DEVELOPMENT OF HERBAL KHLU TEA, CHEMICAL ANALYSIS AND SENSORY ANALYSIS

BY

SURASEE PROMCHUN

562-5121

A SPECIAL PROJECT SUBMITTED TO SCHOOL OF BIOTECHNOLOGY, ASSUMPTION UNIVERSITY IN PART FULFILLMENT OF THE REQUIREMENT OF THE DEGREE OF BACHERLOR OF SCIENCE IN BIOTECHNOLOGY

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Title:	The Development of Herbal Khlu Tea, Chemical and Sensory Analysis
By:	Mr. Surasee Promchun
I.D.	562-5121
Advisor:	A.RoungdaoKlinjapo
Level of study:	Bachelor of Science
Department:	Food Technology
Faculty:	Biotechnology
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(A. RoungdaoKlinjapo)
Advisor

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I would like to express my gratitude to my project, A.Roungdao Klinjapo for her guidance, aid, suggestion and dedication. Additionally, I would like to thank you "Uncle Nid's Kitchen" for providing crucial information regarding Khlu leaves processing and providing leaves for making tea. Furthermore, I would like to thank you my colleagues, both underclassmen and upperclassmen, for aiding in lab preparation, chemical analysis and statistical analysis. Hopefully, this report will guide and useful to all the readers whose work is related to this project.

Surasee Promchun

ABSTRACT

Khlu (*Pluchea indica*) is a plant with anti-inflammatory and antioxidant medicinal properties. Consumption of Khlu leaves as a culinary herb offers significant health-promoting compounds. Khlu tea has been commercially available in Thailand as a health-promoting drink. In this research, herbal Khlu tea were developed with addition of 1%, 2%, 3% and 4% of *Centella asiatica* (Asiatic pennywort or Bua bok) and Aegle marmelos (Bael fruit). Khlu and Asiatic pennywort was dried using tray dryer at 45°C for 48 hours, while bael fruit was for 2 hours. Dried ingredients were ground, and 5 g of solid mixture was packed and sealed in tea sachet. As the results, the best formula was 4% Asiatic pennywort and 3% bael fruit. For total phenolic compound, total flavonoid content, scavenging activity, and IC50, 4% Asiatic pennywort-Khlu tea had as 26.12 ± 1.50 mg GAE/ g sample, 18.02 ± 2.62 mg GAE/ g sample, 71.38 ± 9.77%, 76.04 ± 7.62 µL, respectively, while 3% Bael fruit-Khlu tea had 33.35 ± 4.40 mg GAE/ g sample, 20.56 ± 2.97 mg GAE/ g sample, 83.58 ± 8.87%, and 7.30 ± 2.15 µL, respectively. The pH of 4% Asiatic pennywort-Khlu tea was 5.57 ± 0.01 and 3% Bael fruit-Khlu tea was 5.63 ± 0.02.

KEYWORDS: Pluchea indica/ Khlu tea/ antioxidant/ phenolic/ flavonoid/ IC50/ sensory analysis

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INTRODUCTION

Small to medium sized enterprises (SMEs) has been a discussed topic for the decade. SMEs contributed to the economic growth of Thailand. However, its role and impact on country's economic has been understated. This business gained momentum of its popularity after Tom Yum Kung Crisis during 1997 – 1999. During that period larger enterprises collapsed and Thai Baht currency value plummeted. In purpose of rejuvenating, Thai government has been promoting SMEs ever since with hope to rebuild the devasted economy. Many SMEs are local producer offering product that has strong local identity. This aspect is further highlighted when OTOP was introduced. Many local producers and artisans saw this opportunity to developed and introduce their local products. Coupling the rise of internet network and physical logistic network, the reach to consumer is longer than it has ever been. The first problem arises when a product is in the market for a period or launching the new product into the market. The product needs to be different in a sense of improvement of the former version without deterring its existing benefits.

Several communities and artisans have been selling Khlu tea as their product, especially in the provinces that have larger area of marshland and coastline. Despite of their origin, the product is the fundamentally the same which are redundant and repetitive. This study is set out to explore the possibility of new combination between Khlu and Thai herbs like Asiatic pennywort and bael fruit and to compare their antioxidant activity and sensory evaluation with the commercial herbal Khlu tea; ginger-Khlu tea and lemongrass-Khlu tea.

OBJECTIVES

1. To find a new combination of Khlu based herbal tea.
2. To gain insight of consumers preference of certain attributes.
3. To study chemical properties of every formulations of Khlu based herbal tea.

LITERATURE REVIEW

I. KHLU (*Pluchea indica* (L.) Less)

1.1. Botanical information

Pluchea indica (L.) Less is an evergreen large shrub found abundantly in salt marshes and mangrove swamps. It is commonly known as Indian marsh fleabane or Khlu in Thai, camphorweed, Kuo bao ju (Chinese), Munjhu rukha or Kukrakonda (Bengali), and Beluntas (Bahasa). It is belonged to the family of Asteraceae. Khlu is a perennial shrub plant with small branches (0.5-2 m tall) widely distributed not only in the coastal line of Southeast Asia but also in warm temperature regions of countries such as Philippines, Australia, Taiwan, India, Mexico, and Hawaii.

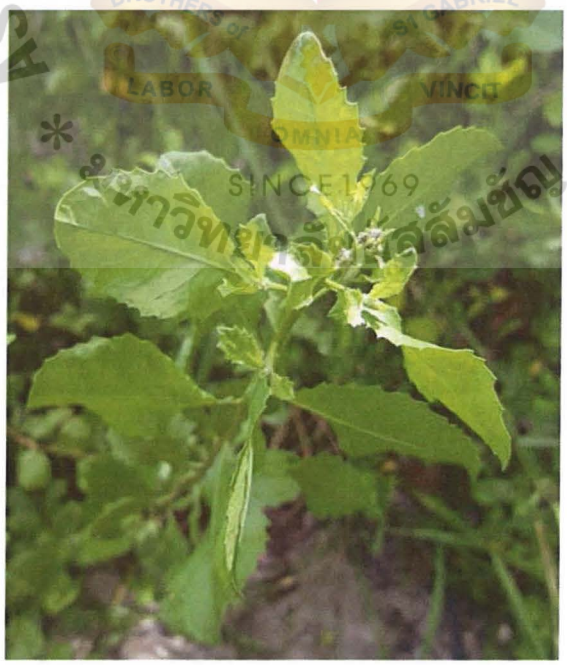

Figure 1 Foliage of *Pluchea indica (L.)* Less or Khlu

This species is a branching shrub up to 2 meters tall. The Khlu leaves are described as simple, sessile, glabrous, obovate, serrated with an acute apex. Moreover, for Khlu leaf, its alternate, stalkless or shortly-stalked leaves have membranous leaf blades that are toothed, usually drop-shaped, and 2.5–8.0 by 1.0–5.0 cm. The toothed oval leaf blades are papery but not thin, and often have a fine coating of hairs. An aroma is produced when the leaf blades are crushed (Suriyaphan, 2014; Polsiri, 2015).

Figure 2 Flowers of *Pluchea indica (L.)* Less or Khlu

The flower heads grow in dense clusters in the leaf axils and at the branch tips. The tubular flowers have pinkish purple florets have long, 7 mm wide, protruding styles. Its flower heads are found together in clusters or on branched shoots, at the leaf axils or the end of leafy twigs. Its flowers have a cup-like structure of white hairs that are spreading and 3.0–4.0 mm long. Each rachilla is composed of violet/white flowers which bear tiny fruits. The fruit body is a millimeter in length with a white pappus. Its indehiscent fruit (fruit that does not open to release its content when it is ripe) is brown, dry, one-seeded, cylindrical, five-ribbed, and 1.0 mm long. The seeds are dispersed on the wind (Suriyaphan, 2014).

The plant often grows in wet saline coastal habitat, such as brackish marshes and mangroves. Though it is not very competitive 32 with other flora, it can easily colonize coastal habitat and impact native and cultivated plants. The plant contains the compounds β-sitosterol and stigmasterol, which have antidiabetic properties. The roots and leaves are reported to possess astringent and antipyretic properties and are given in decoction as a diaphoretic in fevers. In Indo-China the roots in decoction are prescribed in fevers as a diaphoretic and an infusion of the leaves is given internally in lumbago. The root and leaves are used in Patna as astringent and antipyretics (Pramanik *et.al.*, 2007; Suriyaphan, 2014).

1.2 Nutritive value of Khlu leaves

Khlu leaves possess a natural sweet taste and astringent flavor similar to other wild edible vegetables in Southeast Asia. Raw or blanched Khlu leaves are consumed as a side dish with Nam Prik (freshly made chili paste). In addition, Khlu leaves are often used as one of the ingredients in several local dishes such as yum (sour and spicy salad) and kang ped (spicy coconut milk soup). The significant nutrients of Khlu leaves are shown in Table 1. Khlu leaves are clearly a good source of dietary fiber, calcium and β-carotene. One hundred grams of Khlu leaves have 251 mg of calcium, near the 297 mg Ca found in one serving (8 oz.) of 2% fat milk (Suriyaphan, 2014).

Since Khlu can naturally grow in wet saline habitats, it may contain high amounts of sodium (Na) and chloride (Cl) due to osmotic effects and soil salinity. Therefore, over consumption of Khlu leaves during long periods of time may present a health hazard, especially for individuals suffering from hypertension and cardiovascular disease.

Table 1 Nutritive values of Khlu leaves

Nutrients	Content / 100 g
Water	87.53 g
Protein	1.79 g
Fat	0.49 g
Ash	0.20 g
Insoluble dietary fiber	0.89 g
Soluble dietary fiber	0.45 g
Total dietary fiber	1.34 g
Carbohydrates	8.65 g
Calcium	251 mg
B-carotene	1,225 µg
Vitamin C	30.17 µg

Source: Suriyaphan, 2014.

1.3 Medicinal uses

Khlu is used as a natural medicine in three forms: decoction, poultice and infusion. Khlu has been used commonly in Thai medicine to cure variety of diseases such as respiratory disease, fever, rheumatism, anti-ulcer, anti-tuberculosis, anti-diuretic, anti-diabetic properties and potential antiophidian principles (Arsiningtyas *et.al.*, 2014). The plant itself is a source of antioxidant and phytochemicals and reduce the cell damage from oxidative stress. Main antioxidant found in Khlu are tannins, terpenes, lignin glycosides, triterpenoids, polyphenol including some flavonoids; quercetin and quinic acid; and eudesmane derivatives. It was demonstrated that extracts of *P. indica* had the DPPH, ABTS and ferric cyanide free radical scavenging activities which can be found in high quantity in its leaves (Srimoon and Ngiewthaisong, 2015).

Currently, besides of being regarded as medicine, a handful of small and medium-sized enterprises (SMEs) are producing Khlu herbal tea to satisfy the rising demand of healthy product and healthy diet lifestyle.

1.4 Bioactive compounds of Khlu leaves

Bioactive compounds are defined as secondary metabolites of plants which typically occur in small quantities in foods but are highly beneficial to health and are physiologically active when consumed. Bioactive compounds are also known as "Phytochemicals" ("phyto" meaning plant). Examples of typical phytochemicals found in fruits and vegetables are terpenoids, phenolic compounds, glucosinolates and chlorophylls, etc. Phytochemical fractionation studies have revealed that plant extracts obtained from the genus pluchea are comprised of phenolic acids, flavonoids, tannins, monoterpenes, triterpenoids, eudesmane-type sesquiterpenoids, chalocones, phenylpropanoids, benzenoids, ligan glycosides and steroids

In general, chlorogenic acid, caffeic acid and quercetin are found in fruits, vegetables and grains. Khlu leaves also contain significant amounts of chlorogenic acid, caffeic acid and carotenoids. Major flavonoids in Khlu leaves are quercetin and kaemferol, which are found in much higher concentration in Indian mulberry leaves. As expected, all three green leafy herbs contain low amounts of anthocyanins (Suriyaphan, 2014).

II. KHLU "TEA"

2.1 Backgroud of Khlu tea

Around the world, Khlu leaves and numerous leafy herbs, such as rosemary, peppermint, mulberry, lotus, mate, persimmon, bamboo, lemongrass, are commercially available as "teas" and commonly consumed as an infusion. It is well established that consumption of certain herbal teas especially green tea in adequate quantity and with suitable frequency and proper preparation helps promote human health, by boosting immune system, and lower the risk of certain chronic diseases such as allergy, anxiety, arteriosclerosis, depression, dyslipidemia, headache, hypertension,

hypoglycemia, intestinal disorders, insomnia, muscle cramps, sinus, etc. Nonetheless, there has been some concern regarding the misuse of Khlu as one of the main ingredients of commercial herbal teas for weight reduction, due to its diuretic property. This effect is only temporary, because the hypothalamus will soon initiate the feeling of thirst. The retail price of commercial Khlu tea in Thailand ranges between US $ 2-6/100 grams.

2.2 Guidelines for Khlu tea production

First of all, Khlu leaves were removed all blemished leaves from the freshly plucked leaves, then thoroughly washed and removed excess water by using a low speed centrifuge (such as a salad spinner) then place on a perforated stainless tray or plastic bucket for 10-15 minutes. Then, Khlu leaves were dried using sun drying. During drying process, Khlu leaves were constantly turned the leaves upside down. This was a very important step for the manufacture of a high quality Khlu tea. Basically, proper heat treatment contributes four essential benefits to the quality of Khlu tea as follows;

(i) Inactivation of heat resistant deteriorative enzymes such as oxidative and hydrolytic enzymes

(ii) Reduction of microbial load

(iii) Formation of desirable thermally generated aroma and flavor com- pounds

(iv) Enhancement of phytochemical compounds extractability by disruption of cell membranes and cell walls

In order to inhibit growth of spoilage micro-organisms in "tea" the final moisture content should not be higher than 10%. Finally, Khlu tea was packed in a food grade container (~5 grams/tea bag) and properly seal.

Figure 3 Drying process of Khlu tea

Figure 4 Khlu tea bag

2.3 Effects of drying methods on Khlu leaves

Drying is critical part of preserving leaves from biological and chemical changes. As fresh leaves are considered biochemically alive. Through the removal of moisture content, enzymes will be deactivated and same goes for bacterial yeast and mold which water are essential to their growth. As the result, leaves will have extended shelf life and can be logistical transport further to sell outlets, but the drying process hinders to phytochemicals in the leaves as well.

A study was conducted on effect of difference drying methods on medicinal plants by Mahonom *et.al.* (1999). One of its objectives was to see how much of each drying method effect the phytochemical in plants such as celery, Bilimbi, Japanese mint and guava. Drying condition were oven drying 50 ± 1°C for 9 hours, oven drying 70 ± 1°C for 5 hours, and freezing drying compared with fresh plants as a control. The results from freeze drying method shows the least percent loss of phytochemical followed by oven drying 70 ± 1°C for 5 hours and drying 50 ± 1°C for 9 hours, respectively. The results showed that the main factors were time and temperature of drying condition. Freeze drying machine was still relative expensive compare conventional drying oven. As the result, the optimal way to balance between the cost of processing and preservation of phytochemicals was drying the ingredient in lower temperature and using longer period to ensure that the ingredients' moisture decreased to the desired outcome. Using of lower temperature also reduced the burnt that appear when Khlu leaves were exposed higher temperature. As the plant used in the study was less dedicated then Khlu and could tolerate higher heat without burnt appearance.

III. BAEL (*Aegle marmelos* (L.) Correa)

3.1 Botanical information

Bael fruit (*Aegle marmelos* (L.) Correa) is a tropical fruit native to Southeast Asia and belongs to the Rutaceae family. It is grown throughout India as well as in Sri Lanka, Pakistan, Bangladesh, Burma, Thailand, and most of the Southeast Asian countries. In Thailand, it is commonly found growing in many regions, especially the lower north and central part consisting of Phichit, PrachinBuri, and Phitsanulok provinces. The peel of the fruit which is a very hard shell and green to brown in color depends on ripening stage. The appearance of yellow or orange edible

pulp is like a boiled pumpkin, possesses a slightly sweet taste and a characteristic floral, terpene-like aroma, very fragrant and pleasantly flavored. Fruits are 5 to 7.5 cm in diameter, globose, oblong pyriform, rind gray or yellow, pulp sweet, thick yellow, orange to brown in color. Seeds are numerous and arranged in the cells surrounded by a slimy transparent mucilage and have wooly hairs. Additionally, seeds are surrounded by slimy transparent mucilage.

Figure 5 A fruit of *A. marmelos* (L.) Correa; fresh fruit (left) and dried fruit (right)

3.2 Bioactive compounds and nutritive value

The bael fruit pulp contains many functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids, and other antioxidants which may protect us against chronic diseases. Total dietary fiber found in this fruit can be divided into insoluble dietary and soluble dietary fiber (mucilage and pectin). In addition, it also contains many vitamins and minerals including vitamin C, vitamin A, thiamine, riboflavin, niacin, calcium, and phosphorus. Therefore, bael fruit may indicate that it is one of the important plants used for indigenous traditional medicine. There are innumerable references of its uses in traditional

medicine. Table 2 showed some bioactive compounds in bael fruit reported by Charoensiddhi and Anprung (2008).

Table 2 Bioactive compounds of bael fruit (Charoensiddhi and Anprung, 2008)

Bioactive compounds	Mean±SD
Total dietary fiber (TDF) (g/ 100 g dw[a])	19.84±0.01
Soluble dietary fiber (SDF)	11.22±0.06
Insoluble dietary fiber (IDF)	8.62±0.04
Antioxidant activities	
DPPH assay (EC_{50}, µg dw/ µg DPPH)	6.21±0.34
FRAP assay (µM TE[b]/ g dw)	102.74±3.01
Total phenolics (mg GAE[c]/ g dw)	87.34±4.44
Total flavonoids (mg CE[d]/ g dw)	15.20±0.51
Total carotenoids (µg/ g dw)	32.98±0.51
Ascorbic acid (mg/ 100 g dw)	26.17±0.85

[a] dw = dry weight basis, [b] TE = trolox equivalent
[c] GAE = gallic acid equivalent, [d] CE = catechin equivalent
All values were performed in triplicate.

3.3 The use of bael in aspect of food

The uses of bael fruit in aspects of food have many forms in each country. For example, the ripe fruit is consumed fresh and prepared as nectar, squash, sherbet, jam, marmalade, and fruit cream in India. However, in Thailand these fruits are usually cut into pieces and dried, packed in bags or pulverized and packed as tea bags, and preserved in syrup as the bael fruit glacé which is normally used as dessert or an ingredient for cakes. Thai bael fruit in terms of bioactive compounds and characteristic flavor was considered to have a potential for use as functional food and value-added processed products. In recent years, many beverage brands have been incorporated the use of bael as the added ingredient to appeal to health-conscious consumer trend that has been on a steady increase.

IV. ASIATIC PENNYWORT (*Centella asiatica* (Linn.) Urban)

4.1 Botanical information

Asiatic pennywort (*Centella asiatica* (Linn.) Urban) is also known as the Indian pennywort, marsh pennywort, water pennywort, pennyweed, and, occasionally, sheep rot. Asiatic pennywort has the following names in other languages: Bua bok (Thai); ji xue cao, luei gong gen, tung chain (Chinese); daun kaki, kuda (Indonesia); tsubo-kusa (Japanese); or pegaga (Malay) (Peiris and Kays, 1996).

Figure 6 Asiatic pennywort (*C. asiatica* (Linn.) Urban)

Asiatic pennywort is a prostrate, faintly aromatic, stoloniferous, perennial, creeping runner belongs to the family Apiaceae. It is atropical medicine native to Southeast Asian countries. It has long-stalked, green, rounded apices which have smooth texture with palmately netted veins. The leaves are borne on pericladial petioles, around 2 cm (0.79 in). The flowers are white or pinkish to red in color, born in small, rounded bunches (umbels) near the surface of the soil. Each flower is partly enclosed in two green bracts. The hermaphrodite flowers are minute in size, less

than 3 mm (0.12 inch), with five to six corolla lobes per flower. Each flower bears five stamens and two styles. The fruit are densely reticulate, distinguishing it from species of Hydrocotyle which have smooth, ribbed or warty fruit. It usually takes three months to reach maturity and produces flower and fruit during April and June, annually.

4.2 Bioactive compound and nutritive value

Asiatic pennywort contains broad range of bioactive compounds such as triterpenes, carotenoids, glycosides, flavonoids, alkaloids, volatile oils, and fatty oils. Hence, the extract of this plant exhibits wide range of effect. For example, antidepressant effect which comes from triterpenes and antioxidant from carotenoids, flavonoids, and tannin. Generally, Asiatic pennywort is a rich source of minerals (including iron, calcium, potassium, and magnesium) and vitamins, including vitamins K, C, E, and many of the B vitamins.

4.3 Culinary uses and nutritional value

In Sri Lanka, Asiatic pennywort is most commonly used as a green salad vegetable. Finely sliced leaves are mixed with sliced shallot, green chilis, and lime juice and salt are added. It is also cooked as a curry with spices, onion, and coconut milk. Creeping cultivars are used for a porridge preparation. Fresh juice pressed from whole plants, including the roots, is boiled with a small amount of rice and coconut milk to prepare 'gotukola kenda', a thin porridge commonly used as a breakfast drink. Asiatic pennywort is likewise one of the most commonly used and frequently available edible leaves used in leaf concentrate meals, which are prepared as a porridge for feeding preschool children in Sri Lanka to combat nutritional deficiencies. Leaves of the Asiatic pennywort are 87.7% moisture and 2% protein on a fresh weight basis (16.26% dry weight), 0.2% fat, 6.7% carbohydrate, 1.6% fiber, and 1.6% ash. Fresh leaves are an excellent source of vitamin C, containing about 7 mg/100 g and contain 738 IU of vitamin A and 0.09 mg

of vitamin B 1/100 g fresh edible material. When the leaves are allowed to wilt, however, 95% to 99% of the vitamin C is lost. The plant is also a relatively good source of the minerals Ca (171 mg/ 100 g edible), P (32), and Fe (5.6). Leaf composition varies somewhat with location (Peiris and Kays, 1996).

4.4 Use in commercial products

Due to high value of the extracts of Asiatic pennywort, it has been developed into various commercial products and launched in the markets around the world. Oral preparations, capsules, tablets, syrups, tonics, and also have incorporated with some other products such as tea have been launched for various conditions namely, to improves mental abilities, to enhance vascular support, as an anti-stress formulas, to enhance immunity, as a lipid-lowering agent, and for many other valuable properties; some of them are listed below under the health benefits.

METHODOLOGY

1. Materials

Khlu (*Pluchea indica*) were harvested around salt farm at Samutsakorn province, Thailand. Asiatic pennywort and dried Bael were purchased from supermarket and local market, while tea sachet was order from the dealer. All chemicals used for analysis are available in laboratory.

2. Preparation of dried ingredients

Khlu leaves were separated from their stalks, washed with clean tap water and drained them of all excessed water, and dried in tray dryer at 45°C for 48 hours. Dried Khlu leaves were ground and kept in dark airtight packaging at room temperature.

Asiatic Pennywort was purchased from a local market, washed and drained of any excessed water and dried in tray dryer at 45°C for 48 hours and ground before keep in dark-airtight packaging at room temperature.

Dried bael was dried in tray dryer at 45°C for 2 hours in order to remove reabsorbed moisture. All the dried ingredient was grounded using blender and kept in airtight packaging at room temperature before use.

3. Formulate herbal Khlu tea (Asiatic pennywort-Khlu tea and Bael-Khlu tea)

Asiatic pennywort powder or bael powder was added into Khlu powder at 1, 2, 3, and 4% (w/w). The mixture 5 g was added into tea sachet and seal. To brew herbal Khlu tea, the prepared sachet was soaked in 100 mL of hot water (90°C) for 3 minutes. Brewed herbal Khlu tea were evaluated for color, flavor, taste, and appearance using 9-point hedonic score by 30 panelists. Commercial Ginger-Khlu tea and Asiatic Pennywort -Khlu tea were evaluated and compared with the prepared herbal Khlu tea.

4. Chemical and physiochemical properties of herbal Khlu tea

Both Asiatic pennywort-Khlu tea and Bael-Khlu tea were investigated chemical properties and compared with the commercial herbal Khlu tea – Ginger-Khlu tea and Pandan leaf-Khlu tea. Chemical properties of herbal Khlu tea were pH, color, total phenolic compound, total flavonoid content, and radical scavenging activity.

4.1 Determination of total phenolic compound

Total Phenolic compound was determined by modified method from Purushothaman *et al.*, 2013. Folin-Ciocalteau reagent was diluted to 10 times with distilled water. Sample 0.5 ml was added to 2.5 ml of the previously diluted Folin-Ciocalteau reagent and incubated for 3 min at room temperature. Then, 2 ml of 2 % sodium carbonate (w/v) was added into sample. The mixture was incubated at 45°C for 15 minutes. The absorbance was measured at 765 nm using spectrophotometer. Total phenolic compound was determined from a calibration curve prepared with a gallic acid.

4.2 Determination of total flavonoids content

Total flavonoids content was determined by Aluminium trichloride method using gallic acid as standard. A volume of 1.250 mL of sample was added to 750 µL of 5% Sodium nitrite ($NaNO_2$) solution. The mixture was allowed to stand for 6 min, later on 150 µL of Aluminium trichloride (10%) was added and incubated for 5 min, followed in succession by the addition of 7.5 mL of 1 M Sodium hydroxide (NaOH). The final volume of the solution was adjusted to 2.5 mL with distilled water. After 15 min of incubation the mixture turned to pink and the absorbance was measured at 765 nm.

4.3 Determination of free radical scavenging activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was used with modification from Kensornbuakkao and Yasurin (2016). The 1 mL of sample was added to 4 mL DPPH reagent (50 μM). The mixture was shaken vigorously and allow to incubate at room temperature in absence of light for 30 minutes. The absorbance was measured at 517 nm and the results were expressed as percentage reduction of DPPH reagent.

$$Percent\ reduction\ of\ DPPH\ reagent = 100(\frac{A_0 - A_c}{A_0})$$

Where A_0 is the absorbance of DPPH prior to reduction and A_C is the value of absorbance of the mixture of sample and DPPH.

Inhibition concentration (IC_{50}) was investigated using DPPH reagent (25 μM).

5. Statistical analysis and experimental design

Experiment design in use was The Randomized Complete Block Design (RCBD). All the data were analyzed by using the statistic analyze program, SAS enterprise Guide ver. 7.1.

RESULTS AND DISCUSSION

1. Preparation of dried ingredients

Khlu leaves and Asiatic pennywort were separated the foreign materials or dried leaves and washed before dried using tray dryer at 45°C for 48 hours, while bael fruits were dried at 45°C for 2 hours. The dried ingredients were immediately grounded using a blender.

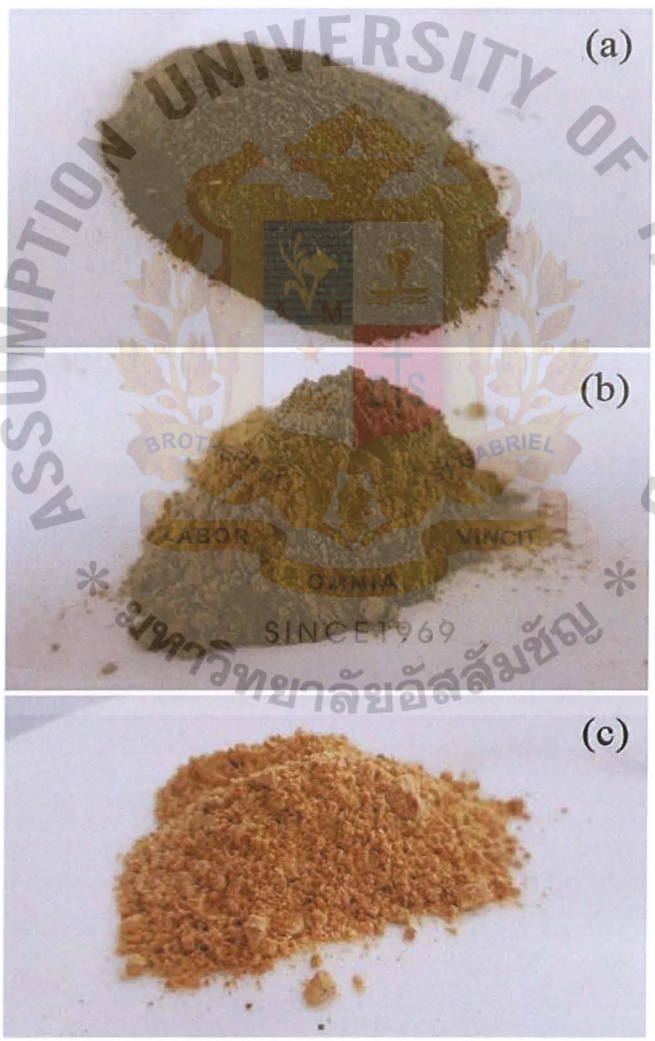

Figure 7 Ground dried ingredients; Khlu leaves (a), Asiatic pennywort (b), and bael fruit (c)

2. Formulate herbal Khlu tea (Asiatic pennywort-Khlu tea and Bael-Khlu tea)

Asiatic pennywort powder or bael powder was mixed with Khlu leaf powder at 1, 2, 3, and 4% (w/w). The mixture 5 g was added into tea sachet and hot sealed using sealing machine. Herbal Khlu tea sachet was soaked in 100 mL of hot water (90°C) for 3 minutes. The tea drink obtained from all the formulated herbal Khlu tea were brown in appearance and gave distinguished aroma from green tea or black tea. The unique aroma was resulting from Khlu natural local biome of estuary where the plant was exposed to brackish water which was absorbed into plant cell. Giving the sweet and salty aroma which characterized Khlu based tea. The intensity of this aroma varied throughout the year from the advancement and retreat of sea water into Khlu's natural habitat. Aroma of added herbs powder, Asiatic pennywort and bael, can be perceived as well. While aroma of Asiatic pennywort blended well, bael fruit aroma stood out. Resulted in another unique combination of pleasant aroma.

(a) (b) (c) (d)

Figure 8 Brewed Asiatic pennywort-Khlu tea with various Asiatic pennywort percentage; 1% (a), 2% (b), 3% (c), and 4% (d)

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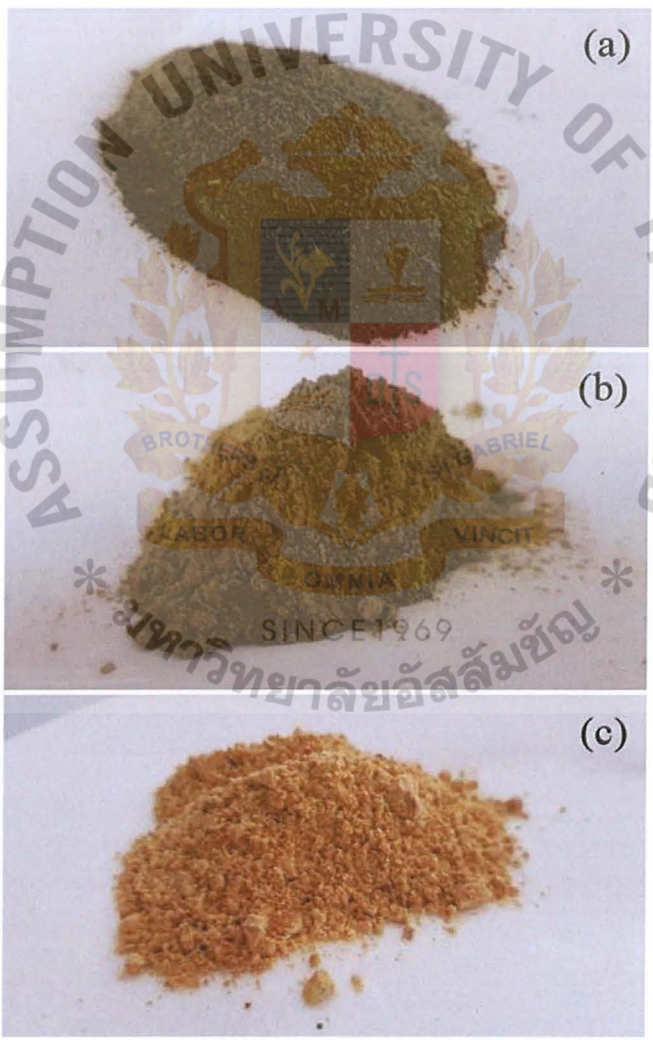

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(a) (b) (c) (d)

Figure 9 Brewed bael fruit-Khlu tea with various bael fruit percentage;

Figure 10 Commercial ginger-Khlu tea (right) and lemongrass leaf-Khlu tea (left)

Brewed herbal Khlu tea were evaluated for color, flavor, taste, and appearance using 9-point hedonic score by 30 panelists. Commercial ginger-Khlu tea and lemongrass leaf-Khlu tea were evaluated and compared with the prepared herbal Khlu tea. The sensory evaluation results showed in Table 3 to 5.

Table 3 Sensory evaluation score of Asiatic pennywort-Khlu tea

Attributes	Asiatic pennywort percentage (w/w)			
	1%	2%	3%	4%
Color	3.93 ± 1.25^{c}	4.43 ± 0.81^{c}	5.06 ± 0.91^{b}	5.83 ± 0.87^{a}
Aroma	4.30 ± 1.25^{b}	4.17 ± 0.81^{b}	4.23 ± 0.91^{b}	5.60 ± 0.97^{a}
Herbal flavor	3.17 ± 1.21^{d}	4.13 ± 1.07^{c}	5.20 ± 1.27^{b}	5.93 ± 0.98^{a}
Bitterness	5.07 ± 0.86^{b}	4.43 ± 0.94^{c}	4.67 ± 1.06^{cb}	5.70 ± 1.18^{a}
Body	5.90 ± 0.71^{a}	5.10 ± 1.26^{b}	5.60 ± 0.97^{ab}	5.33 ± 1.27^{b}
Overall liking	4.06 ± 0.86^{b}	4.40 ± 0.93^{b}	4.00 ± 0.94^{b}	5.70 ± 0.79^{a}

Table 4 Sensory evaluation score of bael fruit-Khlu tea

Attributes	Bael fruit percentage (w/w)			
	1%	2%	3%	4%
Color	5.27 ± 0.79^{c}	5.33 ± 0.88^{c}	7.20 ± 0.76^{a}	5.97 ± 0.85^{b}
Aroma	5.17 ± 0.91^{c}	5.23 ± 0.68^{c}	6.83 ± 0.87^{a}	6.17 ± 0.83^{b}
Herbal flavor	4.43 ± 0.86^{d}	5.30 ± 0.75^{c}	6.93 ± 0.82^{a}	6.40 ± 0.81^{b}
Bitterness	5.40 ± 0.9^{b}	5.80 ± 0.92^{b}	7.07 ± 0.74^{a}	5.83 ± 0.79^{b}
Body	5.40 ± 0.8^{c}	5.97 ± 0.89^{b}	7.00 ± 0.83^{a}	5.67 ± 0.76^{bc}
Overall liking	5.07 ± 1.08^{c}	5.80 ± 0.76^{b}	6.07 ± 1.08^{a}	7.33 ± 0.83^{b}

Table 5 Sensory evaluation score of commercial herbal-Khlu tea

Attributes	Commercial herbal Khlu tea	
	Ginger-Khlu tea	Lemongrass-Khlu tea
Color	**8.00 ± 0.85**	7.00 ± 0.77
Aroma	**8.00 ± 0.79**	6.00 ± 0.80
Herbal flavor	6.00 ± 0.93	**7.00 ± 0.89**
Bitterness	6.00 ± 0.89	6.00 ± 0.69
Body	7.00 ± 0.83	7.00 ± 0.83
Overall liking	6.00 ± 0.85	**8.00 ± 0.82**

Based on the result shown in Table 3, 4% Asiatic pennywort showed the highest score for its color, aroma, herbal flavor, bitterness, and overall liking as 5.80 ± 0.87, 5.60 ± 0.97, 5.93 ± 0.98,

5.70 ± 1.17, and 5.70 ± 0.79, respectively, while 1% Asiatic pennywort showed the highest score for body of tea as 5.90 ± 0.71. In Table 4, sensory evaluation of bael fruit-Khlu tea at 3% bael fruit showed the highest score for color, aroma, herbal flavor, bitterness, body and overall liking score as 7.20 ± 0.76, 6.83 ± 0.87, 7.00 ± 0.82, 7.00 ± 0.74, 7.00 ± 0.83 and 7.33 ± 0.83 , respectively. For the commercial herbal-Khlu tea, ginger-Khlu tea had higher score than lemongrass-Khlu tea for color (8.00 ± 0.85) and aroma (8.00 ± 0.79). Lemongrass-Khlu tea had higher score than ginger-Khlu tea for herbal flavor (7.00 ± 0.89) and overall liking (8.00 ± 0.82), while bitterness and body of commercial herbal-Khlu tea showed same score with no significantly differences (P>0.05).

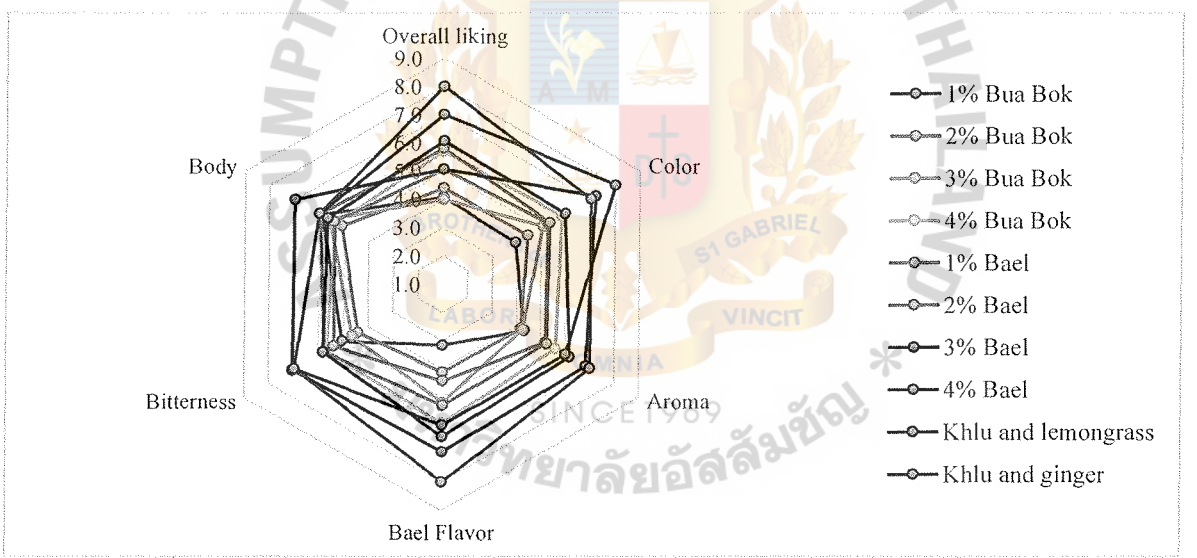

Figure 11 Comparison of sensory profiles composed of mean scores of 6 attributes identified in all herbal-Khlu tea samples.

Figure 11 showed the sensory profiles of all attributes identified in all herbal-Khlu tea samples. As the results, herbal-Khlu tea that showed the highest score for color, aroma, flavor, bitterness, body, and overall liking was lemongrass-Khlu, ginger-Khlu, ginger-Khlu, ginger-Khlu

together with 3% bael-Khlu, 3% bael-Khlu, and ginger-Khlu, respectively. Thus, the best mixture between herbal and Khlu tea was ginger and 3% bael fruit.

Therefore, as the sensory aspect, the best formula for Asiatic pennywort-Khlu tea and bael fruit-Khlu tea was 4% Asiatic pennywort and 3% bael fruit, respectively.

Table 6 Sensory evaluation of selected formula of herbal Khlu tea and commercial Khlu tea

Formula	Liking score
4% Asiatic pennywort-Khlu tea	7.00 ± 0.65
3% bael fruit-Khlu tea	7.00 ± 0.56
Ginger-Khlu tea	7.00 ± 0.70
Lemongrass-Khlu tea	7.00 ± 0.63

To investigate the acceptance of herbal Khlu tea, 30 panelists (18 females and 12 males) were kindly asked to participate in consumer test and evaluated herbal Khlu tea liking score. All herbal Khlu tea had liking score as 7.00. Those results implied that panelists like herbal Khlu tea. Moreover, they accepted all herbal Khlu tea with 100% and the suitable price for one pack of herbal Khlu tea (75 g) was 150 Baht.

3. Chemical properties of herbal Khlu tea

Every formulation of herbal Khlu tea was investigated regarding chemical properties and compared with the commercial herbal Khlu tea – ginger-Khlu tea and lemongrass-Khlu tea. To study the chemical properties of herbal Khlu tea, pH, color, total phenolic compound, total flavonoid content, and radical scavenging activity were investigated.

There were significantly different in pH between all herbal Khlu tea formulations (as shown in Table 7). The pH value from commercial ones were significantly different than the rest

of the formulation, skewing toward neutral pH of 7. Contrarily, all the values from every formulation were all below 6. Those results implied that all herbal Khlu tea had mild acidic properties.

Table 7 The pH value of brewed herbal Khlu tea

Sample	%	pH
Asiatic pennywort-Khlu tea	1	5.58 ± 0.01[a]
	2	5.58 ± 0.01[a]
	3	5.57 ± 0.01[a]
	4	5.57 ± 0.01[a]
Bael fruit-Khlu tea	1	5.57 ± 0.01[a]
	2	5.62 ± 0.03[a]
	3	5.63 ± 0.02[a]
	4	5.54 ± 0.08[a]
Ginger-Khlu tea	-	6.68 ± 0.23[b]
Lemongrass-Khlu tea	-	6.71 ± 0.17[b]

Table 8 The color measurement of brewed herbal Khlu tea

Sample	%	L*	a*	b*
Asiatic pennywort-Khlu tea	1	21.90 ± 0.07	19.99 ± 0.05	34.32 ± 0.12
	2	22.39 ± 0.05	20.13 ± 0.01	35.52 ± 0.00
	3	23.25 ± 0.04	20.29 ± 0.08	37.23 ± 0.43
	4	22.97 ± 0.05	20.44 ± 0.12	38.21 ± 0.13
Bael fruit-Khlu tea	1	23.03 ± 0.04	20.39 ± 0.09	38.66 ± 0.08
	2	23.79 ± 0.68	20.19 ± 0.18	39.66 ± 0.92
	3	24.17 ± 0.03	20.09 ± 0.02	40.13 ± 0.14
	4	22.25 ± 0.05	18.79 ± 0.06	34.98 ± 0.29
Ginger-Khlu tea	-	25.54 ± 0.09	0.44 ± 0.02	28.27 ± 0.12
Lemongrass-Khlu tea	-	27.49 ± 0.08	-1.14 ± 0.07	26.46 ± 0.34

The color description for each sample was expressed as CIE values for L*(lightness) axis, a*(red to green) axis and b*(yellow to blue) axis as shown in Table 8. Lightness of the commercials sample are the highest among all values obtained that are 27.49 ± 0.08 and 25.54 ±

0.09 for Lemongrass-Khlu tea and ginger-Khlu tea, respectively. While Asiatic pennywort juice and bael fruit juice has deeper color of green and red orange, respectively, lemongrass and ginger had paler yellow-green color in them in comparison.

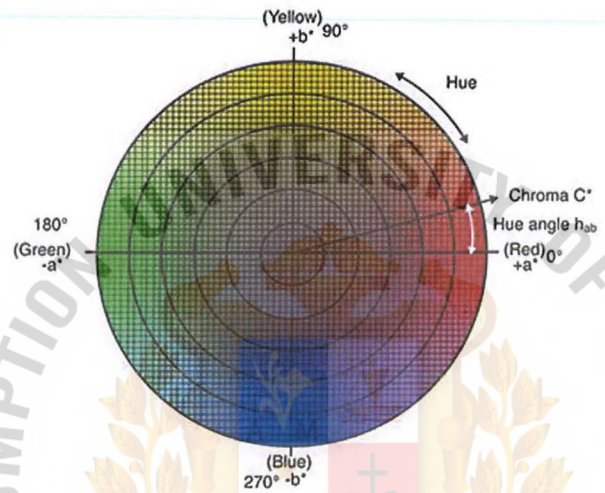

Figure 12 CIELAB color chart

The yellowness (b*) of commercial samples were also significantly different from the rest. Commercial Lemongrass-Khlu tea was in negative value (-1.14 ± 0.07). This can be interpreted that brewed lemongrass-Khlu tea had pale green color. Additionally, for the commercial ginger-Khlu tea, the value of b* was 0.44 ± 0.02. This value indicated that the brewed ginger-Khlu tea exhibited the trace shade of yellow.

Naturally, both lemongrass and Asiatic pennywort has chlorophyll as predominant pigment. However, during the first few seconds of brewing, a tea solution was blue instead of green as it should be. Thorat *et.al* (2018) studied on the effect of drying on color values of

lemongrass (*Cymbopogon citratus*). They found that the high temperature could lead to the replacement of magnesium in the chlorophyll by hydrogen, thereby converting chlorophylls to pheophytins. However, the rate of change varied with the temperature. The discoloration of products was more affected by temperature, time and medium of drying. This might be due to pigment degradation during drying process and browning reaction occurring resulting in the color changes. Color changes could be because of chlorophyll pigments were reduced as a result of photooxidation reaction in the cells. In addition, there is competition between peroxidase enzyme and chlorophyllase as well. It can be suggested that the lemongrass used in making commercial product was dried in the sun according to this study.

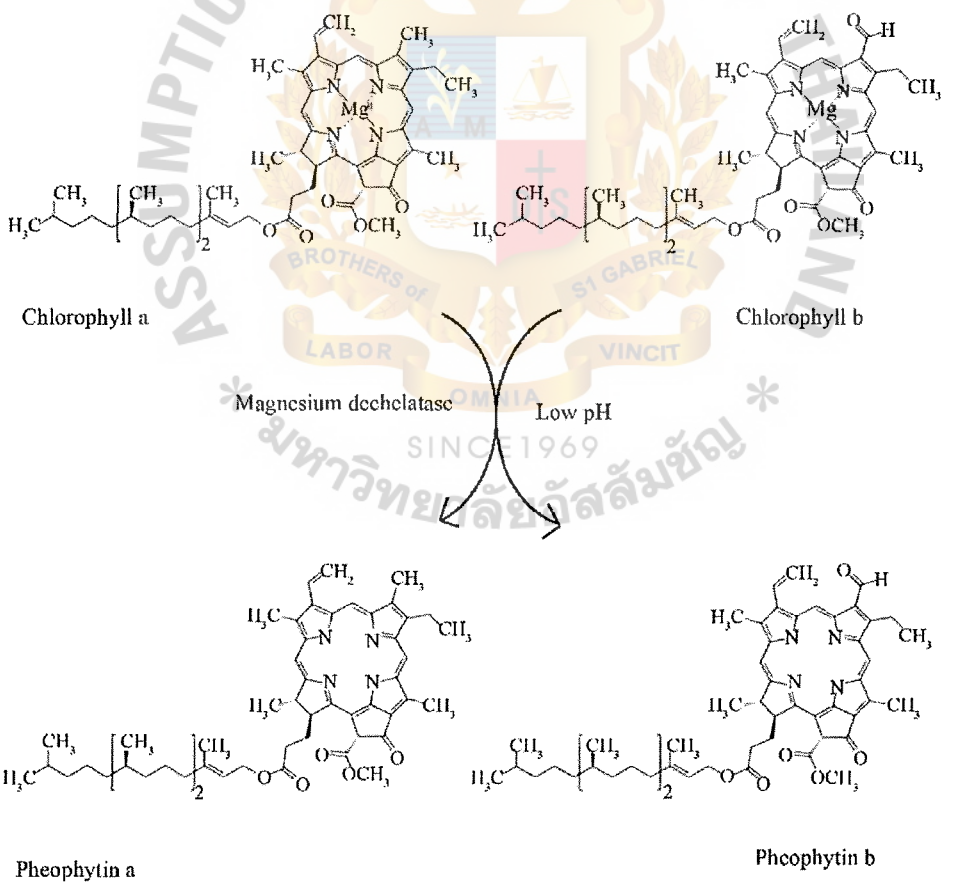

Figure 13 Chlorophyll degradation in the present of magnesium dechelatase and acid

Figure 13 showed the degradation of chlorophyll a and b by the present of enzyme magnesium dehydrogenase and acid. Those factors caused the color changes from bright green of chlorophyll to olive brown of pheophytin. Not only that, chlorophyll can be degraded according to the present of oxygen, thus, the color of brewed lemongrass and Asiatic pennywort changed when they were cooled down.

3.1 Determination of total phenolic compound

All herbal Khlu tea was brewed and investigated the total phenolic content using Folin-Ciocalteau method and gallic acid was used as the standard. As the results showed in Table 6, commercial ginger-Khlu tea and lemongrass-Khlu tea showed the lower total phenolic compound than Asiatic pennywort-Khlu tea and bael fruit-Khlu tea. In Figure 11, Asiatic pennywort at high amount showed the lowest total phenolic compound. The higher the Asiatic pennywort, the lower the total phenolic compound.

Table 9 Total phenolic compound in various brewed herbal Khlu tea

Sample	%	Total phenolic compound (GAE/ g sample)
Asiatic pennywort-Khlu tea	1	35.19 ± 5.59
	2	32.83 ± 8.44
	3	27.87 ± 4.28
	4	26.12 ± 1.50
Bael fruit-Khlu tea	1	32.31 ± 4.03
	2	31.34 ± 3.36
	3	33.35 ± 4.40
	4	16.07 ± 6.29
Ginger-Khlu tea	-	18.38 ± 5.22
Lemongrass-Khlu tea	-	12.70 ± 1.86

Peiris and Kays (1996) reported that Asiatic pennywort had total phenolic compounds 0.78 ± 0.00 mg GAE/ g sample and Suriyaphan (2014) reported the total phenolic compound in Khlu as 28.48

± 0.67 mg GAE/g sample. Therefore, the higher ratio of Asiatic pennywort in herbal Khlu tea mixture caused the lower Khlu leaves ratio resulting for the decreasing of total phenolic compound when the amount of Asiatic pennywort increased. For bael fruit-Khlu tea showed no significantly different among all bael fruit concentrations, except 4% bael fruit that showed the lowest total phenolic compound.

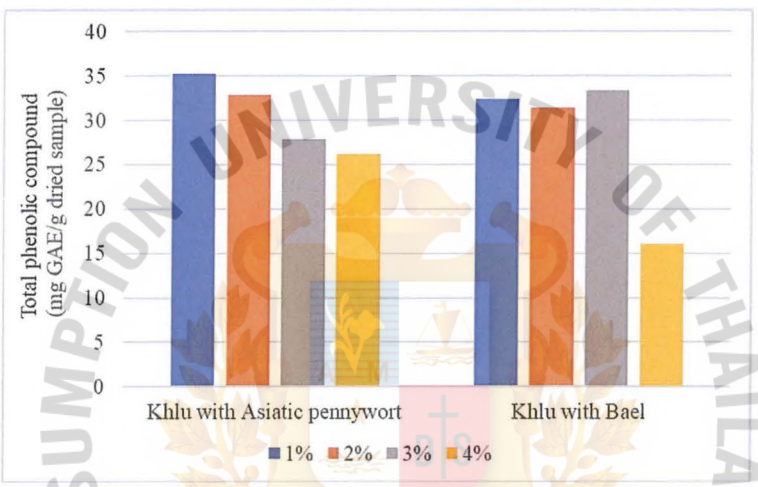

Figure 14 Comparison of total phenolic compound in various brewed herbal Khlu tea

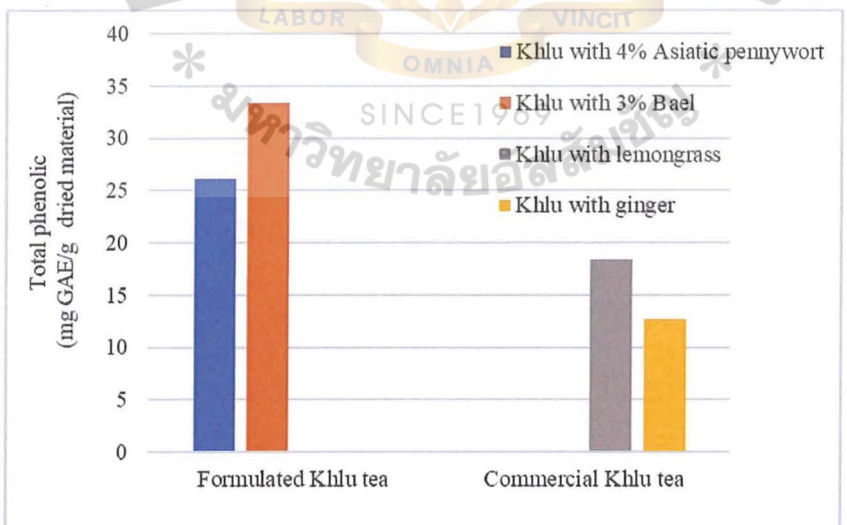

Figure 15 Comparison of total phenolic compound in commercial herbal Khlu tea and formulated Khlu tea

As the results showed in Figure 14, total phenolic compound in formulated herbal Khlu tea both Asiatic pennywort and bael fruit showed the higher amount than ginger-Khlu and lemongrass-Khlu tea. Even though, ginger was reported to contain total phenolic compound about 101.6 ± 0.6 mg GAE/g sample (Maizura *et al.*, 2011) and lemongrass contained total phenolic compound approximately 66.9 ± 1.7 g GAE/ g sample (Khadri *et al.*, 2010). The ginger and lemongrass used to produce commercial herbal Khlu tea was dried before used. Moreover, the commercial herbal Khlu tea was kept for long time before used to investigate the total phenolic compound. The long storage time affected the reduction of total phenolic compound in dried herbal.

Table 10 Total flavonoid in various brewed herbal Khlu tea

Sample	%	Total flavonoid compound (GAE/ g sample)
Asiatic pennywort-Khlu tea	1	20.58 ± 1.66
	2	19.37 ± 1.44
	3	19.72 ± 1.66
	4	18.02 ± 2.62
Bael fruit-Khlu tea	1	18.51 ± 2.48
	2	19.63 ± 2.07
	3	20.56 ± 2.97
	4	18.70 ± 1.51
Ginger-Khlu tea	-	17.41 ± 1.63
Lemongrass-Khlu tea	-	18.19 ± 1.38

To investigate the amount of total flavonoid content in sample, gallic avid was used as the standard. Table 10 showed the total flavonoid content in all formulation of herbal Khlu tea. As the results, 1% Asiatic pennywort and 3% bael fruit showed the high amount of total flavonoid content that were 20.58 ± 1.66 and 20.56 ± 2.97, respectively. In Figure 15, graph showed that 3% bael fruit showed the lowest total flavonoid content when compared with other bael fruit percentages. The results from both total phenolic compound and total flavonoid content indicated that 4% bael fruit was not suit for matching with Khlu tea. There should had some factors affected

or inhibit the expression of antioxidant activity in bael fruit and this needed more proof for further study.

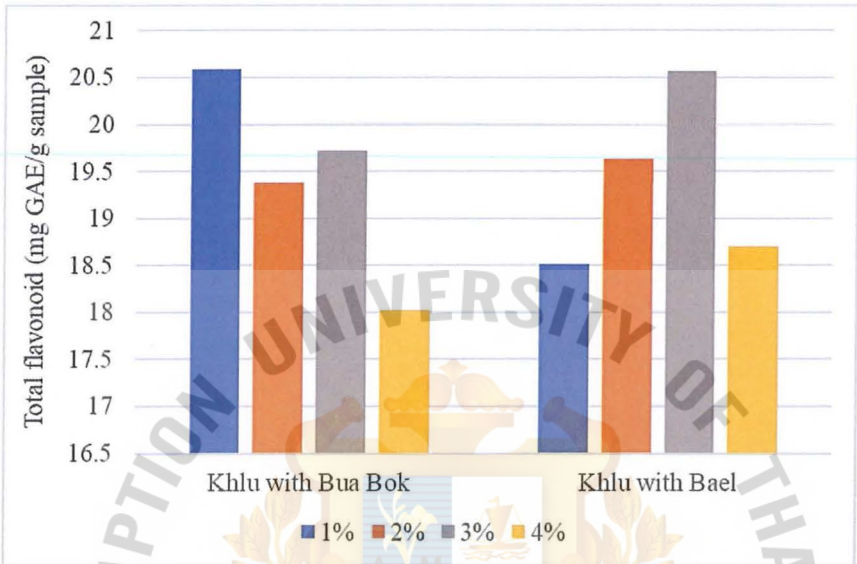

Figure 16 Comparison of total flavonoid in various brewed herbal Khlu tea

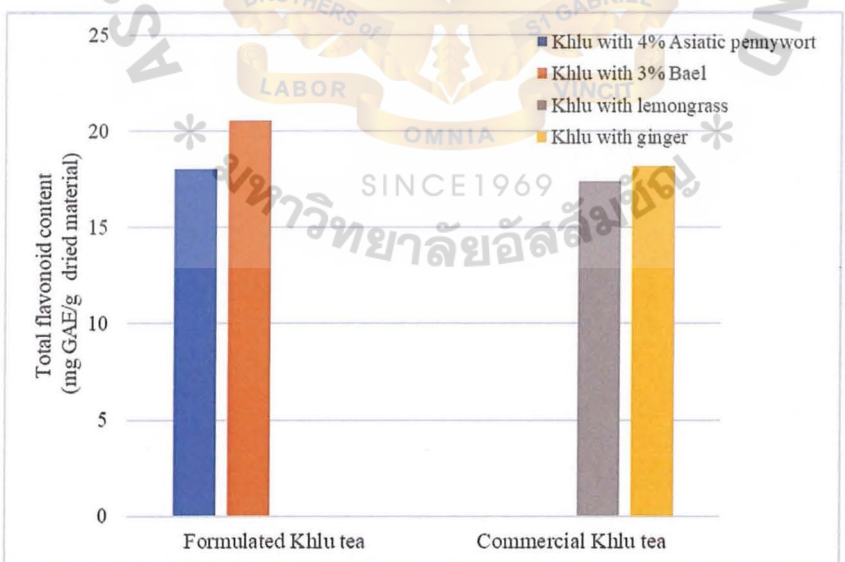

Figure 17 Comparison of total flavonoid content in commercial herbal Khlu tea and formulated Khlu tea

To compare total flavonoid content in all formula of herbal Khlu tea, Figure 17 showed that commercial Khlu tea both ginger and lemongrass-Khlu tea showed the total flavonoid in the same level with 4% Asiatic pennywort-Khlu tea, while total flavonoid in 3% bael fruit-Khlu tea showed the highest content. In 2008, Charoensiddhi and Anprung reported that the fresh Thai bael fruit had total flavonoid content approximately 15.20 ± 0.51 g GAE/ g sample, while Khlu leaves had 6.39 mg GAE/g sample (Suriyaphan, 2014).

Table 11 Scavenging activity in various brewed herbal Khlu tea

Sample	%	Scavenging activity (%)
Asiatic pennywort-Khlu tea	1	71.20 ± 4.24
	2	84.75 ± 3.12
	3	82.67 ± 5.67
	4	71.38 ± 9.77
Bael fruit-Khlu tea	1	84.75 ± 5.31
	2	83.01 ± 2.38
	3	83.58 ± 8.87
	4	62.48 ± 6.39
Ginger-Khlu tea	-	47.48 ± 5.07
Lemongrass-Khlu tea	-	44.64 ± 5.28

To investigate antioxidant in herbal Khlu tea, the scavenging activity of DPPH was investigated. In Table 8, both 2 and 3% Asiatic pennywort showed the high score of scavenging activity at 84.75 ± 3.12 and 82.67 ± 5.67 %, respectively, while ginger-Khlu tea and lemongrass-Khlu tea had scavenging activity as 47.48 ± 5.07 and 44.64 ± 5.28%, respectively. However, all bael fruit percentage showed no significantly different among scavenging activity, except 4% bael fruit which had the lowest scavenging activity as 62.48 ± 6.39%.

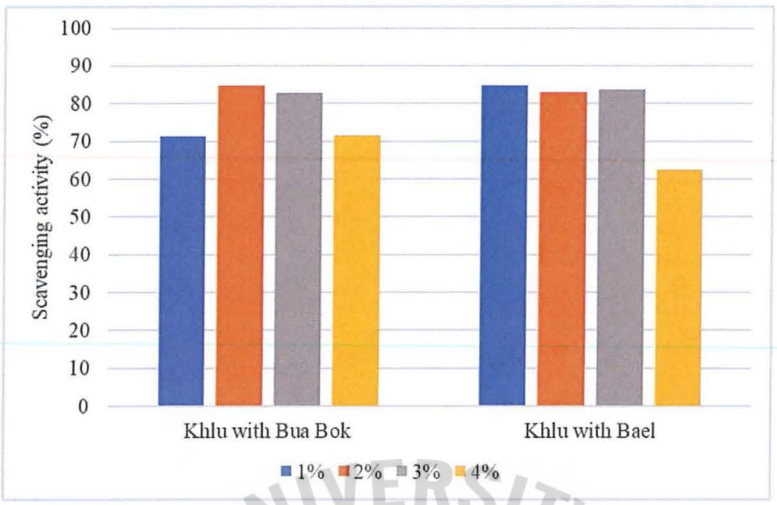

Figure 18 Comparison of scavenging activity in various brewed herbal Khlu tea

Figure 18 compared the scavenging activity of the best formula of Asiatic pennywort-Khlu tea and bael fruit-Khlu tea with commercial herbal Khlu tea. The results showed that commercial herbal Khlu tea both ginger and lemongrass-Khlu tea had the lower scavenging activity than formulated Khlu tea because the scavenging activity also reduced when stored for a period.

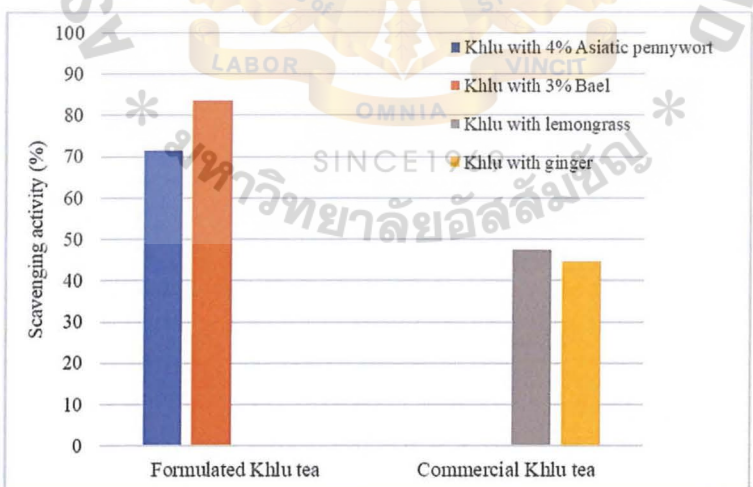

Figure 19 Comparison of scavenging activity in commercial herbal Khlu tea and formulated Khlu tea

Results of total phenolic compound, total flavonoid content, and scavenging activity were following the same trend where all calculated values of commercial samples were significantly lower than those of formulation samples.

In the case of commercial Khlu tea with ginger sample, the possible reason was temperature during was not high enough. According to the study of influence of maturity and drying temperature on antioxidant activity by Samakradhamrongthai and Utama-ang (2019), they founded that the highest total phenolic content was observed in drying at 60°C and the lowest at 40 °C. For optimal phenolic content, shorter time at higher drying temperatures was required while the total phenolic content and antioxidant activities of ginger rhizomes were higher when drying was done at higher temperature. The usage of high temperatures in extraction and food storage leads to the damage of total phenolic content. However, significant changes were confirmed between the different temperatures and the use of lower temperatures caused a slower loss of antioxidant activities. Insufficient drying temperature contributed to lower total flavonoid content and scavenging activity of the sample as well.

Table 12 IC_{50} value in various brewed herbal Khlu tea

Sample	%	IC_{50} (µL)
Asiatic pennywort-Khlu tea	1	75.89 ± 1.81
	2	80.79 ± 2.35
	3	68.02 ± 2.57
	4	76.04 ± 7.62
Bael fruit-Khlu tea	1	73.02 ± 1.91
	2	74.39 ± 2.37
	3	77.30 ± 2.15
	4	75.89 ± 0.94
Ginger-Khlu tea	-	99.55 ± 2.87
Lemongrass-Khlu tea	-	96.23 ± 11.31

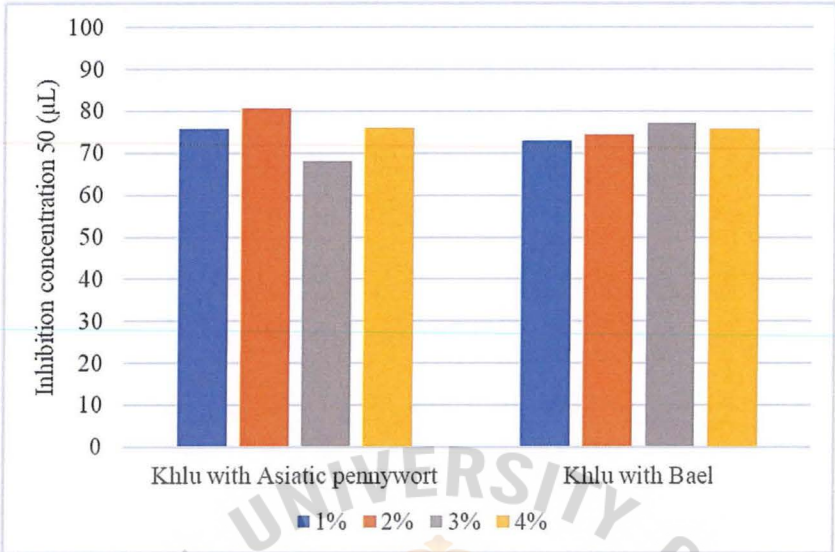

Figure 20 Comparison of IC50 of all formulated Asiatic pennywort-Khu tea and bael fruit-Khlu tea

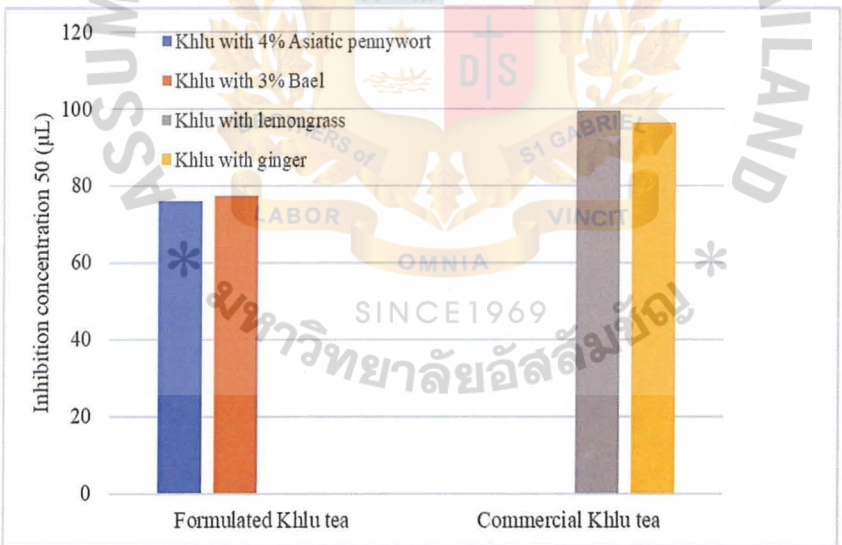

Figure 21 Comparison of IC_{50} of all herbal Khlu tea

In contrast to ginger, commercial Khlu tea with lemongrass sample's low antioxidant was resulted from drying temperature was too high. A study on effect of heat in drying process on antioxidant of lemongrass leaf power conducted by Tran and Nguyen (2018), found that as the temperature of the drying process increased, the total phenolic, total flavonoid, and scavenging activity of the sample decreased.

In Table 12 and Figure 20, all formulated herbal Khlu tea showed non significantly different of IC_{50}. As the results showed in Figure 21, IC_{50} of commercial herbal Khlu tea showed the higher IC_{50} than formulated herbal Khlu tea.

There was a significant different of inhibition concentration (IC_{50}) between formulated herbal Khlu tea and commercial Khlu tea samples. Commercial Khlu tea samples' IC_{50} was significantly higher than the rest of the samples. Because the herbal concentrations were different and affected on drying temperature for both lemongrass and ginger resulting in low antioxidant activity. Commercial herbal Khlu tea was packed lighter in tea sachet to begin with. Therefore, the concentration of antioxidant compound presented in liquid sample was evidently less and those prepared in formulation that were packed in tea sachet. Additionally, too low and too high drying temperature of ginger and lemongrass was another factor that contributed. With less of total phenolic and flavonoid content extracted from the herbs, made IC_{50} values of commercial were higher than the rest.

CONCLUSION

1. The best formula for Asiatic pennywort-Khlu tea and bael fruit-Khlu tea was 4% Asiatic pennywort and 3% bael fruit, respectively.

2. 4% Asiatic pennywort-Khlu tea had total phenolic compound, total flavonoid content, scavenging activity, and IC_{50} as 26.12 ± 1.50 mg GAE/ g sample, 18.02 ± 2.62 mg GAE/ g sample, 71.38 ± 9.77%, 76.04 ± 7.62 µL, respectively.

3. 3% Bael fruit-Khlu tea had total phenolic compound, total flavonoid content, scavenging activity, and IC_{50} as 33.35 ± 4.40 mg GAE/ g sample, 20.56 ± 2.97 mg GAE/ g sample, 83.58 ± 8.87%, and 7.30 ± 2.15 µL, respectively.

4. The pH value of 4% Asiatic pennywort-Khlu tea and 3% Bael fruit-Khlu tea was 5.57 ± 0.01 and 5.63 ± 0.02, respectively.

5. The color values were L*22.97 ± 0.05, a*20.44 ± 0.12, and b* 38.21 ± 0.13 for 4% Asiatic pennywort-Khlu tea and L*24.17 ± 0.03, a*20.09 ± 0.02, and b* 40.13 ± 0.14 for 3% Bael fruit-Khlu tea.

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APPENDIXEs

I Statistical analysis

1.1 Asiatic Pennywort sensory statistical analysis

Asiatic Pennywort

The GLM Procedure

Dependent Variable: y1 (Overall Liking)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	73.4666667	2.2958333	2.69	0.0001
Error	87	74.3250000	0.8543103		
Corrected Total	119	147.7916667			

R-Square	Coeff Var	Root MSE	y1 Mean
0.497096	20.35132	0.924289	4.541667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	56.42500000	18.80833333	22.02	<.0001
rep	29	17.04166667	0.58764368	0.69	0.8724

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	56.42500000	18.80833333	22.02	<.0001
rep	29	17.04166667	0.58764368	0.69	0.8724

Asiatic Pennywort

The GLM Procedure

Duncan's Multiple Range Test for y1 (Overall Liking)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	0.85431

Number of Means	2	3	4
Critical Range	.4743	.4991	.5156

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	trt
A	5.7000	30	4
B	4.4000	30	2
B			
B	4.0667	30	1
B			
B	4.0000	30	3

Asiatic Pennywort

The GLM Procedure

Dependent Variable: y2 (Color)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	80.6666667	2.5208333	2.40	0.0007
Error	87	91.3000000	1.0494253		
Corrected Total	119	171.9666667			

R-Square	Coeff Var	Root MSE	y2 Mean
0.469083	21.26812	1.024415	4.816667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	60.70000000	20.23333333	19.28	<.0001
rep	29	19.96666667	0.68850575	0.66	0.9001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	60.70000000	20.23333333	19.28	<.0001
rep	29	19.96666667	0.68850575	0.66	0.9001

Asiatic Pennywort

The GLM Procedure

Duncan's Multiple Range Test for y2 (Color)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	1.049425

Number of Means	**2**	**3**	**4**
Critical Range	.5257	.5532	.5714

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**trt**
A	5.8333	30	4
B	5.0667	30	3
C	4.4333	30	2
C			
C	3.9333	30	1

Asiatic Pennywort

The GLM Procedure

Dependent Variable: y3 (Aroma)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	86.8666667	2.7145833	2.06	0.0043
Error	87	114.4583333	1.3156130		
Corrected Total	119	201.3250000			

R-Square	Coeff Var	Root MSE	y3 Mean
0.431475	25.07108	1.147002	4.575000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	42.29166667	14.09722222	10.72	<.0001
rep	29	44.57500000	1.53706897	1.17	0.2851

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	42.29166667	14.09722222	10.72	<.0001
rep	29	44.57500000	1.53706897	1.17	0.2851

Asiatic Pennywort

The GLM Procedure

Duncan's Multiple Range Test for y3 (Aroma)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	1.315613

Number of Means	**2**	**3**	**4**
Critical Range	.5886	.6194	.6398

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**trt**
A	5.6000	30	4
B	4.3000	30	1
B			
B	4.2333	30	3
B			
B	4.1667	30	2

Asiatic Pennywort

The GLM Procedure

Dependent Variable: y4 (Herbal Flavor)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	161.6333333	5.0510417	3.63	<.0001
Error	87	120.9583333	1.3903257		
Corrected Total	119	282.5916667			

R-Square	Coeff Var	Root MSE	y4 Mean
0.571968	25.58671	1.179121	4.608333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	132.2916667	44.0972222	31.72	<.0001
rep	29	29.3416667	1.0117816	0.73	0.8325

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	132.2916667	44.0972222	31.72	<.0001
rep	29	29.3416667	1.0117816	0.73	0.8325

Asiatic Pennywort

The GLM Procedure

Duncan's Multiple Range Test for y4 (Herbal Flavor)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	1.390326

Number of Means	2	3	4
Critical Range	.6051	.6368	.6577

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**trt**
A	5.9333	30	4
B	5.2000	30	3
C	4.1333	30	2
D	3.1667	30	1

Asiatic Pennywort

The GLM Procedure

Dependent Variable: y5 (Bitterness)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	60.0333333	1.8760417	1.86	0.0124
Error	87	87.8333333	1.0095785		
Corrected Total	119	147.8666667			

R-Square	Coeff Var	Root MSE	y5 Mean
0.405996	20.23043	1.004778	4.966667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	27.66666667	9.22222222	9.13	<.0001
rep	29	32.36666667	1.11609195	1.11	0.3510

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	27.66666667	9.22222222	9.13	<.0001
rep	29	32.36666667	1.11609195	1.11	0.3510

Asiatic Pennywort

The GLM Procedure

Duncan's Multiple Range Test for y5 (Bitterness)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	1.009579

Number of Means	**2**	**3**	**4**
Critical Range	.5157	.5426	.5605

Means with the same letter are not significantly different.

Duncan Grouping		**Mean**	**N**	**trt**
	A	5.7000	30	4
	B	5.0667	30	1
	B			
C	B	4.6667	30	3
C				
C		4.4333	30	2

Asiatic Pennywort

The GLM Procedure

Dependent Variable: y6 (Body)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	52.1666667	1.6302083	1.51	0.0675
Error	87	93.8000000	1.0781609		
Corrected Total	119	145.9666667			

R-Square	Coeff Var	Root MSE	y6 Mean
0.357388	18.93639	1.038345	5.483333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	10.70000000	3.56666667	3.31	0.0239
rep	29	41.46666667	1.42988506	1.33	0.1591

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	10.70000000	3.56666667	3.31	0.0239
rep	29	41.46666667	1.42988506	1.33	0.1591

Asiatic Pennywort

The GLM Procedure

Duncan's Multiple Range Test for y6 (Body)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	1.078161

Number of Means	2	3	4
Critical Range	.5329	.5607	.5792

Means with the same letter are not significantly different.				
Duncan Grouping		**Mean**	**N**	**trt**
	A	5.9000	30	1
	A			
B	A	5.6000	30	3
B				
B		5.3333	30	4
B				
B		5.1000	30	2

1.2: Bael statistical analysis

Bael

The GLM Procedure

Dependent Variable: y1 (Overall Liking)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	104.2333333	3.2572917	4.63	<.0001
Error	87	61.2333333	0.7038314		
Corrected Total	119	165.4666667			

R-Square	Coeff Var	Root MSE	y1 Mean
0.629936	13.82879	0.838947	6.066667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	80.26666667	26.75555556	38.01	<.0001
rep	29	23.96666667	0.82643678	1.17	0.2794

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	80.26666667	26.75555556	38.01	<.0001
rep	29	23.96666667	0.82643678	1.17	0.2794

Bael

The GLM Procedure

Duncan's Multiple Range Test for y1 (Overall Liking)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	0.703831

Number of Means	2	3	4
Critical Range	.4305	.4531	.4680

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**trt**
A	7.3333	30	7
B	6.0667	30	8
B			
B	5.8000	30	6
C	5.0667	30	5

Bael

The GLM Procedure

Dependent Variable: y2 (Color)

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	32	83.6333333	2.6135417	3.40	<.0001
Error	87	66.9583333	0.7696360		
Corrected Total	119	150.5916667			

R-Square	Coeff Var	Root MSE	y2 Mean
0.555365	14.76503	0.877289	5.941667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	72.29166667	24.09722222	31.31	<.0001
rep	29	11.34166667	0.39109195	0.51	0.9793

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	72.29166667	24.09722222	31.31	<.0001
rep	29	11.34166667	0.39109195	0.51	0.9793

Bael

The GLM Procedure

Duncan's Multiple Range Test for y2 (Color)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	0.7696 36

Number of Means	**2**	**3**	**4**
Critical Range	.4502	.4738	.4894

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**trt**
A	7.2000	30	7
B	5.9667	30	8
C	5.3333	30	6
C			
C	5.2667	30	5

Bael

The GLM Procedure

Dependent Variable: y3 (Aroma)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	78.2333333	2.4447917	3.60	<.0001
Error	87	59.0666667	0.6789272		
Corrected Total	119	137.3000000			

R-Square	Coeff Var	Root MSE	y3 Mean
0.569798	14.08496	0.823970	5.850000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	57.43333333	19.14444444	28.20	<.0001
rep	29	20.80000000	0.71724138	1.06	0.4084

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	57.43333333	19.14444444	28.20	<.0001
rep	29	20.80000000	0.71724138	1.06	0.4084

Bael

The GLM Procedure

Duncan's Multiple Range Test for y3 (Aroma)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	0.678927

Number of Means	2	3	4
Critical Range	.4229	.4450	.4596

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**trt**
A	6.8333	30	7
B	6.1667	30	8
C	5.2333	30	6
C			
C	5.1667	30	5

Bael

The GLM Procedure

Dependent Variable: y4 (Herbal Flavor)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	128.2000000	4.0062500	5.69	<.0001
Error	87	61.2666667	0.7042146		
Corrected Total	119	189.4666667			

R-Square	Coeff Var	Root MSE	y4 Mean
0.676636	14.55217	0.839175	5.766667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	112.7333333	37.5777778	53.36	<.0001
rep	29	15.4666667	0.5333333	0.76	0.7995

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	112.7333333	37.5777778	53.36	<.0001
rep	29	15.4666667	0.5333333	0.76	0.7995

Bael

The GLM Procedure

Duncan's Multiple Range Test for y4 (Herbal Flavor)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	0.704215

Number of Means	**2**	**3**	**4**
Critical Range	.4307	.4532	.4681

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**trt**
A	6.9333	30	7
B	6.4000	30	8

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**trt**
C	5.3000	30	6
D	4.4333	30	5

Bael

The GLM Procedure

Dependent Variable: y5 (Bitterness)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	66.0666667	2.0645833	2.77	<.0001
Error	87	64.8583333	0.7454981		
Corrected Total	119	130.9250000			

R-Square	Coeff Var	Root MSE	y5 Mean
0.504615	14.33066	0.863422	6.025000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	46.89166667	15.63055556	20.97	<.0001
rep	29	19.17500000	0.66120690	0.89	0.6327

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	46.89166667	15.63055556	20.97	<.0001
rep	29	19.17500000	0.66120690	0.89	0.6327

Bael

The GLM Procedure

Duncan's Multiple Range Test for y5 (Bitterness)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	0.745498

Number of Means	2	3	4
Critical Range	.4431	.4663	.4816

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	trt
A	7.0667	30	7
B	5.8333	30	8
B			
B	5.8000	30	6
B			
B	5.4000	30	5

Bael

The GLM Procedure

Dependent Variable: y6 (Body)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	32	62.4000000	1.9500000	2.80	<.0001
Error	87	60.5916667	0.6964559		
Corrected Total	119	122.9916667			

R-Square	Coeff Var	Root MSE	y6 Mean
0.507351	13.88970	0.834539	6.008333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	44.15833333	14.71944444	21.13	<.0001
rep	29	18.24166667	0.62902299	0.90	0.6105

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	44.15833333	14.71944444	21.13	<.0001
rep	29	18.24166667	0.62902299	0.90	0.6105

Bael

The GLM Procedure

Duncan's Multiple Range Test for y6 (Body)

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error

Alpha	0.05
Error Degrees of Freedom	87
Error Mean Square	0.696456

Number of Means	**2**	**3**	**4**
Critical Range	.4283	.4507	.4655

Means with the same letter are not significantly different.

Duncan Grouping		**Mean**	**N**	**trt**
	A	7.0000	30	7
	B	5.9667	30	6

Means with the same letter are not significantly different.				
Duncan Grouping		Mean	N	trt
	B			
C	B	5.6667	30	8
C				
C		5.4000	30	5