

Potential of Utilizing Corn Silk (*Stigma maydis*), Thebu Leaves (*Costus speciosus*), Green and Purple Guava (*Psidium guajava*) Leaves in Formulating Green Tea and Black Tea Blends

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ABSTRACT

Purpose: Underutilized plant materials such as corn silk (*Stigma maydis*), guava leaves (*Psidium guajava*), and 'thebu' leaves (*Costus speciosus*) are rich sources of various phytochemicals with potential health benefits. This study was carried out to investigate the potential of developing green tea and black tea blends by incorporating corn silk, green and purple guava leaves, and 'thebu' leaves.

Research Method: Five green tea and black tea blends were prepared using steamed and dried 'thebu' leaves, corn silk, and green and purple guava leaves. Sensory properties of the samples were evaluated. Antioxidant activity (AOA) and total polyphenol (TPP), total flavonoid (TF), ash and crude fiber contents of the samples were determined.

Findings: The selected green tea blend consisted of 50% green tea, 16.7% corn silk, 16.7% 'thebu' leaves and 16.7% green guava leaves whereas the selected black tea blend consisted of 50% black tea, 12.5% corn silk, 12.5% 'thebu' leaves and 25% purple guava leaves. Comparable TPP contents (gallic acid equivalent %) were found in green tea blends (22.51 ± 0.93) and black tea blends (21.78 ± 0.11). Antioxidant activity (percentage inhibition) of green and black tea blends (85.93 ± 0.44 and 87.38 ± 1.17 respectively) were significantly higher than that of green tea (81.58 ± 0.92) and black tea (81.43 ± 0.88).

Research Limitations: The optimal processing conditions for preserving the properties of selected materials are crucial in formulating herbal tea blends, yet they remain to be established.

Originality/ Value: Corn silk, green and purple guava, and 'thebu' leaves hold great potential for formulating tea blends with improved physicochemical, therapeutic, and sensory properties.

Keywords: Antioxidant activity, Corn silk, Guava leaves, Physicochemical properties, Tea blend, 'Thebu' leaves.

INTRODUCTION

Tea, derived from *Camellia sinensis*, is currently the most widely consumed beverage worldwide; therefore, it is recognized as an important global food product (FAO, 2022). It has specific organoleptic properties – taste, aroma, and color. Further, tea exhibits various health-promoting properties such as antioxidant, anticancer, anti-glycemic, anti-cholesteric, and antimicrobial (Kaur *et al.*, 2007; Prasanth, 2019; Rasheed, 2019; Wang *et al.*, 2022). Mainly the flavonoids are responsible for most of these health benefits of tea (Wang *et al.*, 2022). Corn, commonly

known as maize (*Zea mays* L.), is an annual crop that belongs to the family of grass Poaceae (Shah, 2016). Corn hairs, commonly known as corn silk (*Stigma maydis*), are a bundle of silky, long, yellowish, and purplish strands that can be seen on top of corn fruit.

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It has been consumed for a long time as a therapeutic remedy for various illnesses and is important as an alternative natural-based treatment (Hasanudin *et al.*, 2012; Vijitha and Saranya, 2017). It is an excellent source of many bioactive compounds such as volatile oils, steroids, alkaloids and natural antioxidants such as flavonoids and other phenolic compounds with beneficial effects on human health (Emmanuel *et al.*, 2016; Elkewawy, 2018; Nawaz *et al.*, 2019). Although corn is widely cultivated across many districts in Sri Lanka, corn silk is typically discarded as waste and remains unused for any practical purpose.

'Thebu' (*Costus speciosus*) is a perennial herbaceous plant known for its diverse pharmacological activities, including antibacterial, antifungal, anticholinesterase, antioxidant, anti-hyperglycemic, anti-inflammatory, analgesic, antipyretic, antidiuretic, larvicidal, antistress, and estrogenic (Pawar and Pawar, 2012; Waisundara *et al.*, 2015; Choudhury and Das, 2019; Vaidya and Shingadia 2020). Phenolics and alkaloids found in 'Thebu' are mainly responsible for their therapeutic properties (Choudhury and Das, 2019).

Health benefits of the guava leaves (*Psidium guajava*) are largely attributed to their rich content of phytochemicals, including quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, tannins, carotenoids, triterpenoids and caffeic acid (*Psidium guajava*) are largely attributed the (El-Ahmady *et al.*, 2013; Weli *et al.*, 2019; Kumar *et al.*, 2021). The antioxidant capacity of guava leaf extract more strongly correlates with their phenolic content than their flavonoid content (Chen and Yen, 2007). Purple guava is a new cultivar that has not yet been commercially utilized in Sri Lanka. Further, no reports have indicated any toxic compounds in corn silk, 'Thebu' leaves, or green and purple guava leaves. These materials have yet to be studied for their potential use in the formulation of tea blends.

Therefore, this study aimed to explore the potential of incorporating corn silk, 'Thebu' leaves, and green and purple guava leaves into the formulation of green and black tea blends with improved therapeutic properties.

MATERIALS AND METHODS

Material

Corn silk, 'Thebu' leaves and green and purple guava leaves were collected from Badulla, Sri Lanka. Black tea (Zesta - Broken Orange Pekoe Fannings) was purchased from a supermarket in Badulla, Sri

Lanka. Green tea was prepared at the Tea Technology Laboratory, Uva Wellassa University, Sri Lanka. All the chemicals used in this study were of analytical grade.

Methods

Sample preparation: Freshly collected corn silk, 'Thebu' leaves, green and purple guava leaves were washed properly and spread on a clean surface leaving to drain off surface water. Then these materials were steamed for about 45 seconds at 100-110°C. Steamed samples were spread on a clean surface again leaving to drain off surface moisture and then they were cut into smaller pieces. Then, these materials were dried in an oven at 40-45°C to a moisture content of about 4-5%. Dried samples were ground and sieved through a 500 µm sieve separately.

Fresh tea leaf sample of TRI 2023 variety was collected from the 'Thelbadda' Estate, Badulla, Sri Lanka, and green tea was prepared at the Tea Technology Laboratory of Uva Wellassa, University, Sri Lanka, following the steaming green tea manufacturing process described by Ahmed and Stepp (2013).

As shown in Table 01, five green tea blends and five black tea blends were prepared. The proportions of individual materials were adjusted to ensure each blend totaled 100. All green tea blends contained 50% green tea, 'Thebu' being one-third of the remaining 50% (16.67). Corn silk and green guava leaf proportions varied at 25%, 50%, and 75% of the remaining 33.33% (8.34, 16.67, and 25 respectively). All black tea blends contained 50% black tea, 'Thebu' being one-fourth of the remaining 50% (12.5). Corn silk and green guava leaf proportions varied at 12.5%, 25%, 50%, 62.5% and 75% of the remaining 50% (6.25, 12.5, 25, 31.25, and 37.5 respectively). These samples were prepared in triplicate.

Sensory evaluation: Samples of green tea (1.5 g each) and black tea (2.0 g each) were brewed separately with 150 mL aliquots of boiled distilled water in tea-tasting mugs for five minutes. Infusions were decanted into white ceramic tea-tasting cups labeled with random three-digit codes. The sensory properties like color, aroma, taste, astringency, and overall acceptability of these infusions were evaluated by a sensory panel consisting of 52 untrained panelists using a seven-point Hedonic scale. Having explained the study, the consents of the panelists were obtained for their participation in this sensory analysis.

Determination of antioxidant activity: Each sample

Table 1: Ingredients (%) Used for the Preparation of Green Tea and Black Tea Blends

Blend	Treatment Code	Ingredients (%)					
		Green Tea	Black Tea	Corn Silk	'Thebu' Leaves	Green Guava Leaves	Purple Guava Leaves
Green Tea Blend	142	50		0	16.67	33.34	
	283	50		8.34	16.67	25.00	
	391	50		16.67	16.67	16.67	
	451	50		25.00	16.67	8.34	
	798	50		33.34	16.67	0	
Black Tea Blend	125		50	0	12.50		37.50
	265		50	6.25	12.50		31.25
	348		50	12.50	12.50		25.00
	472		50	25.00	12.50		12.50
	551		50	37.50	12.50		0

(1.0 g) was brewed with 50 mL of boiling water for 5 min separately. After allowing cooling to room temperature, each extract was filtered through a filter paper separately into graduated tubes.

Antioxidant activities of these extracts were estimated as percentage inhibition using the diphenylpicrylhydrazyl (DPPH) assay (Brand-Williams *et al.*, 1995). Solutions were prepared by mixing 34 μ L of each sample extract with 68 μ L of 20 ppm DPPH. Distilled water was used as the control sample. Prepared solutions were kept in dark at room temperature for five min and the absorbance of each sample was measured against the control at 517 nm using micro-plate reader. The following formula was used to calculate the percentage inhibition.

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (1)$$

Determination of chemical composition: Total polyphenol contents (TPP) of the samples were determined as gallic acid equivalent (g GAE/100 g) by the Folin-Ciocalteu colorimetric assay (ISO 14502-1:2005). Total flavonoid contents (TF) of the samples were determined as quercetin equivalent (g QE/100 g) by the aluminum chloride colorimetric method (Shraim *et al.*, 2021) with slight modifications. These samples were also analyzed for total ash content by incineration in a furnace at 525 $\pm$ 25 $^{\circ}$ C (ISO 1575, 1980). Crude

fiber contents of these samples were estimated by weighing the dried residues left after acid and alkali digestion (ISO 15598, 1998).

Determination of color of sample infusions: The colors of the infusions were identified as color codes by using Munsell plant color chart.

Microbial analysis: Total plate count of the sample was determined according to the SLS standard (SLS 516, 1991). This analysis was performed under accelerated storage conditions to assess the microbiological stability of the stored samples.

Statistical analysis: Data from the physicochemical analysis were subjected to analysis of variance and mean separation (Tukey's Pairwise Comparison). Sensory data were statistically evaluated by performing the Friedman test. Statistical analyses were done using the statistical software Minitab 17.

RESULTS AND DISCUSSION

Sensory Properties

The results of the Friedman test indicated that the color, brightness, aroma, taste, astringency, and overall

Table 2: Estimate Means Received for Different Samples

Sensory Attribute	Estimate Means Received for Different Samples ^a					P Value	Highest Sum Ranks
	142	283	391	451	798		
Color	5.2	5.6	6.0	5.1	5.8	0.001	192.5
Brightness	5.0	5.0	6.0	5.0	5.0	0.002	191.5
Aroma	5.1	5.5	6.9	5.1	4.9	0.000	200.5
Taste	5.0	5.4	6.5	5.0	4.8	0.000	210.5
Astringency	4.85	5.55	6.05	4.95	4.35	0.000	203.5
Overall Acceptability	4.7	5.7	6.3	5.0	4.8	0.000	214.5

^a Sample Codes:

142 - Green tea (50%), *Thebu* leaves (16.7%), corn silk (0%) and green guava leaves (33.4%)

283 - Green tea (50%), *Thebu* leaves (16.7%), corn silk (8.4%) and green guava leaves (25%)

391 - Green tea (50%), *Thebu* leaves (16.7%), corn silk (16.7%) and green guava leaves (16.7%)

451 - Green tea (50%), *Thebu* leaves (16.7%), corn silk (25%) and green guava leaves (8.4%)

798 - Green tea (50%), *Thebu* leaves (16.7%), corn silk (33.4%) and green guava leaves (0%)

Table 3: Results of Sensory Analysis of Black Tea Blends

Sensory Attribute	Estimate Means Received for Different Samples ^a					P Value	Highest Sum Ranks
	125	265	348	472	551		
Color	5.8	6.0	6.8	5.8	5.6	0.000	210.0
Brightness	6.0	6.0	7.0	6.0	6.0	0.000	206.5
Aroma	5.0	5.0	6.0	5.0	4.9	0.000	210.0
Taste	5.0	5.2	6.4	4.6	4.3	0.000	216.0
Astringency	5.05	5.25	6.55	4.95	4.45	0.000	224.5
Overall Acceptability	5.0	5.8	7.0	5.2	5.0	0.000	233.0

^a Sample codes:

798 - Black tea (50%), *Thebu* leaves (12.5%), corn silk (0%) and purple guava leaves (37.5%)

125 - Black tea (50%), *Thebu* leaves (12.5%), corn silk (6.25%) and purple guava leaves (31.25%)

283 - Black tea (50%), *Thebu* leaves (12.5%), corn silk (12.5%) and purple guava leaves (25%)

391 - Black tea (50%), *Thebu* leaves (12.5%), corn silk (25%) and purple guava leaves (12.5%)

451 - Black tea (50%), *Thebu* leaves (12.5%), corn silk (37.5%) and purple guava leaves (0%)

acceptability of all prepared green tea and black tea blends differed significantly ($p < 0.05$).

The green tea blend coded as 391 received highest means for all sensory parameters. Therefore, it was selected as the best green tea blend. The chosen green tea blend was consisted of 50% green tea, 16.7% corn

silk, 16.7% 'Thebu' leaves, and 16.7% green guava leaves (Table 02). The black tea blend 348, which received the highest mean scores across all sensory parameters, was selected as the best black tea blend. This blend was consisted of 50% black tea, 12.5% corn silk, 12.5% 'Thebu' leaves, and 25% purple guava leaves (Table 03).

Phenolic Contents and Antioxidant Activity

Polyphenols are anti-oxidants and they are principally responsible for most of the health benefits of plant materials (Engelhardt, 2010; He *et al.*, 2020). As shown in Table 04, the total polyphenol contents (g GAE/100 g) of green guava leaves (22.36 ± 0.48) and purple guava leaves (22.47 ± 0.88) were found to be closely comparable to that of green tea (20.91 ± 0.53) and black tea (23.09 ± 0.26). The total polyphenol content reported in guava leaves (27.9 ± 1.4 g GAE/100 g) by Chen and Yen (2007) is comparable to the findings of the present study. This strongly suggests the potential health benefits of guava leaves. Phenolic compounds such as Guavin A, Galloyl (epi) catechin, Gallic acid, and Catechin were reported to be present in guava leaves in significant amounts (Díaz-de-Cerio *et al.*, 2016).

Furthermore, green guava and purple guava leaves contained significant amounts of flavonoids compared to green tea (Table 04). Quercetin, kaempferol and myricetin and their derivatives are the major flavonoid compounds present in guava leaves (Chang *et al.*, 2012; Díaz-de-Cerio *et al.*, 2016; Kumar *et al.*, 2021).

Higher antioxidant activities found in green and purple guava leaves further confirm their potential health-promoting properties. Chen and Yen (2007) also reported higher antioxidant activity in dried guava leaves compared to the 'polyphenon 60', which is a commercial polyphenol product extracted from green tea. Moreover, the inhibition percentages of 86.0% to 82.2% for sample concentrations of 5 to 25 µg/ml of dried guava leaves reported by Chen and Yen (2007) align closely with the inhibition percentages of $87.94 \pm 0.46\%$ and $88.09 \pm 0.76\%$ observed for green and purple guava leaves respectively in the present study.

Corn silk and 'Thebu' leaves contained significantly less amounts of polyphenols and flavonoids compared to other ingredients (Table 04). Singh *et al.*, (2022) also reported similar amount of polyphenol ($9.4 \pm 0.03\%$) in corn silk. Further, Ku *et al.* (2009) reported low amounts of polyphenols (0.53% - 3.27%) and flavonoid (0.98% - 2.42%) compounds in Korean corn silk. Nevertheless, corn silk exhibited higher antioxidant activity ($87.53 \pm 0.53\%$). This result is consistent with

the antioxidant activity of corn silk ($80.80 \pm 1.84\%$) reported by Singh *et al.* (2022). The high antioxidant activity observed may be attributed to the presence of constituents such as carotenes ($0.022 \pm 0.00\%$) and Vitamin C ($8.84 \pm 0.45\%$) in corn silk, which have significant antioxidant potential (Haslina *et al.*, 2019). Contradictorily, Tian *et al.* (2013) reported that high DPPH scavenging activity in six different corn silk varieties in China were attributed to phenolics and flavonoids.

The antioxidant activity, as well as the total polyphenol and total flavonoid contents of 'Thebu,' were significantly lower compared to those of the other materials. Nevertheless, 'Thebu' is known to be material with antibacterial, antifungal, anti-cholinesterase, anti-hyperglycemic, anti-inflammatory, analgesic, antipyretic, antidiuretic, larvicidal, antistress and estrogenic properties (Pawar and Pawar, 2012). Therefore, incorporating 'Thebu' into tea blends could enhance their health-promoting properties. The higher antioxidant activities (% inhibition) observed in the green tea blend (85.93 ± 0.44) and black tea blend (87.38 ± 1.17) compared to green tea (81.58 ± 0.92) and black tea (81.43 ± 0.88) may be attributed to the greater antioxidant activity of corn silk and guava leaves.

Ash Content and Crude Fiber Content

Ash refers to the residue left after removing moisture and organic matter by heating food at high temperature ($525 \pm 25^\circ\text{C}$). Ash content represents the total mineral matter content in foods. Higher ash content is an indication of contamination of food with mineral matters. Crude fiber is the residue of plant materials except minerals remaining after digestion with dilute acid and alkali. It consists largely of cellulose and lignin. Crude fiber content is an indication of maturity of plant materials. Higher percentages of both ash and crude fiber are undesirable.

As shown in Table 05, ash content and crude fiber content of both the selected black tea blend and the selected green tea blend exceeded the maximum permitted levels stipulated for tea (8% and 16.5% respectively) by the International Standard Organization (ISO11287, 2011; ISO 3720, 20011). However, ash and fiber contents can be reduced by carefully selecting raw materials, preventing sand from entering the tea during processing, removing sand after processing, and extracting fiber from the tea using electrostatic fiber extractors or color sorters.

Table 4: Phenolic contents and antioxidant activity of ingredients and blends

Material	Parameter		
	TPP (g GAE/100g)	TF (g QE/100g)	AOA (% inhibition)
Green tea	20.91±0.53 ^a	16.41±0.74 ^a	81.58±0.92 ^c
Black tea	23.09±0.26 ^a	12.53±0.04 ^b	81.43±0.88 ^c
Corn silk	8.90±1.97 ^b	4.43±0.14 ^d	87.53±0.53 ^{ab}
Green guava leaves	22.36±0.48 ^a	12.35±1.20 ^b	87.94±0.46 ^{ab}
Purple guava leaves	22.47±0.88 ^a	12.95±0.02 ^b	88.09±0.76 ^a
‘Thebu’ leaves	8.44±1.71 ^b	1.26±0.13 ^c	68.60±0.47 ^d
Green tea blend	22.51±0.93 ^a	12.19±0.12 ^b	85.93±0.44 ^b
Black tea blend	21.78±0.11 ^a	10.13±0.28 ^c	87.38±1.17 ^{ab}

*Values are mean ± Standard Deviation (n=3). Values with different superscript letters in a column differ significantly ($p \leq 0.05$).

GAE: gallic acid equivalent; QE: quercetin equivalent; TPP: total polyphenol content; TF: total flavonoid content; AOA: antioxidant activity.

Table 5: Ash and crude fiber content of ingredients and blends

Samples	Parameter (%)	
	Ash content	Crude fiber
Selected black tea blend	12.98±0.23 ^b	19.09±0.50 ^a
Selected green tea blend	12.06±0.81 ^b	17.12±0.28 ^{abc}
Black tea	10.12±0.61 ^c	14.08±0.04 ^{bc}
Green tea	5.17±1.16 ^e	12.61±1.67 ^c
Corn silk	3.67±0.58 ^f	18.53±0.42 ^{ab}
Purple guava leaves	5.00±0.50 ^e	16.10±2.34 ^{abc}
Green guava leaves	6.50±0.87 ^d	16.75±6.86 ^{abc}
‘Thebu’ leaves	14.83±0.29 ^a	18.76±0.55 ^a

*Values are mean ± Standard Deviation (n=3). Values with different superscript letters in a column differ significantly ($p \leq 0.05$).

Table 6: Color of infusions of ingredients and final blends.

Sample	Color code	Color name
Green tea blend	5Y 8/10	Strong yellow
Black tea blend	5R 4/8	Red
Green tea	5Y 8/8	Yellow
Black tea	2.5YR 3/6	Dark red
Corn silk	2.5Y 6/8	Olive brown
Green guava leaves	5Y 8/6	Yellow
Purple guava leaves	10R 4/8	Red
‘Thebu’ leaves	5Y 8/8	Yellow

Color of Infusions

Identification of color of infusion of ingredient is very much useful in optimizing ratios of ingredients during

blending. Colors of the infusions identified as color codes by using Munsell plant color chart are presented

Table 7: Total plate counts of green and black tea blends.

Duration	Total Plate Count (CFU g ⁻¹)	
	Green tea blend	Black tea blend
1 st week	1.0×10 ¹	1.0×10 ¹
3 rd week	1.5×10 ¹	1.3×10 ¹

in Table 06. Diverse colors of infusions of ingredients (Yellow to dark red) indicate the potential usage of these ingredients to achieve desired colors of blends. Color of the tea infusions may vary with different factors such as, processing method (Wang *et al.*, 2022), method of infusion, infusion time and mineral content (Polat *et al.*, 2022). Consumer preference for the developed color of infusions is the most important factor when preparing tea blends.

Total Plate Count

Higher Total Plate Count (TPC) indicates microbial contamination of tea. TPC of both the green and black tea blends remained below the maximum permitted limit for tea (10,000 cfu g⁻¹) (Sri Lanka Tea Board, 2021). Further, De Silva *et al.* (2019) reported that the TPC of tea samples collected from Sri Lankan tea market were within the range of 0.45 × 10³ to 6.64 × 10³ CFU/g.

CONCLUSION

In conclusion, the findings emphasize the superior antioxidant activity (as % inhibition) of green guava

leaves (87.94±0.46), purple guava leaves (88.09±0.76), and corn silk (88.09±0.76) compared to pure green tea (81.58±0.92) and black tea (81.43±0.88). Additionally, ‘Thebu’ leaves exhibit notable antioxidant potential (68.60±0.47% inhibition). The optimal recipe for an herbal green tea blend consists of 50% green tea, 16.7% corn silk, 16.7% ‘Thebu’ leaves, and 16.7% green guava leaves. For an herbal black tea blend, the ideal formulation includes 50% black tea, 12.5% corn silk, 12.5% ‘Thebu’ leaves, and 25% purple guava leaves. The formulated blends of herbal green and black teas, with significantly higher antioxidant activities (85.93±0.44% and 87.38±1.17%, respectively) compared to pure green tea (81.58±0.92%) and black tea (81.43±0.88%), suggest that green guava, purple leaves, corn silk, and ‘Thebu’ leaves are promising herbal materials for developing tea blends with improved physicochemical and therapeutic properties and with appealing organoleptic properties.

Conflicts of Interest

The authors declare no conflict of interest.

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