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Production and Quality Evaluation of Herbal Tea Mixtures from *Phyllanthus Debilis, Osbeckia octandra*, and *Artrocarpus heterophyllus* Leaves

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Abstract.
Introduction: *This study investigated the acceptability and quality of herbal tea mixtures prepared using* Phyllanthus debilis, Osbeckia octandra, *and* Artrocarpus heterophyllus *leaves powders in different proportions. Preference for different sensory attributes of tea samples was evaluated using thirty untrained panelists. Proximate composition, tea quality parameters, and microbial counts of tea samples were evaluated as per AOAC and ISO standard methods. The amount of major and trace minerals was evaluated using atomic absorption spectrophotometer. Results revealed that dark-colored samples were much preferred by the consumers. A significant positive correlation (r=0.920) showed among redness (a*) of teas and color preference. Yet, the overall preference was reduced in more bitter and astringent samples. Overall acceptability scores highest for* A. heterophyllus *tea (M9), yet the color preference was highest in* P. debilis *tea (M8) and mix of* P. debilis and O. octandra *(M4). The tea samples were rich in nutrients, yet crude fiber and moisture contents exceed the required maximum limits. Heavy metal concentrations and microbial counts lay within the standard limits. This study showed mixing herbs can enhance the acceptability and quality of herbal infusions. With such optimizations, these novel tea mixtures can thrive commercially in the tea market.*

Keywords: Herbal tea, Quality parameters, Sensory attributes

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1. Introduction

People have been seeking medicine from natural sources to cure diseases since ancient times.[1] Knowledge of exploiting plants to treat different ailments was passed down from generation to generation, both written and verbal. Even today, 80% of people in developing countries use traditional and complementary medicine, particularly herbal therapies, for primary healthcare.[2]

In addition, the prophylactic benefits of these herbs are being investigated for potential use in novel pharmaceuticals, nutraceuticals, and functional foods.[3]

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Functional foods are foods that offer health benefits beyond their nutritional value and they are becoming increasingly popular as a convenient and inexpensive solution to chronic health problems.[4] Herbal tea or tisanes are a type of functional food that has gained considerable interest recently. They are infusions or decoctions made from fresh or dried roots, stems, leaves, fruits, flowers, seeds, bark, or whole plants from one or more herbal tea plant species.[5] The herbal tea market was valued at USD 3,290.17 million in 2021 and is expected to reach the value of USD 5,445.22 million by 2029, at a compound annual growth rate (CAGR) of 6.50% during the forecast period of 2022-2029.[6] There are plenty of herbal tea products in the Sri Lankan market and tea is the most preferred functional food product among local consumers.[7] Plants used in Sri Lankan traditional medicine systems are gradually being introduced into the market as herbal teas. However, the scientific investigation of their efficacy, safety, and production qualities is scanty.

Incorporating herbs into food matrices will offer a variety of flavors, aromas, visual appeal, texture and antimicrobial properties to food.[8] Some consumers face difficulties in accepting herbal foods due to characteristic flavors and colors.[9] Therefore, it is vital to evaluate the sensory properties before introduce them to the market place. Measuring tea quality parameters is vital to maintain the briskness/astringency, brightness, and safety of herbal teas.

In Sri Lankan traditional medicine and folk medicine, the whole plant of *P.debilis* or its parts is used to prepare porridge or 'decoction' to treat ailments such as liver complications, diabetes mellitus, and skin diseases. Matured *O. octandra* leaves are consumed as porridges and reported to have antidiabetic and hepatoprotective properties.[10-12] *A. heterophyllus* leaves are utilised in Siddha medicine and folk medicine systems to treat diabetes.[13]

This study aimed at producing herbal teas using different proportions of *Phyllanthus debilis*, *Osbeckia octandra*, and *Artrocarpus heterophyllus* leaves and select the best tea sample for mass production based on sensory properties, tea quality parameters, microbial quality, and safety of the herbal tea samples.

2. Materials and methods

2.1 Preparation of herbal tea

Phyllanthus debilis (Niruri), *Artocarpus heterophyllus* (Jak), and *Osbeckia octandra* (Eight Stamen Osbeckia) leaves were screened for production based on their ethnopharmacological importance, Ayurveda claims and previous studies of the same research group (unpublished data). Plants were collected from Balangoda district, Sri Lanka and authenticated by comparison with the voucher specimen deposited at the National Botanical Gardens Peradeniya, Sri Lanka.

Leaves of the plants were separated and air dried in a dry and shady place. Dried leaves were pulverized and sieved (seiver with 600 nm mesh diameter). Powdered plant samples were stored in airtight zip lock bags at -80 ^{0}C (Nove-80 freezer) until use. The powdered leaves were mixed at different ratios to prepare the different herbal infusions (Table 1).

Table 1: Composition of herbal tea products

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Sample code	Leaves powder %		
	Phylanthus debilis	*Osbeckia octandra*	*Artrocarpus heterophyllus*
M3	-	100.00	-
M4	66.67	16.66	16.66
M5	50.00	-	50.00
M7	33.33	33.33	33.33
M8	100.00	-	-
M9	-	-	100.00

Leaves powders (individual/mixtures) were dipped in hot water (80 ^{0}C) for 10 min until attained room temperature and filtered using Watman no.1 filter papers and the filtrate was separated. Freshly prepared infusions were used for the tests. Proximate composition was tested for the dry powders. Plant powder to water ratio was 1:75.

2.2 *Sensory properties*

Sensory properties of selected herbal infusions were evaluated using thirty untrained panelists at Sabaragamuwa University of Sri Lanka. Sensory attributes (Color, aroma, astringency, bitterness, aftertaste, and overall acceptability) were evaluated using a five-point hedonic scale test (5=strongly like and 1=strongly dislike).

2.3 Instrumental color values

The colour of the samples was measured using a Hunter Lab colour meter (CR 400, Conika Minolta, Japan). Measurements were taken directly at three different locations, after standardization with a white calibration plate (L* = 94.12, a* = 0.29, and b*= 2.73). Colour was expressed in Hunter Lab units L*, a* and b*, where L* indicates lightness, a* indicates hue on a green (–) to red (+) axis, and b* indicates hue on a blue (–) to yellow (+) axis.[14]

2.4 Proximate composition, tea quality parameters and mineral content

The crude protein, crude fat and total fibre content were determined in dry basis by using AOAC Method 920.152, 920.39, and 991.43, respectively.[15.16]

Loss in mass at 103 ^{0}C, total ash, and acid-insoluble ash contents were determined as per the methods described in ISO 1573:1980.[17] ISO 1575:1987, and ISO 1577:1987, respectively.[18] Loss in mass at 103 ^{0}C (moisture content) was determined after oven drying to a constant weight at 103^{0}C. Total ash content was obtained by incinerating the sample in a muffle furnace (Obersal) at 550^{0}C for five hours until whitish gray ash was obtained.

Concentration of major elements (Na, K) and trace elements (Ni, Zn, Mn, Cu, Pb, Cd) of selected tea mixtures were measured as per the method utilized by Perera et al. (2020)[19] with slight modifications using Atomic Absorption Spectrophotometer (AAS) (Varian 240FS).

2.5 Microbial quality evaluation

Aerobic plate count, yeast and mould, and *E coli* counts were determined by ISO 4833:2013, ISO 21527-2:2008, and ISO 7251:2005, respectively.[20]

2.6 Statistical analysis

Statistical analysis of the results was performed using Minitab 17 software. The significance of the difference of tested parameters among samples was evaluated using one-way ANOVA method and the Tukey's test, at a significance level of a = 0.05.The relationship between sensory and instrumental data was explored through calculating the Pearson's correlation coefficient at $P \leq 0.05$.

3. Results and discussion

3.1 Sensory properties of selected herbal tea infusions

Sensory attributes of tea infusions occurred due to their chemical compositions.[21] As different herbs have diverse chemical compositions, the consumer preference for the selected samples is significantly different except for the attribute aroma preference (p=0.063) (Table 2).

Even though phytochemicals such as phenols, flavonoids, isoflavones, terpenes, and glucosinolates have numerous health benefits, most of them have a bitter, acrid, or astringent taste and therefore aversive to the consumer.[22] Astringency usually involves the formation of aggregated precipitates between tannins or polyphenols and proteins in the saliva.[23] However, some findings revealed bitter phytocompounds have a higher probability of exerting health benefits.[24]

In the study, M7 and M9 obtained highest preference for bitterness and M7 obtained highest preference to astringency (Table 2) (Figure 1). Consumers much prefer for those samples which having less bitterness and astringency. Overall acceptability also highest in these samples (M9 and M7) which demonstrated bitterness and astringency have a considerable impact in consumer preference for the herbal teas. Sample M8 have the least bitterness and aftertaste preference and overall acceptability while M3 have least astringency preference. By mixing the herbs, overall acceptability and the preference for color, bitterness, astringency and aftertaste can be increased. Hence, polyherbalism have advantageous effect on product optimization as well.

Table 2: Average rank values of consumer preference data

Sample	Color	Aroma	bitterness	Astringency	After taste	Overall acceptability
M3	2.50±0.99[b]	2.94±1.21[a]	2.89±0.78[ab]	2.56±1.20[b]	2.89±1.28[ab]	2.78±1.00[ab]
M4	4.17±0.79[a]	3.61±0.78[a]	2.89±1.07[ab]	2.94±0.80[ab]	2.89±0.96[ab]	2.94±0.96[ab]
M5	3.61±1.04[a]	3.44±0.78[a]	3.28±1.28[ab]	3.22±0.88[ab]	3.22±1.22[ab]	3.33±1.03[ab]
M7	3.56±0.78[a]	3.33±0.84[a]	3.44±0.96[a]	3.56±0.78[a]	3.22±0.94[ab]	3.61±0.85[a]
M8	4.17±1.04[a]	3.61±0.85[a]	2.39±1.04[b]	2.94±1.06[ab]	2.44±0.98[b]	2.56±0.98[b]
M9	**3.33±0.91[ab]**	**2.94±0.73[a]**	**3.72 ±0.83[a]**	**3.33±0.91[ab]**	**3.61 ±1.09[a]**	**3.67 ±1.03[a]**
P value	0.000	0.063	0.002	0.036	0.041	0.002

Mean ± SE (n=30). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

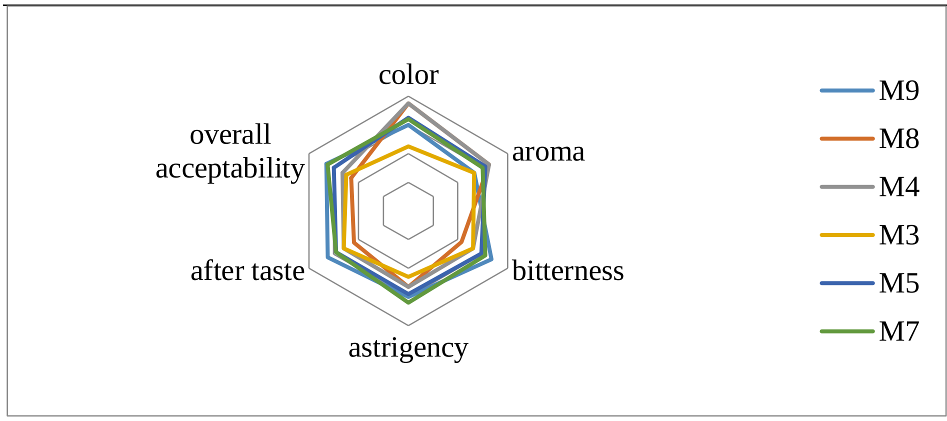

Figure 1: Average rank values of consumer preference data

Food color have largely impact to the purchasing decision of consumer.[21] In here, a correlation analysis was conducted to measure the strength and direction of association between sensory data and instrumental color values. The results showed that statistically significant negative correlation among color preference and both L* (whiteness) and b* (yellowness) values while the correlation was positive for the color preference and a* (redness) value (Table 4). That means darker the tea color (redness increases, whiteness, and yellowness decreases), color preference of the consumer increased. Table 3 showed the lightness (L*) highest in M3 and lowest in M8. By mixing the herbs in different propositions the color values can be adjust.

Table 3: Instrumental color values of the samples

Sample	**L***	**a***	**b***
M3	25.72±0.51^{a}	-0.17±0.01^{e}	11.28±0.06^{a}
M4	13.40±0.03^{d}	4.15±0.01^{b}	2.59±0.01^{e}
M5	15.66±0.19^{c}	3.05±0.02^{c}	7.41±0.05^{c}
M7	15.56±0.19^{c}	4.41±0.14^{b}	6.73±0.03d
M8	13.74±0.20^{d}	5.38±0.03^{a}	1.81±0.04^{f}
M9	**18.32±0.01^{b}**	**1.19±0.16^{d}**	**9.15±0.03^{b}**
P value	0.000	0.000	0.000

Table 4: linear correlation between sensory and instrumental color parameters

Parameters	**Color**	**Overall acceptability**
Lightness	-0.926*	-0.164
a* coordinate	0.920*	-0.18
b* coordinate	-0.876*	0.166

* Correlations are statistically significant (P<0.05)

Based on the sensory quality attributes, particularly the color values, M4, M5, and M8 were selected for further product quality tests.

3.2 Proximate composition and tea quality parameters

The samples studied significantly ($p<0.05$) differed in all the proximate parameters. Study of Rehman et al. (2002) suggested 1-2% of protein and 0.95-1.62% of fat contents for better quality of tea samples.[25] In contrast, in these herbal mixtures, comparatively high fat contents (4.80-6.25%) were observed. Highest crude protein and fat content was observed in sample M4. Crude fiber contents of these herbal teas were slightly exceeding the maximum limit 16.50%. Crude fiber content highest in M5 may be due to high fiber content of *A. heterophyllus*. The moisture contents of the samples studied were above the standard value of 7% and this may have a negative impact on the shelf life of the samples. Total ash and acid insoluble ash contents are in the standard range.[26]

Biomolecules in herbs (phytochemicals, protein and carbohydrate) are responsible for organoleptic, gustatory and medicinal properties of tea. Extraction of these molecules to the tea brew is depend on various parameters such as caffeine content, boiling time and temperature This aspect also should consider in herbal tea production.[25-27]

Table 5: Proximate composition and tea quality parameters

Parameter	**Dry basis %**			**Required maximum limit (%)***
	Mix 4	**Mix 5**	**Mix 8**	
Crude protein	2.91±0.11^a	1.65±0.04^b	2.02±0.01^b	-
Fat	6.25±0.25^a	5.33±0.00^b	4.80±0.07^b	-
Crude fiber	20.67±0.33^b	36.33±2.03^a	18.67±1.76^b	16.50
Moisture	13.83±0.17^b	12.83±0.17^a	11.17±0.17^a	7.00
Total ash	7.08±0.68^a	6.77±0.65^a	8.24±0.74^a	4-8
Acid insoluble ash	0.56±0.02^b	0.09±0.01^c	1.20±0.05^a	1.0

*Source: [26]

3.3 Minerals content

Foods and beverages are key route for human to expose metals. Some studies showed herbal teas contain higher heavy metal contents than traditional teas.[28] As these are totally new formulations and the ingredients are not frequently use by the consumers, it is vital to test for the heavy metal contents.

Table 6 showed the results for the heavy metal content of selected tea infusions. Accordingly, Zn, Cd, Cu, and Pb levels were within the standard limits for herbal teas. Other heavy metals also were in minute amounts. K and Na are highest in sample M4.

Table 6: Mineral content of selected polyherbal compositions

Parameter	ppm				
	M4	M5	M8	Maximum limit *	
				WHO	FDA
Na	0.340±0.00^{a}	0.297±0.00^{c}	0.32±0.02^{b}	-	
K	211.66±0.58^{a}	155±1.00^{c}	185.66±2.08^{b}	-	
Cu	0.032±0.00^{a}	0.023±0.00^{b}	0.030±0.00^{a}	20.00	20.00
Mn	1.242±0.00^{c}	2.058±0.01^{a}	2.002±0.00^{b}	-	
Zn	**0.014±0.00^{c}**	**0.115±0.00^{a}**	**0.1023±0.00^{b}**	**50.00**	**50.00**
Cd	0.006±0.00	BDL	BDL	0.20	0.30
Pb	0.03±0.00^{a}	0.01±0.00^{b}	0.01±0.00^{b}	10.00	10.00
Ni	BDL	BDL	BDL	-	

BDL-Bellow detected limit.WHO-Word Health Organization, FDA- US Food and Drug Administration

*Source: [29]

3.4 Microbial quality of herbal infusions

Poor agricultural, collection, handling and storage practices of herbal products may leads to poor microbial quality. Insufficient drying leads to increased level of microbial contamination, hence, drying time and temperature is a critical control point in herbal tea production. Some studies unveiled that market available herbal teas were contaminated with both bacteria and fungi at various levels may be potential risk for the regular consumer arising public health issue.[30.31]

Table 7 showed the microbiological analysis for the tea mixtures soon after the production. Total viable count of all samples was less than 10^4 cfu/g and yeasts and moulds were within the limits. *E. coli* was not detected. *P. debilis, O. octrandra, A. heterophyllus* were reported to have antimicrobial properties against *Salmonella typhi* and *Escherichia coli*.[32] This antimicrobial property may be reason for the higher microbial quality of tea mixtures. Yet, suitable packaging materials and storing conditions need to select to ensure the keeping quality of the product at mass scale production.

Table 7: Microbial quality of herbal tea infusions

Type of Micro-organisms	M4	M5	M8	**Maximum limits***
Total Viable Count (Aerobic colony count), cfu/g	$1.3*10^3$	$2.0*10^3$	$2.3*10^3$	$2.0*10^5$
Yeasts, cfu/g	<100	<100	<100	$1.0*10^2$

Moulds, cfu/g	<100	<100	<100	$1.0*10^3$
E.coli, cfu/g	ND	ND	ND	Absent

*[26]; ND-not Detected

Mean is average of two replicates and express as CFU/g. Mean with different letters in the same row are significantly different P≤0.05.

4. Conclusion

The results showed sensory qualities, nutrition composition, and tea quality parameters of herbal teas can alter by mixing different herbs. This concept is utilized in Ayurveda to enhance the efficacy of herbal treatments, called polyherbalism. However, the herbal-herbal interactions should be taken into account in such modifications. The sensory evaluation demonstrated samples with high redness (a*) were much preferred by the consumers. Yet, the highest bitterness and astringency reduced the overall acceptability. Overall acceptability scores were highest for *A. heterophyllus* tea (M9) with less astringency and bitterness. However, the color preference was highest in *P. debilis* tea (M8) and mix of *P. debilis* and *O. octandra* (M4). The tea samples were rich in nutrients. Heavy metal concentrations and microbial counts lay within the standard limits stipulated by WHO and FDA. After accessing the functional properties, these novel tea mixtures can thrive commercially in the tea market.

Ethics approval: Sumatera Medical Journal (SUMEJ) is a peer-reviewed electronic international journal. This statement below clarifies ethical behavior of all parties involved in the act of publishing an article in Sumatera Medical Journal (SUMEJ), including the authors, the chief editor, the Editorial Board, the peer-reviewer and the publisher (TALENTA Publisher Universitas Sumatera Utara). This statement is based on COPE’s Best Practice Guidelines for Journal Editors.

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