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Development of Herbal Tea Product Based on *Crossandra infundibuliformis* and *Justicia betonica* Leaves for Functional Drink: Antioxidant Activity, Sensory Evaluation, and Nutritional Value

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ABSTRACT

Crossandra infundibuliformis and *Justicia betonica*, belonging to the Acanthaceae family, are well-known medicinal herbs in India, Sri Lanka, and Thailand. This study aims to determine antioxidant activity in extracts, including sensory evaluation of herbal tea products from both herbs. Leaf extracts were taken to determine total extractable phenolic content (TPC), total extractable flavonoid content (TFC), and antioxidant activity, including compound contents, using the high-performance liquid chromatography (HPLC) technique. The herbal tea products were prepared for sensory evaluation using the 9-point hedonic scale. The selected tea formula was studied for physicochemical properties and nutritional value. The results showed that *C. infundibuliformis* extract exhibited a higher activity value for TPC, TFC, and antioxidant activity than *J. betonica*. Moreover, the *C. infundibuliformis* and *J. betonica* leaf extracts contained 8 and 9 types of phenolic and flavonoid compounds, respectively. The T2 formula of herbal tea provided the highest sensory evaluation. It showed moisture and water activity contents of less than 7% and 0.6, while the nutritional value provided energy, protein, carbohydrates, sugar, vitamins A and B2, sodium, *β*-carotene, calcium, iron, and ash. Therefore, *C. infundibuliformis* and *J. betonica* can be produced as herbal tea for being a source of antioxidants.

Keywords: Antioxidants, *Crossandra infundibuliformis*, functional drink, herbal tea, *Justicia betonica*

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INTRODUCTION

The trend of consuming healthy food and drink products has recently become very popular worldwide, including in Thailand. According to Patathananone et al. (2023), consumers are increasingly aware of the importance of consuming healthy food and raw materials from vegetable groups and herbs, which serve as a source of numerous

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biological substances for producing healthy food products. Herbal tea ranks among the top three most popularly consumed beverages globally, with an increasing market for functional food products in many countries (Salazar-Campos et al., 2023). Herbal tea is a healthy beverage in the same form and method of consumption as tea. It is also convenient for making and drinking, providing many functional properties that benefit consumers (Phakamus et al., 2018). *Crossandra infundibuliformis* and *Justicia betonica*, native to tropical Asia and Africa, spread throughout India, Sri Lanka, and Thailand (Eapen et al., 2019; Hari et al., 2022). *Crossandra infundibuliformis* is a local plant in Thailand that belongs to the Acanthaceae family.

Researchers use this plant's fresh and dried leaves for their hepatoprotective, antioxidant, antibacterial, antifungal, and anticandidal properties (Hari et al., 2022). According to Naik et al. (2022), the *J. betonica* plant, a member of the Acanthaceae family, possesses analgesic, antimalarial, antioxidant, antimicrobial, and anti-inflammatory properties. In Thailand, these plants have been traditionally utilized in Thai folklore medicine for therapeutic purposes. They were often prepared as herbal extracts or boiled in water to create a drink believed to alleviate coughs, act as a tonic, and counteract toxins in the blood. (Kanchanapoom et al., 2004; Wong-arun et al., 2014). Thai folklore medicine has been useful in both healing fields in ancient times, but the application of these herbs in functional drinks remains unexplored. Furthermore, the combination of both herbs could possibly produce superior functional properties and be beneficial for consumers, particularly antioxidant properties.

Therefore, this study aims to develop herbal tea products as a source of antioxidant drinks from both herbs, focusing on the leaf part in the developing stage, which is ideal for the development of herbal tea. It will be achieved by determining antioxidant activity, including identifying phenolic and flavonoid compounds in both extract herbs and evaluating consumers' sensory experience. Hence, the prototype product can be used to create a nutritious and functional drink containing herbs that are appealing to the general consumer, benefiting entrepreneurs and consumers. Furthermore, the insights gained from this research can be used for commercial applications further.

MATERIALS AND METHODS

Chemicals

The determination chemicals for antioxidant activities and High-Performance Liquid Chromatography (HPLC) analysis were purchased from Sigma-Aldrich, Seelze, Germany. The other chemicals used as analytical grades are from Merck, Darmstadt, Germany; LAB-SCAN, Dublin, Ireland, and Fisher Scientific, Leicestershire, England.

Plant Materials and Preparation

The developing stage of all plant leaves was purchased directly from farmers: *C. infundibuliformis* and *J. betonica* from Chai Nat, Thailand, and *Pandanus amaryllifolius* from Nakhon Pathom, Thailand. Then, the leaves were rinsed using tap water, allowed to drain, and air-dried for 5-8 days until the moisture content reached 8-10% (w/w) following traditional methods (Ruangyuttikarn et al., 2013). Finally, they were stored in a sealed, dry container for future analysis.

Sample Extraction

C. infundibuliformis and *J. betonica* dried leaves were ground by blender into a fine powder and passed through a sieve with 60 meshes. Afterward, both leaf powders were soaked in hot water at a temperature of 98±1°C with a ratio of 1 per 10 (w/v) for a duration of 1 hr. The liquid should be strained using three layers of gauze and then passed through filter paper (Whatman No. 4). Both filtrated samples were dried in freeze-dried form and kept at 4°C (Ruangyuttikarn et al., 2013) for further study.

Determination of Total Extractable Phenolic Content (TPC), Total Extractable Flavonoid Content (TFC), and Antioxidant Activity

The determination of TPC for both extracts was done using a modified Folin-Ciocalteu by the method of Tan and Kassim (2011), and the standard curves were generated using gallic acid and stated as milligrams of gallic acid equivalent per gram of dried extract (mg GAE/g dried extract). The TFC was determined using the aluminum chloride colorimetric method (Imam et al., 2011) with some modifications, was determined using catechin as the standard and expressed as milligrams of catechin equivalent per gram of dried extract (mg CE/g dried extract).

The antioxidant activity consisting of DPPH, ABTS, and FRAP was determined. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was assessed in both extracts based on the DPPH assay (Raj et al., 2016). The determination of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid [ABTS]) radical scavenging using the ABTS assay (Pereira et al., 2014) with some modifications and the ferric ion-reducing antioxidant power (FRAP) was determined with some modifications of the method of Tan & Chan (2014). The activity was quantified by comparing the results with a calibration curve using gallic acid as a standard and expressing as milligrams of gallic acid equivalent per gram of dried extract (mg GAE/g dried extract).

Determination of Phenolic and Flavonoid Compounds Using High-performance Liquid Chromatography (HPLC) Technique

The quantitative phenolic and flavonoid compounds of *C. infundibuliformis* and *J. betonica* extracts were measured by HPLC. The determination of the compounds was completed using the HPLC system (Shimadzu, Kyoto, Japan) equipped with an Inersil C18 (250×4.6 mm, GL Sciences, Japan) column. The mobile phase solvent A is composed of 2% (v/v) acetic acid in water, while solvent B consists of 100% (v/v) acetonitrile. The column temperature was controlled at 30ºC, and solvent A was programmed, keeping a consistent flow rate of 1 ml/min while gradually increasing the proportion of solvent B from 5% to 90% over 75 min. (Liaudanskas et al., 2014). Gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, ferulic acid, quercetin, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, rutin, caffeine, syringic acid, vanillin, synaptic acid, myricetin, and *trans*-cinnamic acid were used as standard agents.

Preparation of Herbal Tea

Five formulas of the herbal tea prepared from *C. infundibuliformis*, *J. Betonica*, and *P. amaryllifolius* dried leaves at the following ratios: 4:1:0 (T1), 3:1:1 (T2), 2:2:1 (T3), 1:3:1 (T4), and 0:4:1 (T5). All samples were grounded and sifted through a 60-mesh sieve, then soaked in hot water at 85±2ºC for 4-5 min before being taken to the panelists.

Sensory Evaluation of Herbal Tea

The sensory acceptability of herbal tea products was assessed based on appearance, color, odor, flavor, astringent test, and overall liking. This study used regular consumers for the tested panelists. Each sample was coded with three-digit codes and served randomly (10 ml/sample) to the panelists, and the panelists were provided drinking water between sample tests. A 9-point hedonic scale was used to assess the sensory attributes of product samples (Meilgaard et al., 2007). The rating scale extended from 1 (strongly dislike) to 9 (strongly like) and was evaluated by 50 panelists. This research was considered for ethical approval in human research by the Human Research Ethics Committee, Rangsit University (COA. No. RSUERB2023-069).

Physicochemical Properties of the Selected Herbal Tea

The selected herbal tea product was analyzed for its physicochemical properties, including color values, water activity (a_w), and moisture content (%) (Association of Official Analytical Chemists [AOAC], 2019).

Nutritional Value of the Selected Herbal Tea

The selected herbal tea sample underwent analysis to determine its energy content, proximate compositions, and nutritional value. These included protein, fat, fiber, carbohydrates, ash, sugar, *β*-carotene, vitamins A, B1, and B2, and mineral contents of sodium, calcium, and iron. The analysis method used was outlined in the AOAC (2019).

Statistical Analysis

The data was collected to determine the variance analysis (ANOVA) to set up experimental research. The mean comparison was performed using Duncan's multiple-range tests and the *t*-test. The data was evaluated for statistical significance at a confidence level of 95% ($p<0.05$) using suitable statistical software.

RESULTS AND DISCUSSION

TPC, TFC, and Antioxidant Activity of the *C. infundibuliformis* and *J. betonica* Leaf Extracts

The TPC, TFC, and the activity of antioxidants determined using DPPH, ABTS, and FRAP assays in *C. infundibuliformis* and *J. betonica* leaf extracts are shown in Table 1. This experiment uses the developing leaves of both plants to develop herbal tea as a source of antioxidants. Generally, the stage of plant leaves for making herbal tea was reported to be highly antioxidant in the developing stage of leaves (Chan et al., 2013). The findings indicated that the TPC, TFC, and antioxidant activity of the *C. infundibuliformis* leaf extract was significantly higher than that of the *J. betonica* leaf extract. It may be due to the difference in type and composition, including the quantity of bioactive compounds in plant leaves that lead to antioxidant activity, especially phenolic and flavonoid compounds (Phakamus et al., 2018). In general, phenolic compounds contribute as primary antioxidants, whereas flavonoids serve as primary and secondary antioxidants (Lim et al., 2007). Patil et al. (2014) found that the methanol extract of *C. infundibuliformis* leaves contain high levels of phenolic (98.52 mg of gallic acid/g of extract) and flavonoid (84.59 mg of rutin/g of extract) while, the methanol extract of the entire *J. betonica* plant was observed to have an inhibitory concentration 50 (IC50) of free radical formation at 31.1%, which corresponds to 1671 µg/ml (Manokari et al., 2019). Moreover, the result showed that *C. infundibuliformis* leaf extract was found to have higher TPC and TFC (177.46±3.11 mg GAE/g dried extract and 254.81±1.78 mg CE/g dried extract) than *Thunbergia laurifolia* leaf extract, herbal tea in Thailand, which was reported with the TPC and TFC as 123.68±2.94 GAE/g dried extract and 62.83±2.85 mg CE/g dried extract (Junsi et al., 2017).

Table 1
TPC, TFC, and antioxidant activity of Crossandra infundibuliformis *and* Justicia betonica *leaf extracts*

Activities	**Leaf extracts**	
	Crossandra infundibuliformis	***Justicia betonica***
TPC (mg GAE/g dried extract)	177.46±3.11^{a}	115.88±4.19^{b}
TFC (mg CE/g dried extract)	254.81±1.78^{a}	48.54±1.62^{b}
DPPH (mg GAE/g dried extract)	7.50±0.02^{a}	4.45±0.16^{b}
ABTS (mg GAE/g dried extract)	23.40±0.91^{a}	12.42±0.89^{b}
FRAP (mg GAE/g dried extract)	45.32±0.86^{a}	20.17±0.22^{b}

Note. TPC = Total extractable phenolic content, TFC = Total extractable flavonoid content, GAE = Gallic acid equivalent, CE = Catechin equivalent, $^{a-b}$ mean within the given range is significantly different ($p<0.05$), values presented are in the form of mean ± standard deviation ($n = 3$)

Furthermore, Nor and Mohd (2013) reported that the TPC of the ethanol extraction from shoots of the green tea and black tea resulted in 80.27±0.61 and 76.93±1.72 mg GAE/g of dry sample weight, which showed the lower TPC than *C. infundibuliformis* leaf extract. However, compared to other research, it highlighted the challenge of reaching a solid conclusion about TPC, TFC, and antioxidant determination in individual plants when using different factors. Generally, variations in TPC and TFC contents, as well as antioxidant activity, can be influenced by several factors such as planting location, extract preparation and determination, and leaf development stage (Pyankov et al., 2001; Stein et al., 2016). The *C. infundibuliformis* and *J. betonica* leaf extracts demonstrated the highest antioxidant activities in the FRAP assay, followed by the ABTS and DPPH assays. The use of boiled water at 98±1°C in this study likely aided in solubilizing and extracting antioxidant compounds from the plant cell structure by disrupting their polarity. This made it easier to measure these compounds using the FRAP and ABTS assays, whereas the DPPH assay was better suited for detecting lipophilic compound groups (Xiao et al., 2017). Cheng et al. (2023) added that the use of hot water extraction ranging from 5 to 120 minutes on various teas, including green, Oolong, black, and scented teas, improved extraction efficiency with increasing water temperature. The study indicated that the highest levels of antioxidants were obtained, particularly at 100ºC. These circumstances can potentially degrade antioxidant compounds that are sensitive to heat (Sharma et al., 2015), and they could impact a greater number of compounds with strong heat tolerance in the sample, making them suitable for tea production. Hence, these results suggest that *C. infundibuliformis* and *J. betonica* leaves are suitable sources of antioxidants.

Phenolic and Flavonoid Contents of *C. infundibuliformis* and *J. betonica* Determined by HPLC

The study utilized HPLC to analyze the phenolic and flavonoid compounds in leaf extracts of *C. infundibuliformis* and *J. betonica*. The analysis involved using 18 standard compounds reported and found in tea and herbal plants (Chandrasekara & Shahidi, 2018; Shaik et al., 2023; Tungmunnithum et al., 2018). The quantification of compounds is based on their respective standard calibration curves (Table 2). The results found that the leaf extract of *C. infundibuliformis* contained eight phenolic and flavonoid compounds in descending order: chlorogenic acid, myricetin, protocatechuic acid, gallic acid, vanillin, caffeic acid, epicatechin, and *p*-coumaric acid. On the other hand, the leaf extract of *J. betonica* contained nine compounds: myricetin, sinapic acid, ferulic acid, gallic acid, protocatechuic acid, chlorogenic acid, *p*-coumaric acid, *trans*-cinnamic acid, and vanillic acid. The type and content of compounds containing phenolic and flavonoid substances influence various biological activities, particularly antioxidant activity.

The study found that the quantity of bioactive compounds in *C. infundibuliformis* leaf extract was generally higher, but the variety of compounds was less than in *J. betonica* leaf extracts. Furthermore, the findings of this research indicate that the leaf extract of

Table 2
Phenolic and flavonoid contents of Crossandra infundibuliformis *and* Justicia betonica *leaf extracts determined by HPLC*

No.	**Compounds**	**Contents (mg/g extract)**	
		Crossandra infundibuliformis	***Justicia betonica***
1	Gallic acid	0.70±0.01	0.39±0.01
2	Protocatechuic acid	2.19±0.04	0.35±0.02
3	*p*-Hydroxybenzoic acid	ND	ND
4	Catechin	ND	ND
5	Chlorogenic acid	37.41±0.04	0.31±0.04
6	Caffeine	ND	ND
7	Vanillic acid	ND	ND
8	Caffeic acid	0.43±0.05	ND
9	Syringic acid	ND	ND
10	Epicatechin	0.33±0.01	ND
11	Vanillin	0.63 ± 0.02	0.13±0.02
12	*p*-Coumaric acid	0.04±0.01	0.17±0.03
13	Ferulic acid	ND	0.91±0.03
14	Sinapic acid	ND	0.53±0.06
15	Rutin	ND	ND
16	Myricetin	9.46±0.14	2.85±0.01
17	Quercetin	ND	ND
18	*Trans*-cinnamic acid	ND	0.14±0.01

Note. ND = Not detected, Values presented are in the form of mean ± standard deviation (n = 3)

C. infundibuliformis contains chlorogenic acid and myricetin as its major components. According to Liang and Kitts (2016), chlorogenic acid has been identified as a potent antioxidant. Barzegar (2016) noted myricetin as one of the most significant flavonoids in food with the highest antioxidant activity. In addition, both extracts contain bioactive compounds that offer not only antioxidants but also other biological activities, such as gallic acid, caffeic acid, protocatechuic acid, and sinapic acid, reported to demonstrate properties that fight inflammation, cancer, tumors, mutations, and it also protects the nervous system and has antibacterial effects (Chen, 2016; Sato et al., 2011). However, both extracts reported some bioactive compounds as not detected (ND). The effect in phenolic and flavonoid profiles may be due to several factors, such as plant preparation and extraction, the limit of quantitation (LOQ) of equipment, and the form of the standard compound referenced in the experiment (Tungmunnithum et al., 2018).

Sensory Qualities of Herbal Tea

The sensory attributes of the herbal tea made from dried leaves of *C. infundibuliformis* and *J. betonica*, including appearance, color, odor, flavor, astringent test, and overall liking, were assessed and are shown in Figure 1. The *P. amaryllifolius* dried leaves (10%) were used in this experiment to improve the overall odor and flavor. It is well-known that a characteristic of this herb is used in Southeast Asian cuisines for food and beverage flavorings (Bhatt et al., 2021). Our preliminary tests revealed that added *P. amaryllifolius* mixed with *C. infundibuliformis* and *J. betonica* dried leaves in herbal tea formulas seemed to exhibit more consumer acceptability. The results of sensory acceptability from the consumer test showed a ranged score of each attribute between neither like nor dislike and moderate scales. The acceptability scores differed significantly ($p<0.05$) for all attributes except for the astringent test. Moreover, the scores indicated that the panelists preferred the herbal tea from formula T2 more than the other formulas. It aligns with the panelists' comments indicating that the T2 tea has a better flavor and odor. This phenomenon may be due to the ratio of leaves in herbal tea products, in which the T2 tea contains most *C. infundibuliformis* leaves (*C. infundibuliformis: J. betonica: P. amaryllifolius*, 3:1:1). In addition, the T2 tea can provide benefits for consumers who drink this herbal tea for health, which is the previous analysis result of antioxidant activity, which showed that the *C. infundibuliformis* leaf extract provides more antioxidant activity than *J. betonica* leaf extract. Therefore, the herbal tea product from the T2 formula was selected to assess the physical and chemical properties and nutritional values.

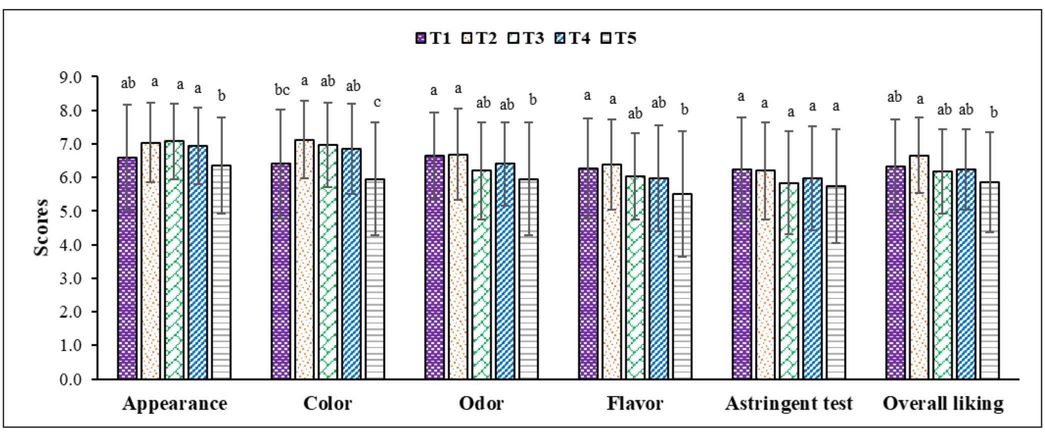

Figure 1. Sensory quality of the five formulas of herbal tea

Note. $^{a-b}$ mean within the given range is significantly different ($p<0.05$), values presented are in the form of mean ± standard deviation ($n = 50$)

Physicochemical Properties of the Selected Herbal Tea

The physicochemical properties of the selected herbal tea, *C. infundibuliformis,* and *J. betonica* leaf powders are shown in Table 3. The analysis of the color values revealed that the selected herbal tea powder did not display a significant difference ($p\geq0.05$) compared to *C. infundibuliformis* leaf powder. This result may be due to the ratio of leaf content

Table 3
Physicochemical properties of the leaf powder

Contents	**Leaf powder**		
	Selected tea	***Crossandra infundibuliformis***	***Justicia betonica***
Color values			
*L**	42.41±0.22[b]	42.72±0.49[b]	44.70±0.40[a]
*a**	-2.69±0.18[a]	-2.78±0.13[a]	-6.39±0.16[b]
*b**	24.13±0.45[b]	21.05±0.18[c]	29.73±0.49[a]
Water activity (a_w)	0.55±0.01[b]	0.55±0.00[b]	0.56±0.00[a]
Moisture content (%)	6.72±0.03[b]	6.46±0.03[c]	6.85±0.03[a]

Note. [a-b] mean within the given range is significantly different ($p<0.05$), values presented are in the form of mean±standard deviation ($n = 3$)

in the selected tea formula, which contained the highest content of *C. infundibuliformis* compared with other leaf powders. Moreover, the moisture content in all leaf samples was below 7%, which follows the criteria outlined in the notification of the Ministry of Public Health on tea from plants in Thailand (Thai-Food and Drug Administration, 2021) that specify the dried tea must have a moisture content less than 10% by weight. Furthermore, it was found that the water activity (a_w) content of all samples was less than 0.6. Thus, these conditional properties of the dried leaf powder, especially the selected tea product, could help to prevent the growth of some microorganisms that cause deterioration in the product.

Table 4
Nutritional values of the selected herbal tea

Nutrition information	**Quantities (per 100 g of powdered tea)**
Total energy (kcal)	334.07
Total energy from fats (kcal)	18.99
Total fat (g)	2.11
Saturated fat (g)	0.75
Cholesterol (mg)	ND
Protein (g)	19.60
Total Carbohydrate (g)	59.17
Dietary fiber (g)	41.58
Sugar (g)	1.22
Sodium (g)	0.08
Vitamin A (mg)	2.44
β-Carotene (mg)	14.62
Vitamin B1 (mg)	<0.03
Vitamin B2 (mg)	0.34
Calcium (mg)	1,541.01
Iron (mg)	10.03
Ash (g)	12.03

Note. ND = not detected

Nutritional Values of the Selected Herbal Tea

The nutritional values of the selected herbal tea are detailed in Table 4. Analyses revealed that the product provided 334.07 kcal of total energy per 100 g of powder and contained protein, carbohydrates, sodium, sugar, and ash at 19.60 g, 59.17 g, 85.31 mg, 1.22 g, and 12.03 g, respectively. The result showed that the product revealed various nutrients such as vitamin A, vitamin B2, β-carotene, calcium, and iron. The β-carotene is a substance that can be turned into vitamin A and acts as an antioxidant. It is used in the food industry to provide natural color (Liyanaarachchi et al., 2021). Vitamin A acts as an antioxidant and has been found to protect against Deoxyribonucleic acid (DNA) damage caused by reactive oxygen species (ROS), which is linked to the development of cancer (Fagbohun et al., 2023). Furthermore, Ca is the most prevalent stored nutrient in the human body and is crucial for living organisms because of its vital involvement in a variety of functions (Szlacheta et al., 2020). Fe has also been identified as a crucial trace element for nearly all living organisms, as it plays a crucial role in different metabolic activities, such as DNA creation, respiration, and photosynthesis (Rout & Sahoo, 2015). Moreover, herbal tea contains plant-based nutrients and is low in calories, sugar, and salt, making it suitable for a healthy diet. As a result, herbal tea can provide beneficial nutrients that are appropriate for customers.

CONCLUSION

The research showed that the leaf extract from *C. infundibuliformis* showed greater TPC, TFC, and antioxidant activity when compared to the leaf extract from *J. betonica*. In addition, *C. infundibuliformis* leaf extract found 10 phenolic and flavonoid compounds, ranked from high to low contents as follows: chlorogenic acid, myricetin, protocatechuic acid, gallic acid, vanillin, caffeic acid, rutin, epicatechin, syringic acid, and *p*-coumaric acid, while *J. betonica* leaf extract found 15 compounds, which are as follows: myricetin, sinapic acid, ferulic acid, gallic acid, rutin, *p*-hydroxybenzoic acid, protocatechuic acid, chlorogenic acid, epicatechin, p-coumaric acid, *trans*-cinnamic acid, vanillic acid, vanillin, catechin, and syringic acid, respectively. The T2 formula of herbal tea with mixing *C. infundibuliformis* and *J. betonica* dried leaves and *P. amaryllifolius* at 3:1:1 provided the highest sensory evaluation of the appearance, color, odor, flavor, and overall linking. The nutritional value of selected herbal tea (T2) is the most accepted formula, providing energy, protein, carbohydrates, sugar, vitamin A, B2, sodium, *β*-carotene, calcium, iron, and ash. At the same time, the moisture and water activity contents of the product showed less than 7% and 0.6, respectively. This experiment supported using both herbs as herbal teas for functional drinks. However, the study of the potential single or combined leaf activity that affects other functional properties such as anti-inflammatory, antimicrobial, anti-diabetic, and anti-dote should be further investigated both *in vitro* and *in vivo*.

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REFERENCES

Association of Official Analytical Chemists (AOAC). (2019). *Official methods of analysis.* (21st ed). Washington DC.

Barzegar, A. (2016). Antioxidant activity of polyphenolic myricetin in vitro cell-free and cell-based systems. *Molecular Biology Research Communications*, *5*(2), 87-95.

Bhatt, V., Barvkar, V. T., Furtado, A., Henry, R. J., & Nadaf, A. (2021). Fragrance in *Pandanus amaryllifolius* Roxb. despite the presence of a betaine aldehyde dehydrogenase 2. *International Journal of Molecular Sciences*, *22*(13), 6968. https://doi.org/10.3390/ijms22136968

Chan, E. W. C., Lye, P. Y., Eng, S. Y., & Tan, Y. P. (2013). Antioxidant properties of herbs with enhancement effects of drying treatments: A synopsis. *Free Radicals and Antioxidants*, *3*(1), 2-6. http://doi.org/10.1016/j.fra.2013.02.001

Chandrasekara, A., & Shahidi, F. (2018). Herbal beverages: Bioactive compounds and their role in disease risk reduction - A review. *Journal of Traditional and Complementary Medicine*, *8*(4), 451-458. https://doi.org/10.1016/j.jtcme.2017.08.006

Chen, C. (2016). Sinapic acid and its derivatives as medicine in oxidative stress-induced diseases and aging. *Oxidative Medicine and Cellular Longevity*, *2016*(1), 3571614. http://doi.org/10.1155/2016/3571614

Cheng, Y., Xue, F., & Yang, Y. (2023). Hot water extraction of antioxidants from tea leaves-optimization of brewing conditions for preparing antioxidant-rich tea drinks. *Molecules*, *28*(7), 3030. https://doi.org/10.3390/molecules28073030

Eapen, J., Ansary, P. Y., Hameed, A. S., Indulekha, V. C., & Resny, A. R. (2019). Phamacognosy of Leaf, Stem and Root of *Justicia betonica* Linn. *International Journal of Research in Ayurveda and Pharmacy*, *10*(1), 44-48. https://doi.org/10.7897/2277-4343.100111

Fagbohun, O. F., Gillies, C. R., Murphy, K. P. J., & Rupasinghe, H. P. V. (2023). Role of antioxidant vitamins and other micronutrients on regulations of specific genes and signaling pathways in the prevention and treatment of cancer. *International Journal of Molecular Sciences*, *24*(7), 6092. https://doi.org/10.3390/ijms24076092

Hari, V. P, Abdul, A. A, Vanitha, M., Ganesan, S., & Gokulnath, K. (2022). Pharmacognostical studies and pharmacological activities of *Crossandra infundibuliformis*. *International Journal of Research and Development in Pharmacy & Life Sciences*, *8*(5), 140. http://doi.org/10.4172/2278-0238.1000140

Imam, M. Z., Akter, S., Mazumder, M. E. H., & Rana, M. S. (2011). Antioxidant activities of different parts of *Musa sapientum* L. ssp. *Sylvestris* fruit. *Journal of Applied Pharmaceutical Science*, *1*(10), 68-72.

Junsi, M., Siripongvutikorn, S., Yupanqui, C. T., & Usawakesmanee, W. (2017). Efficacy of *Thunbergia laurifolia* (rang jued) aqueous leaf extract for specific biological activities using RAW 264.7 macrophage cells as test model. *International Food Research Journal*, *24*(6), 2317-2329.

Kanchanapoom, T., Noiarsa, P., Ruchirawat, S., Kasai, R., & Otsuka, H. (2004). Triterpenoidal glycosides from *Justicia betonica*. *Phytochemistry*, *65*(18), 2613-2618. https://doi.org/10.1016/j.phytochem.2004.06.029

Liang, N., & Kitts, D. D. (2016). Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. *Nutrients*, *8*, 16. http://doi.org/10.3390/nu8010016

Liaudanskas, M., Viškelis, P., Jakštas, V., Raudonis, R., Kviklys, D., Milašius, A., & Janulis, V. (2014). Application of an optimized HPLC method for the detection of various phenolic compounds in apples from Lithuanian cultivars. *Journal of Chemistry*, *2014*(1), 542121. https://doi.org/10.1155/2014/542121

Lim, Y. Y., Lim, T. T., & Tee, J. J. (2007). Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry*, *103*(3), 1003-1008. https://doi.org/10.1016/j.foodchem.2006.08.038

Liyanaarachchi, V. C., Premaratne, M., Ariyadasa, T. U., Nimarshana, P. H. V., & Malik, A. (2021). Two-stage cultivation of microalgae for production of high-value compounds and biofuels: A review. *Algal Research*, *57*, 102353. https://doi.org/10.1016/j.algal.2021.102353

Manokari, M., Latha, R., Priyadharshini, S., Cokul, R. M., Beniwal, P., Manjunatha, R. Y., Rajput, B. S., & Shekhawat, M. S. (2019). A comprehensive review on a less explored medicinally important plant *Justicia betonica* L. *World Scientific News*, *131*, 110-122.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007). *Sensory evaluation techniques* (4^{th} ed.). CRC Press.

Naik, S. K., Manjula, B. L., Balaji, M. V., Marndi, S., Kumar, S., & Devi, R. S. (2022). Antibacterial activity of *Justicia betonica* Linn. *Asian Pacific Journal of Health Sciences*, *9*(4), 227-230. https://doi.org/10.21276/apjhs.2022.9.4.45

Nor, Q. I. M. N., & Mohd, F. A. B. (2013). Phytochemicals and antioxidant properties of different parts of *Camellia sinensis* leaves from sabah tea plantation in Sabah, Malaysia. *International Food Research Journal*, *20*(1), 307-312.

Patathananone, S., Pothiwan, M., Uapipatanakul, B., & Kunu, W. (2023). Inhibitory effects of *Vernonia amygdalina* leaf extracts on free radical scavenging, tyrosinase, and amylase activities. *Preventive Nutrition and Food Science*, *28*(3), 302-311. https://doi.org/10.3746/pnf.2023.28.3.302

Patil, K., G., Jaishree, V., & Tejaswi, H. P. (2014). Evaluation of phenolic content and antioxidant property of *Crossandra infundibuliformis* leaves extracts. *American Journal of Plant Sciences*, *5*(9), 1133-1138. http://doi.org/10.4236/ajps.2014.59126

Pereira, A. C. S., Dionísio, A. P., Wurlitzer, N. J., Alves, R. E., Brito, E. S., Silva, A. M. O., Brasil, I. M., & Filho, J. M. (2014). Effect of antioxidant potential of tropical fruit juices on antioxidant enzyme profiles and lipid peroxidation in rats. *Food Chemistry*, *157*(15), 179-185. http://doi.org/10.1016/j.foodchem.2014.01.09012

Phakamus, S., Chokrathok, S., Woraratphoka, J., & Innok, S. (2018). Development of Yanang mixed herb tea products. *Burapha Science Journal*, *23*(3), 1682-1695.

Pyankov, V. I., Ivanov, L. A., & Lambers, H. (2001). Chemical composition of the leaves of plants with different ecological strategies from the boreal zone. *Russian Journal of Ecology*, *32*(4), 221-229. https://doi.org/10.1023/A:1011354019319

Raj, V. K., Kumar, J. R., Balasubramanian, S., Swamy, S. N., Keerthini, D., Shambhavi, N., Kanthesh, B. M., & Avinash, K. O. (2016). Comparative evaluation of hepatoprotective effects of exotic fruits and common vegetables extracts on CCl_4 induced hepatotoxicity: An *in vitro* study. *International Journal of Pharmaceutical Sciences and Research*, *7*(8), 3388-3393. http://doi.org/10.13040/IJPSR.0975-8232.7(8).3388-93

Rout, G. R., & Sahoo, S. (2015). Role of iron in plant growth and metabolism. *Reviews in Agricultural Science*, *3*, 1-24. https://doi.org/10.7831/ras.3.1

Ruangyuttikarn, W., Chattaviriya, P., Morkmek, N., Chuncharunee, S., & Lertprasertsuke, N. (2013). *Thunbergia laurifolia* leaf extract mitigates cadmium toxicity in rats. *Science Asia*, *39*(1), 19-25. https://doi.org/10.2306/scienceasia1513-1874.2013.39.019

Salazar-Campos, J., Salazar-Campos, O., Gálvez-Ruiz, O., Gavidia-Chávez, H., Gavidia-Chávez, M., Irigoin-Guevara, M., & Obregón-Domínguez, J. (2023). Functional properties and acceptability of potentially medicinal tea infusions based on *Equisetum arvense*, *Desmodium molliculum*, and *Mentha piperita*. *Preventive Nutrition and Food Science*, *28*(4), 444-452. https://doi.org/10.3746/pnf.2023.28.4.444

Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., Sugawara, M., & Iseki, K. (2011). *In vitro* and *in vivo* antioxidant properties of chlorogenic acid and caffeic acid. *International Journal of Pharmaceutics*, *403*(1-2), 136-138. https://doi.org/10.1016/j.ijpharm.2010.09.035

Shaik, M. I., Hamdi, I. H., & Sarbon, N. M. (2023). A comprehensive review on traditional herbal drinks: Physicochemical, phytochemicals and pharmacology properties. *Food Chemistry Advances*, *3*, 100460. https://doi.org/10.1016/j.focha.2023.100460

Sharma, K., Ko, E. Y., Assefa, A. D., Ha, S., Nile, S. H., Lee, E. T., & Park, S. W. (2015). Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *Journal of Food and Drug Analysis*, *23*(2), 243-252. https://doi.org/10.1016/j.jfda.2014.10.005

Stein, R. J., Horeth, S., Melo, J. R. F., Syllwasschy, L., Lee, G., Garbin, M. L., Clemens, S., & Krämer, U. (2017). Relationships between soil and leaf mineral composition are element-specific, environment-dependent and geographically structured in the emerging model *Arabidopsis halleri*. *New Phytologist*, *213*(3), 1274-1286. https://doi.org/10.1111/nph.14219

Szlacheta, Z., Wąsik, M., Machoń-Grecka, A., Kasperczyk, A., Dobrakowski, M.., Bellanti, F., Szlacheta, P., & Kasperczyk, S. (2020). Potential antioxidant activity of calcium and selected oxidative stress markers in lead- and cadmium-exposed workers. *Oxidative Medicine and Cellular Longevity*, *2020*, 8035631. https://doi.org/10.1155/2020/8035631

Tan, K. W., & Kassim, M. J. (2011). A correlation study on the phenolic profiles and corrosion inhibition properties of mangrove tannins (*Rhizophora apiculata*) as affected by extraction solvents. *Corrosion Science*, *53*(2), 569-574. http://doi.org/10.1016/j.corsci.2010.09.065

Tan, Y. P., & Chan, E. W. C. (2014). Antioxidant, antityrosinase and antibacterial properties of fresh and processed leaves of *Anacardium occidentale* and *Piper betle*. *Food Bioscience*, *6*, 17-23. http://doi.org/10.1016/j.fbio.2014.03.001

Thai-Food and Drug Administration. (2021). *Announcement of the Ministry of Public Health: Tea from plants*. https://food.fda.moph.go.th/media.php?id=509438370968117248&name=P426.PDF

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, *5*(3), 93. https://doi.org/10.3390/medicines5030093

Wong-arun, W., Jongkolnee, W., Mengkwang, R., Kwangswat, A., Sukaphat, S., & Duangmala, E. (2014). *The diversity of medicinal plants in Sam Roi Yod FreshWater Marsh*. Rajamangala University of Technology Rattanakosin.

Xiao, H. W., Pan, Z., Deng, L. Z., El-Mashad, H. M., Yang, X. H. Mujumdar, A. S., Gao, Z. J., & Zhang, Q. (2017). Recent developments and trends in thermal blanching: A comprehensive review. *Information Processing in Agriculture*, *4*(2), 101-127. http://doi.org/10.1016/j.inpa.2017.02.001