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# The Use of Mangosteen Pericarp (*Garcinia mangostana* L.) Extract to Fortify the Green Tea Drink Enchanced Antioxidant Activity.

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## Abstract

Mangosteen pericarp and tea contain high polyphenol content that has disease preventive properties. This study aimed to utilize mangosteen pericarp for producing added-value product, to evaluate polyphenols stability in green tea drink with mangosteen pericarp extract, and to evaluate the effect of citric acid to the polyphenols stability in green tea drink with mangosteen pericarp extract. Mangosteen pericarp was hot air dried at 50ºC until the moisture content reached 7.43±0.10%. Dried mangosteen pericarp was soaked in distilled water, then the total polyphenol content, DPPH, and FRAP of the extracted solution were analyzed. The extract was added into green tea drink at seven different concentrations (0.1-0.7%). The optimum concentration into green tea was evaluated by sensory test, and then it was mixed with gradual concentrations of citric acid (0.06, 0.1, and 0.2%). The results showed that the total phenolic compound, DPPH and FRAP of the extract were 127.39±1.19 mg GAE/ml sample, 44.92±0.68 mmol TE/ml sample, and 21.49±0.13 mM asorbic acid/ml sample, respectively. The optimum concentration evaluated by sensory test was 0.4%. The mixture of green tea drink with 0.4% mangsteen pericarp extract and 0.2% citric acid showed the lowest pH (2.79), the highest total phenolic compound (25.48±0.38 mg GAE/ml sample), DPPH (57.86±1.25 mmol TE/100 ml sample), and FRAP (5.68±0.33 mM asorbic acid/ml sample) compared with the other treatments ($p<0.05$). Therefore, mangosteen pericarp extract can be applied in green tea drink and provided more polyphenol content for the product. In addition, citric acid enhanced the stability of polyphenol in green tea drink.

**Keywords:** *Antioxidant activity, Citric acid, Green tea, Mangosteen pericarp, Polyphenol*.

## 1. Introduction

*Garcinia mangostana* L. or mangosteen is a tropical fruit from the family Guttiferae (1). Thailand and Indonesia are one of the high mangosteen production countries, where the production is approximately three and two million tons, respectively (2).

Mangosteen consists of endocarp and pericarp. Mangosteen pericarp is the largest part of the fruit, which comprises 66.67% of the total fruit (3). The pericarp is usually discarded as waste due to its unpleasant taste of bitterness, however, it can be a good source of antioxidant according to its rich polyphenol content, such as xanthone (4). The mangosteen pericarp also contains tannin that may interfere protein absorption in digestive system when it is consumed in high content (5). Thus, the consumption of mangosteen pericarp has increased as dietary supplement due to their potential pharmacological properties (4). The extraction of mangosteen pericarp can also contain high polyphenol content, therefore it can easily be mixed into food products and/or beverage like tea.

Tea is one of the most popular drinks in the world. More than three billion cups of tea are consumed per day (6). Recently, popularity of tea has increased due to its potential health benefits against cardiovascular diseases and cancer from its antioxidant activities (7). Mangosteen pericarp extract and green tea drink are matched due to the acidity properties. The phytochemical screening performed on the mangosteen pericarp extract showed that the extract is acidic with pH 5 (8). In tea drink, polyphenols are stable when pH is below 4 and they are unstable in solutions with pH above 6 (9). Commercially, the pH of tea drinks is usually adjusted to pH 3.8-4.0. Addition of mangosteen pericarp extract may add bitter taste to the green tea drink and yet may increase functional properties like antioxidant activity because of its high polyphenol content. Additionally, the tea condition may affect the stability of the polyphenol in both mangosteen pericarp extract and green tea.

The objectives of this research were to utilize mangosteen pericarp for producing added-value product, to evaluate polyphenols stability in green tea drink with mangosteen pericarp extract, and to evaluate the effect of citric acid to the polyphenols stability in green tea drink with mangosteen pericarp extract.

## 2. Material and methods

Mangosteens and dried green teas were purchased from Baan Duu Market, Chiang Rai, Thailand. Sucrose and fructose syrup (Mitr Phol®, Thailand) were also purchased from a local market.

Folin-Ciocalteu's phenol reagent and trichloroacetic acid from LobaChemi®, India; gallic acid, 2,2-Diphenyl-1-picrylhydrazyl, and Trolox-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid and potassium hexacyano-ferrate (III), from Sigma Aldrich®, Canada; sodium carbonate, ascorbic acid and sodium dihydrogen phosphate from Ajax Finechem®, New Zealand; iron (III) chloride hexahydrate and disodium hydrogen phosphate from Fisher®, UK were used as chemical regents.

### 2.1 Preparation of Extract from Mangosteen Pericarp

Extraction methods were modified from Cheok et al. (10) and Zarena and Sangkar (11). Mangosteen was washed and separated between fruit and pericarp, and then weighed. Mangosteen pericarp was hot air dried at 50°C until the moisture content

reached 7%, then ground into powder using a hammer mill (Baan Pramong Co. Ltd, Thailand).

Mangosteen pericarp powder (20 g) was accurately weighed and placed in a 250 ml Erlenmeyer flask, then distilled water (100 ml) was added. The mixture was stirred for 20 hours on a shaking water bath at 25°C, then centrifuged at 5,000 rpm for 10 min and filtered using Whatman filter paper No. 1. The filtrate was concentrated using a rotary evaporator controlled at 40°C for 1 hour at 120 rpm, and then the volume of each extract was collected. The extracts were stored in refrigerator 4°C until they were analyzed for total polyphenols content, DPPH, and FRAP.

**2.2 Tea Drink Fortified with Mangosteen Pericarp Extract**

Dried green tea leaves (2 g) were brewed with 150 ml boiled water (95°C) for 2 min and filtered. Green tea infusion was mixed with 8% fructose and 3% sucrose (w/v). Varying concentrations of extract (0-0.7%) were then added to the green tea drink as shown in Table 1. The optimum concentration of mangosteen pericarp extract applied in green tea drink was obtained from sensory test.

**Table 1.** Formulation of tea drink fortified with mangosteen pericarp extract.

| Ingredients | Control | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
|---|---|---|---|---|---|---|---|---|
| | Concentration (%) | | | | | | | |
| Tea infusion | 88.7 | 88.7 | 88.7 | 88.7 | 88.7 | 88.7 | 88.7 | 88.7 |
| Fructose | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Sucrose | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Extract | 0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 |

Next, gradual concentrations of citric acid (0.06, 0.1, and 0.2% w/v) were added to green tea drink (Table 2). Mangosteen pericarp extract was then added and the mixture stirred until homogenized. Tea drink was then pasteurized at 70°C for 2 min and hot-filled in pasteurized bottles and cooled to 40°C (12).

**Table 2.** Formulation of tea drink fortified with mangosteen pericarp extract and citric acid.

| Ingredients | Control | T4 | TC1 | TC2 | TC3 |
|---|---|---|---|---|---|
| | Concentration (%) | | | | |
| Tea infusion | 88.7 | 88.7 | 88.7 | 88.7 | 88.7 |
| Fructose | 8 | 8 | 8 | 8 | 8 |
| Sucrose | 3 | 3 | 3 | 3 | 3 |
| Extract | 0 | 0.4 | 0.4 | 0.4 | 0.4 |
| Citric acid | 0 | 0 | 0.06 | 0.1 | 0.2 |

**2.3 pH measurement**

pH of green tea drinks with mangosteen pericarp extract were measured according to AOAC using a pH meter (13).

**2.4 Moisture content**

Moisture content of the dried pericarp powder was determined using an oven dryer for overnight or to constant the weight (14).

**2.5 Total polyphenol content**

Total polyphenol content was assayed according ISO 14502-1 using gallic acid as a standard (15). Mangosteen pericarp extract was diluted 1:200 and tea drink was diluted with ratio 1:100, both with distilled water. Diluted mangosteen pericarp extract and green tea drinks (1.0 ml) were put into a tube. Then, 5.0 ml of 10% v/v Folin-Ciocalteu reagent and 4.0 ml of 7.5% w/v sodium carbonate solution was added with mixing. The mixture was left for 1 hour at room temperature and the absorbance measured at 765 nm using water as blank. The total polyphenol content was reported as gallic acid equivalents (GAE) in mg/ml sample extract.

**2.6 DPPH Radical Scavenging Activity**

DPPH radical scavenging activity was assayed by Molyneux with few of modification using trolox as standard (16). Mangosteen pericarp extract was diluted 1:200 and tea drink was diluted with ratio 1:100, both with distilled water. Diluted mangosteen pericarp extract and green tea drinks (50 µl) were mixed with 1.95 ml 60 µM DPPH solution and left in the dark place for 30 min at room temperature, and absorbance measured at 517 nm using methanol as blank and Trolox as standard. The radical scavenging activity was expressed as % inhibition.

**2.7 Ferric Reducing Antioxidant Power (FRAP)**

Ferric reducing antioxidant power was assayed by Benzie and Strain with few of modification using ascorbic acid as standard (17). Mangosteen pericarp extract was diluted 1:200 and tea drink was diluted with ratio 1:100, both with distilled water. Diluted mangosteen pericarp extract and green tea drinks (1.0 ml) were mixed with 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml 1% potassium hexacyanoferrate (III). The mixture was incubated at 50ºC for 30 min, then 2.5 ml of 10% trichloroacetic acid was added and the mixtures centrifuged for 10 min. Supernatant (2.5 ml) was mixed with 2.5 ml of water and 0.5 ml of 1% ferric chloride. Then, absorbance was measured at 700 nm.

**2.8 Sensory Evaluation**

Sensory qualities of green tea drink with mangosteen pericarp extract were evaluated by 30 panelists. Then, 9-point hedonic scale was used for assessing color, aroma, tea flavor, sweetness, sourness, bitterness, taste, and overall likely (17).

**2.9 Statistical Analysis**

All data was subjected to analysis of variance to identify differences among means by Duncan's multiple range test using SPSS program (version 16.0) at $p<0.05$.

## 3. Results and discussion

**3.1 Chemical Properties of Mangosteen Pericarp Extract**

Mangosteen pericarp was hot air dried at 50ºC. Previously, temperature 75ºC was an appropriate drying temperature to preserve polyphenol in mangosteen pericarp (8). However, drying at temperature lower than 75ºC could prevent the damage to

polyphenols, which are more stable in low temperature. Mangosteen pericarp was dried for 53 hours, then the moisture content of the dried mangosteen pericarp powder was reached 7.43±0.10%.

Table 3 shows the amount of total polyphenol content, DPPH and FRAP values of mangosteen pericarp extract. Total polyphenol content of mangosteen pericarp extract was 127.3±0.19 mg GAE/ml sample. DPPH value of mangosteen pericarp extract was 44.92±0.68 mmol TE/100 ml sample. Furthermore, FRAP value of mangosteen pericarp extract was 21.49±0.13 mM asorbic acid/ml sample.

**Table 3.** Chemical properties of mangosteen pericarp extract.

| Chemical properties | Concentration |
|---|---|
| Moisture content (%) | 7.43±0.10 |
| Total polyphenol content (mg GAE/ml sample) | 127.3± 0.19 |
| DPPH antioxidant activity (mmol Trolox/100 ml sample) | 44.92±0.68 |
| FRAP antioxidant activity (mM asorbic acid/ml sample) | 21.49±0.13 |

Results were expressed as mean±SD, n=3.

### 3.2 Chemical and Sensory Properties of Green Tea Drink Fortified with Mangosteen Pericarp Extract

Polyphenols are the main compound in mangosteen pericarp extract and tea. Table 4 shows the total phenolic content of tea drink fortified with mangosteen pericarp extract. The highest phenolics was found at the tea drink with 0.7% (w/v) mangosteen pericarp extract, which was significantly different from the others ($p<0.05$). Evidently, total phenolic content in tea drinks increased with an increased mangosteen pericarp extract.

Polyphenols were one of the main antioxidants (18). The more mangosteen pericarp extract added in the tea drinks, the more polyphenols were found. As a result, the antioxidant activity expressed with DPPH and FRAP increased (Table 4).

**Table 4.** Chemical content of green tea drink fortified with mangosteen pericarp extract.

| Sample | TPC (mg GAE/ml sample) | DPPH (mmol Trolox/ml sample) | FRAP (mM asorbic acid/ml sample) |
|---|---|---|---|
| control | 25.24±0.80$^{h}$ | 8.61±0.36$^{g}$ | 51.88±1.03$^{h}$ |
| T1 | 27.49±0.24$^{g}$ | 11.29±0.39$^{f}$ | 55.95±0.21$^{g}$ |
| T2 | 32.19±0.80$^{f}$ | 13.01±0.21$^{e}$ | 58.00±0.29$^{f}$ |
| T3 | 34.90±0.39$^{e}$ | 13.18±0.54$^{e}$ | 59.84±0.29$^{e}$ |
| T4 | 37.00±0.23$^{d}$ | 19.73±0.14$^{d}$ | 67.88±0.71$^{d}$ |
| T5 | 38.96±0.21$^{c}$ | 20.81±0.25$^{c}$ | 70.75±0.48$^{c}$ |
| T6 | 44.43±0.32$^{b}$ | 23.19±0.44$^{b}$ | 73.88±0.15$^{b}$ |
| T7 | 52.03±2.15$^{a}$ | 25.76±0.55$^{a}$ | 85.24±0.75$^{a}$ |

Results were expressed as mean±SD, n=3.
a-h in superscript in column was significantly different

Table 5 shows the sensory evaluation of green tea drink fortified with varying concentrations of mangosteen pericarp extract (0-0.7%). Not only was the color likely accepted (6.27-6.67), but also the sweetness (6.27-6.57). However, the concentration of mangosteen pericarp extract higher than 0.4% inversed the sensory test for aroma, bitterness, taste, and overall likely. Therefore, the mangosteen pericarp extract at 0.4% was selected as an acceptable level to the panelists.

**Table 5.** Sensory analysis of green tea drink fortified with mangosteen pericarp extract.

| Sample | Color | Aroma | Tea flavor | Sweetness | Bitterness | Taste | Overall |
|---|---|---|---|---|---|---|---|
| Control | 6.67±0.76$^{a}$ | 6.63±0.76$^{a}$ | 6.40±0.97$^{a}$ | 6.43±0.97$^{a}$ | 6.07±0.99$^{a}$ | 6.63±0.85$^{a}$ | 6.60±0.77$^{a}$ |
| T1 | 6.57±1.14$^{a}$ | 6.30±0.92$^{ab}$ | 6.60±1.13$^{b}$ | 6.53±1.14$^{a}$ | 6.33±0.84$^{a}$ | 6.23±1.01$^{ab}$ | 6.30±1.02$^{ab}$ |
| T2 | 6.33±0.92$^{c}$ | 6.10±0.71$^{c}$ | 6.53±1.07$^{b}$ | 6.47±0.97$^{b}$ | 6.27±0.83$^{a}$ | 6.07±0.78$^{c}$ | 6.03±0.72$^{b}$ |
| T3 | 6.47±0.97$^{b}$ | 6.27±1.01b$^{c}$ | 6.33±0.80$^{bc}$ | 6.40±0.97$^{b}$ | 6.27±1.05$^{a}$ | 6.27±1.05$^{c}$ | 6.23±0.97$^{bc}$ |
| T4 | 6.60±1.00$^{a}$ | 6.07±0.64$^{d}$ | 6.33±0.96$^{bc}$ | 6.27±1.08$^{c}$ | 6.07±0.74$^{a}$ | 6.10±0.71$^{d}$ | 6.03±0.67$^{b}$ |
| T5 | 6.47±0.97$^{b}$ | 5.47±0.82$^{d}$ | 6.03±0.81$^{c}$ | 6.27±1.01$^{c}$ | 5.50±0.86$^{b}$ | 5.33±0.76$^{d}$ | 5.37±0.76$^{cd}$ |
| T6 | 6.27±1.08$^{d}$ | 5.47±0.73$^{d}$ | 5.97±1.07$^{c}$ | 6.30±0.79$^{bc}$ | 5.37±0.76$^{bc}$ | 5.47±0.73$^{d}$ | 5.43±0.77$^{c}$ |
| T7 | 6.30±1.15$^{cd}$ | 4.93±0.83$^{e}$ | 6.10±0.96$^{c}$ | 6.57±0.77$^{a}$ | 5.03±0.81$^{c}$ | 5.07±0.78$^{e}$ | 4.97±0.81$^{d}$ |

### 3.3 Chemical and Sensory Properties of Green Tea Drink Fortified with Mangosteen Pericarp Extract and Citric Acid

Gradual concentrations of citric acid significantly affected the pH of tea drink ($p<0.05$) (Table 6). Adding mangosteen pericarp extract decreased the pH of tea drink. The phytochemical screening performed on the mangosteen pericarp extract showed the extract was acidic with pH 5 (8). This means the pH of tea was decreased with the addition of pericarp extract. Citric acid a food grade acidulant

can also lower the pH of solution (19). Thus, the mixture of higher concentration of citric acid and pericarp extract decreased pH of tea.

In the green tea making process, citric acid was added before addition of mangosteen pericarp extract. Acidifying such mixtures increases polyphenol's stability by preventing the oxidation reaction of polyphenol in the solution (20). Total polyphenol content was significantly increased with increasing citric acid concentration ($p<0.05$). The green tea drink fortified with 0.4% mangosteen pericarp extract and 0.2% citric acid showed the highest total polyphenol content (Table 6).

Total polyphenol content increased with added higher citric acid, resulting in an decreased pH. The lower pH did not favor polyphenol oxidase, resulted in stabilizing polyphenol in acidic condition (8). As reported previously, the low pH could maintain the principal polyphenol catechin in the tea (21).

Table 6 shows that citric acid significantly affected antioxidant capacity in tea drink measured by DPPH and FRAP ($p<0.05$). The antioxidant capacity increased with increasing of citric acid. Tea drink fortified with 0.4% mangosteen pericarp extract and 0.2% of citric acid gave the highest antioxidant capacity. As the highest total polyphenols, the principal antioxidants larger existed in the tea (22), thus the antioxidant capacity should be largest in the employed conditions.

**Table 6.** Chemical content of green tea drink fortified with mangosteen pericarp extract and citric acid.

| Treatment | pH | TPC (mg GAE/ml sample) | DPPH (mmol Trolox/100 ml sample) | FRAP (mM asorbic acid/ml sample) |
|---|---|---|---|---|
| control | 5.42±0.01$^{a}$ | 13.66±0.27$^{e}$ | 1.61±0.38$^{e}$ | 41.37±0.22$^{e}$ |
| T4 | 4.95±0.02$^{b}$ | 17.19±0.08$^{d}$ | 14.49±0.45$^{d}$ | 45.62±0.19$^{d}$ |
| TC1 | 3.58±0.01$^{c}$ | 20.02±0.18$^{c}$ | 42.47±0.92$^{c}$ | 48.40±0.39$^{c}$ |
| TC2 | 3.12±0.01$^{d}$ | 22.51±0.12$^{b}$ | 46.44±2.21$^{b}$ | 50.04±0.36$^{b}$ |
| TC3 | 2.97±0.01$^{e}$ | 25.48±0.38$^{a}$ | 57.86±1.25$^{a}$ | 56.81±0.33$^{a}$ |

Results were expressed as mean±SD, n=3.
a-e in superscript in column was significantly different

Table 7 shows the result of sensory evaluation for the green tea drink fortified with 0.4% mangosteen pericarp extract and citric acid (0.06, 0.1, and 0.2% w/v). All sensory qualities were assessed as likely (6-7 scores from 9-scale), except for sourness. All scores for citric acid adding sample were slightly smaller than the score of control, but no statistically significant differences were found. Citric acid mostly affected to the sourness of green tea drink (21), therefore, the green tea drinks fortified here with mangosteen pericarp extract and citric acid should be likely acceptable.

**Table 7.** Sensory analysis of green tea drink fortified with mangosteen pericap extract and citric acid.

| Sample | Color | Aroma | Tea flavor | Sweetness | Sourness | Bitterness | Taste | Overall |
|---|---|---|---|---|---|---|---|---|
| Control | 7.17±1.29 | 6.30±1.34 | 7.07±1.11 | 6.40±2.01 | 5.90±1.56 | 6.50±1.48 | 6.87±1.38 | 7.00±1.23 |
| T4 | 6.90±1.24 | 5.97±1.47 | 7.13±1.51 | 6.47±1.59 | 6.00±1.26 | 6.40±1.04 | 6.87±1.20 | 6.90±1.18 |
| TC1 | 6.60±1.48 | 6.07±1.48 | 6.73±1.51 | 6.20±1.83 | 6.70±1.70 | 6.03±1.85 | 6.67±1.94 | 6.93±1.57 |
| TC2 | 6.65±1.31 | 6.23±1.22 | 6.67±1.37 | 6.10±1.49 | 6.53±1.70 | 6.43±1.52 | 6.70±1.53 | 6.90±1.42 |
| TC3 | 6.30±1.54 | 6.03±1.54 | 6.70±1.51 | 6.30±1.56 | 6.53±1.80 | 6.47±1.28 | 6.87±1.31 | 6.97±1.50 |

Results were expressed as mean±SD, n=3.
In no case were the results in a column significantly different from each other.

## 4. Conclusion

Mangosteen pericarp extract showed high functional properties, such as polyphenol content and antioxidant capacity. The addition of higher mangosteen pericarp extract resulted in increasing of total polyphenols and antioxidant activity in the tea drink. An optimal concentration of pericarp extract in the green tea drink evaluated by sensory test was 0.4%. Addition of citric acid significantly affected on pH, total polyphenols, DPPH, and FRAP. Green tea drink with the highest concentration of citric acid gave the lowest pH. The lowest pH green tea drink showed the highest total polyphenols, DPPH, and FRAP ($p<0.05$), which was acceptable as slightly likely (6.97±1.50) by the sensory test. These results suggested that the addition of mangosteen pericarp extract could fortify the tea drink with favorable evaluation.

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