Determination of Phenolic Compounds and Antioxidant activity of the Leaves of Soursop (Annona muricata L.) mixed with Various Herbal

T T Y Nhi1,2,3, N N Quyen4, P V Thinh1,2, N T Bay5 and T T Truc6,*

1Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.
2NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.
3Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, Vietnam
4Faculty of Environmental and Food Engineering, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
5TRAVIPHA Co., Ltd., Tien Giang Province, Vietnam
6College of Agriculture, Can Tho University, Can Tho City, Vietnam

*Corresponding author: tttruc@ctu.edu.vn, labasm2013@gmail.com

Abstract. Food diversification is increasingly approaching customer needs. Tea processing field brings a lot of health. The present study has mixed the soursop leaves tea with stevia, chrysanthemum, rose, and licorice to produce an effective mixture with improved biological activities and flavors. Survey results showed that high TPC content was present in chrys (4.01 ± 0.03 mg/g DW) entailed an increasing concentration when mixed and vice versa. In most mixtures, free-radical scavenging by DPPH and ABTS in soursop leaves tea mixtures remained unchanged (ranging from 0.5 - 1.5 mg/g DW). The color brightness value in CIE system is highly dependent on soursop leaves tea (34.52 ± 0.71 in L*). These findings provide helpful insight to combinations of tea and common herbs for daily use. Further studies on toxicology and health benefits of these tea combinations would be on demand.

1. Introduction
Recently, herbs have received a great deal of public attention due to their benefits for human health [1]–[3]. Thanks to the presence of numerous secondary metabolites such as caffeine, polyphenols, vitamins and flavonoids, herbal tea is considered as the best healthcare product [4], [10]. Tea can be made from various sources of tea plant or dried plant leaves. In particular, the tea from soursop (Annona muricata L.) leaves which enriches in vitamins and minerals have exhibited effective anti-oxidant and anti-cancer activities, as shown in previous studies [5]–[11]. The plant is originated from the Americas and the Caribbean, then extended to some Asian countries such as Malaysia and Vietnam [12], [13]. According to Caleja1 et al. (2019), mixing different types of herbs is supposed to result in synergism which could enhance the nutritional values and flavors [14]. Therefore, the present study has mixed soursop leaves tea with four different types of herbs, namely, Stevia rebaudiana, Glycyrrhiza uralensis, Chrysanthemum morifolium and Rosa chinensis. Steavia (Stevia rebaudiana) is a herb with potential sweeteners, capable of increasing glucose tolerance and reducing plasma glucose levels, suitable for...
people with Type II diabetes [15]. Stevia mixed with black tea has given a high antioxidant combination [16]. In addition, licorice (Glycyrrhiza uralensis) has anti-inflammatory, antioxidant and regulatory genes [17]. A study by Kim et al. (2019) revealed that the mixture of G. uralensis, Liriope platyphylla and Cinnamomum cassia synergistically showed a potential laxative effect by targeting the mucosal tissues [18]. Chrys (Chrysanthemum morifolium) was used as a drink to aid in detoxification, strengthen the lungs and alleviate red, itchy eye [19]. Blending of C. morifolium with ascorbic acid-2-glucoside (AA2G) reduced the skin damage caused by UV radiation [20]. Rose (Rosa chinensis) was added to beverages to increase the antioxidants content [21].

By mixing and extracting the samples with ethanol using on previous testing methods [8], [22], [23], free-radical scavenging capability in tea bag was quantified based on total phenolic content (TPC), total flavonoids content (TFC), DPPH and ABTS assays. In addition, an experiment was conducted to evaluate the consumer's favorite value for the best mixing ratio.

2. Material and method

2.1. Preparation of dried leave

Based on the previous study, soursop leaves drying parameters to retain the highest concentration of antioxidant compounds were selected. Stevia, licorice, rose and chrys were purchased from Oriental Medicine, Hai Phong (Vietnam) at a ratio of 1:1, 1:2, 1:3 and 1:4, respectively. Uniform dimensions 0.25mm < material < 0.75mm. Each 2g of tea was extracted with 100 ml of hot water (80 °C), then cooled naturally at room temperature for 20 min.

![Figure 1](image_url)

**Figure 1.** Visual image of (A) Soursop leaves, (B) *S. rebaudiana*, (C) *G. uralensis*, (D) *R. chinensis*, (E) *C. Morifolium*

2.2. Determination of total phenolic content (TPC)

The TPC of soursop mixtures were performed using the method described by Chandra et al. 2014 [23]. Soursop mixture samples were mixed with 10% Folin-Ciocalteu solution and let stand for 5 min in the dark. Na₂CO₃ solution 7.5% was added and the mixtures were left for 1 h. TPC is expressed in milligrams of gallic acid equivalent in 1g of dry matter (mgGAE /g DM)

2.3. Determination of total flavonoid content (TFC)

The total flavonoids were determined by the colorimetric method described by Ghasemi (2009) [24]. 0.5 ml of post-extract leave fluid is added to a test tube with 0.1 ml of 10% AlCl₃ reagent, homogeneous for 5 min. Add 0.1ml of CH₃COOK 1M solution with 4.3 ml of ethanol. Allow to stand 30 minutes and measure the level of optical absorption. Optical density reflecting TFC was measured at 415 nm. Quercetin is used as a reference standard. TFC was expressed in milligrams of quercetin equivalent per
gram of extracted compounds (mgQE/g DW).

2.4. Determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) assay
The samples were diluted to an appropriate concentration, then added with DPPH solution (OD517 nm = 1.1 ± 0.02) and left for 30 min without light. The optical absorbance was measured at 517 nm on UV-Vis spectrophotometer. Ethanol (99.5%) was used as control. Vitamin C (ascorbic acid) was used as the reference standard [25].

2.5. Determination of ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay
Test to assess the ability to prevent the formation of colored oxidized products (734 nm) of ABTS [2,2’-azisbonis (3-ethylbenzothiazolin-6-sulfonic acid) diammonium] of the test substances. The degree of decrease in absorption intensity was measured at 734 nm [26]. The scavenging ability of the tea pulp was calculated using the standard equation: 

\[ y = 20.36x - 1.3855 \]

with Regression coefficients R=0.9985.

2.6. Determination of Lab* system
Select collate values L*, a*, b* based on CIE Lab* color space previously described by Torres B et al., 2011 [27]. The brightness is measured using a colorimeter Chroma Scanner (NR60CP model). Results were displayed as L* (brightness ranges from 0-100), a* (from green to red) and b* (from blue to yellow).

2.7. Sensory evaluation
2 g of samples were diluted with boiling water 80 °C (1:50 ratio). Three-digit encoded samples avoid being affected by user senses. The appropriate table is based on the given score corresponding to the standard description term (TCVN 3215 - 79 test).

Table 1. The sensory description of blended tea

| Critical coefficient | Flavor                        | Color                        | Aroma                        | Favorite         |
|----------------------|-------------------------------|------------------------------|------------------------------|------------------|
| 5                    | Taste cool, tea flavor, enjoy delicious | Pellucid, vivid yellow, typical for tea | Specific for type lightly | Like extremely |
| 4                    | Taste cool, enjoy delicious   | Pellucid, slightly yellow    |                              | Like             |
| 3                    | Tea flavor                    | Cloudy yellow                | Aromatic, not characteristic | Accept           |
| 2                    | Acrid, enjoy delicious        | Not typical tea color        | Odorless                     | Neither like nor dislike |
| 1                    | Acrid, enjoy poorly           | Strange color                | Strange smell                | Dislike          |

2.8. Statistical analysis
Microsoft Excel and Stagraphic software (The Plains corporate, Virginia) were used to analyze the results based on three replicates. The difference was assessed ANOVA a factor with significance level between means with an error of 0.5%

3. Results and discussion

3.1. The change in antioxidant content according to the mixing ratio
The preliminary results of the main input materials, SL after drying and combination with herbs with values of the content of bioactive compounds. As shown in Figure 2, with a total ascorbic acid (TAA) content of 1.09 ± 0.07 mg/g dry materials, reduced more than 50%. Notably, total phenolic content was high but significantly decreased after hot water exposure. Compounds sensitive to heat and light decay
during heating, in accordance with the announcement of Kauhila et al. [28]

After extraction with hot water (80 °C), then cooled, the bioactivity was measured at different wavelengths for the results shown in Figure 2. Soursop leaves with lower polyphenol content (2.41 ± 0.03 mg/g dry weight) lower than chrys (4.01 ± 0.03) (A1) and stevia (2.97 ± 0.03) (C1), however higher than roses (1.92 ± 0.03) (B1) and licorice (0.71 ± 0.11) (D1). This value increased significantly when the mixing ratio of stevia increased (5.81 ± 0.02) (C1). There were no significant differences in sample 4 and 5 regarding TPC (C1); 5 and 6 about TFC (C2), respectively. The mix almost does not affect the TAA value of stevia and SL tea (C2). The TPC value is dominated by the difference in herb type, highest stevia with 5.74 ± 0.07 mg (C1), rose accounting for 1.92 ± 0.03 mg (B1) TPC in 1g of dry matter, the remaining chrys and licorice with 4.01 ± 0.03 (A1) and 0.71 ± 0.11 mg (D1), respectively. The reported data is higher than the chrys material in a recent study, that determined the TPC concentration of chrys accounted for more than 0.001% on DW [29].

The difference in antioxidant activity is dominated by herbs. TFC content is likely to be obscured relatively wavelength by chlorophyll in leaves. Typically, the value of stevia leaf and SL reached the highest TFC of 2.19 ± 0.05 mgQE/g DW (C2). In chrys tea, increasing the percentage of this ingredient in a bag increases TFC (A2). In 2 g of tea provides a large amount of DPPH and ABTS (about 1 mg/g DW) (Figure 2-A2, B2, C2 and D2). The difference in the antioxidant activity comes from samples 3 and 5 in Figure 2-B1, which is most likely caused by the particle size in the tea bag. The larger size limits the ability to contact with water and at the same time this loss is affected by high temperatures [30, [31].

3.2. Lab* value and commercial sensory

After color statistics and sensory evaluation of blended tea samples, the results are recorded in Table 2. L* brightness negligible in samples combining Stevia and SL. This low value was mixed by SL (34.52 ± 0.71) with dark brown color, because the heating during drying, the color and chlorophyll components in SL degraded to form pheophytin and pheophorbide [32].

Make leaf colors from green to brown. On the other hand, the flavonols in the phenolic group are glycosides involved in the typical coloring reaction in tea processing [32]. The increase of the chrys ratio did not significantly affect the tea color, indicated by color brightness (L*) 49.99 ± 3.39, green gamuts (a*) -2.64 ± 0.07 and light yellow gamuts (b*) 3.94 ± 0.27, and the TCD value at each rate compared to chrys (p < 0.05) was the same. The temperature and extraction time did not significantly affect the color of the chrys [33]. Stevia has a low color value similar to SL, with L* (36.73 ± 2.36), a* (1.04 ± 0.41) and b* (13.99 ± 1.25), respectively. Understandably, the recognition of the color of stevia is from leaves, while the remaining is from flowers and wood [34]. The desired feature of tea was a yellow-colored and transparent solution, which is dominated by b*. This highest value was 1:4 stevia samples (15.82 ± 0.88), followed by licorice blending. Because of the vivid colors in SL, most tea colors are generally accepted when mixed. Unexpected color may possibly be a sign of low-quality tea.

The descriptive score for all flavors is from 3-4, showing that the tester is able to identify and describe in accordance with the flavor characteristics of the tea. While stevia did not provide difference in taste. It is possible that the stevia's outstanding features include the type of sugar and the typical sweet taste, which makes it difficult to identify [14]. Suitable for Favorite in licorice (1:2) and Chrys (1:1) with 4.55 and 4.27, respectively. The combination of SL and rose did not bring a high score compared to the original flower pattern (16.65), but it was still in the favored range (>15). 1:1 (Chrys), 1:2 (Rose), 1:3 (Stevia), and 1:2 (Licorice) are determined to be appropriate when Critical coefficient scores are high (15.71; 15.56; 16.65; and 16.91, respectively).
Figure 2. Biological activity of herbal blending ratio with sample 1 (soursop leave), 2 (herbal types A1-2: Chrysanthemum, B1-2: Rose, C1-2: Stevia; D1-2: Licorice, respectively), 3 (1:1), 4 (1:2), 5 (1:3), 6 (1:4).

a-f differed significantly according to Duncan’s multiple range test (p < 0.05)
### Table 2: Color system and sensory scores of herbal tea

| Soursop leave | Herbal types | 1:1 | 1:2 | 1:3 | 1:4 |
|---------------|--------------|-----|-----|-----|-----|
|               |              | C*  | R   | S   | L   |
| L*            | 34.52 ± 0.71 | 49.99 ± 3.39 | 49.63 ± 5.68 | 49.16 ± 1.68 | 48.49 ± 1.39 | 50.19 ± 1.82 |
| a*            | -1.49 ± 0.09 | 4.36 ± 0.67 | 4.01 ± 2.21 | 4.12 ± 1.25 | 4.71 ± 1.19 | 41.18 ± 5.02 |
| b*            | 12.83 ± 0.46 | 3.94 ± 0.27 | 3.68 ± 0.64 | 10.00 ± 0.38 | 11.62 ± 0.38 | 9.24 ± 0.26 |
| TCD           | 0            | 1.70 ± 0.22 | 11.27 ± 0.49 | 6.85 ± 0.49 | 8.25 ± 0.27 | 10.41 ± 2.64 |
|               |              | 13.99 ± 1.25 | 13.35 ± 0.29 | 14.41 ± 0.22 | 14.80 ± 0.29 | 15.82 ± 0.84 |
|               |              | 6.92 ± 0.43 | 13.27 ± 0.21 | 11.39 ± 0.77 | 11.64 ± 0.84 | 10.55 ± 0.45 |
| Flavor        | 3.36 ± 0.67  | C*  | R   | S   | L   |
|               |              | 3.82 ± 0.75 | 3.82 ± 0.75 | 3.82 ± 0.75 | 3.64 ± 0.67 | 3.36 ± 0.50 |
|               |              | 4.27 ± 0.79 | 3.64 ± 0.67 | 4.00 ± 0.63 | 3.91 ± 0.83 | 3.64 ± 0.67 |
|               |              | 3.91 ± 0.83 | 3.45 ± 1.04 | 3.82 ± 0.60 | 4.00 ± 0.89 | 3.82 ± 0.60 |
|               |              | 3.55 ± 0.69 | 3.55 ± 0.52 | 4.09 ± 0.54 | 3.64 ± 0.67 | 3.64 ± 0.50 |
| Color         | 3.55 ± 0.52  | C*  | R   | S   | L   |
|               |              | 3.73 ± 0.65 | 4.00 ± 0.77 | 3.73 ± 0.47 | 4.00 ± 0.63 | 3.64 ± 0.67 |
|               |              | 4.00 ± 0.63 | 3.73 ± 0.65 | 3.82 ± 0.87 | 3.64 ± 0.50 | 3.64 ± 0.67 |
|               |              | 3.91 ± 0.83 | 4.00 ± 0.63 | 3.91 ± 0.30 | 4.36 ± 0.67 | 4.00 ± 0.45 |
|               |              | 3.55 ± 0.52 | 3.64 ± 0.67 | 4.36 ± 0.50 | 3.55 ± 0.69 | 3.64 ± 0.50 |
| Aroma         | 4.36 ± 0.67  | C*  | R   | S   | L   |
|               |              | 4.09 ± 0.54 | 4.00 ± 0.63 | 3.91 ± 0.54 | 3.82 ± 0.40 | 3.91 ± 0.70 |
|               |              | 4.18 ± 0.75 | 3.73 ± 0.74 | 3.82 ± 0.60 | 3.82 ± 0.87 | 3.91 ± 0.70 |
|               |              | 3.36 ± 0.92 | 3.73 ± 0.79 | 4.00 ± 0.63 | 4.18 ± 0.60 | 4.00 ± 0.63 |
|               |              | 3.64 ± 0.50 | 3.91 ± 0.54 | 4.27 ± 0.47 | 3.91 ± 0.54 | 3.55 ± 0.52 |
| Favorite      | 3.82 ± 0.75  | C*  | R   | S   | L   |
|               |              | 3.82 ± 0.60 | 4.27 ± 0.47 | 3.91 ± 0.54 | 3.91 ± 0.54 | 4.09 ± 0.30 |
|               |              | 4.27 ± 0.65 | 3.91 ± 0.54 | 4.09 ± 0.70 | 4.09 ± 0.30 | 4.18 ± 0.75 |
|               |              | 3.64 ± 0.50 | 3.82 ± 0.60 | 3.27 ± 0.90 | 4.00 ± 1.26 | 3.36 ± 1.03 |
|               |              | 3.64 ± 0.52 | 3.73 ± 0.47 | 4.53 ± 0.52 | 3.36 ± 1.05 | 3.55 ± 0.52 |
| Critical coefficient scores | 14.87 | C   | R   | S   | L   |
|               |              | 15.49 | 14.71 | 15.27 | 15.20 | 14.87 |
|               |              | 16.65 | 14.76 | 15.56 | 15.20 | 15.33 |
|               |              | 14.98 | 14.80 | 15.60 | 16.65 | 15.71 |
|               |              | 14.29 | 14.73 | 16.91 | 14.00 | 14.11 |

**Means different between rows. With C: Chrysanthemum, R: Rose, S: Stevia, L: Licorice**

4. Conclusion

The TPC values of most soursop mixtures were greater than 1 mg/g DW, indicating that this is a valuable source of phenolic compounds. Incorporation of licorice and rose into tea enhanced the sensory value, with a focus on aroma and flavor. For antioxidant activity, it is only supportive when combined with soursop leaves tea. Evaluation results from testers show that most Critical coefficient scores are greater than 11.2 (scores for commercial response). The data of the study is considered as a tool to provide information for tea-loving consumers. The combinations diversify the products and understand the ingredients they bring about. However, omission in the extracted temperature range of hot water has not been investigated. Furthermore, as the habit of consuming these blended tea greatly influences these values, the toxicology and health benefits of these tea products require further studied.
Acknowledgment
This study was supported by Tien Giang Department of Science and Technology, Tien Giang Province, Vietnam.

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