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The Potency of Green Tea Powder in Enhancing the Antioxidant Activity and Sensory Properties of Pluchea-Butterfly Pea Flower Herbal Tea

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ABSTRACT

Pluchea herbal tea, derived from pluchea leaf powder infusion, exhibits an unappealing colour and low antioxidant activity. A previous study combining pluchea leaf powder with butterfly pea flower powder in a 40:60% w/w ratio improved sensory preference but failed to significantly enhance bioactive constituents and antioxidant activity. Given the well-documented antioxidant properties of green tea, this study aimed to investigate the effects of incorporating green tea on the antioxidant activity, bioactive constituents, and sensory properties of herbal tea composed of green tea-pluchea leaf and butterfly pea flower powders in a 40:60% w/w ratio. A randomized block design was employed with a single factor: the proportion of green tea powder to pluchea leaf powder (0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, and 100:0% w/w) in four replicates. Among these formulations, the 80:20% ratio of green tea to pluchea leaf powder demonstrated the most significant improvement in DPPH free radical scavenging activity with value of 981±12 mg GAE/kg, bioactive compounds content, with total phenol content of 37,469±413 mg GAE/kg and total flavonoid content of 3406±29 mg CE/kg. The panelist preference of colour parameter was also improved, but ferric reducing antioxidant power and other sensory properties, such as aroma and taste were not significantly different compared with the control (pluchea leaf powder with butterfly pea flower powder in 40:60% ratio). These results indicate that the formulation of green tea, pluchea leaf, and butterfly pea flower powders at 28:12:60% w/w ratio have strong potential for development as a functional herbal beverage.

Keywords: Antioxidant activity, Bioactive compounds, Green tea, Pluchea-butterfly pea flower herbal tea, Sensory properties.

Introduction

The leaves of *Pluchea indica* Less have been processed into an herbal drink by packaging approximately 2 g of the dried leaves in tea bag. A study by Widyawati *et al.*[1] reported that steeping 2 g of pluchea leaves in 100 mL of hot water resulted in the highest consumer acceptance, however, the antioxidant activity and bioactive compounds content remained low. Previous research suggested that the addition of honey improved the total phenolic and total flavonoid contents of pluchea herbal tea.[2] Additionally, studies have shown that black tea reduces the antioxidant activity, total flavonoids, and total phenols,[3] while green tea increases these parameters when incorporated into pluchea leaves at 50% concentration.[4] Attempts to enhance pluchea herbal tea with moringa leaves, rosella petals, and basil leaves have not significantly improved its bioactive properties. Although, incorporating butterfly pea flowers into pluchea leaves at a 60% (w/w) proportion enhanced panelist acceptance due to its vibrant colour, this treatment did not significantly boost bioactive constituents or antioxidant activity. Therefore, adding other herbs or ingredients, such as green tea into the formulation is essential for enhancing these properties.

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Green tea (*Camelia sinensis*) is well known for its antioxidant activity and bioactive compounds. The bioactive constituents of green tea include steroids, alkaloids, tannins, and flavonoids, with total phenols, total flavonoids, and reducing capacity reported at 0.8 g/g, 16 mg/g, and 0.13 g/g, respectively.[5] Habiburohman and Sukohar[6] emphasized that green tea contains polyphenol compounds, including catechins, theaflavins, and thearubigins, which are known to possess antioxidant activity. Polyphenolic compounds in green tea primarily include gallic acids, gallocatechin, catechins, epicatechins (6.4%), epigallocatechins (19%), epigallocatechin gallate (59%), *p*-coumaroylquinic acids, epicatechin gallate (13.6%), and gallocatechin-3-gallate. According to Hasan *et al.*[7], the methanol extract of green tea has strong antioxidant activity, with an IC_{50} value of 69.51 µg/mL in the DPPH radical scavenging assay, and also a caffeine content of about 74.47%. in addition, the antioxidant activity of epicatechins, epigallocatechin, epigallocatechin gallate, and epicatechin gallate has been demonstrated through various *in vitro* assays involving hydrogen atom donation or electron transfer.[8] The polyphenolic compounds in green tea can chelate metal ions such as iron and copper, potentially preventing them from acting as catalysts in the free radical formation process.
Therefore, incorporating green tea into pluchea leaf-butterfly pea flower herbal tea is expected to enhance its bioactive compounds content and antioxidant activity without compromising sensory characteristics. This study aims to determine the effects of different proportions of green tea and pluchea leaf powders on the bioactive compounds content (total flavonoids and total phenols), antioxidant activities (ferric ion reducing power and DPPH free radical scavenging activity), and sensory properties (colour, taste, and aroma) of herbal tea formulated with green tea-pluchea leaf powders and butterfly pea flower powder.

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Materials and Methods

Chemicals and equipment

The chemicals used included methanol (CH_3OH, Fulltime), distilled and double distilled water and (PT. Akua Surabaya, Indonesia), bottled drinking water was supplied from a local store in Surabaya, Indonesia, (+)-catechin (Sigma-Aldrich), aluminum chloride ($AlCl_3$, Merck), sodium nitrite ($NaNO_2$, Merck), sodium hydroxide (NaOH, Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich), gallic acid (Riedel-deHaen), follin-ciocalteau (Merck), disodium hydrogen phosphate (Na_2HPO_4, Merck), sodium dihydrogen phosphate (NaH_2PO_4, Merck), potassium ferricyanide ($K_3[Fe(CN)_6]$, Merck), ferric chloride ($FeCl_3$, Merck), and sodium carbonate (Na_2CO_3, Riedel-deHaen). The main equipment used included an electric blender (Philips), 45-mesh sieve (W. S. Tyler), sealer, water heater (Philips), digital balance (Mettler Toledo), and UV-Vis spectrophotometer (Shimadzu UV-1800).

Collection of plant materials

Pluchea leaves were collected from Surabaya's Mangrove Garden. Butterfly pea flower and green tea were purchased from a local store in Surabaya, Indonesia. Tea bags were purchased from CV Peri Akas, Godean, Yogyakarta, Indonesia. The plant materials were identified in the Plant Systematics Laboratory, Biology Study Program, Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic University with the following taxonomic IDs: 175518 for Pluchea leaves, 15501 for Butterfly pea flower, and 4442 for green tea.

Sample preparation

Green tea, pluchea leaves, and butterfly pea flowers were dried, powdered, and sieved through a 45-mesh sieve. The powder was further dried in an electric oven at 120ºC for 10 minutes.[9] The herbal tea were filled with green tea and pluchea leaf powder at different proportions (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100% w/w). Each tea bag contained 0.8 grams (40%) of the mixture [green tea (28%) and pluchea leaf powder (12%)] along with 1.2 grams (60%) of butterfly pea flower powder (Table 1 and Table 2). The filled tea bags were then packaged in plastic pouches laminated with aluminum foil. The tea was brewed in 95ºC water for 5 minutes, and thereafter used for bioactive compounds, antioxidant activity, and sensory evaluations.

Table 1: Formulation of pluchea-butterfly pea flower herbal tea with various proportion of green tea-pluchea leaf powder

Ingredient	Composition										
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**
Green tea powder (%)	0	4	8	12	16	20	24	28	32	36	40
Pluchea leaf powder (%)	40	36	32	28	24	20	16	12	8	4	0
Butterfly pea flower (%)	60	60	60	60	60	60	60	60	60	60	60
Water (mL)	200	200	200	200	200	200	200	200	200	200	200

Note: Percentage was based on 2 g of herbal tea per tea bag

Table 2: Proportion (%) of green tea to pluchea leaf powder

Ingredient	Composition										
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**
Green tea powder (%)	0	10	20	30	40	50	60	70	80	90	100
Pluchea leaf powder (%)	100	90	80	70	60	50	40	30	20	10	0

Note: Percentage was based on 40% of pluchea leaf powder in herbal tea

Determination of total phenol content

The total phenol content (TPC) of the herbal tea was evaluated using the Folin-Ciocalteu colourimetric method with gallic acid (100 – 500 ppm) as the reference standard.[10,11] The mixture was prepared by combining 0.1 mL of steeped herbal tea with 1 mL of Folin-Ciocalteu reagent, and shaken to achieve a homogeneous solution. Afterward, 2 mL of 7.5% sodium carbonate and distilled water were added to reach a final volume of 10 mL. The mixture was left to react for 5 minutes at room temperature. The absorbance was measured at 760 nm using a spectrophotometer. TPC was expressed as milligrams of gallic acid equivalent per kilogram of sample (mg GAE/kg sample).

Determination of total flavonoid content

The total flavonoid content (TFC) was measured using aluminum chloride colourimetric methods.[10,11] Approximately 0.1 mL of steeped herbal tea was added to 0.3 mL of 5% sodium nitrite and allowed to react for 5 minutes. Then, the solution was mixed with 0.3 mL of 10% aluminum chloride for 1 minute. About 1 mL of 1 M sodium hydroxide was subsequently added to the mixture, followed by the addition of distilled water to a final volume of 10 mL. After 10 minutes of incubation, the absorbance of the mixture was measured spectrophotometrically at 510 nm using (+)-catechin (100 – 600 ppm) as a reference standard. The results were reported as milligrams of catechin equivalent per kilogram of sample (mg CE/kg sample).

DPPH free radical scavenging assay

The DPPH free radical scavenging assay was carried out using the method outlined by Baliyan *et al.* (2022)[12] with minor adjustment. About 1 mL aliquot of steeped herbal tea was mixed with 1 mL of methanol and 3 mL of 60 mM DPPH in methanol. The mixture was allowed to react in a dark room for 30 minutes, after which the absorbance was measured at 517 nm using a spectrophotometer. Gallic acid (5 – 40 ppm) was used as the standard. The percentage inhibition of DPPH radical was determined using the formula below.

$$\text{DPPH Inhibition (\%)} = \frac{A0-As}{A0} \quad x\,100$$

Where;

A0 = Absorbance of the control (DPPH solution in methanol) and As = Absorbance of the sample (DPPH solution + sample or standard).

Gallic acid standard curve was obtained by plotting percentage inhibition of DPPH versus concentration of gallic acid. The antioxidant activity of the preparation was reported as milligrams of gallic acid equivalent per kilogram of sample (mg GAE/kg sample).

Ferric reducing antioxidant power (FRAP) assay

The ferric-reducing capacity of the steeped herbal tea was determined using a spectrophotometric method following the procedure outlined by Okafor *et al.* (2024)[13] with slight modifications. The capability of bioactive compounds in the steeped herbal tea to reduce Fe^{3+} ions to Fe^{2+} ions was indicated by the formation of a blue coloured complex. Approximately 0.1 mL of steeped herbal tea was mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferric cyanide. The mixture was incubated at 50°C for 20 minutes, after which 2.5 mL of 10% mono-chloroacetic acid was added. After homogenization, 2.5 mL of distilled water and 2.5 mL of 0.1% ferric chloride were added, and the mixture was allowed to react for 10 minutes. The absorbance was measured using a spectrophotometer at 700 nm, with gallic acid (200 – 1000 ppm) as the reference standard. The results were expressed as milligrams of gallic acid equivalent per kilogram of sample (mg GAE/kg sample).

Sensory analysis

The sensory properties of the steeped herbal tea were analyzed using the method described by Widyawati *et al*. (2022).[14] The sensory test used was a hedonic scoring method for aroma, taste, and colour parameters. The sensory testing involved 60 untrained panelists, who had previously been informed about the assessment procedure. The panelists, aged between 17 and 25 years, consisted of both men and women, students of the Food Technology Study Program, Faculty of Agricultural Technology, Widya Mandala Catholic University, Surabaya. Each panelist received 11 samples that had been assigned a random three-digit code. The panelists assigned a score of 1–15 to each sample, with the following categories: 1–3.0 strongly dislike, 3.1–6.0 dislike, 6.1–9.0 neutral, 9.1–12.0 like, and 12.1–15 strongly like. Each portion of the herbal tea was steeped with hot water at 95°C for 5

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minutes, then poured into 10 mL shot glasses for serving and tested. The best treatment of the steeped herbal tea was determined using a spider web graph.

Statistical analysis

The study was conducted using a randomized block design, with a single factor of green tea-pluchea leaf powder proportions, at 11 levels (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100% w/w). All samples for each treatment were analyzed four times, and data obtained were expressed as the mean ± standard deviation. All data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test using SPSS (SAS/STAT version 23.0, SAS Institute Inc., Cary, NC, USA). Differences between mean values were regarded as significant at $P < 0.05$.

Results and Discussion

Herbal tea made from a 40:60% w/w mixture of pluchea leaf and butterfly pea flower powder exhibited high antioxidant activity, however, its consumer acceptance was lower than that of other proportions. The addition of green tea, which has a variety of bioactive compounds, can increase the bioactive compounds content and antioxidant activity of the herbal tea without decreasing consumer acceptance.

Bioactive compounds content of pluchea leaf-butterfly pea flower herbal tea

The effect of incorporating green tea powder at various proportions into pluchea leaf powder on the bioactive compounds content of steeped pluchea leaf-butterfly pea flower herbal tea was investigated. The result indicated that incorporating green tea powder at proportions of 10 to 60% (w/w) significantly decreased the total phenolic content (TPC) in steeped tea samples, whereas proportions between 70 and 100% w/w significantly ($p \leq 0.5$) increased TPC (Figure 1). This observation indicates that the addition of green tea powder in the concentration range of 10 to 60% w/w may have caused an antagonistic interaction between the phenolic compounds of green tea, pluchea leaves, and butterfly pea flowers, resulting in reduced TPC.

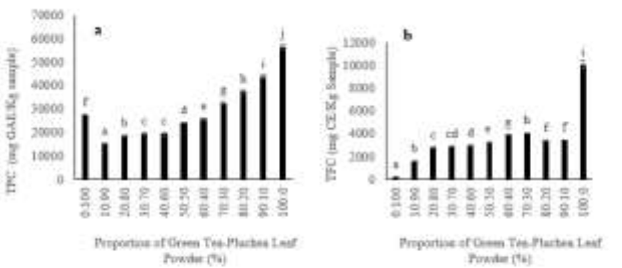

Figure 1: Total phenol content **(a)** and total flavonoid content **(b)** of steeped pluchea leaf-butterfly pea flower herbal tea with various proportions of green tea-pluchea leaf powder

The measurement of TPC is a reflection of the quantity of free hydroxyl groups in the benzene ring of phenolic compounds that can react with Follin-Ciocalteu's reagent. It has been reported that the quantification of TPC in a herbal sample is affected by the number of aromatic structures, their spatial distribution and hydroxyl groups.[15] While some interactions, such as the synergistic effects between protocatechuic and gallic acids or chlorogenic and gallic acids, may occur, others such as those between chlorogenic acids and protocatechuic or chlorogenic and vanillic acids do not exhibit interactions. Additionally, antagonism can occur between vanillic and gallic acids, whereas combinations of gallic, vanillic, and chlorogenic acids may result in synergistic interaction. The mixing of all four organic acids (gallic, vanillic, chlorogenic, and protocatechuic acids) could also lead to synergistic interactions.

Pluchea leaves have been shown to contain a variety of compounds, including quercetin (5.21 ± 0.26 mg/100 g wet weight), chlorogenic acid (20.00 ± 0.24 mg/100 g wet weight), caffeic acid (8.65 ± 0.46 mg/100 g wet weight), kaempferol (0.28 ± 0.02 mg/100 g wet weight), total anthocyanin (0.27 ± 0.01 mg/100 g weight wet), myricetin (0.09 ± 0.03 mg/100 g wet weight), β-carotene (1.70 ± 0.05 mg/100 g wet weight), total carotenoids (8.70 ± 0.34 mg/100 g wet weight), 3-*O*-*O*-dicafeoylquinic acid, 3,4-*O*-dicaffeoylquinic, 4,5-*O*-*O*-dicaffeoylquinic acid, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, and 5-*O*-caffeoylquinic acid.[14] On the other hand, green tea is rich in flavanols, flavonols, and phenolic acids including catechins, theaflavins, and thearubigins.[16,17] Catechins account for approximately 30 - 40% of green tea, primarily epicatechins (6.4%), epicatechin gallate (13.6%), epigallocatechin (19%), and epigallocatechin gallate (59%).[17] Green tea contains a broader range of phenolic compounds compared to other teas, including catechins, epicatechins, gallocatechin, epigallocatechin, catechin gallate, gallocatechin gallate, epigallocatechin gallate, ellagic acid, gallic acid, quercetin, astragalin, kaempferol, chlorogenic acid, caffeine, myricetin, and theaflavin.[17] The richness of butterfly pea flowers in phenolic compounds, such as gallic acid, ternatin, kaempferol, quchesol, and myricetin has also been highlighted.[18] Given the presence of phenolic compounds in pluchea leaves, green tea, and butterfly pea flowers, there is significant potential for both antagonistic and synergistic interactions.

Additionally, the interaction between proteins and phenolic compounds have been documented. Phenolic compounds can form complex compounds with protein through covalent or non-covalent bonds, such as hydrophobic, electrostatic, van der Waals, or hydrogen interactions.[19] The binding of polyphenols to proteins can alter protein conformation, leading to either soluble or insoluble complexes. Factors such as polyphenol concentration, molecular structure and weight, the presence of ions or cofactors, and system conditions influence the precipitation or solubility of these complexes. This, in turn, affects their functional and nutritional properties, including bioactivity. Pluchea leaves contain approximately 1.79 g/100 g wet weight of protein[14], while butterfly pea flowers contain 18 - 28 g/100 g dry weight of protein,[18] and green tea contains 20.54% protein.[20]

Likewise, the addition of green tea to pluchea leaves with a proportion of 10-100% w/w resulted in an increase in TFC levels, but the addition of 10-90% w/w resulted in an increase in TFC that was not as drastic as the proportion of 100% green tea, this indicates the existence of synergistic and antagonistic interactions between the bioactive components in the herbal tea drink.

Hidalgo *et al*.[21] reported that kaempferol and myricetin, myricetin and quercetin, and quercetin and catechin exhibit antagonistic interactions, while quercetin-3-β-glucosidase and epicatechin demonstrated a synergistic effect. Flavonoids, a class of polyphenol compounds, are among the most potent natural antioxidants. Pluchea leaves contain flavonoids such as quercetin (5.21 ± 0.26 mg/100 g wet weight), myricetin (0.09 ± 0.03 mg/100 g wet weight), kaempferol (0.28 ± 0.02 mg/100 g wet weight), and total anthocyanins (0.27 ± 0.01 mg/100 g wet weight).[14] Green tea is also rich in flavanols and flavonols, including epicatechin, catechin, gallocatechin, catechin gallate, epigallocatechin, epigallocatechin gallate, gallocatechin gallate, quercetin, astragalin, kaempferol, myricetin, caffeine, and theaflavin.[6,16,17] Butterfly pea flowers on the other hand, contain flavonoids such as ternatin, kaempferol, quercetin, and myricetin.[18]

Antioxidant activity of Pluchea leaf-Butterfly pea flower herbal tea

The bioactive compounds in the steeped herbal tea, particularly with the addition of green tea in various proportions, exhibited strong antioxidant activity as shown in Figure 2. The results indicate that incorporating green tea in various proportions to pluchea leaf-butterfly pea flower herbal tea enhanced the DPPH free radicals scavenging activity of the herbal tea. Although, the addition of green tea at 10 to 60% w/w reduced TPC, while proportions of 10 to 100% w/w increased TFC, these changes positively impacted the DPPH free radical scavenging activity. This means that adding TFC in various proportions to herbal tea drinks plays a greater role in donating hydrogen atoms to DPPH free radicals. However, this did not rule out the possibility that this finding suggests that the interaction between phenolic and flavonoid compounds in green tea, pluchea leaves, and butterfly pea flowers enhances their ability to donate hydrogen atom to DPPH radicals effectively. This interaction leads to the formation of DPPH-H molecule, changing the colour of the solution from purple to yellow. DPPH is a stable free radical with a single free electron on the nitrogen atom, allowing it to accept hydrogen atoms and form hydrazine compounds.[22] The stability of this radical is attributed to the push-pull effect caused by the picryl group as an electron acceptor and the diphenyl amino group as an electron donor, and the steric effect on the divalent nitrogen atom.

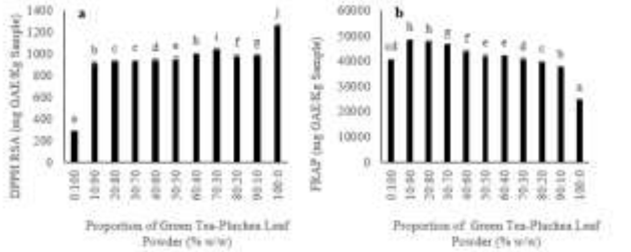

Figure 2: DPPH free radical scavenging activity (a) and ferric ion reducing antioxidant power (b) of steeped pluchea leaf-butterfly pea flower herbal tea with various proportions of green tea powder

The addition of green tea did not consistently enhance DPPH free radical scavenging activity of the herbal tea at all tested proportions (10 to 90% w/w). Certain compounds in green tea, particularly catechins, possess strong hydrogen-donating potential. Catechins play a major role in antioxidant activity, and the interactions between catechins and other phenolic compounds can lead to polymer formation, which enhances the ability of these bioactive compounds to donate hydrogen atoms to DPPH free radicals.[20] Powdering green tea leaves increases the concentration of catechins, making them more effective DPPH-free radical scavengers. Liang *et al*.[23] noted that compounds such as pyrogallol, protocatechuic acid, caffeic acid, gallic acid, and propyl gallate exhibit DPPH free radical scavenging activity. Hydroxylated cinnamic compounds, such as ferulic and caffeic acids, are among the most effective free radical scavengers, surpassing vanillic and protocatechuic acids. Approximately 80% of phenolic compounds demonstrate potential as free radical scavenging activity due to the high reactivity of their hydroxyl radicals.

In a similar vein, the ferric ion reducing antioxidant power (FRAP) of pluchea leaf-butterfly pea flower herbal tea significantly increased by incorporating 10-60% w/w green tea powder. However, adding 70-80% w/w green tea powder did not enhance FRAP activity and the proportion of green tea more than 80% w/w could reduce the ferric ion reducing power. The highest increase in FRAP activity was observed at lower green tea proportions (10-20% w/w) compared to 90-100% w/w green tea powder. Based on the ferric ion reducing power of steeped herbal tea showed synergistic and antagonistic effects of phenolic and flavonoid compounds.

The capacity to reduce ferric ions serves as an indicator of the antioxidant activity of polyphenolic compounds. This reducing power is linked to the presence of reductones, which break radical chains by donating hydrogen atoms.[24] Reducing ability depends on a compound's capacity to reduce Fe^{3+} ions to Fe^{2+} ions using potassium ferrocyanide, forming a blue complex detectable at maximum absorption wavelength of 700 nm.[24] Compounds capable of reducing ferric ions also act as antioxidants by stabilizing free radicals through electron or hydrogen atom donation.[25] The reduction of Fe^{3+} ions indicates that an antioxidant compound has strong antioxidant activity and is polar. FRAP analysis of steeped pluchea leaf-butterfly pea flower herbal tea with various green tea proportions confirmed that the bioactive components of green tea effectively reduce ferric ions. Chlorogenic acid, in particular, binds to Fe^{3+} ions to form complexes, demonstrating high efficiency in chelating Fe^{3+} ions, neutralizing hydroxyl radicals, and promoting iron oxidation, facilitating iron release from ferritin. Chlorogenic acid also reduces Fe^{3+}-induced polymerization.[26] It has been reported that caffeic acid and chlorogenic acid first form complexes with Fe^{3+} ions before reducing them, whereas naringin, ferulic acid, and sinapic acid directly transfer electrons to Fe^{3+} ions, converting them to Fe^{2+} ions without complex formation.[27]

Based on Pearson correlation analysis, the DPPH RSA of steeped herbal tea showed a strong and positive correlation with TPC, but a weak and positive correlation with TFC. In addition, a weak and positive correlation was found between DPPH antioxidant activity and FRAP. However, the content of bioactive compounds (TPC and TFC) in steeped herbal tea showed a very strong and negative correlation with FRAP, and there was a strong and positive correlation between TPC and TFC (Table 3).

Table 3: Pearson correlation coefficients between bioactive contents (TPC and TFC) and antioxidant activity (DPPH RSA and FRAP) of steeped pluchea leaf-butterfly pea flower herbal tea with various proportions of green tea-pluchea leaf powder

	FRAP	TFC	DPPH RSA	TPC
FRAP	1			
TFC	-0.814**	1		
DPPH RSA	-0.357*	0.775*	1	
TPC	-0.936**	0.742*	0.386**	1

Correlation significant at $p < 0.05$ (2-tailed).

In summary, the addition of green tea in various proportions contributed to enhance DPPH scavenging activity, driven by flavonoid compounds capable of donating hydrogen atoms. However, the ability to reduce iron ions in steeped herbal tea was thought to be influenced by non-phenolic phytochemical compounds. Green tea contains a range of bioactive non-phenolic compounds, including alkaloids (caffeine, theophylline, and theobromine), amino acids (theanine and γ-aminobutyric acid), organic acids (acetic, oxalic, ascorbic, citric, succinic, lactic, malic, tartaric, formic, butyric, and hexanoic acids), vitamins (B, C, and E), enzymes (glucosidase and lypoxidase), and chlorophyll.[28] These compounds are believed to play a significant role in the observed DPPH and FRAP antioxidant activities. Notably, the addition of green tea at a 10% w/w proportion resulted in a considerable increase in DPPH free radical scavenging activity, surpassing the ability to reduce ferric ions (FRAP). However, increasing the proportion of green tea beyond this point, did not lead to a substantial increase in antioxidant activity, suggesting that the phenolic components in green tea, pluchea leaves, and butterfly pea flowers may interact with the primary contributor to DPPH and FRAP antioxidant activities coming from non-phenolic compounds. Therefore, the antioxidant activity among bioactive compounds can be given in the following order; polyphenols > theanine > caffeine.[28] Additionally, the chelating ability of copper metal is one of the mechanisms through which green tea prevents peroxidation.

Sensory properties of pluchea leaf-butterfly pea flower herbal tea

The addition of green tea in various proportions significantly affected the colour (Figure 3a) and taste (Figure 3c) of pluchea leaf-butterfly pea flower herbal tea but did not result in any significant changes to its aroma (Figure 3b). Specifically, the preference for the colour of the herbal tea increased with the addition of green tea, particularly at proportions of 10 to 30% w/w. However, higher proportions of 40, 50, and 70% w/w did not show significant differences in preference, while the preference increased again with the addition of 60, 80, 90, and 100% w/w. The highest panelist acceptance for colour occurred with an 80% (w/w) green tea addition, yielding a preference score of 5.37 ± 0.98, which falls within the 'dislike' category. The colour of the herbal tea was primarily influenced by the blue hue of the butterfly pea flower, which is attributed to the anthocyanin compound delphinidin-3-glucosidase.[29]

The variations in colour preference among the steeped herbal tea may be attributed to interactions between the polyphenol compounds in the mixture. These interactions likely contributed to the observed pattern between the level of panelist acceptance of colour and the ability to reduce ferric ions. Polyphenol content, processing methods, and types of additional ingredients has been shown to affect the functional

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properties, nutritional value, and sensory characteristics of a beverage.[30] Additionally, the colour of the steeped herbal tea was influenced by the yellowish-green hues from the brewed green tea and pluchea leaves. The combined contributions of these three components shapened the overall appearance of the formulation.

Regarding aroma, the level of panelist preference for the steeped herbal tea did not differ significantly across the various proportions of green tea added (Figure 3b). This is likely because the contribution of volatile compounds from pluchea leaves, green tea, and butterfly pea flowers remained consistent, resulting in a similar level of panelist acceptance. Wintergreen tea has been reported to contain 18 volatile components, including geranyl acetone, geraniol, jasmone, phenethyl alcohol, 1-octen-3-ol, β-ionone, and longifolene, whereas summer green tea has a different set of 13 components such as indole, 2-methylfuran, ethanethiol, D-limonene, and 1-octanol.[31] On the other hand, 66 volatile components has been reported in pluchea leaves, with (10S,11S)-himachala-3-(12)-4-diene being the most prevalent at 17.13%.[9] According to Marpaung,[29] butterfly pea flowers have a grassy aroma, although some claim that butterfly pea flowers do not have a distinct aroma, which does not affect their use in herbal mixtures. Prihandini *et al*.[32] also stated that while butterfly pea flowers do not have a distinct aroma, their water extract may produce a slightly unpleasant scent. Thus, increasing the proportion of green tea did not significantly alter the panelists' aroma preference, as the proportion of butterfly pea flowers (60% w/w) dominated the blend compared to the green tea (0-40% w/w) and pluchea leaves (0-40% w/w).

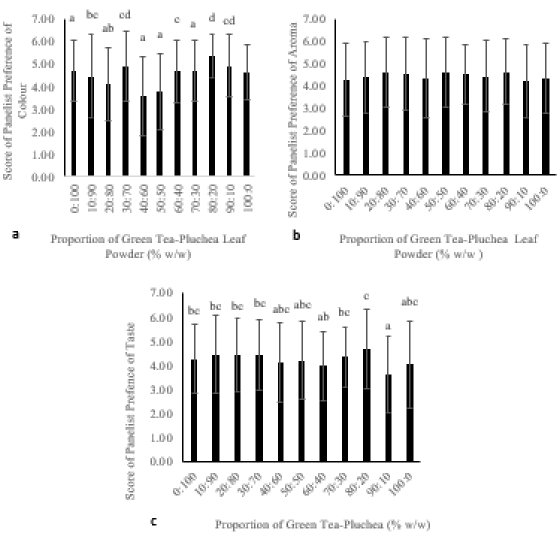

Figure 3: Score of panelist preference; **(a)** Colour **(b)** Aroma, and **(c)** Taste of steeped pluchea leaf-butterfly pea flower herbal tea with various proportions of green tea powder

Panelist preferences for taste showed that adding green tea powder at proportions of 0-30 and 70% w/w did not significantly differ. However, when green tea powder was added at proportions of 40-60% w/w and 100% w/w, there was a significant decrease in taste preference, with the decrease being more pronounced at the 90% w/w level of green tea powder. The addition of 80% w/w green tea yielded the highest taste preference among the panelists (Figure 3c). According to Prihandini *et al*.,[32] the taste of food ingredients is influenced by various factors, including concentration, chemical composition, temperature, and interaction with other taste components. Based on the taste preference results, the pluchea leaf-butterfly pea flower herbal tea exhibited a bitter taste with an astringent sensation. This bitterness stemmed from the tannin compounds in pluchea leaves and the catechin compounds in green tea, while butterfly pea flowers contributed a sweet, sour, and slightly bitter taste. The interaction among the active components in each ingredient produced a unique taste and sensory experience. Results from spider web graph analysis, based on attributes of taste, colour, and aroma of pluchea leaf-butterfly pea flower herbal tea with various proportions of green tea powder, indicated that the best treatment was 80% w/w proportion of green tea, covering an area of 20.7497 cm^2 (Table 4 and Figure 4).

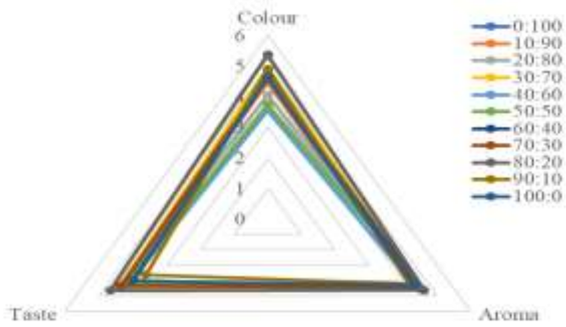

Figure 4: Spider web graph of sensory properties of steeped pluchea leaf-butterfly pea flower herbal tea with various proportions of green tea-pluchea leaf powder

The herbal tea with 80% w/w green tea resulted in the following sensory attributes: colour measured at 7.2177 cm^2, aroma at 6.2640 cm^2, and taste at 7.2680 cm^2 (Table 4). This particular formulation had TPC of 42,007 ± 1,599.49 mg GAE/kg of dried herbal tea, TFC of 307 ± 4.09 mg CE/kg of dried herbal tea, DPPH free radical scavenging activity of 0.293 ± 0.0053 mg GAE/kg of dried herbal tea, and FRAP of 4,200 ± 34.18 mg GAE/ kg of dried herbal tea.

The above observations led to the conclusion that adding green tea at a proportion of 80% w/w to pluchea leaf-butterfly pea flower herbal tea increased the amounts of bioactive compounds (TPC and TFC) and enhanced antioxidant activity (DPPH and FRAP). Furthermore, this combination improved the panelists' preferences for the colour, taste, and aroma of the herbal tea made with green tea and pluchea leaf powders at a 40:60% w/w ratio.

Table 4: Spider web graph area of sensory properties of pluchea leaf-butterfly pea herbal tea with various proportions of green tea-pluchea leaf powder

Proportion of green tea:pluchea leaf powder (% w/w)	Area (cm^2)			
	Colour	Aroma	Taste	Total
0:100	5.8038	5.2835	5.7819	16.8693
10:90	5.6696	5.6490	5.7321	17.0507
20:80	5.4240	5.8975	5.2146	16.5361
30:70	6.4514	5.7723	6.2447	18.4684
40:60	4.4897	5.1398	4.2226	13.8521
50:50	5.0285	5.6354	4.5555	15.2194
60:40	6.1330	5.2205	5.3691	16.7227
70:30	6.0229	5.5867	5.8915	17.5010
80:20	7.2177	6.2640	7.2680	20.7497
90:10	5.9692	4.3984	5.1427	15.5104
100:0	5.8088	5.0397	5.3970	16.2456

Conclusion

The mixture of green tea and pluchea leaf powders at 80:20% w/w resulted in the best sensory properties of green tea-pluchea leaf-butterfly pea flower herbal tea. Compared to the 40:60% w/w ratio, this combination significantly increased the content of bioactive compounds in the herbal tea, including total phenolic content (TPC) and total flavonoid content (TFC), as well as antioxidant activity, as measured by

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DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) in steeped herbal tea. These findings indicate that the incorporation of green tea into pluchea leaf and butterfly pea flower powders have strong potential for development as a functional herbal beverage.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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