512	-	-	-	-	-	-	-	-
675	seeds	water	female	fresh-frozen	-	-	-	-	-	-	-	-	-	-
676	seeds	rape seed oil	female	fresh-frozen	512	-	-	-	-	-	-	-	256	-
677	seedcoats	EtOH	female	fresh-frozen	-	512	64	512	-	-	128	-	128	-
678	immature seed	EtOH	female	fresh-frozen	-	512	64	64	64	-	128	512	128	-
679	branches	EtOH	female	fresh-frozen	-	-	64	64	64	-	-	-	128	-
680	leaves	EtOH	male	fresh-frozen	256	512	128	256	128	-	256	-	512	-
681	leaves	decoction	male	fresh-frozen	-	-	-	-	-	-	-	-	-	-
682	leaves	water	male	fresh-frozen	-	-	-	-	-	-	-	-	-	-
683	branches	EtOH	male	fresh-frozen	-	-	16	16	32	-	32	128	64	-
687	branches	water	female	fresh-frozen	-	-	-	-	512	-	512	-	512	-
688	branches	water	male	fresh-frozen	-	-	-	-	-	-	-	-	-	-
689	seeds	water	female	fresh-frozen	-	512	-	-	256	-	-	-	128	-

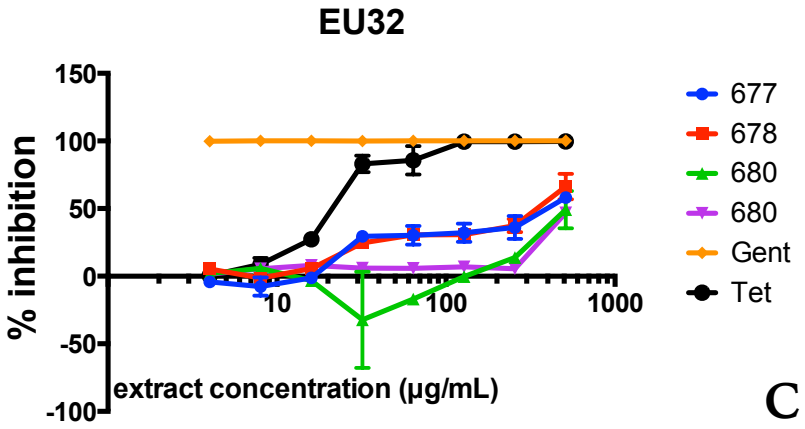
Table 6. Summary chart of MIC results for Ginkgo extracts. Dashes indicate no activities.


EU27
150
100
50
0
-50
-100
% inhibition
1
2
3
extract concentration (µg/mL)
666
676
680
Gent
Tet

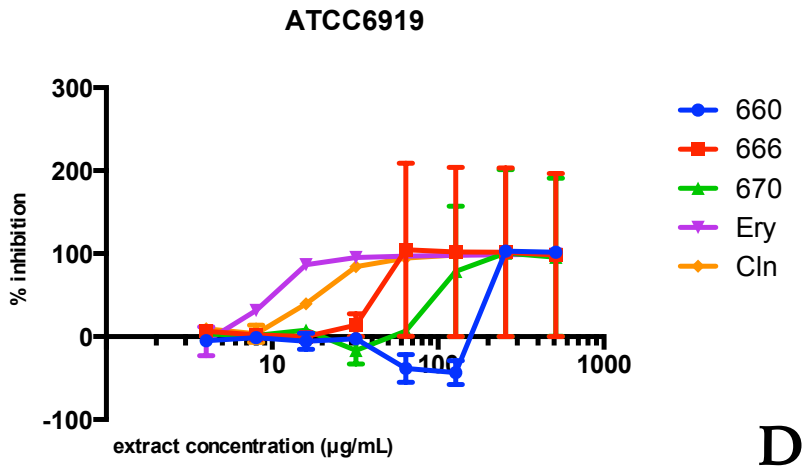
A


EU32
150
100
50
0
-50
% inhibition
10
100
1000
extract concentration (µg/mL)
660
666
674
677
Gent
Tet

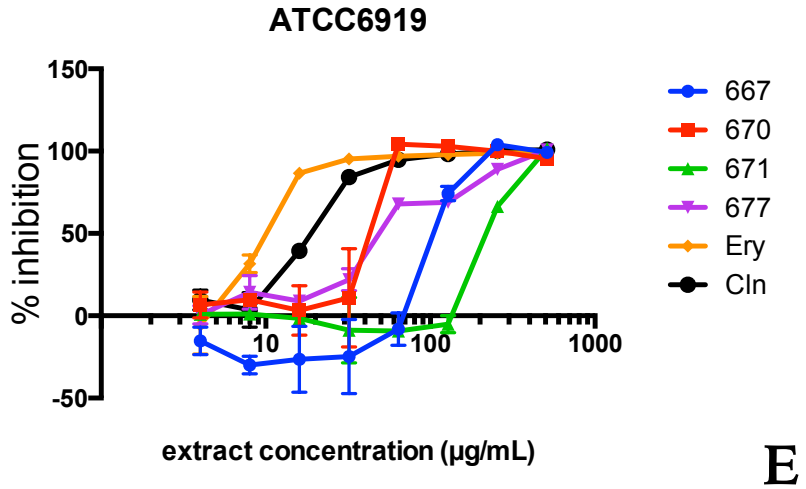
B


EU32
150
100
50
0
-50
-100
% inhibition
10
100
1000
extract concentration (µg/mL)
677
678
680
680
Gent
Tet

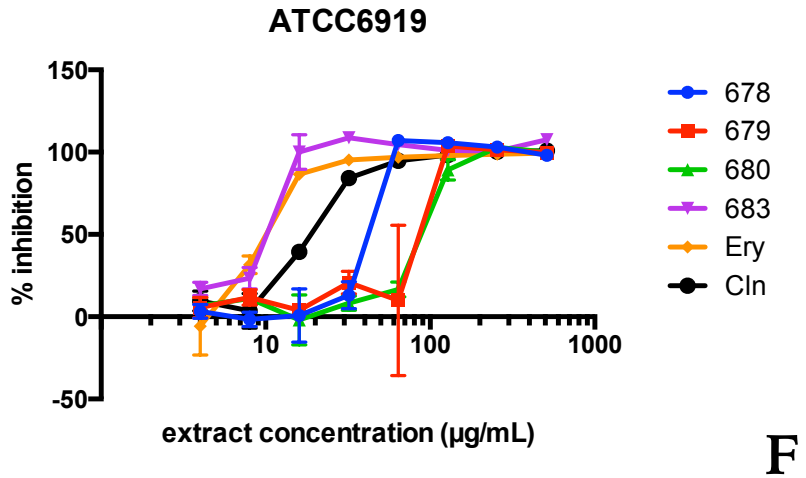
C


ATCC6919
% inhibition
300
200
100
0
-100
10
100
1000
extract concentration (µg/mL)
660
666
670
Ery
Cln

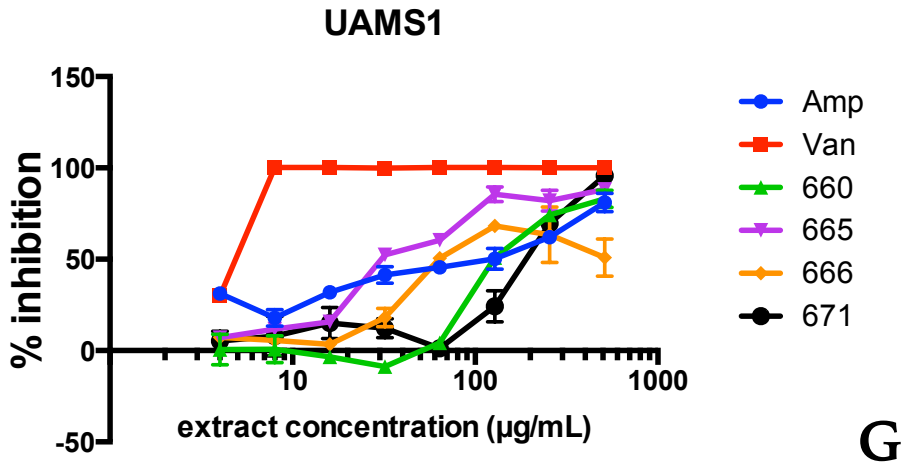
D


ATCC6919
% inhibition
150
100
50
0
-50
10
100
1000
extract concentration (µg/mL)
667
670
671
677
Ery
Cln

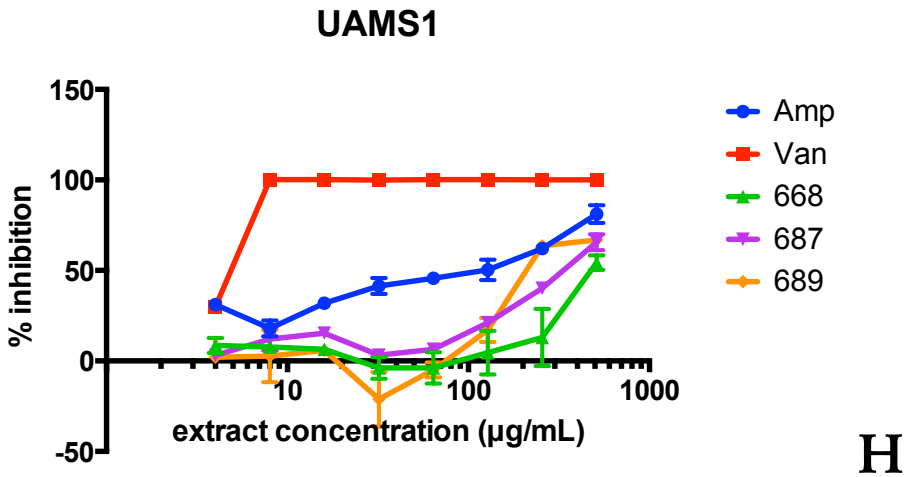
E


ATCC6919
% inhibition
150
100
50
0
-50
10
100
1000
extract concentration (µg/mL)
678
679
680
683
Ery
Cln

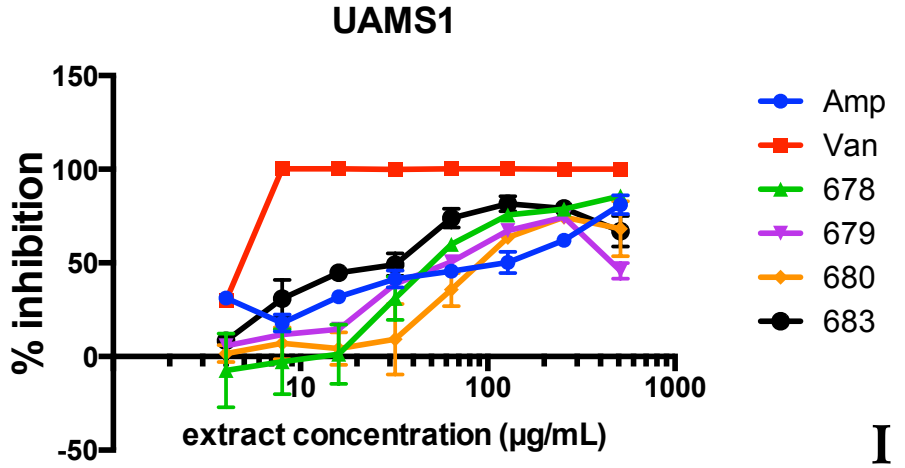
F


UAMS1
% inhibition
150
100
50
0
-50
10
100
1000
extract concentration (µg/mL)
Amp
Van
660
665
666
671

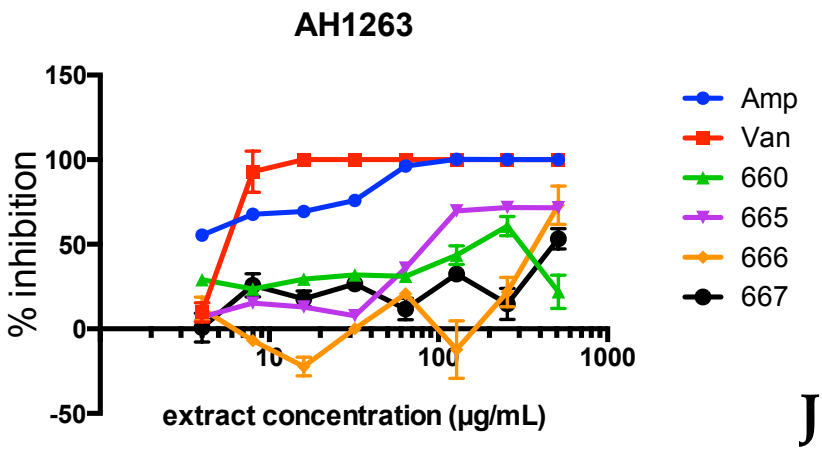
G


UAMS1
% inhibition
150
100
50
0
-50
10
100
1000
extract concentration (µg/mL)
Amp
Van
668
687
689

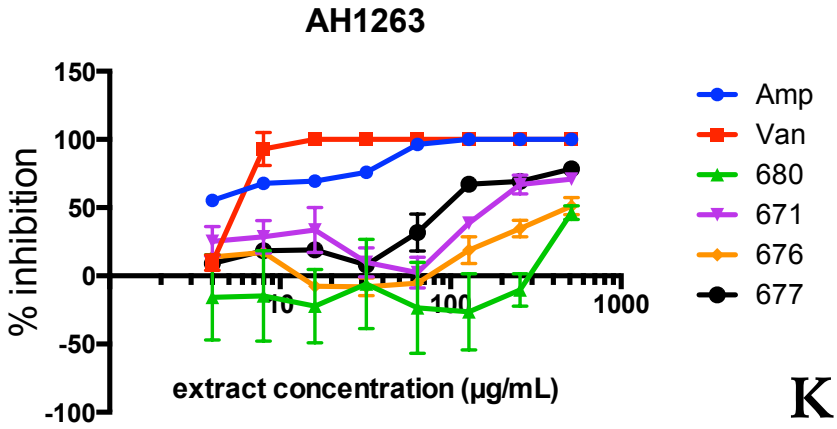
H


UAMS1
% inhibition
150
100
50
0
-50
10
100
1000
extract concentration (µg/mL)
Amp
Van
678
679
680
683

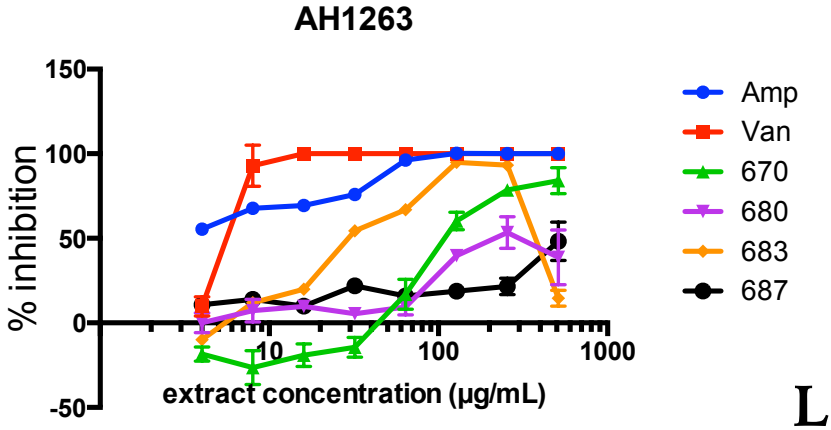
I


AH1263
% inhibition
150
100
50
0
-50
10
100
1000
extract concentration (µg/mL)
Amp
Van
660
665
666
667

J


AH1263
% inhibition
150
100
50
0
-50
-100
10
100
1000
extract concentration (µg/mL)
Amp
Van
680
671
676
677

K


AH1263
% inhibition
150
100
50
0
-50
10
100
1000
extract concentration (µg/mL)
Amp
Van
670
680
683
687

L

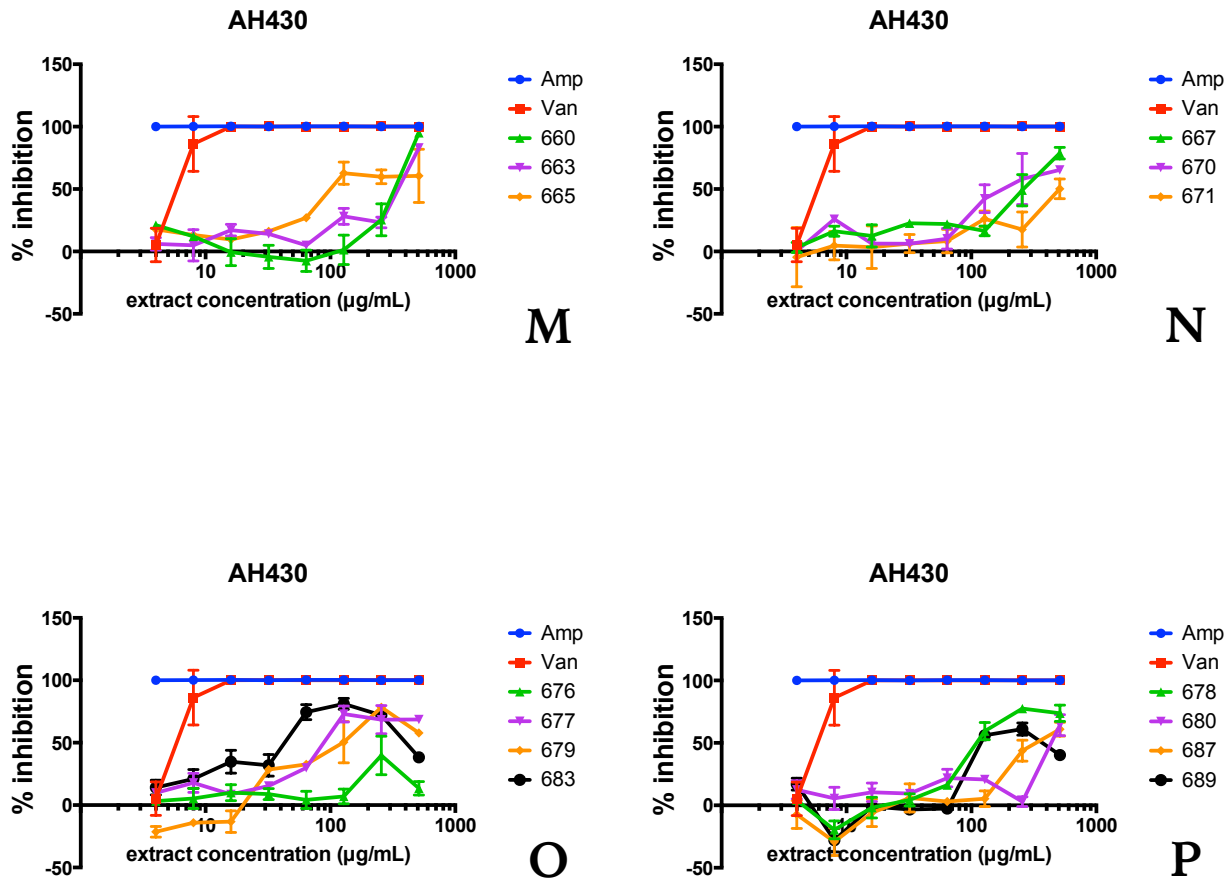


Figure 7. Dose response curves of active Ginkgo extracts. Figure 5A, dose response curve for extracts that actively inhibit growth of bacteria strain EU27. Figure 5B-5C, dose response curves for EU32. Figure 5D-5F, for ATCC6919. Figure 5G-5I, for UAMS1. Figure 5J-5L, for AH1263. Figure 5M-5P, for AH430. Active extracts are grouped in numerical order for each bacteria strain. Data for antibiotics for each strain are the same in each graph.

4.3 LDH cytotoxicity results

Active ginkgo extracts, chemical standards and DMSO as the vehicle were used to treat HaCaT cell line from their active concentration up to 512 μg/mL. The toxicity of Ginkgo extracts to human keratinocyte varies heavily between each extract (see Table 6 and Figure 8). All chemical standards, ginkgolide A, bilobalide, ginkgotoxin, quercetin, isorhamentin and kaemferphol, are not toxic to human keratinocyte (cytotoxicity<20%). DMSO, extracts 663, 668, 674, 677, 678 and 689 are not toxic to human keratinocyte (cytotoxicity<50%) through out the tested concentrations. For extracts 665, 679 and 683 cytotoxicity (>50%) were accountable at 4~8 times greater than their highest active concentration. Extract 660, 666, 670 and 680 are toxic to human cell at their highest MIC50 concentration. Extract 687 is generally toxic to both bacteria and human cell.

Extracts that were active but were not included in cytotoxicity result require further investigation in the future.

Cytotoxicity <50%		**Cytotoxicity >50%**		**Toxic in active range**		**generally toxic**	
extract	**tree parts**	**extract**	**tree parts**	**extract**	**tree parts**	**extract**	**tree parts**
663	seeds	665	seedcoats	660	leaves	687	branches
668	leaves	679	branches	666	branches		
674	seeds	683	branches	670	branches		
677	seedcoats			680	leaves		
678	immature seeds						
689	seeds						
DMSO							
Chemical standards							

Table 7. Summarization of cytotoxicity results.

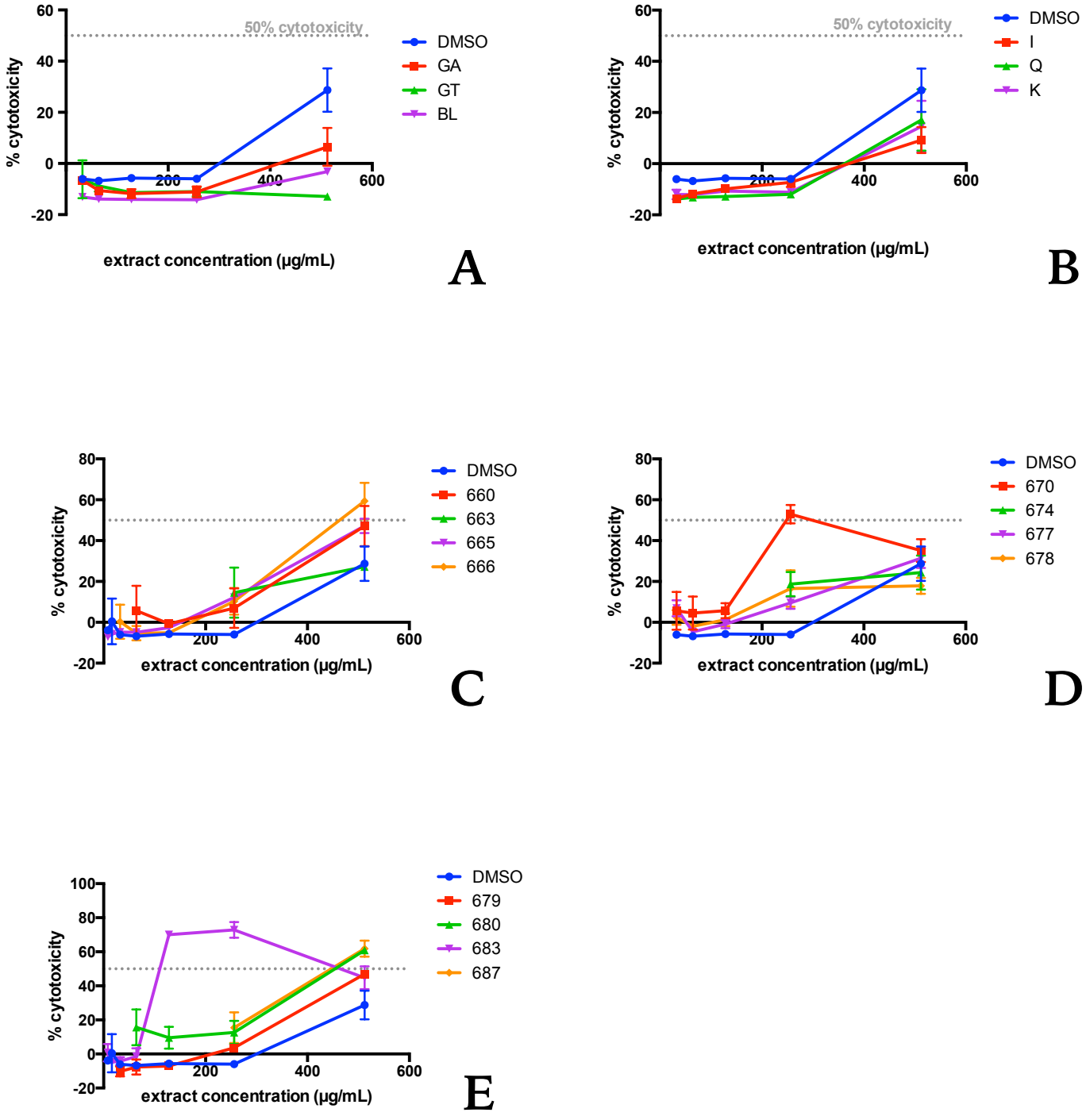


Figure 8. Dose response plots for cytotoxicity assays. The plots represent percentage cytotoxicity of extracts versus their concentration. A horizontal reference line was added to each graph to indicate the level where we started to account cytotoxicity, which is 50%. Figure 8A-8B, dose repose plots for chemical standards. Figure 8C-8E, dose response plots for the active Ginkgo extracts.

Chapter 5 Discussion

Data analysis

Twenty-seven Ginkgo extracts were tested against 6 skin pathogenic bacteria strains, generating 162 activity result in total. Seventeen of the 27 extracts were actively against bacteria growth at concentration 512 μg/mL or lower. Out of the 17 extracts, there is one that is active against all bacteria selected, which is 680, the ethanolic extract of fresh-frozen male leaves; three that are active against 5 of them, which is 660, 666 and 678, which are ethanolic extracts of dry female leaves, ethanolic extracts of dry female branches, and ethanolic extracts of fresh-frozen immature fruits. However, we cannot simply conclude here whether extraction methods, tree parts and genders of the trees contribute to the effectiveness of the extracts.

The percentages of inhibition of the 27 extracts at 512 μg/mL were grouped by extraction methods, tree parts, and gender of the Ginkgo tree, in order to determine whether there are significant differences of percent inhibitions within each group. All 162 activity results were included in ANOVA analysis of extraction method and tree parts, as well as unpaired t-test for storage method (GraphPad Prism6). Extracts associated with seed were not included in the unpaired t-test for gender; only the 108 activity results generated from branch and leaf extracts were included. A summary of the comparison is shown in Table 8 and Figure 9.

The differences of percent inhibition between ethanol maceration, water sonication, water decoction and oil infusion is significant, with p value<0.0001 (α=0.05). The comparison of tree parts also show significant differences, with with p value<0.0001 (α=0.05). Thus, those comparisons were carried out further (see Figure 10 and Figure 11). There are no significant

differences between the percent inhibition of extracts that were from male and female trees, or the extracts that were dry or fresh-frozen before test.

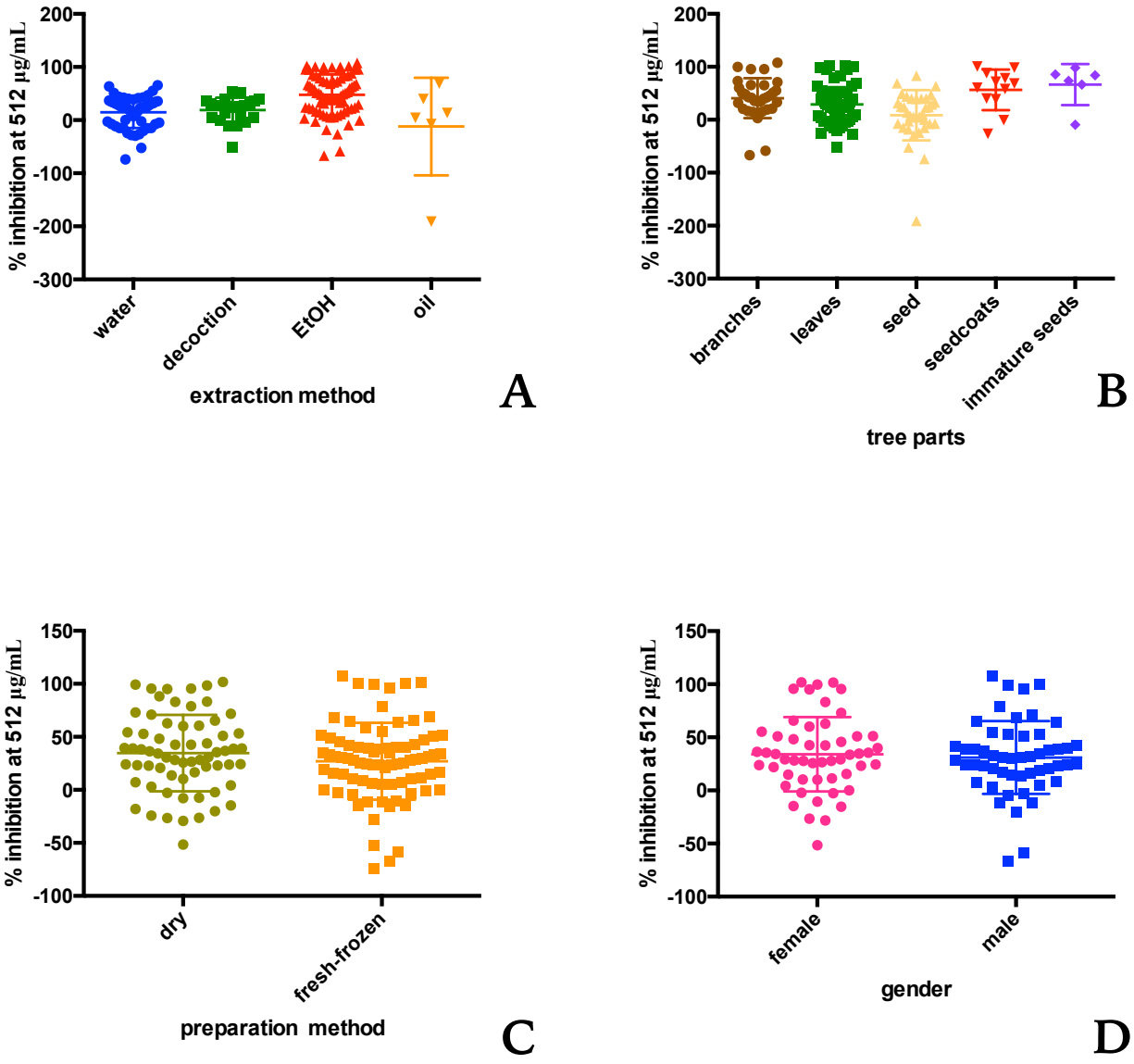


Figure 9. Graphs of data analysis on factors that contribute to effectiveness of Ginkgo extracts. Figure 9A, ANOVA analysis of extraction method. Figure 9B ANOVA analysis of Ginkgo tree parts. Figure 9C unpaired t-test of preparation method. Figure 9D, unpaired t-test of gender of the tree collected from.

	Comparison groups	Statistical test	P values	Significance of the differences
Extraction method	Water decoction, water sonication, EtOH maceration, oil infusion	ANOVA	<0.0001	significant
	Dry vs. fresh-frozen	Unpaired t	0.1988	Not significant
Tree parts	Branches, leaves, seeds, seedcoats, immature seeds	ANOVA	<0.0001	significant
Gender of the tree	Male vs. female	Unpaired t	0.6452	Not significant

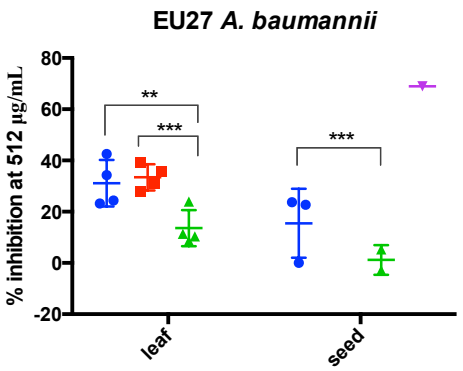
Table 8. Summary of statistical analysis for factors contribute to effectiveness of Ginkgo extracts. The differences of percent inhibition within each group of factor that may contribute to the effectiveness of Ginkgo extracts are compared.

The effect from extraction methods and tree parts to the extracts' inhibition activities were analyzed for each bacteria strain using Student's t-test (Microsoft Excel 2016). Notice here that the statistical analysis was specific to an individual bacteria strain. Also notice that a factor contributes to effectiveness of Ginkgo extract when two conditions were meet: one, the mean of its percentage inhibition was significantly different from other factors (see charts in Figure 10 and 11); two, its percentage inhibitions were higher than other factors (judging form scatter plots in Figure 10 and 11).

When analyzing different extraction methods, the percentage inhibitions at highest concentration were grouped by tree parts separately in order to exclude any effect caused by different tree parts. In other words, percentage inhibition data generated form same strain, same tree parts (leaves or seeds) but extracted by different method were compared. As a general trend for all 6 strain (see Figure 10), ethanolic extracts' inhibition activities were significantly different

from extracts made other way, and they were higher. Thus, ethanol maceration is the extraction method generating most effective Ginkgo extracts.

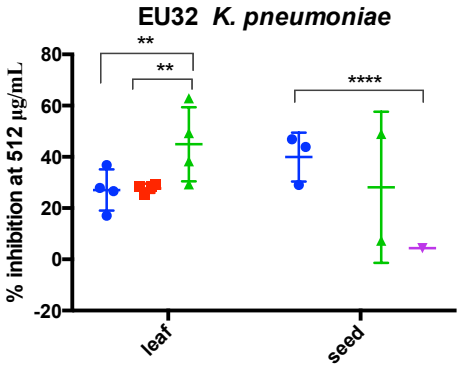
When analyzing different tree parts, only ethanolic extracts' inhibition activity data were included in order to exclude any effect caused by different extraction method. Because the significances of differences between growth inhibition of extracts made from all tree parts varies strongly among each bacteria strain, we cannot conclude which single tree part that generated the most effective Ginkgo extracts. For the comparisons of effectiveness of Ginkgo extracts made from different tree part to each bacteria strain, see Figure 11.



A

EU27	EtOH	sonication	decotion
EtOH		0.001176961	0.000255293
sonication			0.445140095
decotion			
all from same strain, same plant part (leaves)			

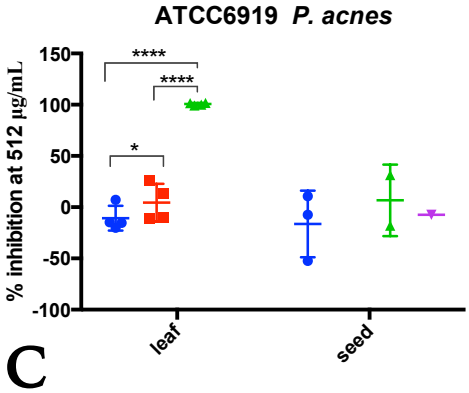
EU27	EtOH	sonication	oil infusion
EtOH		0.000467484	0.167591862
sonication			0.256481768
oil infusion			
all from same strain, same plant part (leaves)			



B

EU32	EtOH	sonication	decotion
EtOH		0.003019412	0.0026988
sonication			0.90072672
decotion			
all from same strain, same plant part (leaves)			

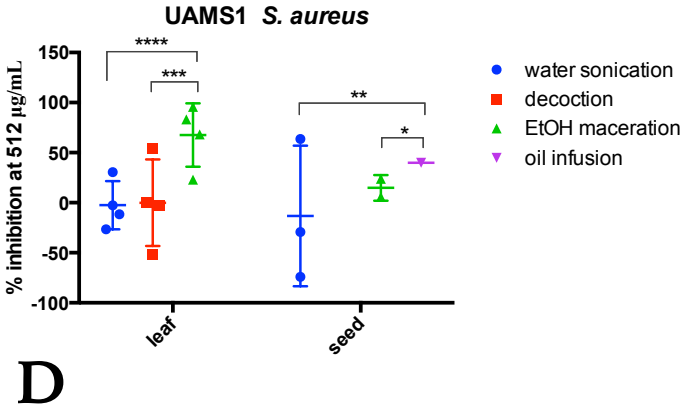
EU32	EtOH	sonication	oil infusion
EtOH		0.318303989	0.06912758
sonication			4.7578E-06
oil infusion			
all from same strain, same plant part (seeds)			



C

ACC6919	EtOH	sonication	decotion
EtOH		1.52E-12	7.3377E-10
sonication			0.02190463
decotion			
all from same strain, same plant part (leaves)			

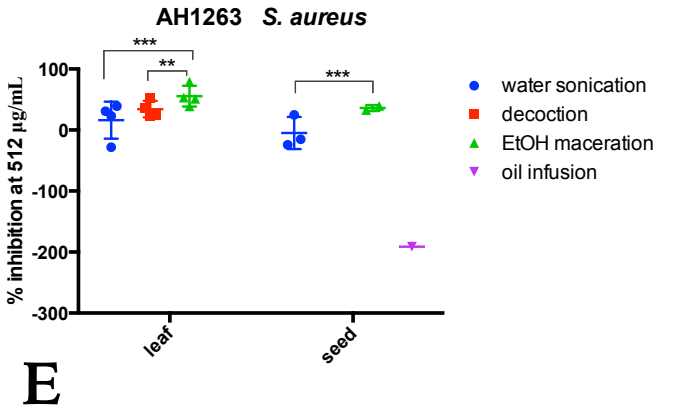
ATCC6919	EtOH	sonication	oil infusion
EtOH		0.09293374	0.16709636
sonication			0.38063681
oil infusion			
all from same strain, same plant part (seeds)			



D

UAMS1	EtOH	sonication	decotion
EtOH		3.341E-06	0.00013714
sonication			0.32456967
decotion			
all from same strain, same plant part (leaves)			

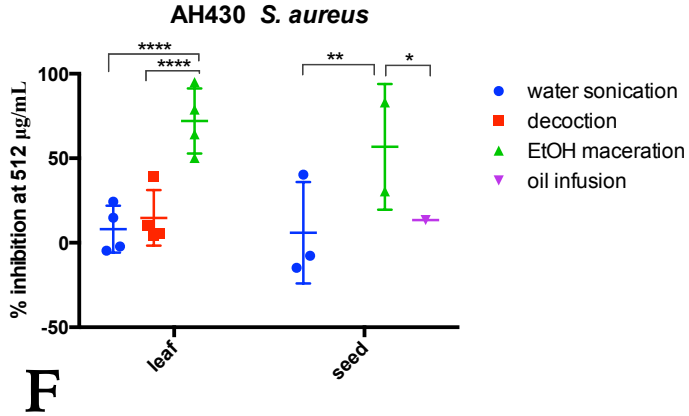
UAMS1	EtOH	sonication	oil infusion
EtOH		0.24074181	0.00445736
sonication			0.03685472
oil infusion			
all from same strain, same plant part (seeds)			



E

AH1263	EtOH	sonication	decotion
EtOH		0.0003926	0.00151251
sonication			0.05900433
decotion			
all from same strain, same plant part (leaves)			

AH1263	EtOH	sonication	oil infusion
EtOH		0.00070222	0.09532947
sonication			0.14600318
oil infusion			
all from same strain, same plant part (seeds)			

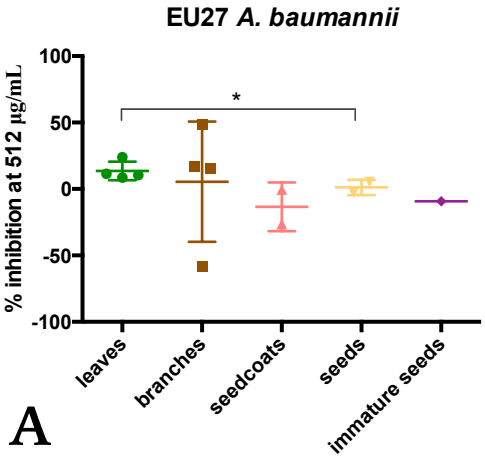


F

AH430	EtOH	sonication	decotion
EtOH		3.96739E-09	3.88927E-08
sonication			0.268464227
decotion			
all from same strain, same plant part (leaves)			

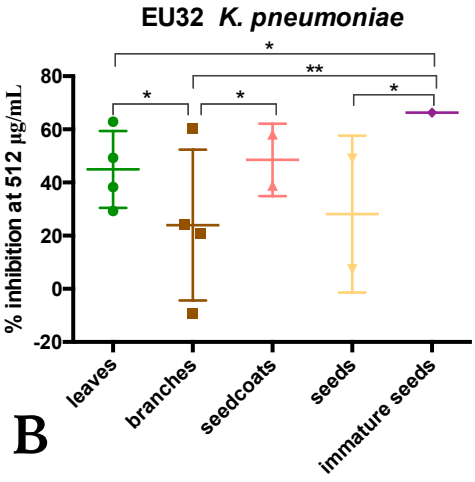
AH430	EtOH	sonication	oil infusion
EtOH		0.00603157	0.01328071
sonication			0.44116412
oil infusion			
all from same strain, same plant part (seeds)			

Figure 10. Comparison of extraction methods in MIC assays. The significance levels of each comparison were based on the student's t-test results on the right of each scatter plots. * stands for p≤ 0.05, ** stands for p≤ 0.01, *** stands for p≤ 0.0001, **** stands for p≤ 0.00001.



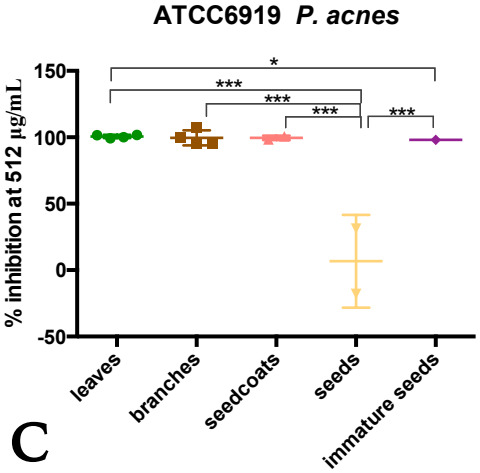
EU27	leaves	branches	seedcoats	seeds	immature seeds
leaves		0.53747155	0.27955614	0.01773202	0.201702535
branches			0.48500978	0.74050005	0.430517643
seedcoats				0.55086449	0.897636596
seeds					0.493324733
immature seed					

all from same strain, same extraction methods (only EtOH)



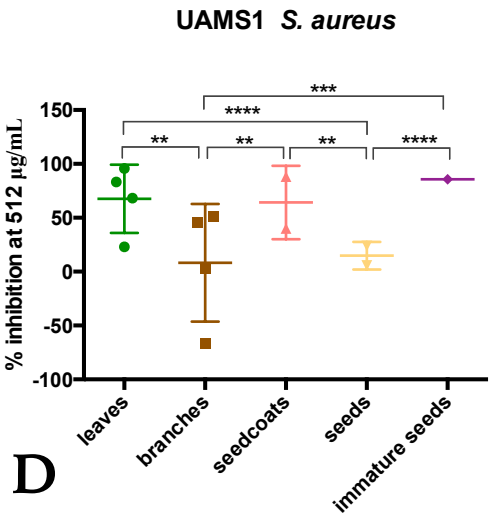
EU32	leaves	branches	seedcoats	seeds	immature seeds
leaves		0.027060349	0.586413598	0.1783806	0.027970545
branches			0.013143749	0.75108689	0.001000962
seedcoats				0.11424278	0.056537693
seeds					0.013955528
immature seeds					

all from same strain, same extraction methods (only EtOH)

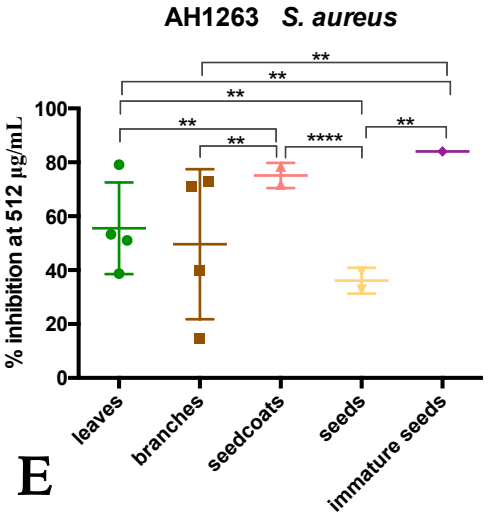


ATCC6919	leaves	branches	seedcoats	seeds	immature seeds
leaves		0.50572292	0.12939246	0.00037849	0.024782293
branches			0.87663964	0.00035028	0.394612486
seedcoats				0.00039815	0.243726285
seeds					0.000426975
immature seed					

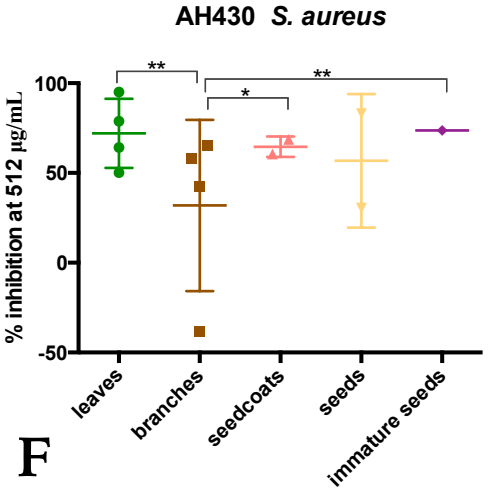
all from same strain, same extraction methods (only EtOH)



UAMS1	leaves	branches	seedcoats	seeds	immature seeds
leaves		0.00233161	0.8127169	9.58209E-05	0.061445567
branches			0.0066919	0.672432536	0.000209274
seedcoats				0.004145877	0.102393357
seeds					1.98394E-05
immature seed					
all from same strain, same extraction methods (only EtOH)					



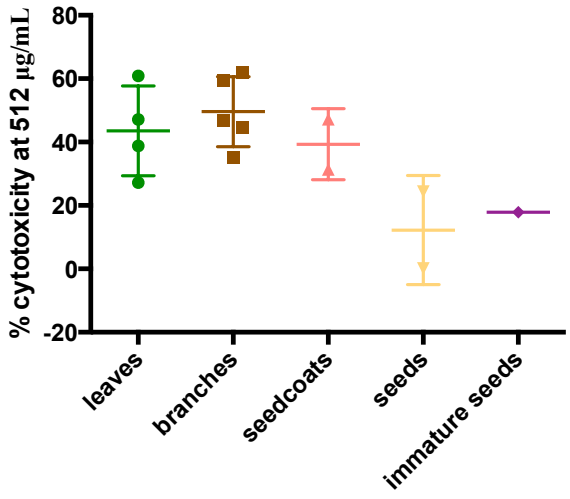
AH1263	leaves	branches	seedcoats	seeds	immature seeds
leaves		0.42982508	0.00428733	0.001489635	0.003887921
branches			0.00754359	0.114984537	0.002254279
seedcoats				2.94494E-08	0.169375359
seeds					0.00325521
immature seed					
all from same strain, same extraction methods (only EtOH)					



AH430	leaves	branches	seedcoats	seeds	immature seeds
leaves		0.00971173	0.35744535	0.27475481	0.804118395
branches			0.03136439	0.16880377	0.007134102
seedcoats				0.56949155	0.227791075
seeds					0.220905825
immature seed					
all from same strain, same extraction methods (only EtOH)					

Figure 11. Comparison of tree parts in MIC assays. The significance levels of each comparison were based on the student's t-test results on the right of each scatter plots. * stands for p≤ 0.05, ** stands for p≤ 0.01, *** stands for p≤ 0.0001, **** stands for p≤ 0.00001.

The percentages cytotoxicity of active extracts at 512 μg/mL were grouped by tree parts, in order to determine whether there are significant differences of toxicity to human keratinocyte among each part of Ginkgo tree. Student's t-test was used to compare extracts from every tree part to each other. The differences of cytotoxicity were significant between three parts, leaves, branches, seedcoats and the other two parts, seeds and immature seeds (all p value<0.002, α=0.05). The differences were not significant among leaves, branches and seedcoats, or between seeds and immature seeds. The percentage cytotoxicity at 512 μg/mL of leaf, branch and seedcoat extracts were higher than the other two tree part extracts (see Figure 12). Thus, we can conclude that active Ginkgo extracts made from leaves, branches and seedcoat are more toxic to human keratinocyte; Ginkgo seeds and immature seeds are safer in topical uses. This result resonates with the traditional Chinese remedies: as mentioned in the introduction and background section, it was the seed of the Ginkgo tree that was used topically to skin diseases historically.



cytotoxicity	leaves	branches	seedcoats	seeds	immature seeds
leaves		0.24237046	0.4709154	0.00139304	0.000136918
branches			0.0553169	0.00056414	6.09633E-06
seedcoats				0.00415281	0.001990131
seeds					0.402707176
immature see					

Figure 12. Comparison of tree parts in cytotoxicity assays. Student's t-test results were also included in the right of the scatter plots of percentage cytotoxicity at highest tested concentration.

Conclusion

Twenty-seven Ginkgo extracts were made based on traditional Chinese remedies and were tested against 4 pathogenic bacteria, *P. canes*, *S. aureus*, *A. baumannii* and *K. pneumonia*. Seventeen of them were active against bacteria growth. Therefore, this study demonstrated efficacy of this traditional Chinese medicinal use of *Ginkgo biloba.* Further investigation can take place in partitioning the crude Ginkgo extracts to isolate the fractions that have higher antibacterial activities. We also conclude that ethanolic extracts were the most effective Ginkgo extracts against skin pathogenic bacteria, which was suggested by previous studies, e.g., Sati et al. (Sati and Joshi 2011).

Also, according to the MIC result summary, the Ginkgo extracts were more effective against Gram-positive bacteria than Gram-negative ones. There were more the Ginkgo extracts show activities against *P. canes* and *S. aureus*, which are both Gram-positive bacteria species; They are also more effective, since several extracts inhibited bacterial growth over 90% (MIC90 activities) for the two Gram-positive bacteria. For the Gram-negative strains, *A. baumannii* and *K. pneumoniae*, less Ginkgo extracts show activities and none of them have MIC90. This result resonates with what previous researches have shown; for example, aqueous Ginkgo leaf extract and anacardic acid mixture from Ginkgo shown no activity against Gram-negative bacteria, but only to the Gram-positive ones (Boonkaew and Camper 2005). However, previous studies have not explored whether Ginkgo extracts have activities against the formation of biofilm. There is still chances that Ginkgo extracts can work against the Gram-negative bacteria strains by inhibit or eradicate their biofilm.

Moreover, the single chemical compounds known to present in Ginkgo plant were not very effective against the skin pathogenic bacteria; only the flavone glycosides (quercetin,

isorhamentin and kaemferphol) shown activities against *S. aureus* strains. Therefore, further studies can explore whether the combination of the chemical standards, or their synergetic effects, can inhibit bacteria growth more than they do individually.

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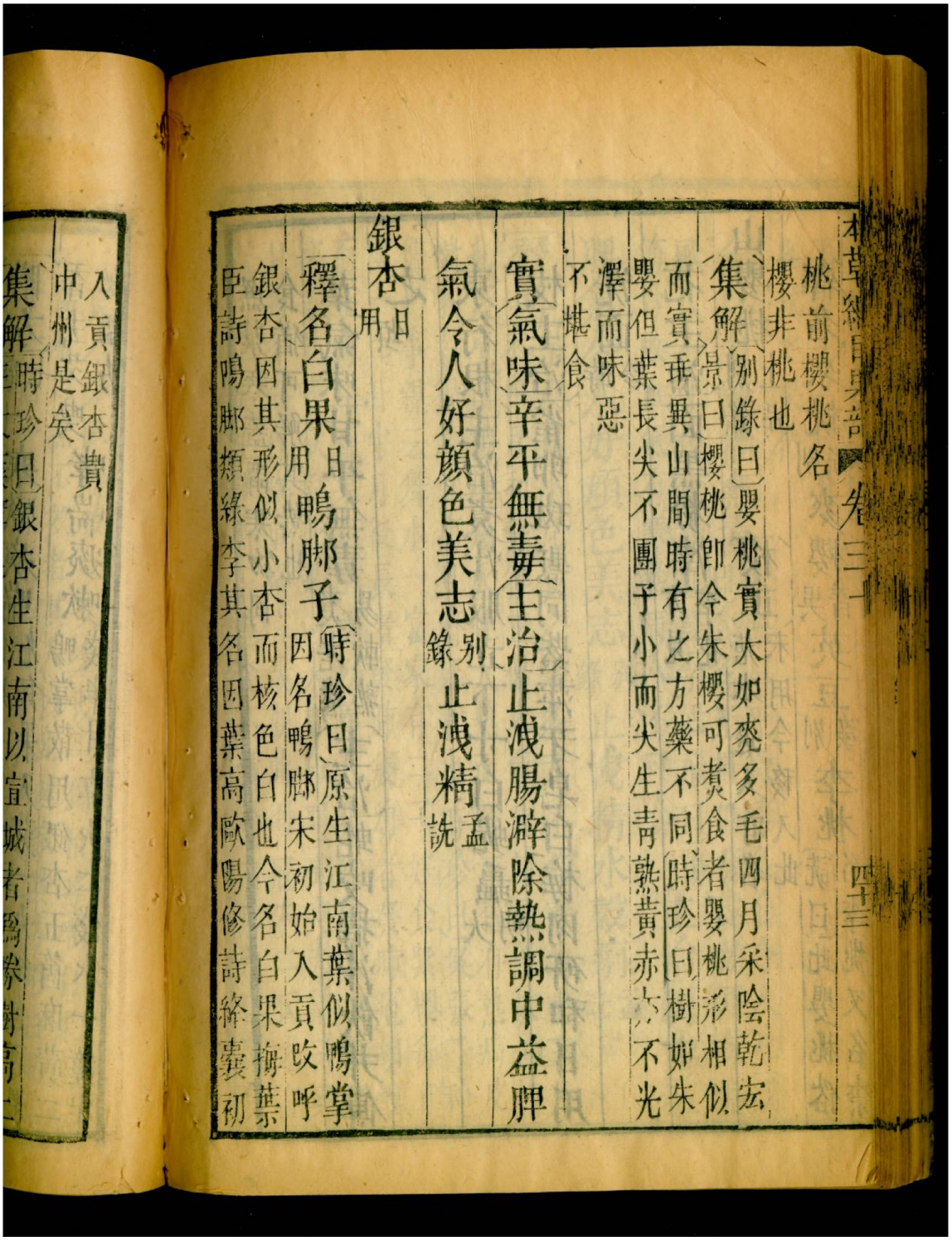
Appendix

1. ***Ben cao gang mu*** (5 pages)



銀杏因其形似小杏而核色白也今名白果按
臣詩陽脞類緣李其名因葉高歐陽修詩絳囊初

入貢銀杏貴
中州是矣

〔集解〕〔時珍曰〕銀杏生江南以宣城者爲勝樹高二三丈葉薄縱理似鴨掌形有刻缺可綠背淡二月開花成簇青白色二更開花隨即卸落人罕見之一枝結子百十枚如棟子經霜乃熟爛去肉取核爲果其核兩頭尖三稜爲雄二稜爲雌其仁嫩時綠色久則黃須雌雄同種其樹相望乃結實或雌樹臨水亦可或鑿一孔內雄本一塊泥之亦結陰陽相感之妙如此其樹耐久肌理白膩術家取刻符印云能召使也文選吳都賦註平仲果其實如銀未知即此果否

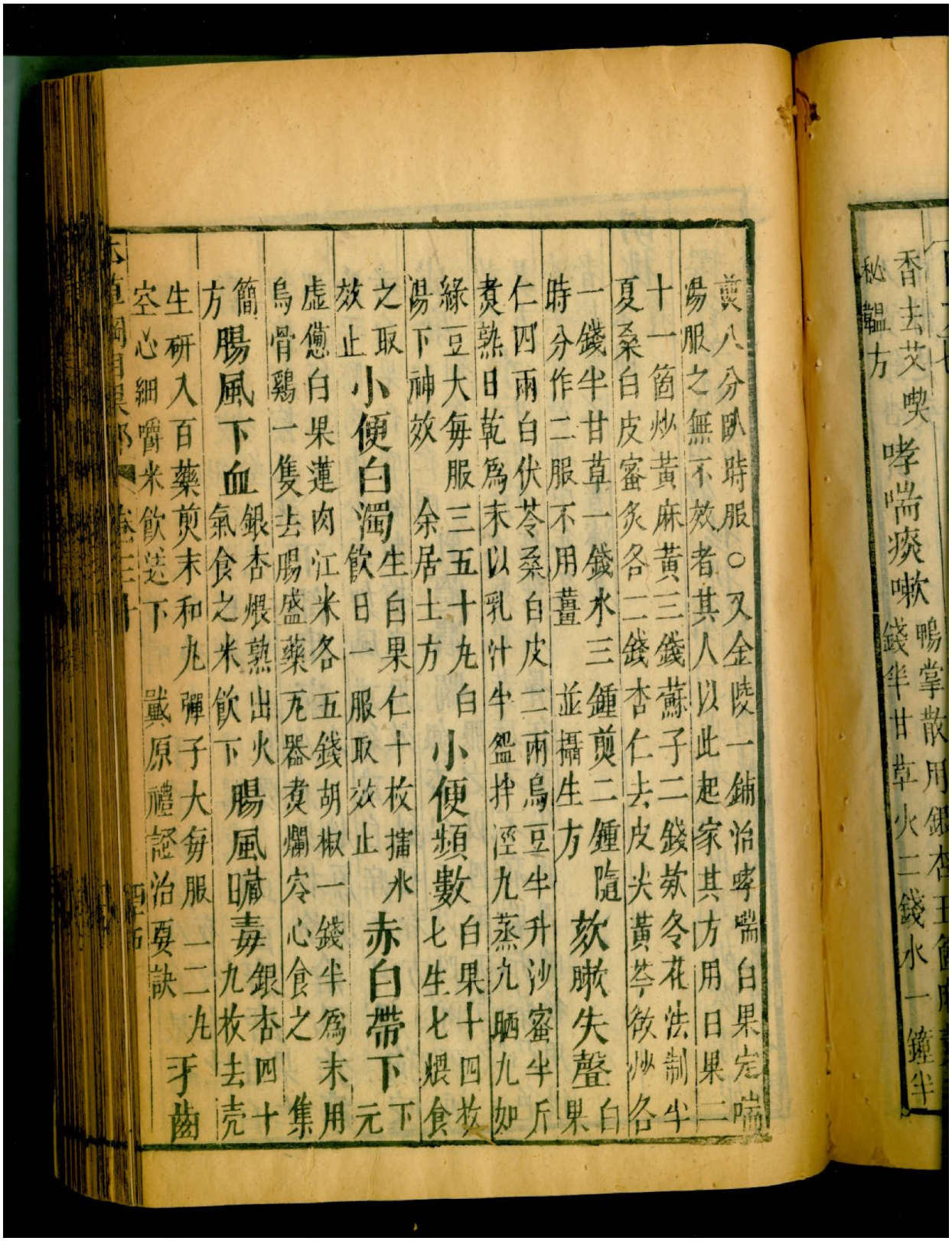
〔核仁〕〔氣味〕甘苦平澁無毒〔時珍曰〕熟食小苦微甘性溫有小毒多食令人盧脹瑞曰多食壅氣動風小兒多食昏霍發驚引疳同鰻鱺魚食患軟風〔主治〕生食引疳解酒熟食益人李廷飛熟食溫肺益氣定喘嗽縮

小便止白濁生食降痰消毒殺虫嚼漿塗鼻面手足去皰皯黯皺皺及疥癬疳蟲陰蝕時珍

時珍曰銀杏宋初始著名而修本草者不收發明近時方藥亦時用之其氣薄味厚性濇而收色白屬金故能入肺經益肺氣定喘嗽縮小便生攙能浣油膩則其去痰濁之功可類推矣其花夜開人不得見蓋陰毒之物故又能殺虫消毒然食多則收令人氣壅臚脹昏頓故物類相感志言銀杏能醉人而三元延壽書言白果食滿千箇者死又云昔有饑者同以白果代飯食飽次日皆死也

附方新十七

寒嗽痰喘白果七箇煨熟以熟艾作七丸紙包再煨香去艾嚼服用銀杏五箇麻黃二錢半甘草火二錢水一鐘半煎八分臥時服○又金陵一鋪治哮喘白果定喘湯服之無不效者其人以此起家其方用白果二十一箇炒黃麻黃三錢蘇子二錢



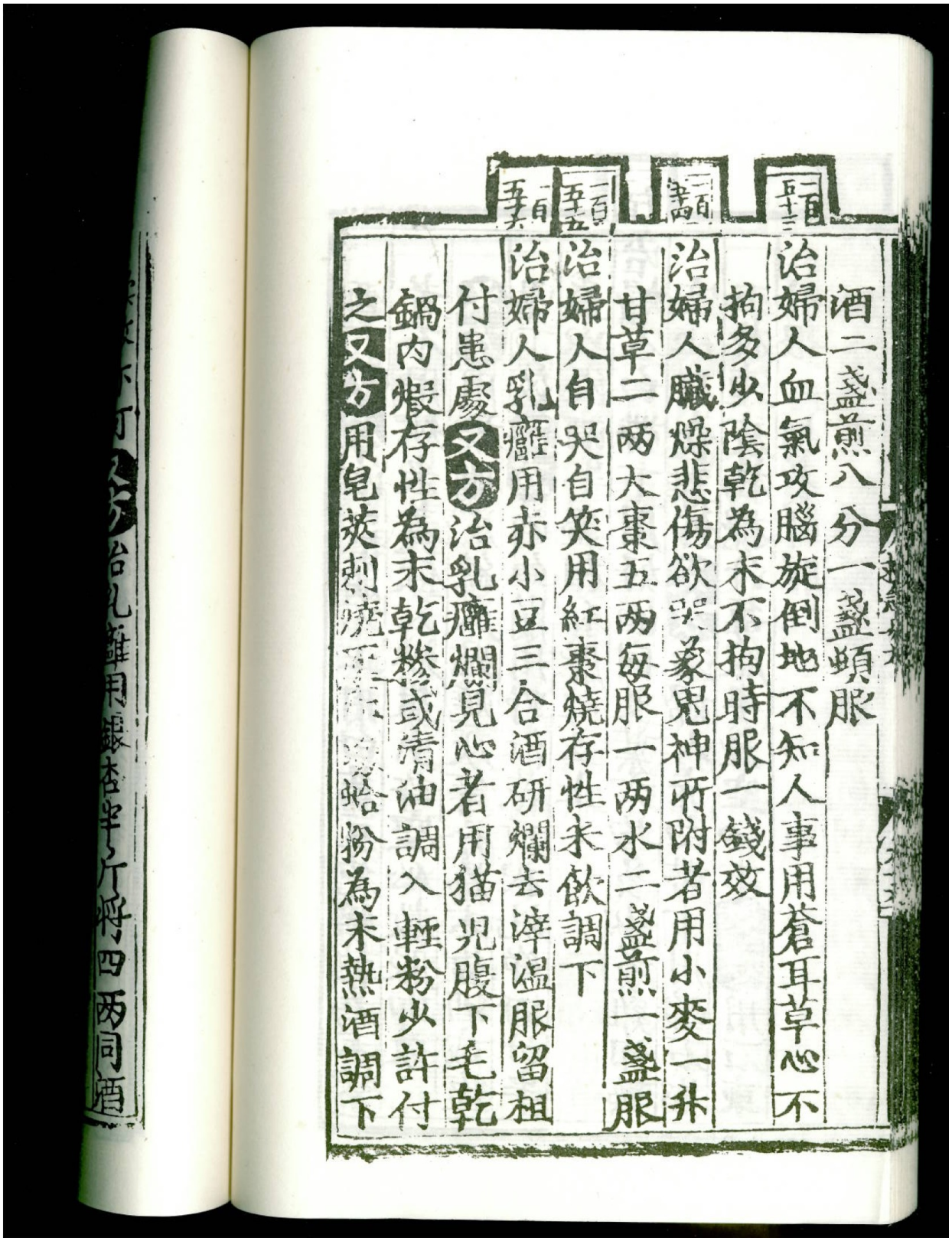
虫齧生銀杏肉食後嚼一手足皴裂生白果嚼爛
二個良 永類鈐方 手頭面癬瘡生白
鼻面酒皶銀杏酒醉糟同嚼爛夜夜塗之
塗旦洗 醫林集要 果仁
切斷頻擦取效 下部疳瘡生白果杵塗之 趙原陽 陰虱作痒
邵氏經驗方 陰毛際肉中生虱如風或紅或白痒不可忍
者白果仁嚼細頻擦之取效 劉長春方 狗咬
成瘡白果仁嚼細塗之 乳癌潰爛銀杏半斤以四兩研酒
服之以四兩研傅之 救急易方 水疔暗疔水疔色黃麻木不痛暗疔瘡凸色
紅使人昏狂並先刺四畔後用銀杏去壳浸油中年久者擣敷之 普濟方

胡桃 宋開寶

〔釋名〕羌桃 名物志 核桃〔頌曰〕此果本出羌胡漢時張騫使西域始得種還植之秦

中漸及東土改名之〔時珍曰〕此果外有青皮肉包之其形如桃故桃乃其核也羌音呼核如胡名或以此或作羌

2. ***Jiu ji yi fang*** (3 pages)



酒二盞煎八分一盞頓服

治婦人血氣攻腦旋倒地不知人事用蒼耳草心不拘多少陰乾為末不拘時服一錢效

治婦人臟燥悲傷欲哭象鬼神所附者用小麥一升甘草二兩大棗五兩每服一兩水二盞煎一盞服

治婦人自哭自笑用紅棗燒存性末飲調下

治婦人乳癰用赤小豆三合酒研爛去滓溫服留相付患處 又方 治乳癰爛見心者用猫兒腹下毛乾鍋內煅存性為末乾糝或清油調入輕粉少許付之 又方 用皂莢刺燒存性為末蛤粉為末熱酒調下

揉散亦可又方治乳癰用銀杏半斤將四兩同酒研服將四兩木研付癰上又方用黃瓜蒌一二箇連皮穰子切碎以無灰酒一碗於瓶內煮半碗去粗時時温服酒盡再煮粗服初時便服比藥即時痛止更不成瘡如已成瘡服之其瘡自穿而痛止又方治乳上結覺硬腫作痛以葱早熟之其法用中樣閙口瓶以炭火入瓶內上以熱灰填滿瓶口用葱葉及葱白搥損令偏覆瓶口以手帕裹瓶倒執將瓶口向腫處任意輕輕熨之有驗又方治乳癰將漬以小長罐燒紙錢在內急以罐口安妳上

3. ***Summary of National Herbs*** (2 pages)

葡球菌、痢疾杆菌及绿脓杆菌均有抑制作用。7.银杏肉质外种皮的浸剂经注射于小白鼠后可引起惊厥。银杏肉质外种皮内含有引起皮肤炎的银杏毒。直接接触此种毒质后即可发生皮肤炎。从皮肤吸收，通过肠与肾排泄，引起胃肠炎与肾炎，有溶血作用。

性味功能　种子(白果)：甘、苦，平。有毒。润肺，定喘，涩精，止带。**叶**：微苦，平。活血止痛。

主治用法　种子：支气管哮喘，慢性气管炎，肺结核，尿频，遗精，白带；外敷治疥疮。**叶**：冠状动脉硬化性心脏病心绞痛，血清胆固醇过高症，痢疾，象皮肿。用量种子、叶 1.5～3 钱。

附方　1. 慢性气管炎：银杏 250 毫克、地龙、黄芩素各 150 毫克（每片含量）。加适量淀粉，制粒，压片。每服 5 片，每日 2 次，早晚空腹服。10 天为一个疗程，中间休息 5 天。

2. 肺结核：秋后采嫩银杏（带肉质外种皮）浸入菜油中 100 天，即为油浸白果，每次服 1 粒，每日 3 次，连服 30～100 天。

3. 冠状动脉粥样硬化性心脏病心绞痛：(1) 舒血宁片：每片含银杏叶总黄酮量约 2 毫克。每次舌下含服 1～2 片，每日 3 次。(2) 复方银杏片：银杏叶、何首乌、钩藤各 1.5 钱，制成片剂，为 1 日量。(3) 舒血宁注射液：每 2 毫升含银杏叶黄酮甙元 0.3 毫克及聚乙二醇 30%。肌肉注射，每次 4 毫升，每日 1 次。疗程 6～10 周。目前北京市生产的银杏叶注射液每毫升含总黄酮量 1.5 毫克。

4. 血清胆固醇过高症：银杏叶提取主要成分黄酮，制成糖衣片，每片含黄酮 1.14 毫克。每次服 4 片，每日 3 次。

5. 小儿肠炎：银杏干叶 1～3 钱，加水 2 碗，煎成 1 碗，擦洗小儿脚心，手心，心口（巨阙穴周围），严重者擦洗头顶。每日 2 次。

制剂　1. 舒血宁片（舒心酮片）：取银杏叶 1,000 克，加水约 8,000 毫升，煮沸 30 分钟，过滤，再加水约 5,000 毫升，煮沸 30 分钟，过滤，合并两次滤液，浓缩至稠浸膏状，加淀粉(约为浸膏重量的 70%)搅匀，70～80℃ 干燥，用小磨磨成颗粒过 30～40 目筛，加 1% 硬脂酸镁混匀压片，片重按每片含总黄酮量 2 毫克计算（约相当于生药 0.5 克）。包糖衣。本品每片黄酮含量限度应在 1.7～2.5 毫克之间。

2. 舒血宁注射液：(1) 提取：将银杏叶拣去杂质，秤取 10 公斤捣碎，投入回流提取罐中，加 90～95%乙醇 120,000 毫升，回流 3 小时，放出提取液，再加 90～95% 乙醇 100,000 毫升，回流 3 小时，放出提取液，两次提取液合并，过滤，滤液回收乙醇，得黑色粘稠物，置水浴中蒸去残留乙醇，加注射用水 3,000 毫升，加热搅拌 30 分钟，放冷水中冷却，待黑色粘稠物结成硬块浮在液面上，倾出黄色混悬液，残留的黑色粘稠物再按上法分别加注射用水 3,000 毫升、4,000 毫升洗两次，(第 3 次水洗时，加热后经冷却若黑色粘稠物不结块，可用滤纸过滤）弃去残渣。将黄色混悬液放冷冻室过夜，用纸浆过滤至透明，加硅胶 500 克，充分搅拌后放置 30 分钟，用纸浆过滤，得精制浓提取液。测定含量。

(2) 注射液配制：取精制浓提取液，按每毫升含黄酮 1.5 毫克投料，加 0.1% 焦亚硫酸钠，0.02%依地酸二钠，加热溶解后，加 0.1% 活性炭煮沸 5 分钟，用滤纸过滤，放置近室温，加入乙醇 5%，注射用水适量，用 10% 碳酸氢钠调 pH 至 6.4，加水至足量，用 4 号垂熔漏斗过滤，灌装，并于安瓿中填充氮气，熔封，100℃流通蒸汽灭菌 15 分钟。本品按总黄酮量计，每毫升为 1.3～1.7 毫克。(药液应注意不能和金属接触，以免变色。本品有一定毒性，因此，在利用不同地区，不同生长期的银杏叶时，须经动物毒性试验后，决定能否使用)

附注　误服过量银杏中毒时，可出现发烧、呕吐、腹泻、惊厥、抽搐、肢体强直、皮肤青紫、瞳孔散大、脉弱而乱，甚至昏迷不醒。解救方法，可洗胃，导泻，服鸡蛋清、活性炭，并加对症处理；如皮肤青紫可给氧气或人工呼吸；出现抽搐可给镇静剂，遇有昏迷可吸入氨水，注射兴奋剂。

847. 银柴胡（附：山银柴胡）

来源　为石竹科繁缕属植物**银柴胡** Stellaria gypsophiloides Fenzl. 的根。

形态特征　多年生草本，高 20～40 厘米。主根圆柱形，直径 1～3 厘米，外皮淡黄色，根头处有许多疣状的茎部残基。茎直立，上部二叉状分枝，节略膨大，密被短毛或腺毛。单叶对生，无柄；叶片披针形，长达 3 厘米，宽达 4 毫米，先端锐尖，基部圆形，全缘，上面疏被短毛或几无毛，下面被短毛。夏末秋初开白色花，花单生；花萼外侧疏被短绒毛；花瓣 2 深裂；雄蕊 10；子房上位，有 3 条花柱。蒴果近球形，成熟时顶端 6 齿裂。种子 1～2 粒。(图 819-1)

生境分布　生于干草原，在石缝中亦有生长。分

4. Pu ji fang (2 pages)

普　济　方

第　七　册

诸　疮　肿

（卷二七二至卷三一五）

明　朱　棣等编

人民卫生出版社

治疔腫出本草

用苦苣折取莖中白汁敷之。疔腫出根。又方以苦苣擣汁。敷疔瘡殊驗。青苗陰乾。以備冬月爲末。水調敷。

治疔瘡出本草

以蜆子活浸取汁。洗瘡口。以糟煮蜆食之。

治疔瘡出本草

以白狗屎水絞。取汁服之。

治疔腫惡瘡出本草

用銅鏃石末。敷瘡上良。

治疔腫出本草

以槐葉煎湯服。皮莖同用。

治疔腫出本草

擣莠蔚莖敷之。仍服其汁。使疔腫毒內消。及治諸毒腫。服之亦效。一方用子。

破疔腫開出本草

以新人屎封之。一日根爛。

療疔腫惡瘡骨疽出本草

以蝮蛇皮作灰。敷之。

治疔腫瘡出本草

以前藥爲細末。用清油調。塗之瘡口內。立效。

又方

以拔毒散。水煎服。

又神效方

千金草四兩 明礬五錢 乳香一兩 右爲末。酒調下一錢。出汗爲度。

又方 治緩疔。

兩尖一兩係兩頭尖 巴豆四箇 右爲細末。貼瘡上。紙封。

又方 治冷疔瘡。

青靛一兩 明礬七錢 右爲末。井花水調服五錢。仍飲熱葱湯一餞。被蓋汗出即愈。

治赤根疔出千金方

熬白粉冬黑蜜和。敷之良。

治一切疔瘡出本草

以蠟蟷燒爲末。和臘月猪脂。敷之。

治疔瘡方

櫻桃者用佛掌花根蜜蓋三件。研汁服。用天茄藥繫瘡口上。連珠者用鴨脚青、燕蘇研。糖水拌刷之。水晶者用水莧、天茄。

治疔瘡方

用荔枝或三箇。或五箇。不用雙。復用狗食過米糞。淘淨爲末。復用糯米厚粥。一處研擣成膏。用好皮紙攤上。貼之患處。紙上留了一竅。只痊可。

又方 治疔瘡。

凡黑者中央凹。四畔黑腫。用頭髮繩箚住。將尖葉薜荔葉擣細取汁。和蜜調一餞服之。又用葱白和蜜擣。敷患處。

又方 治水疔。瘡色黃黑。麻木不疼。

用刀刺四畔。將柜樹根經行路者取二尺許。去皮擣細。井花水調一餞服。待瀉。用三角銀杏去殼。浸在油水年久者。擣敷患處。亦治暗疔瘡頭凸。紅色。使人昏憒狂惶者。

又方 治冷疔瘡。

用生烏頭切片。醋熬成膏子塗瘡上。絹帛貼。次日去絹。疔根自出。

又方 治疔瘡。

取未結子柜樹根細嫩者洗淨。去黑皮。取白皮擂細。以無灰生酒調開。濾去滓。用藥吞下解毒雄黃丸。瘡化黃水而散。

奪命丹 治一切疔腫。

血竭一錢 蟾酥 銅綠 明礬 硃砂 輕粉 大黃各半錢 麻黃半兩去根節 麝香三字 海羊十五箇去媽半 一方用龍腦二字 右研爲細末。將海羊研細爛爲丸。如雞頭大。每服先嚼葱白三寸。然後用好酒送下一丸。如重車行七里。汗出爲效。

走馬丹

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