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The Antioxidant Content of Wong Lo Kat Tea

Valerie Kwong

ABSTRACT

Wong Lo Kat (or Wanglaoji in Mandarin) is a popular soft drink containing herbal tea that is often used as a Chinese folk remedy for mild ailments. The herbs included in the drink are the following: Chinese Mesona, White Frangipani, Microcos, Chrysanthemum, Japanese Honeysuckle, Heal All, and Chinese Licorice. While there is research on the individual plants, there is limited information on the combined mixture of the herbs included in the Wong Lo Kat drink, particularly in published English literature. Antioxidant content for the individual herbs and a combined mixture of equal parts of each herb was tested using the TEAC test. To prepare the combined herb solution, each herb's phenolics were sonicated, centrifuged, and concentrated using a rotary evaporator, then combined in equal parts using 1 mL each. The antioxidant activities were found to be the following: Chinese Mesona was 1.5586 ± 0.1217; White Frangipani was 1.4277 ± 0.2351; Microcos was 1.2813 ± 0.1398; Chrysanthemum was 1.2183 ± 0.1961; Japanese Honeysuckle was 1.4751 ± 0.1702; Heal All was 1.4905 ± 0.1702; Chinese Licorice was 1.1554 ± 0.1483; and combined was 1.2463 ± 0.1892. All values were reported in mmol Trolox equivalency/g sample. ANOVA test was used with Tukey's test to determine that the means of the herbs and combined mixture were not statistically significant from each other. Comparing the Wong Lo Kat mixture with teas available in the market, the antioxidant activity is comparable to several high-quality iterations of green tea and other teas in the market.

Keywords: *antioxidants, herbal tea, TEAC, Wong Lo Kat, Wanglaoji*

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1. INTRODUCTION AND THEORETICAL APPROACH

1.1 Wong Lo Kat and Chinese Medicine

Wong Lo Kat (or Wanglaoji in Mandarin) is an herbal drink popularized in Chinese communities since its creation in Southern China. In Chinese medicine, it is said that the ingredients in the drink assist individuals to recover from minor ailments such as colds and nosebleeds. The ingredients of the commercially available drink are as follows: Water, Sugar, Mesona (Chinese), White Frangipani, Microcos, Chrysanthemum, Japanese Honeysuckle, Heal All, and Chinese Licorice.

Along with many Chinese medicine techniques, there is limited scientific literature about the benefits of the drink's use. While there are studies published about several of the individual ingredients, there is particularly little research done on the Wong Lo Kat beverage as a whole. During its creation, it was believed by many of the Chinese population that the Wong Lo Kat herbal mixture had the ability to provide a "cooling" effect on the body which would assist in fighting minor ailments. The mixture gathered so much acclaim that Wong Lo Kat, the man who created the prescription tea, was named an Imperial Doctor by the Qing Emperor, Man Chung. Furthermore, it gained so much popularity that after the first tea shop was created in Guangzhou, China in 1853, individuals would flock to the teahouse instead of going to the overcrowded hospitals from the tension-filled atmosphere during the Japanese occupation (Evans & Tam, 2004). The belief that herbal teas serve as medicine has continued throughout the decades that media from mainland China has boasted that consuming the product can increase the lifespan by 10 percent. Experts, however, have denied this claim (Shen, 2017; Ke, 2021).

Foods in Chinese medicine are categorized due to their innate nature in terms of thermal categories. However, there is evidence lacking in scientific literature backing up the medicinal benefits of these techniques in western literature. This is due to the nature of these beliefs. What is western and eastern medicine? "Western Medicine Versus Eastern Medicine" written by Stefanov et al. (2020) referred to the NCI Dictionary of Cancer and reviewed the definitions of western and eastern medicine. Western medicine was defined as "a system in which medical doctors and other healthcare professionals (such as nurses, pharmacists, and therapists) treat symptoms and diseases using drugs, radiation, or surgery." Eastern medicine/oriental medicine was referred to as a "medical system that has been used for thousands of years to prevent, diagnose, and treat disease, which is based on the belief that Qi (the body's vital energy) flows along meridians (channels) in the body and keeps a person's spiritual, emotional, mental, and physical health in balance. Oriental medicine includes mainly acupuncture, diet and herbal therapy, but also meditation, physical exercise, and massage." In a sense, western medicine cannot be entirely separated from eastern medicine because many pharmaceutical drugs include some plant-derived compounds for use in some pharmaceutical drugs. The effectiveness of herbs, physical exercises, and massage are known but not exactly accepted. Acupuncture, in particular, has not been thoroughly scientifically reviewed in western medicine literature. However, the main difference between eastern and western medicine is their substantiation by the scientific communities in question, not how effective the treatments are to patients. This is why some physicians/researchers are not as accepting towards acupuncture, because of a lack of acceptable scientific explanation detailing the physical/anatomical aspects of the meridian system. Recently, only scientific theories have been used to explain the functions used in traditional eastern medicine to cure disease, and western medicine, without a known anatomical foundation for the meridians used in acupuncture, is still unlikely to accept acupuncture as an adequate form of treatment.

In a paper written by a graduate student named Xiaoyu (Jennifer) Zhang from the University of San Francisco titled "Blurred Boundaries between Food and Medicine: Traditional Chinese Medicine and Its Impact on Contemporary Chinese Self-Care" in 2020*,* it is discussed how nutrition and dietetics are considered "western-centric." In Chinese, nutrition and dietetics translate to a lifestyle or ideology of health or mental well-being. Traditional Chinese medicine has been around for more than 2000 years and has had an important influence on cultural heritage in China. Many restaurants create dishes involving various herbal medicines due to changing seasons. Hospitals also have integrated traditional medicine along with western medicine techniques as methods of treatments. From interviews with three practicing physicians and fifteen graduate students that have experienced both Chinese and western medicine, it was found that those that had experienced both methods preferred traditional Chinese medicine over western medicine techniques. Those that were exposed to traditional Chinese medicine claimed to use these techniques as forms of self-care that they consciously applied to their eating habits on a daily basis.

Although traditional Chinese medicine is widely accepted in China, western medicine is less likely to adopt these methods without scientific proof using western research methods. Nonetheless, it is not about which style of medicinal practice is better or more effective than another. In reference to "What is integrative medicine? It is a coordinated, multidisciplinary health approach integrating Western and Eastern medical practices" (2016), integrative medicine, the combined treatment of both eastern and western practices uses methods with a patient centered philosophy. This may include a treatment plan using the preventative methods of eastern medicine while also prioritizing the essential prescription medications from western medicine. Maintaining a healthy lifestyle, however, is key to the treatment plan. Sometimes, the symptoms and treatment are obvious, other times, it is not. One such case occurred with one of Dr. Laube's patients, a 60 year old Iranian woman who had immigrated recently from Iran. When looking at her chart, she was consuming a long list of medications as treatment for her constant migraines which did not provide much relief. She reported having a heaviness in her head and when asked about herself, she described being a caregiver to her husband who suffered from complex medical problems. Her symptoms originated mainly from stress, and because of this, Dr. Laube encouraged her to see a psychologist. Once taking his advice, the patient reported that some of her symptoms were alleviated and was grateful to no longer have to rely on so many prescription medications. Integrating both practices may be the most effective method of treatment of ailments.

As such, the analysis of antioxidants used in Chinese herbal remedies is crucial for the acceptance of traditional Chinese medicine into western medicine. According to the National Center of Complementary and Integrative Health (NCCIH), antioxidants are substances that may prevent or delay cell damage. It is said that antioxidants may assist in preventing diseases -a claim very similar to that made in traditional Chinese medicine.

Further, in Lourenço et al (2019)'s study "Antioxidants of natural plant origins: From sources to food industry applications," they discussed the possible negative effects of consuming synthetic antioxidants and the current applications of natural antioxidants in the food industry. Antioxidants have the capability of protecting cells from converting reactive oxygen species into non-radical species. Oxidation reactions damage can also occur in food products when they are exposed to oxygen, heat, or light. These reactions play a significant role in the food products' rate of deterioration over time. Because food is not immediately consumed after harvesting and product, food manufacturers are often searching for methods to increase shelf life and preserve food quality. With this in mind, natural antioxidants have the potential to serve as a method to delay oxidation in food. Synthetic antioxidants were created to simulate the effects of natural antioxidants because they are highly stable, are low cost, and are readily available. Despite their benefits, synthetic antioxidants have been known to cause adverse effects to the human body. Long term consumption may lead to health issues. Likewise, high doses may also cause DNA damage and increase aging. Limited knowledge is also known about their environmental impacts. Despite their increased use, consumers strongly prefer natural antioxidants over their synthetic counterparts opting often for all natural ingredients.

In determining the antioxidant content in the herbs included inside the Wong Lo Kat beverage, perhaps it can provide western audiences a better understanding and be more receptive to eastern medicinal practices. Furthermore, with what is known about synthetic antioxidants, the Wong Lo Kat mixture may have the potential to serve as a useful natural antioxidant. As for the ingredients for the Wong Lo Kat concoction, many of the individual herbs have research investigating their backgrounds and uses.

1.2 *Mesona chinensis*/ Chinese mesona

Beginning with *Mesona chinensis*, or Chinese mesona, it is a plant generally found in China and Southeast Asia. In a study by Huang et al., 2012, M. chinensis was transplanted to both a paddy field and a dry field, then compared for growth, yield, and quantity. The survival rate of M.chinensis was relatively equivalent in both scenarios, however, M.chinensis fared better in the paddy field compared to the dry field. This assertion corresponds with Dave's Garden, one of many blog posts describing the best growing environment for Chinese Menona (*Plantfiles: Platostoma species…,* n.d.). Dave's garden claimed that M. chinensis required consistently moist soil and that it is best for the soil never to dry out for maximum output. M.chinensis is mainly grown in China and East Asia, and likewise, individuals that have posted on Dave's garden's website have expressed finding it difficult to find seeds for this plant. Therefore, while the plant is used extensively in East Asia, research of growth of the plant in Western circles is lacking.

This plant is often used to create grass jelly and is known for its ability to prevent heat-stroke in Chinese medicine (Li et al., 2020). In China, grass jelly is a well-known ingredient used to produce herbal teas and desserts. The plant has also been studied extensively in terms of its functions as an antioxidant. One such study was conducted is called "Polysaccharide from *Mesona chinensis*: Extraction optimization, physicochemical characterizations and antioxidant activities" written by Lin et al. in 2017. The study pointed out initially that a main aspect of *Mesona chinensis, Mesona chinensis* polysaccharide (MCP), has been receiving many accolades for its antioxidant, immunoregulation, and antidiabetic effects. Much of the research done on this aspect of the plant has been done using either the crude or purified polysaccharide from *Mesona chinensis*. Therefore, a variety of extraction parameters were altered in order to determine which is the best method to obtain the highest yield of polysaccharide. It was determined that antioxidant activities of MCP are concentration-dependent and that there is potential for further study of MCP in a role as a functional food.

In another study involving the antioxidant content of *Mesona chinensis* called "Sulfated modification enhanced the antioxidant activity of *Mesona chinensis Benth* polysaccharide and its protective effect on cellular oxidative stress" by Huang et al. published in 2019, a sulfated modification was created and used to see its effects on the physiochemical and antioxidant activities of the polysaccharide from *Mesona chinensis Benth*. Using spectroscopy and chromatography, the chemical composition and structure was altered. From this modification, *Mesona chinensis* polysaccharides demonstrated a protective effect by increasing superoxide dismutase activity and decreasing malondialdehyde content. This protective effect allowed for the improvement of antioxidant activity in *Mesona chinensis* polysaccharides.

"The Antioxidant and Hypolipidemic Effects of *Mesona Chinensis Benth* Extracts" by Xiao et al. published in 2022 also describes the antioxidant and hypolipidemic values of *Mesona chinensis,* particularly, *Mesona Chinensis Benth* (MCB). The MCB was first divided into seven factions and separated by macroporous resin to analyze different components. Each of the components were analyzed for antioxidant, hypoglycemic and hypolipidemic levels with high-performance liquid chromatography (HPLC) and high-performance liquid chromatography–mass spectrometry (HPLC–MS). With those factions with antioxidant effects, the amount of flavonoids was higher compared to polysaccharide values. MCB extracts contained mainly caffeic acid, quercetin 3-O-galactoside, isoquercetin, astragalin, rosmarinic acid, aromadendrin-3-O-rutinoside, rosmarinic acid-3-O-glucoside and kaempferol-7-Oglucoside, indicating potential application for treatment of hyperlipidemia. Among the extracts with antioxidant effects, the content of flavonoids was higher, and the content of polysaccharides was relatively low. Also, MCB total flavonoid (MTF) values were better once enriched or purified, demonstrating better antioxidant and hypolipidemic effects than other fractions.

In "Evaluation of morphological and phytochemical characteristics of *mesona chinensis* populations in southern China" by Li et al. (2020), the morphological and phytochemical characteristics of thirty four different populations of *M. chinensis* were examined. Because of its use in herbal teas and grass jelly, there has been increasing domestication of this herb but without a superior strain in its varying planting areas. In China, the strains of *M. chinensis* are chosen based on their locality. The issue, however, is that in some cases the strains have low yield and poor quality. With this in mind, the purpose of this study was to determine the highest quality cultivars of the *M. chinensis* species to meet the increasing demand for this herb. 34 varying populations were collected between 2016 to 2017 from the four provinces and cultivated in a greenhouse in South China Agricultural University (Guangzhou, Guangdong, China). For the antioxidant activity, species were evaluated by ABTS 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays with values found between 63.91 to 223.41 and 30.35 to 137.84 mmol Trolox equivalents, respectively, after an ethanolic extraction.

1.3 White frangipani (*Plumeria alba L.*)

White frangipani (*Plumeria alba L.*) is a tropical plant that is used in both traditional medicine and aromatherapy. The flower aspect of the plant in particular is often used. Latex from the frangipani plant has been analyzed in research to act as an antitumor, hypotensive, and purgative. White Frangipani is not native to North America, however, seems to grow well in hot, humid environments. It is known to be found in tropical, subtropical regions such as Hawaii and South East Asia. The plant is susceptible to frost and grows well in full, direct sunlight. If left alone, it can grow up to 7 m tall into a small tree or shrub. However, it is recommended to prune the shrub in order to develop a more stable structure. The plant emits a perfumy, fragrant smell and blossoms with many white colored flowers (Barry et al., 2020; University of Florida, n.d.).

A literature review written by Barry et al. called “Future molecular medicine from White frangipani (*Plumeria Alba L.*): A Review. Journal of Medicinal Plants Research " published in 2020 analyzed White frangipani and its pharmacological, toxicological, and biological (at a molecular level) activity. For the purpose of this study, only the relevant information on antioxidants will be included. To determine the antioxidant activity, the flower was extracted by using ethanol and acetic acid. Using ethanol, it was found that there was a 18.19% antioxidant capacity against 2,2-diphenyl-1-picrylhydrazyl (DPPH). Likewise, using acetic acid, there was a 12.74% capacity. The vitamin C content was determined by using the 2,4-

dinitrophenylhydrazine (DNPH) method with 3.08% found for the ethanolic extract and 3.13% found for the acetic acid extract. For polysaccharides, at 3 mg/ml concentration a fluorescence recovery after photobleaching (FRAP) number of 0.0915 and 0.0901 µmol Fe (II)/g for methanolic extract and *Plumeria alba* polysacharrides (PAP) was found, respectively. With the DPPH method at 3 mg/ml concentration, the inhibition percentage was found to be 94.28 and 84.18 for the methanol extract and PAPs, respectively. This indicates that the reducing power of the methanol extract was higher than the PAPs at a 3 mg/ml concentration.

"Testing Antioxidant Activity of *Plumeria Alba* and *Plumeria Rubra* Ethanolic Extracts Using DPPH and Frap Methods and Determining Their Total Flavonoid and Phenolic Levels" by Wiyono & Muhtadi (2020) also tested *Plumeria sp.* or Frangipani's antioxidant levels with multiple parts of the plant using DPPH and FRAP methods. The flower parts, leaves, and stem bark of *Plumeria alba L*. and *Plumeria rubra L*. from Surakarta, Indonesia in 2019 were taken and concentrated with ethanol. Using the DPPH method, the antioxidant levels are as follows: *P. ruba* leaves as 203.59 IC50 (ppm); *P. ruba* flowers as 150.20 IC50 (ppm); and P. alba flowers as 204.81 IC50 (ppm). With the FRAP method, the average antioxidant activity (mg AA equivalent//g extract) are as follows: *P. ruba* leaves with 79.75 ± 5.70; *P.ruba* flowers with 79.67 ± 4.84; and *P.alba* flowers with 79.01 ± 4.09. In the DPPH method, the highest antioxidant activity was from the *P.ruba* flowers with 150.20 IC50 (ppm), and in the FRAP test, the *P. ruba* leaves had the highest antioxidant activity with 79.75 ± 5.70 mg AA/g extract. This disparity was claimed to potentially be due to the gap between testing methods where there could be different conditions.

In Dawood & Hassan's article "Antioxidant activity evaluation of methanolic extract and crude polysaccharides from *Plumeria Alba L.* leaves. (2015), the benefits of natural sources of antioxidants were highlighted, while the detriments of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) added to food were mentioned in favor of more natural sources. Therefore, the goal of this study was to test *Plumeria alba L.*'s antioxidant activity and polysaccharide content. Samples were collected in Egypt in 2013. For extraction, the material was dried, ground into a powder, soaked in ethanol, and concentrated in a rotary evaporator. Using the FRAP assay, polysaccharides at 3mg/ml showed a FRAP value of 0.0915 and 0.0901 µmol Fe (II)/g for MeOH extract and polysaccharides (PAPs), respectively. DPPH free-radicals were compared with standard Trolox concentrations of 0.25, 0.5, 1.5, 2, 2.5. At 3 mg/ml the percentage inhibition was 94.28 and 84.18 for MeOH extract and PAPs, respectively.

1.4 Microcos

In a broader sense, Microcos is a genus for a plant distributed throughout South East Asia (*Microcos paniculata L,* n.d.). It is a shrub which can grow into a medium sized tree up to 27 m tall. The plant develops both fruit and flowers. The flowers develop into clusters and are a white-yellow color meanwhile the fruit are small and oval shaped. Once the fruit matures, it turns a yellow-orange color and can be eaten raw. To grow the plant, stem cutting or from seed works equally as well. It prefers full sun and has a moderate water preference. (*Microcos antidesmifolia (King) Burret,* 2021). Microcos paniculate in particular is a popular variety that is more commonly used for its health benefits (Aziz, 2015). *Microctis Folium,* or the dried leaves of *Microcos paniculata,* is often used in Chinese herbal tea. Much of the current research indicates several bioactive secondary metabolites in the leaves. As a result, it is often used in general tonics and insecticides in traditional Chinese medicine (JIANG & LIU, 2019).

In a study conducted by Fan et al. called "Chemical constituents with free-radical-scavenging activities from the stem of *Microcos paniculate*" published in 2010, four solvent extracts (P.E., EtOAc, n-BuOH, and water) from *Microcos paniculate* plant stems were analyzed with three different in vitro systems (DPPH, ABTS and Molecules 2010, 15 5558 Co (II) EDTA-induced luminol chemiluminescence by flow injection) to determine the free-radical scavenging capabilities of *Microcos paniculate* stems. From the extracts, EtOAc demonstrated the greatest free-radical scavenging capability. As a result, by being divided using chromatography, five compounds, named methyl3β-O-phydroxy-E-cinnamoyloxy-2α,23-dihydroxy-olean-12-en-28-oate (1), epicatechin (2), 3-trans-feruloyl maslinic acid (3), maslinic acid (4) and sucrose (5) were identified. All compounds were purified except sucrose and measured in the three in vitro model systems. The free-radical scavenging activities are as follows: 2>3>1>4. Based on the results, further isolation and investigation of other bioactive components of *Microcos paniculate* would be beneficial to further understand the uses of this plant.

In another study involving the use of *Microcos paniculata* is called "Cholinesterase, protease inhibitory and antioxidant capacities of Sri Lankan medicinal plants" written by Samaradivakara et al. in 2016. The study used the ethanol extracts of seventeen plants from Sri Lanka to examine their Acetylcholinesterase (AChE), Butyrylcholinesterase (BChE), and protease enzyme inhibitory and antioxidant activities. In this study, along with *Microcos paniculata,* the *Toona ciliata Roem., Osbeckia octandra DC., Fluggea leucopyrus Wild.,* and *Evolvulus alsinoides Linn*. plants were all assessed.

The DPPH, FRAP, and ORAC tests were performed in order to determine antioxidant activity. While TPC and TFC were also used to determine phytochemical content, namely to determine total phenolic and total flavonoid content. Because of the multifunctional nature of the plants, multiple tests were performed on the samples. Microcos paniculata in particular had a DPPH value of 44.6 ± 1.17, a FRAP value of 715 ± 4.45, and ORAC value of 280.08 ± 0.28, a TPC value of 79. 65 ± 0.66, and a TFC of 47.08 ± 0.45. From the seventeen extracts, four plants indicated particularly high antioxidant capabilities as well as phenolic contents: *T. ciliata, O. octandra, F. leucopyrus,* and *E. alsinoides.*

From Aziz et al. (2018)'s article "Antioxidant and antidiarrheal activity of the methanolic extract of *Microcos paniculata* roots," the roots of *M. paniculata* (RME) were evaluated with the total antioxidant capacity (TAC), DPPH free radical scavenging assay (DPPHFRSA), nitric oxide scavenging capacity assay (NOSCA), lipid peroxidation by thiobarbituric acid assay (LPTAA), reducing capacity assessment (RCA), cupric reducing antioxidant capacity (CRAC), castor oil and MgSO4 induced diarrheal tests in order to measure the antioxidant and antidiarrheal components of the roots. The benefits of natural antioxidants and also diarrhea's potential in causing malnutrition and mortality within children in developing countries under five years old were highlighted. With this in mind, the purpose of this investigation was to investigate the beneficial qualities of the M. paniculata plant's roots. The roots were harvested from Bangladesh in November, 2013 and powderized for testing. Total phenols and flavonoids from TAC were 182.78 ± 0.12 mg/g RME (in gallic acid equivalent) and 43.5 ± 0.32 mg/g RME (in quercetin equivalent) and 40.83 ± 0.69 mg/g RME (in ascorbic acid equivalent) respectively. The IC50 values of the RME in DPPHFRSA, NOSCA and LPTAA were 158.47 ± 2.66 µg/mL, 157.91 ± 4.56 µg/mL and 148.29 ± 6.48 µg/mL correspondingly.

1.5 Chrysanthemum

Chrysanthemum is a perennial, flowering plant that has often been used in herbal teas in East Asian countries such as China, Korea, Japan, and Vietnam for more than 2000 years. Chrysanthemums are widely cultivated as ornamental flowers. In fact, it is one of the most popular plants in the United States used for ornamental horticulture. (Verma et al., 2012). Nonetheless, its origins are from East Asia. The flowers are considered a perennial, and due to its popularity, varieties vary immensely in both shapes and colors. Chrysanthemum flowers are often grown as young plants or from root cuttings and prefer well-drained soil. Once planted, the flowers prefer areas with constant sunlight. Some varieties may grow into a low-growing bush so they must be planted further apart. Areas with too much shade and water may cause chrysanthemum flowers to generate disease (Utah State University, n.d.) (*How to grow chrysanthemums / RHS gardening,* n.d.).

In Chinese medicine, almost all parts of the plant are utilized for treatments and is one of the most commonly prescribed plants. Extensive research has been done on different varieties of chrysanthemum. For Chinese medicine, the *C. morifolium* and *C. indicum L*. varieties are said to treat inflammation and cure infectious diseases. *C. morifolium* flowers also have been known to purify the blood and is used as a treatment for angina for the coronary artery. The Pharmacopoeia of the People's Republic of China also has stated that *C. morifolium* has the ability to remedy dizziness, headaches, and the common cold. For the *C. indicum* variety, it is also used to detoxify the liver and heart vessels (Hadizadeh et al., 2022).

In a study called "Phytochemical composition and antioxidant activities of two different color Chrysanthemum flower teas" published in 2019 by Han et al., a γ-irradiated mutant chrysanthemum cultivar with dark purple petals (cv. ARTI-Dark Chocolate) was tested with commercial chrysanthemum with yellow petals and compared. For both varieties, the phytochemical composition and antioxidant activity were tested using high-performance liquid chromatography with diode array detector and electrospray mass spectrometry (HPLC-DAD-ESIMS) along with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. Chrysanthemums with yellow petals are often consumed in the Korean herb market with Gamguk and Sanguk being some of the more popular varieties. Nonetheless, little research has been done on chrysanthemum with other colors of flowers. Because of this, the purpose of this study was to determine the active compounds in different varieties of chrysanthemum for future cultivar breeding that would be beneficial for commercialization. A chrysanthemum flower with dark purple petals (cv. ARTI-Dark Chocolate) was recently developed and obtained plant protection rights through the Korea Seed and Variety Service. In this study, the ARTI-Dark Chocolate and the Gamguk varieties were analyzed. Relevant antioxidant information will only be included for the purposes of this review. Each tea was investigated in various water temperatures and infusion times. To test scavenging, the DPPH test was used. Based on their data, varying temperatures did not affect the Gamguk variety significantly despite allowing for various times and temperatures. Meanwhile, ARTI-Dark Chocolate demonstrated a significant change in antioxidant activity from differing time and temperatures. Based on the DPPH radical scavenging assay, ARTI-Dark Chocolate demonstrated higher antioxidant activity compared to Gamguk. The ABTS assay test, however, indicated that both chrysanthemum tea varieties exhibited high radical scavenging effects under all conditions. Based on the data, there is a high indication that the ABTS chrysanthemum variety would be a worthwhile plant to introduce as a new tea.

Another study involving chrysanthemum is called “Comparison of phenolic compounds and the antioxidant activities of fifteen chrysanthemum Morifolium Ramat CV. ‘hangbaiju’ in China,” written by Gong et al. and published in 2019. During different stages of the plant’s growth, it is considered “Hangju,” “Boju,” “Chuju,” and “Gongju,” the four variations cited in the Chinese Pharmacopeia. “Hangbaiju” is a variety of Chrysanthemum morifolium mostly planted in the Zhejiang Province of China. “Hangbaiju” can then be divided into “Duoju” (“DJ”) and “Taiju” (“TJ”) depending on when they are harvested. The “Duoju” are harvested when the flower heads with their ray florets and tubular florets are opened fully. This variety is usually harvested in November. “Taiju” are harvested with the ray florets opened but the tubular florets closed. This variety is usually harvested in October. Once they are harvested, they are dried and given their specific designation. It was hypothesized that the “Duoju” and “Taiju” varieties may have different levels of phenols and antioxidants, as a result, six “Duoju” and nine Taiju” were compared using HPLC. Antioxidants were tested using DPPH, ABTS, and FRAP assays. From the results, fourteen phenolic compounds were found within the flowers in varying concentrations and amounts. “Duoju” was found to be higher in caffeoylquinic acids and stronger antioxidant activities than “Taiju.” With higher caffeoylquinic acid, there is a strong correlation of antioxidant activity. Furthermore, with a principal component analysis (PCA), there demonstrated a clear disparity between phenolic compounds between the two. The comparison of “Duoju” and “Taiju” could be useful for quality control for use of “Hangbaiju” in the future.

In 2019, Li et al. published a study called “Chemical compositions of chrysanthemum teas and their anti-inflammatory and antioxidant properties" analyzing seventeen commercial chrysanthemum teas and their specific chemical compositions from hot water extraction. Much of the research involving the benefits of chrysanthemum have been derived from methanol extraction. However, for many individuals, chrysanthemum is consumed through hot water extraction. Nonetheless, much of the research involves testing methanol extraction. Thus, with this in mind, the purpose of this study is to compare the hot water extraction of chrysanthemum with their 75% methanol extraction counterparts in order to improve the future production and consumption of chrysanthemum tea. From the data, hot water extraction indicated a higher level of total phenolics and less flavonoids per weight of each chrysanthemum flower than overall menthol extraction. The hot water extract of Kunlunmiju 1 displayed the greatest total phenolic value with DPPH and oxygen radical absorbance capacity values of 12.72 mg gallic acid equivalents/g, 105.48 and 1222.50 μmol Trolox equivalents/g, respectively.

1.6 Lonicera japonica (Japanese Honeysuckle)

For the Japanese Honeysuckle plant or *Lonicera japonica*, it originates from East Asia but was introduced to the United States in 1806 for both ornamental and erosion control uses. It is a perennial vine that generates fruit and develops yellow-cream-colored, tubular flowers. Once its establishment in the United States, it quickly was documented to be an "invasive" species throughout much of the Eastern portion of the United States -invasive meaning that it grows extremely quickly and crowds out native species from growing. The plant is able to grow in both full sun and shade. Likewise, because of its many flowers and fruits, seeds are able to be quickly spread by birds. (*Japanese Honeysuckle,* 2010) (*Lonicera japonica,* 2022)

In Lee & Chang's paper "Nutraceuticals and antioxidant properties of *lonicera japonica* thunb. as affected by heating time" (2019), *Lonicera japonica* was heated at 100°C for 30, 60, 90, 120, or 150 minutes in a dry oven then stored in a freezer until it was time for analysis. *Lonicera japonica thumb* is a plant native to East Asia and is often used in the food industry for its antioxidant, anti-inflammatory, and antibacterial activities. Bound phenolic compounds are not able to function as antioxidants. However, Acosta-Estrada et al. has noted that food processing methods are able to release phenolic compounds and improve biological activity in food. Therefore, while heating, a common processing technique, may reduce nutritional quality, it may function as a way to release bound phenolic compounds to increase antioxidant properties. Two main goals of the study were to identify varying individual phenolic acid contents (chlorogenic acid, caffeic acid, 4,5-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid) and individual flavonoids (rutin, quercetin, and luteolin) and while also determining the antioxidant properties (DPPH radical scavenging activity, ABTS radical scavenging activity, FRAP value, and reducing power). From the results, it was found that total phenolic, phenolic acid, and flavonoids increased a significant amount based on the heat processing treatments. Antioxidant activity also increased after being exposed to heat treatment. Particularly, a 60-minute time served as an adequate time for an increase in levels. For antioxidant analysis, the DPPH radical scavenging activity, ABTS radical scavenging activity, FRAP value, and reducing power were analyzed. At time zero, the phenolic and flavonoid values were 2013 ± 23 ug GAE/100 mg and 4495 ± 99 ug RE/100 mg, respectively. For the results, heating for DPPH and ABTS activity had similar patterns. Once L. japonica was heated for 60 minutes, these levels increased significantly by about 29 to 35%. At 60 minutes, the levels were at maximum levels of total phenolics of 2594 ± 73 ug GAE/100 mg and total flavonoids of 5644 ± 57 ug RE/100 mg. At 90 minutes, DPPH and ABTS levels retained significant increases. At heating times above 120 minutes, antioxidant levels decreased significantly with phenolics levels of 2303 ± 80 ug GAE/100 mg and flavonoids of 4995 ± 73 ug RE/100 mg, however, these levels were still significantly above 0 minutes of heating time. Based on these values, heating at 100°C at 60 minutes may be used to improve nutraceutical and antioxidant values in *L. japonica*.

Another study involving *Lonicera japonica* is called “Extraction optimization, antioxidant activity, and tyrosinase inhibitory capacity of polyphenols from *Lonicera japonica*” published in 2019 by Fan et al. The study’s goal was to optimize polyphenol extraction from *Lonicera japonica* with surface methodology and also to determine antioxidant activity from different purities. Some of the most common methods to extract polyphenols are solvent extraction, enzymatic hydrolysis, microwave extraction, and ultrasonic-assisted extraction.

However, these processes may take a long time, partially destroy the polyphenol structure, and reduce the total extraction yield. In the study, a relatively new extraction technology called high speed shearing homogenization extraction is used. The claims are that it involves using less time to process, uses less energy, and uses a lower temperature, thereby being more efficient than other common methods. To determine the antioxidant activity, the DPPH and ABTS assays were used. Using the shearing technology, 3.30 min, an ethanol percentage of 57%, and a solid-liquid ratio of 1:58 were found to most optimally extract the polyphenols from L. japonica. The plant demonstrated both adequate antioxidants and an ability to inhibit tyrosinase. However, a microporous resin prevented monomer substances from being unable to be separated and it is recommended that further experiments be conducted for the most adequate monomer substance.

"Antioxidant and anti-inflammatory activities of *Lonicera japonica thunb. var. Sempervillosa Hayata* flower bud extracts prepared by water, ethanol and supercritical fluid extraction techniques" by Hsu et al. (2016) also analyzed the beneficial antioxidant activities of *L. japonica*. The *Lonicera japonica* Thunberg (*LJ*) species is often used throughout East Asia for its antioxidant, anti-inflammatory, antibacterial, antiviral, and other beneficial characteristics had their antioxidant activity analyzed through extracts from their flower buds. *L. japonica Thunb*. var. *sempervillosa Hayata* (*LJv*), a variant of Lj that mainly grows in Taiwan, also had their antioxidant activity analyzed through extracts from their flower buds. Depending on which part of the plant is analyzed, it may contain different activities. In Taiwan, the flowers and flower buds of Lj are the most often used. Because of this, the flower buds for Lj and Ljv were used in this study. Depending on the extraction method, moisture content in the flower, harvest time, and habitat, it may alter the quality of the extract, which may alter the results of the antioxidant content. Flower buds were extracted through water, ethanol and supercritical-CO2 fluid extraction (SFE). Water and ethanol extraction are traditional methods of extraction while the SFE extraction method was included because it is nontoxic and has high separation selectivity. The CO2 gas can also be reused, avoiding greenhouse effects. Extracts used both dry and fresh flower buds from Ljv but only dry flower buds from Lj because it was difficult to purchase during the same season as Ljv. The dried Lj were purchased in Henan, China and the Ljv were harvested from a local farmer in Jhutian Township, Pingtung (Taiwan). All the samples were harvested in April and authenticated by the Department of Food Science and Technology, Tajen University of Technology, Pingtung, Taiwan. When testing for antioxidants, the DPPH and SOD scavenging activities were measured. The highest concentration tested was 1000 µg/ml and with water extracts demonstrating higher activity over ethanol and SFE extracts. Lj extracts had higher antioxidant activities compared to the Ljv extracts. Also, Lj extracts prepared by 75% ethanol had the lowest IC50 values of DPPH (56.8 ± 0.5 µg/ml) and SOD (134.1 ± 5.7 µg/ml) scavenging activities. All SFE extracts had low antioxidant activities. IC50 values of the Ljv extracts at 150, 250 and 350 bar were 69.3 ± 6.0, 71.0 ± 6.8, 64.3 ± 7.5 µg/ml, respectively. The IC50 values of the dLJ extracts prepared at 150, 250 and 350 bar were 184.7 ± 13.1, 177.6 ± 8.9, 218.9 ± 18.3 µg/ml, respectively. Both the water and ethanol extracts of Lj demonstrated higher anti-inflammatory activity, however, the SFE extract for Ljv was higher than other SFE levels.

1.7 Prunella vulgaris (Heal-all)

Heal-all or *Prunella vulgaris* is often used in medicinal applications and is native throughout Europe and Asia. It is a perennial herb and was widely distributed in both Canada and the United States before European settlers began their exploration. It prefers moist soil but can grow in dry soils as well. It requires a medium amount of water and can grow in both full sun and part-shade conditions. Because of its hardiness, it is often considered a "weed." The plant can grow up to 1 to 2 feet and develops purple, purple-white flowers which bloom between May and September. (*Plant of the Week: Prunella vulgaris, Heal-All, Self-Heal,* n.d.) In Chinese medicine, it is occasionally used for the treatment of the thyroid gland, mastitis (inflammation of breast tissue), pulmonary tuberculosis, infectious hepatitis, and hypertension. Along with its medicinal uses, it is often used in herbal tea, its seedlings are consumed in dishes, and often serves as ornamental plants in southern China. The plant is also notably abundant in flavonoids, phenolic acids, triterpenes, and polysaccharides.

Recent solar UV exclusion research has indicated that growth, yield, and quantity of plants can be greatly affected by various wavelengths. Physical, external factors such as soil components, weather, UV-B radiation, and heavy metal exposure can also affect the growth of plants.

In a study by Chen et al. published in 2019 called "Plant morphology, physiological characteristics, accumulation of secondary metabolites and antioxidant activities of *Prunella vulgaris L*. under UV solar exclusion," it notes that no literature has investigated UV solar exclusion on the *P. vulgaris* plant and has decisively decided to analyze the effects on development, photosynthetic pigment content, cellular strength, the sequence of reactions, and antioxidant activity on the extracts from *P. vulgaris*. Only relevant antioxidant information will be included for the purpose of this review. In order to determine antioxidant activity of *P. vulgaris spicas*, ABTS scavenging DPPH radicals were investigated with ethanol extracts. From the results, despite undergoing various treatments, all ethanol extracts from *P. vulgaris spicas* indicated antioxidative properties. Under UV solar exclusion, *P. vulgaris* ethanol extracts more efficiently scavenged DPPH free radicals, demonstrating *P. vulgaris'* defense systems caused by photo-oxidative damage.

Another study involving *Prunella vulgaris* is called "Ultrasonic extraction and structural identification of polysaccharides from prunella vulgaris and its antioxidant and antiproliferative activities" by Li et al. published in 2019. Researchers of the study have recognized the positive uses of prunella vulgaris. Therefore, extraction is an extremely important part of retaining the beneficial polysaccharide content of the plant. Hot water is one of the most popular and extensively used extraction processes, however, it lacks efficiency in both time and temperature. As such, ultrasonic extraction, a relatively novel extraction process that saves both time and money, was used to extract polysaccharides from *P. vulgaris*. To optimize extraction conditions, surface methodology was used to determine both polysaccharides and antioxidant activity of *P. vulgaris*. 4.0 g of *P. vulgaris* was used with ultrasonic extraction under a 210 W power, 50 min time, and 70-degree temperature. The yield was then compared to conventional extraction methods. From the results, the ultrasonic extraction method indicated a higher extraction yield. Antioxidant content was determined through DPPH, oxygen radical absorbance capacity assay, and cellular antioxidant activity assay. In testing for antiproliferative activities, however, experimental yield was not higher than the predicted value.

"Scrutinizing the antioxidant potential of *Prunella vulgaris L.* : A medicinal plant from central Himalayan region" published in 2015 by Singh et al. also analyzed the antioxidant activity of *Prunella vulgaris.* Singh et al. mentioned that the development of synthetic antioxidants has increased in popularity, however, they are not accepted as therapeutic agents because of their potential toxicity. Therefore, the use of natural antioxidant compounds continues to be of significant value. *P.vulgaris* is popular within European, Asian, and Chinese medicine. Further, it is particularly high in triterpenes, ursolic acid and oleanolic acid, which act as a significant portion of *P.vulgaris's* antioxidant activities. Under this observation, the study aims to analyze the correlation between phytochemicals and the antioxidant activity of *P. vulgaris*' extract. Samples were collected from the Central Himalayan region (Nainital), altitude ranging from 1500-1900 m and their identity was verified by the Botanical Survey of India (BSI) in Dehradun, Uttarakhand. To prepare, the entire plant was washed and oven dried for 72 hours at 50 degrees celsius. It was then blended into a fine powder and soaked and shaken in an 80% methanol solvent for 48 hours at 100 rpm at 35 degrees celsius. Then, it was centrifuged at 10,000 rpm for 10 min. The resulting supernatant was later stored at 4 degrees celsius for later use. To determine radical scavenging activity, DPPH, MCA, FRAP, and ABTS methods were conducted with extracts at varying concentrations. Once testing was completed, total phenol and flavonoid contents were 17.200±0.306 mg gallic acid equivalent (GAE)/g dry weight and 3.920±0.042 mg quercetin equivalents (QE)/g dry weight) were found respectively. Using the

FRAP method, the reducing power potential was found to be 31.209±0.150 mg ascorbic acid equivalent (AAE)/g dry weight. With the DPPH method, the methanolic extract was 30.958 µg/ml. ABTS' testing method with standard ascorbic acid and methanolic extract was 30.558µg/ml and 52.651µg/ml respectively. The MCA assay with the positive control EDTA was found to be 6.3913µg/ml and the methanolic extract was 11.839 µg/ml. Scavenging effects of the methanolic extracts of *P. vulgaris* were found to be four times greater than those found in the synthetic antioxidant ascorbic acid. From the data found, there appears to be a close linear correlation between the polyphenols, DPPH, ABTS, reducing power, and superoxide activity, thereby indicating that *P. vulgaris* is a source of natural antioxidants.

1.8 Glycyrrhiza uralensis (Chinese licorice)

Chinese licorice or *Glycyrrhiza uralensis,* is the root of *Glycyrrhiza uralensis* and originates from East Asia (Wang et. al, 2013). It is often cultivated for use in Chinese medicine to enhance the effectiveness of other ingredients or to reduce bitterness in Chinese herbal remedies. Chinese liquorice is a perennial and can grow between 0.6 to 0.4 m. From June to August, it produces flowers and between July to October, its seeds become the ripest. During cultivation, the plant is often prevented from flowering in order for it to develop stronger roots (U.S. Department of Health and Human Services, n.d.). It prefers moist, sandy soil and either semi-shade or no shade. It is slow to grow from seed so transplanting in late spring to early summer is ideal (*Glycyrrhiza uralensis – Fisch,* n.d.). It is often used to ease excess mucus production, treat heartburn, mitigate inflammatory disorders, and treat issues with the skin or liver.

In a study written by Zhang et al. published in 2015 called “Purification, partial characterization and antioxidant activity of polysaccharides from *Glycyrrhiza uralensis*,” the characterization of antioxidants from a purified and isolated component of *G. uralensis* was analyzed. The physiochemical properties and antioxidant activities of the polysaccharides were also researched to gain a better understanding of the structure of the plant. *G. uralensis* was divided into three polysaccharide fractions (GUPs-1, GUPs-2 and GUPs-3). They were then purified from the root of *G. uralensis* using DEAE-52 and Sephadex G-100 column chromatography. The antioxidant activity of the purified polysaccharides indicated that those GUPs with a lower value for molecular weight and a higher glucose value had a higher antioxidant value.

Another study involving *Glycyrrhiza uralensis* is “Optimized extraction, preliminary characterization, and in vitro antioxidant activity of polysaccharides from *glycyrrhiza uralensis* Fisch" written by Chen et al. and published in 2017. In order to optimize extraction, the temperature, water to raw material ratio, and time were altered with response surface methodology and Box Behnkin design. Root powder was first purified in ethanol then vacuum filtrated in a centrifuge. Then, it was then analyzed using chromatography. The second variation indicated the most absorbance and was therefore used to measure antioxidant activity. After tweaking different parameters, the optimum extraction conditions were found to be at 99°C, a ratio of water to material of 15: 1, and a 2 hour extraction time. The *Glycyrrhiza uralensis* portion that was extracted indicated high levels of antioxidants and immune boosting properties. However, the extraction time must still be optimized further.

"Chemometric analysis of active compounds and antioxidant and α‑glucosidase inhibitory activities for the quality evaluation of licorice from different origins" by Zhang et al. (2021) analyzed the active compounds, antioxidant, and α‑glucosidase inhibitory activities for multiple samples of Chinese licorice by testing for the contents of total flavonoids (TFc), total phenolics (TPc), and total crude polysaccharide (TCPc). The Glycyrrhiza genus contains 30 species native to indigenous to Eurasia, northern Africa, and western Asia. Because of this, the chemical compounds in licorice may vary depending on where the plant was harvested and it may be beneficial to identify metabolites based on area of origin. With this in mind, the purpose of this study was to evaluate multiple samples of Chinese licorice from varying regions, which may be beneficial in choosing the most ideal planting areas and determining quality. 23 samples of licorice were identified and collected from Inner Mongolia, Gansu, and Xinjiang. Once collected, the samples were ground into a powder and double extracted in a methanol solution. After extraction, the supernatants were centrifuged and collected into a flask for later use. For the antioxidant content, the DPPH, OH, and ABTS assays were conducted. Antioxidant percentages of scavenging free radicals of DPPH ranged from 64.38 ± 3.68 to 89.15 ± 0.25; OH ranged from 79.02 ± 2.99 to 98.74 ± 0.99; and ABTS ranged from 85.33 ± 0.41 to 91.82 ± 0.10. From this data, antioxidant capacity from Inner Mongolia appeared to be the highest, followed by Gansu, then Xinjiang. Xinjiang samples demonstrated the highest OH activity values while the lowest values were also from Xinjiang. For the ABTS assay, samples from Gansu demonstrated the best scavenging activity. In general, samples reached values no less than 64 percent and no higher than 98 percent.

1.9 Wong Lo Kat Research

In terms of any research investigating the antioxidant content of Wong Lo Kat as a whole, there were limited studies available. One study called "Total Phenolic Contents and Antioxidant Capacities of Herbal and Tea Infusions" written by Fu et al. in 2011 analyzed the phenolic and antioxidant contents of 51 different types of herbal tea combinations. For the phenolic contents, the Folin-Ciocalteu method was used. Likewise, for the antioxidant activity, the ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays were used. From the results, there displayed a significant correlation between the FRAP and TEAC values, thereby indicating that the 51 beverages tested had the capability to scavenge free radicals. There was also a high correlation between antioxidant capabilities and total phenols. In general, the beverages demonstrated a high source of antioxidant phenolics which could assist in disease prevention.

In another study called "Antioxidant effects and cytoprotective potentials of herbal tea against H_2O_2-induced oxidative damage by activating heme oxygenase1 pathway" written by Zhang et al. in 2020, both the antioxidant activity and its potential protective effects against a H_2O_2 damaged model were studied. Six phenolic acids were identified from the Wanglaoji drink and each of them had their antioxidant activity measured. The herbal tea and target in this study was specifically to investigate Wanglaoji's antioxidant activity and identify its protective effects on a H_2O_2-induced cell damage model. The extract of Wanglaoji herbal tea was provided by Wanlaoji Pharmaceutical Co., Ltd. located in Guangzhou, China, consisting of seven traditional Chinese herbs: Mesona chinensis, *Plumeria rubra, Microctis folium, Chrysanthemum indicum,* *Lonicera japonica, Prunella vulgaris,* and *Glycyrrhiza uralensis*. 1 g of the extract contained 12.89 g of the herbal material. The DPPH and FRAP assays were conducted for the antioxidant activity. Phenolic acids tested were the following: rosmarinic acid, isochlorogenic acid C, neochlorogenic acid, cryptochlorogenic acid, protocatechuic acid, and caffeic acid. DPPH levels for each are the following (μM): Rosmarinic acid (15.2), Isochlorogenic acid C (11.7), Neochlorogenic acid (33.1), Cryptochlorogenic acid (29.1), Protocatechuic acid (17.9), and Caffeic acid (22.0). FRAP levels were the following (μM): Rosmarinic acid (10.0), Isochlorogenic acid C (11.1), Neochlorogenic acid (26.8), Cryptochlorogenic acid (23.1), Protocatechuic acid (25.3), and Caffeic acid (10.8). Each of the activity levels were dose dependent. In the FRAP assay, rosmarinic acid had the highest activity with the order being rosmarinic acid, caffeic acid, isochlorogenic acid C, cryptochlorogenic acid, protocatechuic acid, and neochlorogenic acid. The phenolic acid to total antioxidant activity ratio of the Wanglaoji herbal tea was between 0.2% to 4.8% for DPPH and between 0.4% to 8.3% for FRAP activity. For all six phenolic acids, the total contribution ratio was 9.6% for DPPH and 13.6% for FRAP. From the results, rosmarinic acid, a main component of Prunella vulgaris, contributed to the highest antioxidant activity. The H_2O_2 damaging model is a common model used to test oxidative stress. A young human fetal lung diploid fibroblast cell (2BS cell) was used against H_2O_2 then treated with Wanglaoji and compared with an untreated cell. The treated cell was shown to increase expression of the antioxidant enzyme HMOX1 and the related upstream and downstream genes such as ATF3, NFE2L2, and BLVRB. Total antioxidant activity may have been contributed by the phenolic acids combined with the HMOX1 enzyme and may need to be studied further.

From the literature review, all main components of Wong Lo Kat have had their antioxidant content analyzed in some manner. All studies included in the review have noted that the plants that were analyzed displayed a significant antioxidant content that may assist in preventing oxidative stress. Wong Lo Kat (or Wanglaoji), however, had only a few studies which analyzed antioxidants as a whole. The study written by Zhang et al. in 2020 "Antioxidant effects and cytoprotective potentials of herbal tea against H_2O_2-induced oxidative damage by activating heme oxygenase1 pathway" pointed out that the after analyzing the total antioxidant activity, a main component of the antioxidant activity contribution was from Prunella vulgaris. While the study addressed the Wong Lo Kat beverage directly and an extract of the beverage was directly obtained from the manufacturer, the phenolic acids were the main target of antioxidant testing rather than the individual components and herbal mixture.

Although there are varying levels of research available on individual herbs in the Wong Lo Kat beverage, additional research is still needed for western acceptance of herbs used in traditional Chinese medicine. The effectiveness of the Wong Lo Kat beverage and cooling teas as a whole continues to be more anecdotal and lacking in evidence. Because of this, the analysis of antioxidant content may assist in a greater understanding of the beverage's use.

2. MATERIALS AND METHODS

2.1 Research Ethics

The Belmont report lists that any study must demonstrate a respect for persons, beneficence, and justice. Respect for persons indicates that all participants have the right to choose whether or not they would like to participate in the study. Beneficence indicates that anyone participating in the study will not be harmed and that researchers would try their best to minimize any harm to participants. Justice indicates that participants will be treated fairly. This study, however, does not include the use of human subjects, therefore restricting any complications due to research ethics.

2.2 Research Design

Quantitative antioxidant data was collected from both the herbal mixture and the main ingredients using the Trolox Equivalency Antioxidant Capacity (TEAC) test.

Research was conducted at a food science lab inside University Hall at Montclair State University located in Montclair, New Jersey, United States.

The herbs were gathered from two shops. For the White frangipani, Microcos, Chrysanthemum, Japanese honeysuckle, Heal all, and Chinese licorice herbs, they were collected from an herbal shop located in Chinatown, Man hattan, New York called Ton Ren Herb Supply. The shop was chosen from word of mouth from a local as a reliable location to purchase medicinal herbs. Many of the patrons who frequent the store arrive at the location and inform one of the doctors on site of their issues. The doctor will then prescribe a treatment plan using a concoction of certain herbs. Ton Ren Herb Supply also offers acupuncture and massage depending on your ailment. Mesona (Chinese) was not purchased at this location because according to the doctor on site, it is considered an ingredient rather than an herb. This herb is often found in supermarkets combined with a thickening agent such as agar agar or gelatin to produce the jelly. Because of this, a google search was performed, and Yun Hai Shop, a Taiwanese General Store, was chosen as a location where the Chinese Mesona herb could be purchased in its raw form but also as USDA organic. The shop is located in Williamsburg, Brooklyn, New York and the herb is from Taiwan.

Once the herbs were gathered, they were ground using a coffee grinder, an isolation of phenolics, water based antioxidants, for dry weight assessment was performed on each herb. Samples were prepared in the following order: Mesona (Chinese), White frangipani, Microcos, Chrysanthemum, Japanese honeysuckle, Heal all, Chinese licorice, and combined.

2.3 Isolation of Phenolics of Individual Herbs

For the phenolics process, 0.3 g of the dried powder of Chinese Mesona was combined with 2.5 ml of a 4/1 acetone/water solution. This is equivalent to 2 ml acetone and 0.5 ml water. Once combined, the mixture was poured into a 15 ml centrifuge test tube along with the dried powder and covered with a cap. The mixture was then sonicated for 10 minutes. Once sonicated, the tube was centrifuged at 1000g for 10 minutes. After sonication, it was observed that despite being ground down, a significant amount of the dried powder absorbed the liquid. Furthermore, much of the Chinese Mesona powder was still floating on the top of the centrifuged tube. Because of this, the 4/1 acetone/water solution was doubled in a second trial. The solution became the equivalent of a total of 5 ml with 4 ml of acetone and 1 ml of water. This solution was then sonicated for 10 minutes then centrifuged for 10 minutes at 1000g. The supernatant was then removed from the tube and set aside for later use. Another 4/1 acetone solution with 4 ml of acetone and 1 ml of water was poured into the centrifuged tube, then sonicated for 10 minutes and centrifuged for 10 minutes at 1000g. After completion, both supernatants were combined. The supernatant was labeled and set aside for later.

Next, 0.3 g of the dried powder of White frangipani was combined with 2.5 ml of a 4/1 acetone/water solution. This is equivalent to 2 ml acetone and 0.5 ml water. Once combined, the mixture was poured into a 15 ml centrifuge test tube along with the dried powder and covered with a cap. The mixture was then sonicated for 10 minutes. Once sonicated, the tube was centrifuged at 1000g for 10 minutes. After sonication, it was again observed that despite being ground down, a significant amount of the dried powder absorbed the liquid. Likewise, much of the White frangipani powder floated to the top of the centrifuged tube. Because of this, the 4/1 acetone/water solution was doubled. The solution became the equivalent of a total of 5 ml with 4 ml of acetone and 1 ml of water. It was then sonicated for 10 minutes then centrifuged for 10 minutes at 1000g. The supernatant was then removed from the tube and set aside for later use. Another 4/1 acetone solution with 4 ml of acetone and 1 ml of water was poured into the centrifuged tube, then sonicated for 10 minutes and centrifuged for 10 minutes at 1000g. After completion, both supernatants were combined. The supernatant was labeled and set aside for later.

For Microcos, 0.3g of the dried powder was combined with 2.5 ml of the 4/1 acetone/water solution into a 15 ml centrifuge tube. In other words, this would be equivalent to 2 ml of acetone and 0.5 ml of water. After being combined, the tube was sonicated for 10 minutes and subsequently centrifuged for 10 minutes at 1000g. It was once again observed that much of the dried sample of Microcos absorbed the acetone/water solution so the 4/1 acetone/water solution was doubled to 5 ml. Some particles of Microcos also floated to the top of the liquid despite being centrifuged. Therefore, acetone was increased to 4 ml and water increased to 1 ml. Then, the 5 ml solution along with the dried powder was sonicated for 10 minutes and centrifuged for 10 minutes at 1000g. After being centrifuged, the supernatant was set aside. For the second round, 4 more ml of acetone was added along with 1 ml of water to the 15 ml centrifuge tube. The tube was once again sonicated for 10 minutes and then centrifuged at 1000g for 10 minutes. Following the second centrifugation, both supernatants were combined and set aside for later use.

Chrysanthemum was prepared in the same method as Microcos. 0.3g of the dried powder was combined with 2.5 ml of the 4/1 acetone/water solution at first. Then, once seeing that Chrysanthemum absorbed a large amount of the supernatant after being sonicated and centrifuged, the 4/1 acetone/water solution was doubled to 5 ml. After doubling to 5 ml, the supernatant was set aside to be combined for the second round. Once the second round was completed, both supernatants were combined and set aside.

Japanese honeysuckle and Heal All also demonstrated the same qualities as Mesona (Chinese), White frangipani, Microcos, Chrysanthemum: after sonication and centrifugation, many particles still remained in the supernatant or most of the supernatant was absorbed. For these instances, the acetone/water solutions were doubled and those supernatants were set aside after two iterations.

Chinese licorice proved to be the only herb that did not need to have the acetone/water solution to be doubled. Once the herb was powderized, sonicated, and centrifuged, the majority of the powder stayed to the bottom and the liquid remained mostly separated from the herb.

For some instances, when double the acetone and water had to be added, this may have occurred because the herbs were not ground down enough or that the herb in question is simply very porous.

Each of the combined supernatants were then evaporated in a rotary evaporator at 40 degrees celsius at about 307 mBar until the pressure reached approximately 100 mBar in order to isolate the phenolic from the acetone.

2.4 TEAC Analysis

The antioxidant (AOX) assessment by TEAC analysis was then performed. This method was chosen because it is a relatively standard method in measuring AOX potential. TEAC analysis compares the sample to Trolox in slowing oxidative reactions. Trolox is often known for its powerful antioxidant capacity and is considered standard in antioxidant testing. The comparison of these two compounds is called Trolox equivalency.

To begin this section, a 99.7 to 0.3% 5 mL formic acid solution (15 µL of formic acid with 4 mL of water) was formulated as a solvent for the sample. The phenolics were then further dissolved with 0.3 mL of the formic acid solution combined with water until it reached a total of 3mL. A 101.4405 μM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution was then prepared by mixing 1 mg of DPPH with a 25 mL 80/20 methanol/water (20 mL methanol/5 mL water) solution. Once the DPPH solution was prepared, it was poured into a 50 mL centrifuge tube and sonicated for 4 minutes. More DPPH solutions were prepared based on an as needed basis. A diluted Trolox solution was then prepared. 34 mL of a 1/1 acetone/water solution (17mL acetone/ 17 mL water) was first measured. Then, to make the 1.5 mM Trolox solution, 6 mg of Trolox was combined with 16 mL of the 1/1 acetone/water solution.

To prepare for the test, diluted solutions of 0 mM, 0.3 mM, 0.6 mM, 0.9mM, 1.2 mM, and 1.5 mM were combined. 0 nM contained 0 mL 1.5 mM Trolox solution and 5 mL acetone/H2O; 0.3 mM contained 1 mL 1.5mM Trolox solution and 4 mL acetone/H2O; 0.6 mM contained 2 mL 1.5mM Trolox solution and 3 mL acetone/H2O; 0.9 mM contained 3 mL 1.5mM Trolox solution and 2 mL acetone/H2O; 1.2 mM contained 4 mL 1.5mM Trolox solution and 1 mL acetone/H2O; and 1.5 mM contained 5 mL 1.5mM Trolox solution and 0 mL acetone/H2O.

A microplate was prepared by simultaneously pipetting 290 μL DPPH (101.4405 μM) into 7 wells in a row representing all six of the diluted Trolox solutions and a sample blank. The DPPH acted as a reagent for the Trolox solution. 10 μL of the full strength dissolution was then pipetted into the second column and 5 μL + 5 μL water was pipetted into the third column. Both dissolutions were pipetted into 3 different rows in the case of any abnormalities. The DPPH solution was also pipetted into the sample dissolutions rows. The microplate was then placed into a microplate reader (VersaMax Microplate Reader) and the SoftMax Pro Software was used for microplate analysis. Absorbance was set to 517nm and the loss of absorbance was measured after 30 minutes of microplate incubation at 27˚C.

This process was repeated using each herb individually.

2.5 Combined Mixture

To prepare the supernatant for the combined mixture, 0.3g of dried powder was measured out for each herb. The 4/1 acetone/water solution was again prepared but with 4 mL acetone and 1 mL water for each herb. This was to prevent any discrepancies. Once the 5 mL was combined with each herb, the mixture was again sonicated for 10 minutes and then centrifuged at 1000 g for 10 minutes. The supernatant was then stored for later use and a new 5 mL acetone/water was poured into the tube. Once poured, it was again sonicated for 10 minutes and centrifuged at 1000 g for 10 minutes. The supernatants were then combined together and evaporated in a rotary evaporator at 40 degrees celsius at about 307 mBar until the pressure reached approximately 100 mBar in order to isolate the phenolic from the acetone. Once again, a microplate was prepared with 290 µL DPPH pipetted into 7 wells. The combined solution contained equal parts of isolated phenolics. To prepare the combined herb dissolution, 1 mL of each concentrated herb was combined into one 50 mL centrifuge tube. The tube was then sonicated for 10 minutes and centrifuged for 10 minutes. The full strength 10 µL dissolution and 5 µL dissolution combined with 5 µL water were then pipetted into 2 separate columns. Both dissolutions were pipetted into 3 separate rows and 290 µL of the DPPH solution were also pipetted into these wells. That solution was then tested using the same methods and read through the microplate reader.

2.6 Statistical Analysis

To analyze the raw data, results were expressed as mean values ± standard deviation. One-way analysis of variance (ANOVA) test along with Tukey's test was used to determine if the means of each herb were statistically significant from each other. The level of significance was $\alpha \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1

Sample	TEAC (mmol trolox equivalency/g sample)				
	Rep 1	Rep 2	Rep 3	Mean	SD
Chinese Mesona	1.4989	1.4782	1.6986	1.5586	0.1217
White Frangipani	1.2967	1.2872	1.6991	1.4277	0.2351
Microcos	1.2054	1.196	1.4427	1.2813	0.1398
Chrysanthemum	1.1177	1.093	1.4443	1.2183	0.1961
Japanese Honeysuckle	1.3217	1.4454	1.6582	1.4751	0.1702
Heal All	1.4289	1.3847	1.6578	1.4905	0.1466
Chinese Licorice	1.1334	1.0194	1.3135	1.1554	0.1483
Combined	1.1844	1.0958	1.4586	1.2463	0.1892

Three repetitions were made to test for herb and the combined mixture. The mean and standard deviations are as follows: Chinese Mesona was 1.5586 ± 0.1217; White Frangipani was 1.4277 ± 0.2351; Microcos was 1.2813 ± 0.1398; Chrysanthemum was 1.2183 ± 0.1961; Japanese Honeysuckle was 1.4751 ± 0.1702; Heal All was 1.4905 ± 0.1702; Chinese Licorice was 1.1554 ± 0.1483; and combined was 1.2463 ± 0.1892. All values were reported in mmol Trolox equivalency/g sample.

When comparing the TEAC values, the ANOVA test along with Tukey's test was used to determine if the means of each herb were statistically significant from each other. The level of significance was $\alpha \leq 0.05$. It was found that when looking at all combinations of each sample, no significant synergies or antagonistic differences were able to be identified.

3.2 Discussion

When looking at the antioxidant potential found from each of the individual herbs or the herbs combined together, there did not appear to be any significant difference between each of the values. Therefore, it may be beneficial to compare the values with other teas commonly consumed in the market.

In Rusaczonek et al. (2010)'s study, the goal was to determine the antioxidant content and total polyphenols of the following herbal teas: green, black, pu-erh, white, lemon balm, peppermint, and chamomile. The teas were obtained from retail trade and prepared by adding 200 mL of deionized boiling water over 2 g of tea. Infusions were brewed for 5 minutes for teas and 10 minutes for herbal teas. Once complete, the mixture was poured onto a filter paper and the extract was used for analysis. Looking at the results, the teas had the following TEAC mean values: green (772 ±320.9); black (328 ±209.1); pu-erh (494±116.3); white (742 ±331.5); lemon balm (610±83.0); peppermint (409±125.3); and chamomile (180±10.2). All values were reported in µmol of Trolox / g.

Bartoszek et al. also analyzed the antioxidant content of various types of teas in their study "Comparison of antioxidant capacities of different types of tea using the spectroscopy methods and semi-empirical mathematical model" (2017). The teas analyzed in this study include green, black, and earl gray. All of the teas are derived from the young leaves of *Camellia sinensis* and are developed from a variety of methods. Green and white tea are derived from unfermented; oolong from fermented; and black and red from fermented leaves of the plant. Methods in which the tea is prepared also may affect the antioxidant activity of the tea such as in brewing time and temperature. Adding enhancements such as milk, sugar, lemon, and honey may also cause contradictory effects and may need to be researched further. The purpose of the study was to use the Electron paramagnetic resonance (EPR) model which would allow researchers to reduce the time taken to determine the TEAC value. 18 samples were chosen by 6 manufacturers and purchased from local retail stores. The teas tested were black, green, and earl grey. Each of the teas were also infused with refined sugar, lemon juice, and wildflower honey, and milk. The antioxidant capacities of the substances themselves were also determined. Infusions were prepared with 100 mL of boiling water poured over a tea bag. Some of the tea bags weighed more than others so the results were recalculated as 1 g of tea. 20 mL of fresh lemon juice was added to 100 mL of the tea infusion. 20 g of sugar was added to 100 mL of the infused tea. 20 mL of 3.2% milk was added to 200 mL of infused tea. The 20 mL/200 mL ratio was chosen because that is the amount typically consumed by consumers. 30g of honey was added for 100 mL of infused tea. The honey was individually tested with 3 g of honey in 5 mL of water. All values were converted to µmol of Trolox per 1 g of tea. To find the TEAC values, the EPR method was used. TEAC values for the infusions (µmol/100 ml infusion) were the following:

black tea (592.5 ± 54.1); green tea (754.9 ± 225.8); earl grey tea (407.7 ± 83.9). TEAC values for the tea (µmol/g tea) were the following: black tea (335.4 ± 34.0); green tea (494.8 ± 156.8); earl grey tea (248.4 ± 58.9). All TEAC values in literature varied between 143 and 3100 (µmol Trolox/100 mL) for black tea; 480 and 6200 (µmol TE/100 mL) for green tea; and 728 and 1205 (µmol Trolox/100 mL) for earl grey tea. The values found in this study were at the lower end of this range.

Zhao et al. (2019) also analyzed the antioxidant activities of various teas in their study "Phenolic profiles and antioxidant activities of 30 tea infusions from green, black, oolong, white, yellow and dark teas." In their study, the group aimed to test antioxidant activity through a simulation mimicking tea drinking. 30 well known teas from China were selected in this study: 4 black teas, 5 dark teas, 9 green teas, 4 oolong teas, 3 white teas, and 5 yellow teas. The activities of the infusions (water extracts) were tested using the FRAP, TEAC, and TPC tests with the Folin-Ciocalteu method. Tea infusions were chosen because they might contain different values of antioxidants compared to fat, water, or insoluble-bound portions and the results might be helpful for consumers or nutritionists in assessing intakes of antioxidants from teas. To prepare each sample, the teas were purchased from China and 1 g of each sample was extracted with 10 mL of boiling distilled water then placed in a 98 degrees celsius water bath for five minutes. After repeating the process six times, the infusions were combined and evaluated. For the results, FRAP values varied from 504.80 ± 17.44 to 4647.47 ± 57.87 µmol Fe2+/g DW. The highest FRAP values were Dianqing Tea, Lushan Yunwu Tea, Yuan'an Luyuan Tea, Weishan Maojian Tea, and Xihu Longjing Tea with FRAP values of 4647.47 ± 57.87, 4099.47 ± 105.10, 4088.80 ±

118.39, 3967.47 ± 87.76 and 3872.80 ± 38.16 μmol Fe2+/g DW, respectively. In each category, the FRAP values were green tea (3663.32 ± 535.63 μmol Fe2+/g DW), yellow tea (3582.93 ± 433.94 μmol Fe2+/g DW), oolong tea (1539.13 ± 351.86 μmol Fe2+/g DW), dark tea (1472.27 ± 691.91 μmol Fe2+/g DW), black tea (1283.47 ± 858.62 μmol Fe2+/g DW), and white tea (1160.80 ± 190.32 μmol Fe2+/g DW). TEAC values of the tea infusions ranged from 166.29 ± 24.48 to 2532.41 ± 50.18 μmol Trolox/g DW. The top 5 TEAC values were all 5 green teas (μmol Trolox/g DW): Dianqing Tea (2532.41 ± 50.18), Lushan Yunwu Tea (2353.21 ± 50.68), Xihu Longjing Tea (1935.89 ± 26.32), Dongting Biluochun Tea (1889.22 ± 12.33), and Duyun Maojian Tea (1880.54 ± 45.15). For each category, the TEAC values were the following: green tea (1899.19 ± 315.79 μmol Trolox/g DW), yellow tea (1622.77 ± 190.92 μmol Trolox/g DW), oolong tea (1211.28 ± 176.81 μmol Trolox/g DW), black tea (809.97 ± 237.04 μmol Trolox/g DW), dark tea (715.99 ± 352.02 μmol Trolox/g DW) and white tea (635.42 ± 227.85 μmol Trolox/g DW). In general, green tea demonstrated the highest antioxidant content overall and Dianqing Tea, Lushan Yunwu Tea, and Xihu Longjing Tea displayed the best source of antioxidants.

Samaniego-Sánchez et al.'s paper "The influence of domestic culinary processes on the trolox equivalent antioxidant capacity of green tea infusions" (2011) investigated the effects of different preparation methods on green tea. The factors investigated included water temperature, infusion time, stirring form, and dosage. Green tea is extracted from *Camellia sinensis* with unfermented green tea obtained by drying and pressing the leaves; semi fermented oolong tea obtained from partially fermented green tea leaves before they fry; and fermented red and black tea from leaves dried after fermentation. Unfermented green tea has demonstrated the highest antioxidant capacity because fermentation reduces polyphenol levels. The plant often grows in India, China, Japan, Taiwan, Sri Lanka, Indonesia and Central Africa. Because of its widespread production, the composition may vary depending on when it is harvested, how long the plant has been in production, seasonal conditions, manufacturing techniques, and storage methods. For tea production, infusion methods may also alter polyphenol levels. Therefore, the purpose of this study was to investigate the differences in preparation methods, particularly in water temperature, infusion time, stirring, and dosage form (loose leaf vs tea bag) on antioxidant activity. 20 commercial samples were collected with green tea and green tea with combinations of flowers or fruits. Teabag samples were purchased from supermarkets in either Spain or Mexico. Those samples obtained from Mexico might have deteriorated because the samples were analyzed in Spain. Samples obtained from either Mexico or Spain notably had a Chinese or Japanese origin. 1.5 g of tea was used with 180 mL of distilled water with temperatures measured at 70 °C, 80 °C and 90 °C and the infusion times used were 1, 3 and 5 min. Once the water reached the desired temperature, the tea was immediately added into the water and removed from the heat. After completing the specified time with the correct stirring conditions, the tea was placed into an ice bath and the pH was altered to 3.2 with 1M of citric acid in order to minimize changes to the samples' polyphenol levels. After stabilization, the sample was analyzed immediately. ABTS, TEAC, and TPC methods of measurements were chosen for this experiment, primarily because the ABTS/TEAC method with TPC has shown to produce higher correlation coefficients over the DPPH method. From the results, temperature and infusion time demonstrated the strongest effects on polyphenol and antioxidant levels. TEAC (antioxidant capacity) levels and TPC (polyphenol) levels increased between 70 °C and 80 °C but lowered at 90 °C. This may be because temperatures over 80 °C may alter the stability of polyphenol levels. The highest overall TEAC levels were at 80 °C × 5 (66.24 ± 11.86 μmol trolox/100 g tea) for all conditions but temperatures between 70–80 °C with a 3–5 min infusion time demonstrated the greatest overall outcomes. Although 90 °C may lead to faster infusion, too long of an infusion temperature may lead to the loss of polyphenols. Stirring and dosage did not appear to have much influence on the results. Furthermore, pure green tea appeared to have higher levels of antioxidants compared to those samples mixed with herbs or fruits. This may likely be because there was less green tea in those samples.

Kazimierczak et al. (2013) analyzed the antioxidant activity of green teas by comparing those teas originating from organic versus non-organic production methods. In their paper, Kazimierczak et al. noted how food produced with organic farming methods is characterized by having more phytochemical concentrations due to being produced in areas with lower pollution levels. Furthermore, it was noted that organic farming methods led to an approximately 30% increase in antioxidant levels in raw materials. This increase is again due to practices used in organic food production such as the use of compost which can increase bioactive content in the soil. Bioactive compounds, however, can be affected by soil quality, weather, plant genetics, harvest time, storage, processing, and preparation of raw materials. With a growing interest in organic foods, the study aimed to compare the antioxidant activity and active compounds in green tea infusions through both organic and non-organic methods with the goal of determining the impact of organic farming methods on antioxidant activity. Green tea bags and loose leaf green tea were acquired from two different locations. Organic teas were purchased from a retail store with a certified organic food label and non-organic teas were purchased from a typical grocery store located in Warsaw, Poland. With a comparison of the two types of tea, organic and non-organic teas did not display a significant difference in antioxidant activity. The mean for organic teas was 1687.11± 194.22 μmol Trolox/ g; non-organic teas was 1582.63± 148.62 μmol Trolox/ g; tea bags was 1632.96± 379.18 μmol Trolox/ g; and loose leaf teas was 1636.78± 181.93 μmol Trolox/ g. There was additionally no significant difference between antioxidant activity between tea bags and loose leaf teas.

Alasalvar et al. (2013) compared the compositional, nutritional, and functional characteristics of instant teas from both low and high quality black teas. Black tea was chosen because it is believed to be one of the most popular tea consumed in the world. To process black tea, a commo n method includes withering, rolling, fermentation, then firing. In the market, there are a variety of teas produced, some of which include blended tea, loose tea, tea bags, tea packets, instant tea, and ready-to-drink tea. Instant tea powder has been increasingly more popular. To produce instant tea powder, it is produced through spray, freeze, or vacuum drying. Spray drying is often used to create a powder from semi liquid and dried with hot air because of its ability to maintain the quality of the tea. A variety of varying quality teas were obtained from the ÇAYKUR Tea Processing Plant from Rize, Turkey in June 2011. The teas obtained were low quality with grades between 4-7 and high quality teas with grades between 1 and 3. All teas were obtained from the same production in order to prevent any discrepancies and processed 3 months before storing. Once obtained, they were stored in a temperature controlled cabinet until use.

Antioxidant activity was measured with the TEAC assay and extracted with 70% methanol and expressed in micromoles of TE per gram. Low quality instant tea had an antioxidant content of 1455 ± 184 and high quality instant tea had an antioxidant content of 1442 ± 140. Despite the quality differences, there were no major differences in antioxidant content.

Lin et al (2013) analyzed tea infusions from green, oolong, and black teas. These teas are all derived from the leaves of *Camellia sinensis L*. but produced with different fermentation processes. Green tea is unfermented, oolong tea is partially fermented, and black tea is fermented. Black tea is most common in the west and green tea is very popular in Asia. Oolong tea is preferred in China and Taiwan. The goal of this study was to determine the quality and antioxidant content of tea infusions of green, oolong, and black tea from the same variety of leaves. Sensory characteristics were also measured. All teas were prepared with the leaves of *Camellia sinensis L. i*n the autumn from a tea farm in Mingjian, Nantou County, Taiwan. For green tea, young leaves were "parched" or fire blanched at 280 to 300 degrees celsius for 5 to 6 minutes, rolled, then dried. Oolong teas had young leaves dried indoors for 5 hours with a hand mixing for 1 min at 1 hour intervals then stored for 15 minutes and fermented for 3.5 hours; they were then parched, rolled, and dried. Black tea had young leaves dried indoors for 10 hours, rolled, then dried. All dried leaves were pulverized and sieved. For the tea infusions, 3 g were used of each tea and mixed with 150 mL of boiling water for 5 minutes. After 5 minutes, the mixture was strained in a tea strainer and cooled until it reached room temperature. The remaining liquid was used for analysis. To determine the antioxidant content, the infusion was freeze dried. Results of the TEAC antioxidant content was expressed in μmol Trolox/g. Green tea had a TEAC value of 1.02 ± 0.02 µmol Trolox/g, oolong tea 1.09 ± 0.05 µmol Trolox/g, and black tea 2.16 ± 0.01 µmol Trolox/g. For strength of reducing power and scavenging, nonfermented was the strongest followed by semi-fermented then fully fermented. Black tea had the highest Trolox equivalent antioxidant capacity value followed by oolong tea then green tea.

In 2017, Kart & Çağındı analyzed the antioxidant activity of rose tea in their study "Determination of antioxidant properties of dry rose tea." Components of the rose are used in food, perfume, and in cosmetics. Additionally, the rose has been known to induce sedative, anti-stress properties, hemostatic, stomach, liver, intestines, fever and skin disease therapeutic and anti-inflammatory functions. One function that antioxidants serve in food is to prolong the shelf life of food without deterioration. With this in mind, man made antioxidants have been developed with the purpose of prolonging shelf life in foods. Conscious consumers have preferred natural ingredients in their foods, therefore, natural antioxidants are in demand. Antioxidants assist in maintaining the sensory characteristics in food such as the color, taste, and smell of food. Some common uses of roses in products include rose oil, rose syrup, rose jam, rose water, in flavoring, or as coloring. While the antioxidant levels of various rose species have been examined more thoroughly, there was limited information on rose tea in particular. Therefore, the study aimed to quantify the antioxidant activity of various components of the rose used for rose tea. 3 different varieties of dried rose buds as well as 3 different varieties of dried rose petals were obtained from the Isparta and İzmir markets to be used in this study. To prepare the sample, the dried rose bud or petal was kept in boiling water at 98 degrees celsius for five minutes then filtered. The three different rose petals had the following TEAC values (µM trolox/200 mL):10.78±0.08, 8.40±1.44, and 8.11±0.69. Rose bud TEAC values were (μM trolox/200 mL): 2.38±0.07, 2 0.75±0.19, and 0.64±0.08. All rose bud antioxidant levels were lower than rose buds and a statistically significant difference was found between different varieties of roses.

Referring to the extraction method and solvent type through the use of chamomile tea, Karaaslan (2021) analyzed the antioxidant and total phenolic contents of white and yellow chamomile teas in their study “Investigation of antioxidant properties of chamomile consumed as Herbal Tea.” The teas were investigated through various extraction and solvent methods. For the extraction method, polar organic solvents such as water, ethanol, acetone, methanol, and acetonitrile are preferred organic solvents. It is notable that both the extraction method and the solvent used will alter the analysis results. Both the white and yellow chamomile were extracted with the following extraction methods: soaking, stirring, sharking, and sonication assisted stirring. The solvents used were water, acetonitrile, and methanol. Chamomile samples were collected throughout different locations in Tunceli, Turkey. Once collected, the samples were washed in tap water and ultrapure water, dried at 50°C, crushed with a mortar, then stored in polyethylene storage bags until analysis. Each process was repeated three times. Samples weighed 2 g each with 20 mL of solvent and extracted at room temperature for 120 minutes then filtered with filter paper. For the results, the best extraction method which produced the most yield was sonication assisted stirring. As for extraction solvents, water extracts demonstrated the best yield while acetonitrile demonstrated the least amount of yield. DPPH levels for white chamomile with sonication assisted stirring were 10.886 ± 0.244 mg TEAC/g for water extracts, 0.121 ± 0.014 mg TEAC/g for acetonitrile extracts, and 3.478 ± 0.168 mg TEAC/g for methanol extracts. For yellow chamomile with sonication-assisted stirring, the DPPH levels were 11.709 ± 0.693 mg TEAC/g for water extracts, 0.237 ± 0.003 mg TEAC/g for acetonitrile extracts, and 4.465 ± 0.074 mg TEAC/g for methanol extracts. Comparing water extraction with other extraction methods, there was a statistically significant difference between water and both acetonitrile and methanol extraction methods. Yellow chamomile displayed a higher overall antioxidant capacity.

In Karaaslan (2021)'s study, the difference between extraction methods and solvents were examined on chamomile. A notable factor to keep in mind when comparing studies is not only the type of antioxidant test performed, but also the extraction and solvent method. In analyzing the results, water extraction with sonication assisted stirring demonstrated to be the most effective method of extraction. While some researchers used water as their solvent in an effort to simulate the tea drinking experience, others used different methods which may have increased or decreased their antioxidant results.

In a direct comparison of the antioxidant activity of the combined Wong Lo Kat herbal mixture (1.2463 ± 0.1892 mmol Trolox equivalency/g sample) with other teas, green tea was frequently identified as a tea that displayed high levels of antioxidant activity. However, while this is true, depending on a variety of factors, this may alter the levels of antioxidants in the tea. For instance, in Bartoszek et al. (2017)'s study, they found a TEAC green tea antioxidant value of 754.9 ± 225.8 for infusions and 494.8 ± 156.8 for tea. All samples were purchased from a commercial retail store. Meanwhile, in Zhao et al. (2019)'s study, all samples were obtained from

China but not specified exactly where. The top highest values out of 30 varieties were all green teas with antioxidant values of Dianqing Tea (2532.41 ± 50.18), Lushan Yunwu Tea (2353.21 ± 50.68), Xihu Longjing Tea (1935.89 ± 26.32). Rusaczonek et al. (2010) also obtained their teas from a local retail stores with mean TEAC mean values of green (772 ±320.9); black (328 ±209.1); pu-erh (494±116.3); white (742 ±331.5); lemon balm (610±83.0); peppermint (409±125.3); and chamomile (180±10.2) teas. These values also appear to be in the low range.

In Samaniego-Sánchez et al (2011)'s study, they analyzed dosage type (loose vs tea bag), water temperature, infusion time, and stirring form on the antioxidant values of green tea. From their study, they found that whether the leaves were in a loose form or tea bag, it did not make much of a difference. Kazimierczak et al. (2013)'s study comparing green tea from tea bags or loose also found that there was no significant difference between the two antioxidant contents. Furthermore, Alasalvar et al. (2013) analyzed both low and high quality instant black teas obtained from the same location and production line. Nonetheless, the low quality tea had a 1455 ± 184 micromoles of TE per gram and high quality tea had a 1442 ± 140 micromoles of TE per gram antioxidant content, demonstrating no major differences in antioxidant content.

With these factors in mind, perhaps such a large range between antioxidant levels in tea exists because of how old the sample is rather than whether the sample was purchased from a retail store. In a retail store, storage conditions, drying methods, and how long ago the product was dried may vary immensely. However, if the specific herb was directly harvested and dried for analysis purposes, this has the potential to retain much more of the polyphenols, therefore allowing for a higher antioxidant capacity. Likewise, from Alasalvar et al.'s study, when two different quality teas were compared, both teas were taken from the same production line yet the antioxidant content did indicate any significant difference. In addition to the tea's age, the production methods must be considered as a factor rather than the quality of the tea.

When comparing the antioxidant capacity of the individual herbs and combined mixture (mmol Trolox equivalency/g sample)(Chinese Mesona was 1.5586 ± 0.1217; White Frangipani was 1.4277 ± 0.2351; Microcos was 1.2813 ± 0.1398; Chrysanthemum was 1.2183 ± 0.1961; Japanese Honeysuckle was 1.4751 ± 0.1702; Heal All was 1.4905 ± 0.1702; Chinese Licorice was 1.1554 ± 0.1483; and combined was 1.2463 ± 0.1892, the herbs as well as the combined mixture appear to have antioxidant activity levels on par with certain preparations of green tea.

Despite green tea being named as one of the highest levels of antioxidants, in Lin et al (2013)'s study, when green, oolong, and black tea's antioxidant value was compared, it was found that black tea (2.16 ± 0.01 µmol Trolox/g) had the highest value followed by oolong tea (1.09 ± 0.05 µmol Trolox/g) then green tea (1.02 ± 0.02 µmol Trolox/g). All samples were obtained from the same species of plant, from the same location in Taiwan. Nonetheless, black tea was found to have the highest TEAC value. In this manner, it is notable that all teas should be considered and have the capability to add value.

Kazimierczak et al. (2013)'s study compared the difference between organic and non-organic green tea. It was found from their study that there was no significant difference between those teas that were organic versus non-organic. Chinese Mesona, purchased from Yun Hai Shop, was the only herb that was obtained with packaging labeled as USDA organic.

Although Chinese Mesona's antioxidant content did not vary significantly from other herbs in the combined mixture, referring to Kazimierczak et al.'s study, the herb's organic origins would not have made a significant impact on their antioxidant content. Therefore, comparing teas or herbs with their organic counterparts would not have significantly impacted the antioxidant content.

Kart & Çağındı (2017) analyzed the antioxidant content of rose tea and pointed out how antioxidants are used to preserve the sensory characteristics of food. Synthetic antioxidants are created in order to exhibit the same characteristics in natural antioxidants and are often added to foods to demonstrate these characteristics. They are often used as a substitute over natural antioxidants because they tend to be more stable, lower cost, and are widely distributed. However, according to Lourenço et al. (2019), the use of synthetic antioxidants are concerning and high doses may have the ability to cause DNA damage and/or also lead to faster deterioration over time. Furthermore, long term use of synthetic antioxidants have led to several health issues such as skin allergies, digestive issues, and for some individuals an increased risk of cancer. Therefore, natural antioxidants such as those from roses or the herbs from the Wong Lo Kat mixture may have a beneficial use in the food industry.

3.3 Limitations and Biases

One point of concern may be the source for the individual ingredients. Excluding Chinese Mesona, ingredients were purchased from Ton Ren Herb Supply, a local vendor in New York City, Manhattan Chinatown. While Ton Ren Herb Supply is trusted within the local community, adulteration and chemical contaminants should be considered. Whether it is truly a point of concern is inconclusive. Authenticity tests, however, may be too ambitious of a task to complete as a master's thesis must be completed in a short timeframe. Therefore, a quality control check was excluded from this study. Chinese Mesona was purchased from Yun Hai Shop, however, a notable difference between this herb and the herbs obtained at Ton Ren Herb Supply may be that the product was labeled as USDA organic.

Another point of concern may potentially be which part of each plant was tested and the origins of the plant. While the herbs analyzed are supposedly the correct plant species, when referring to other studies, the antioxidant content may vary depending on where the plant originated from, which cultivar of the plant that was chosen, which part of the plant that was tested, which methods were used to dry the herb, and the length of time between when the herb was harvested to when it was used. Because the herbs included in this study originated from two different herbal shops, the development and growth of each plant were not monitored. Furthermore, when obtaining the ingredients from the shops, the specific parts of each plant were not clearly specified. For instance, perhaps the flowers from Japanese Honeysuckle were the constituents tested, and because of this, antioxidant levels may be more or less potent.

Furthermore, when testing the combined herbal mixture, it may not be an accurate representation of the Wong Lo Kat herbal tea. This is because the ratios of each herb are not known to the public. Without knowing the formula, it is unclear whether or not this causes a significant difference in the antioxidant content compared to the actual formula of the Wong Lo Kat canned drink.

Disclaimer: the results were not skewed towards any researcher bias as concrete data will compensate for this. At this time, Montclair State and the researcher are not sponsored or affiliated with any producers of Wong Lo Kat tea.

3.4 Future Implications

Current literature on specific cooling tea formulations is limited. The commercial version of Wong Lo Kat, however, has gradually gained mainstream popularity within Chinese communities. An antioxidant investigation of the beverage may allow western audiences to grow more accepting of Chinese herbal tea formulations. Because of a lack of understanding within western populations, further research must be conducted to fully understand Chinese herbal formulations' effects and interactions with existing western medicine. As of now, consumption of herbal teas is generally considered safe. However, currently, herbal teas are generally consumed without any clear time or dosage. The main attitude towards herbal teas is that they are food with potential preventative effects. Because of this, overconsumption of various formulations along with herbal teas combined with western medicine must be investigated further (Fu, 2018). Furthermore, a more detailed study on the specific plants sourced, processed, and authenticated as well as identifying which part of the plant specifically is used in the Wong Lo Kat tea may be points of interest for additional study. When comparing the antioxidant levels with other known teas popular in western countries such as green, black, white, chamomile, and peppermint, the antioxidant levels appear to be on the higher end along with some varieties of green tea. With green tea often touted as a significantly high source of antioxidants, it is notable to indicate that the Wong Lo Kat mixture along with each of the individual herbs taken separately are on par with many varieties of herbal teas.

4. CONCLUSIONS

Based on the TEAC method, the herbs Chinese Mesona, White Frangipani, Microcos, Chrysanthemum, Japanese Honeysuckle, Heal All, Chinese Licorice as well as the combined mixture of the herbs did not display any significant difference from each other. The antioxidant activities were found to be the following: Chinese Mesona was 1.5586 ± 0.1217; White Frangipani was 1.4277 ± 0.2351; Microcos was 1.2813 ± 0.1398; Chrysanthemum was 1.2183 ± 0.1961; Japanese Honeysuckle was 1.4751 ± 0.1702; Heal All was 1.4905 ± 0.1702; Chinese Licorice was 1.1554 ± 0.1483; and combined was 1.2463 ± 0.1892. All values were reported in mmol Trolox equivalency/g sample. ANOVA test was used with Tukey's test to determine that the means of the herbs and combined mixture were not statistically significant.

While this is true, in comparison to other teas in the retail market, the Wong Lo Kat mixture as well as the individual herbs display an antioxidant content on par with several other high quality iterations of green tea and other teas in the market. Furthermore, despite Chinese Mesona being labeled as USDA organic, the organic origins would not have made a significant impact on the results as well as whether the tea was packed in a tea bag or in its loose form. Even when comparing two teas derived from the same location but processed in different methods, despite green tea being touted as having high antioxidant content, black tea was found to have a higher content. When comparing various teas' antioxidant levels, it is important to note that each herb has their own benefits and that various methods such as place of origin, time of harvest, and processing methods can make an enormous impact. Therefore, the antioxidant results found do not necessarily represent all combinations of the herbs included in the Wong Lo Kat drink.

5. TABLES

Table 1

Sample	TEAC (mmol trolox equivalency/g sample)				
	Rep 1	Rep 2	Rep 3	Mean	SD
Chinese Mesona	1.4989	1.4782	1.6986	1.5586	0.1217
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Chrysanthemum	1.1177	1.093	1.4443	1.2183	0.1961
Japanese Honeysuckle	1.3217	1.4454	1.6582	1.4751	0.1702
Heal All	1.4289	1.3847	1.6578	1.4905	0.1466
Chinese Licorice	1.1334	1.0194	1.3135	1.1554	0.1483
Combined	1.1844	1.0958	1.4586	1.2463	0.1892

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