RESEARCH ARTICLE

Evaluation of physicochemical and organoleptic properties of wild tea (*Camellia sinensis*) compared to selected commercially grown tea cultivars in Sri Lanka

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ABSTRACT

"Ceylon tea", has a special place as one of the best quality tea among the tea drinkers worldwide. However, Sri Lankan tea industry is facing some challenges associated with lower productivity and increasing cost of production. Therefore, higher price should be gained through value addition and product differentiation. Production of wild tea is one such strategy associated with higher demand in niche markets. An experiment was conducted to evaluate qualities of wild tea with compared to commercially cultivated tea and free growing cultivar in the Low Country region in Sri Lanka. Wild tea, free growing cultivar (TRI 2025), two commercial cultivars from the Tea Research Station in Rathnapura (TRI 4049 and TRI 2023) and one cultivar from a commercial grower (TRI 2023) were used for evaluation. Phytochemical investigation was carried out on methanol crude extracts of the made tea leaves. The made tea samples were subjected to organoleptic evaluation to determine its quality and preference. Significantly highest total phenolic content, total flavonoid content and the highest $ABTS^+$ free radical scavenging activities were recorded in wild tea than other commercial cultivars. Organoleptic qualities such as colour, aroma, taste, strength and overall acceptability were favorably higher in wild tea, over the other commercial cultivars.

***Keywords**: Antioxidant properties, total polyphenol content, total flavonoid content, $ABTS^+$ free radical scavenging activity, organoleptic qualities, wild tea*

INTRODUCTION

Tea (*Camellia sinencis*) is one of the most popular natural beverages and unique to the tropical weather and monsoons in Sri Lanka which enables tea industry to flourish. At present tea has become a key industry in the economy of Sri Lanka. As the highest net foreign exchange generator for many decades, tea is considered to be the most important agribusiness in the country (Ariyawardana, 2001). According to the Central Bank Report (Anon, 2017), tea production recorded a positive growth in 2017 but in a declining trend in the past four years. Accordingly, production recovered with a growth of 5.2%, resulting in an output of 307.7 million kilograms in 2017, compared to 292.6 million kilograms in 2016.

However, at present, the Sri Lankan tea industry is facing some challenges such as increasing cost of production and low productivity in factory and in field level rising competition of newly emerging low-cost producer countries (Hilal and Mubarak, 2016). These weaknesses of the tea industry mean that there is less possibility of pursuing a low-cost strategy in competing with other countries. Therefore, product differentiation achieved through value-addition should be considered as a better strategy in positioning country within the global tea industry (Ariyawardana, 2001).

Wild tea is tea produced from unattended tea plants which are usually older than 100 years (Julian, 2016). There is a good chance to develop wild tea as a new product under the brand of Ceylon Tea. It is grown from wild tea seeds within the natural forests or cultivated in upland fields where it is mixed with other crops or transplanted to tea gardens. Yunnan province in China and Laos make wild tea product and they have obtained high market value and a niche market is being developed for wild tea products globally (Pedersen *et al.*, 2016).

In Sri Lanka wild relatives of the tea plant cannot be found. However, unproductive abandoned tea cultivations, which are more than 50-100 years old, can be found in the Sri Lanka. These cultivations have freely grown with other vegetative cover and converted to the natural forests at present.

Due to the increasing cost of production and rising competition of newly emerging low-cost producer countries, it strongly needs to maintain the unique price for Ceylon tea. Therefore, evaluations of the existing variability of wild tea within the country by exploring variation of quality and antioxidant properties are very essential.

Present study was undertaken to evaluate antioxidant properties and organoleptic qualities of wild tea in comparison to commercially cultivated cultivars in the Low Country Region of Sri Lanka.

MATERIALS AND METHODS

Experimental design and plant material

Experiment was conducted according to Complete Randomized Design with five replications. Shoots with a bud and two leaves were collected from each tea plant (*Camellia sinensis)*. Five tea cultivars were used for the study as follows;

Wild tea leaves were harvested from a 130 y abandoned tea field, which now appears as a forest in Erathna (06° 83’ N, 80° 44’ E) (828 m amsl), Sri Lanka. Leaves of TRI 2025 were harvested from 10 y abandoned tea field (free growing) in St. Joachim estate in Ratnapura, Tea Research Institute (06° 73’ N, 80° 36’ E) (29 m amsl). Meanwhile, the leaves of TRI 4049 were harvested from normal trained field in St. Joachim estate in Ratnapura, Tea Research Institute (06° 72’ N, 80° 36’ E) (29 m amsl). Leaves of TRI 2023 were harvested from normal trained field in St. Joachim estate in Ratnapura (06° 72’ N, 80° 36’ E) (29 m amsl). Finally, the leaves of TRI 2023 were harvested from commercially cultivated field in Erathna (06° 83’ N, 80° 40’ E) (710 m amsl).

Processing

Tea leaves were carefully transported to Forest hill (Pvt.) Ltd. in Erathna for processing. Then, tea shoots were laid out to wither indoors for 24 h at room temperature, and then rolled by hand for 15 min. Thereafter rolled tea leaves were allowed to ferment by covering a wet clean cloth for 1.30 h and the fermented shoots were dried at 80 °C for 4 h.

Sample preparation for antioxidant assay

Dry solid crude extracts were obtained from 4 g of made tea leaf of each sample following the method described by Dissanayake *et al*. (2018). The dried solid crude extracts were labeled and stored at -80 °C until used.

Total polyphenol content (TPC) assay

The Folin-Ciocalteu method (Singleton *et al*., 1999) was adopted to determine Total Polyphenolic Content (TPC) in crude extract of processed tea leaves. The TPC was estimated as Gallic Acid Equivalents (GAE) using Gallic acid standard curve and expressed as mg GAE/g of dried crude extracts.

Total flavonoid content (TFC) assay

Total flavonoid content in crude extract of processed tea leaves was determined by aluminum chloride method (Siddhuraju and Becker, 2003). Total Flavonoid Content (TFC) of tea crude extracts of processed tea leaves were estimated as Quercetin Equivalents (QE) using Quercetin standard curve and expressed as mg QE/g of dried crude extracts.

$ABTS^+$ free radical scavenging assay

$ABTS^+$ free radical scavenging assay of crude extract of processed tea leaves was determined using 2,2-azinobis-3-ethylbenzothiozoline-6-sulfonic acid ($ABTS^+$) radicals according to the method described by Re *et al.* (1999).

Sensory evaluation for made tea

The made tea samples were subjected to organoleptic evaluation to determine its quality and preference by thirty untrained panel members in the age group of 20 – 28 y from the faculty of Agricultural Sciences in Sabaragamuwa University of Sri Lanka. Tea infusions were prepared by brewing processed tea leaves (100 g) in 5 L of boiling water for five min. The samples were evaluated for Colour, Aroma, Taste, Strength and overall acceptability on a nine-point hedonic scale, where score of nine point stood for excellent and one for highly unacceptable.

Data analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by comparison of means by Fisher's Least Significant Difference (LSD) test. All results presented in tables and graphs are mean of samples with a standard error.

RESULTS AND DISCUSSION

Total polyphenolic content, total flavonoid content and $ABTS^+$ radical scavenging activity

Average TPC of tea crude extracts were significantly different ($P<0.05$) among wild tea, free growing, abandoned TRI 2025 and commercial cultivated TRI 4049, TRI 2023 (Rathnapura), TRI 2023 (Erathna) (Table1). The significantly ($P<0.05$) highest TPC resulted in wild tea crude solid extracts (149.5±0.74 mg GAE/g of crude solid extracts) compared with free growing and commercially cultivated tea. The lowest resulted in TRI 2023 (E) extracts (112.4±1.12 mg GAE/g of crude solid extracts) but it was not significantly ($P>0.05$) different from free growing TRI 2025 (B) and TRI 4049 (C), TRI 2023 (D) commercial cultivated tea (Table 1).

Average TFC of tea crude extracts were significantly ($P<0.05$) different among each extracts of wild tea, free growing TRI 2025 and commercial cultivated TRI 4049 (C), TRI 2023 (D), TRI 2023 (E) tea. The results from mean comparison among them revealed that the highest TFC resulted in wild tea extracts (14.9±0.29 mg Quercetin equivalent/g of crude solid extracts) and lowest resulted in TRI 2023 extracts (10.3±0.10 mg Quercetin equivalent/g of crude solid extracts), but it was not significantly ($P>0.05$) different from free growing TRI 2025 and TRI 4049, TRI 2023 commercial cultivated tea.

$ABTS^+$ free radical scavenging activity detected by IC_{50} of all tea extract was significantly ($P<0.05$) different among wild tea, Free growing TRI 2025 and commercially cultivated TRI 4049, TRI 2023, TRI 2023 (Table 1). The results from mean comparison among them revealed that the lowest IC_{50} resulted in wild tea extracts (1.1±0.05 mg trolox/g of extract) and the highest IC_{50} resulted in TRI 4049 tea extracts (2.8±0.22 mg trolox/g of extract) but it was not significantly ($P>0.05$) different from free growing TRI 2025 and TRI 2023, TRI 2023 commercial cultivated tea. The IC_{50} of a compound is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant required to decrease the ABTS concentration by 50%. A lower IC_{50} indicates a higher antioxidant activity of a compound.

TPC content, TFC content and $ABTS^+$ free radical scavenging activity, in wild tea (Table 1) were significantly ($P<0.05$) high compared to other tea samples.

This indicated that TPC and TFC directly related to the antioxidant activity of tea. This is aligned with previously recorded study (Saeed *et al.*, 2012). Phenolic compounds are essential plant compounds and are major factors responsible for biological activities such as antioxidant, antimicrobial, antiviral and anticancer activities (Katiyar *et al.*, 1996). Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids (Wojdyło *et al.*, 2007).

Previous studies suggest that consumption of green and black tea beverages may bring positive health effects (Peter *et al.*, 2001). One hypothesis explaining such effects is that the high levels of flavonoids in tea which can protect cells and tissues from oxidative damage by scavenging oxygen-free radicals. Rietveld and Wiseman (2003) mentioned that tea flavonoids are potent antioxidants that are absorbed from the gut after consumption and significantly increase the antioxidant capacity of the blood.

Table 1: Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and $ABTS^+$ free radical scavenging activity (as IC_{50} value) of wild tea and commercial growing tea cultivars. Values are presented as mean (±SE) of 5 independent replicates. Values with different superscript in column are significantly different ($P<0.05$).

Tea plant	TPC (mg GAE/g of crude extract)	TFC (mg QE/g of crude extract)	IC_{50} (mg trolox/g of crude extract)
Wild tea (A)	149.5±0.74[a]	14.9±0.29[a]	1.1±0.05[b]
Free growing TRI 2025 (B)	114.5±1.65[b]	11.0±0.28[b]	2.7±0.04[a]
TRI 4049 (C)	112.8±1.20[b]	10.4±0.27[b]	2.8±0.22[a]
TRI 2023 Rathnapura (D)	112.7±1.80[b]	10.3±0.10[b]	2.6±0.06[a]
TRI 2023 Erathna (E)	112.4±1.12[b]	10.9±0.13[b]	2.5±0.13[a]

In this context wild tea showed highly positive impact as a healthy tea for consumers compared to other commercially grown tea. Accordingly, wild tea plants studied showed high standard in antioxidant activities compared to other commercial varieties used in the study. According to some studies, green tea was found to be more beneficial than black tea when consider the antioxidant activities, but some studies described that both have same effect. Our present results proved that wild tea was in ahead of other black teas in commercial use and can be considered more health benefit over other. However, reason behind this variation of antioxidant activities of wild tea over commercial varieties to be investigated further.

Sensory evaluation

There was a significant (*P*<0.05) difference in colour of made tea among wild tea and tea cultivars. The highest mean rank was recorded in wild tea (6.15±0.587) whereas the lowest was from both TRI 2023 from Rathnapura (2.85±0.489) and Erathna (3.95±0.887) (Table 2). The highest sum of rank for colour was recorded in wild tea (123) and lowest for commercial TRI 2023 (57) (Table 3). Aroma, also behaved in similar trend with significant difference between tea cultivars. Mean rank of aroma in wild tea was significantly (*P*<0.05) higher (6.35±0.671) than that of other cultivars. Significantly (*P*<0.05) lowest mean rank was recorded in commercially grown TRI 2023 (3.8±0.768). The tea cultivar from 10 y abandoned land also showed significantly (*P*<0.05) higher mean rank than all cultivated varieties such as TRI 4049 and both TRI 2023 (Table 2).

Taste was also significantly (*P*<0.05) different between the five tea cultivars. Higher mean rank for taste recorded in wild tea and lowest in TRI 2023. However, tea cultivar from free growing land TRI 4049 was also statistically similar to wild tea (Table 2). The mean rank of strength of the made tea was significantly (*P*<0.05) higher in wild tea whereas that of TRI 2023 was the lowest. TRI 2025 and TRI 4049 received intermediate mean rank for strength. Overall acceptability was significantly (*P*<0.05) different between tea cultivars. Wild tea got significantly (*P*<0.05) highest overall acceptability than any other tea cultivars and TRI 2023 got the lowest overall acceptability (Table 2).

Table 2: Mean rank of organoleptic properties of tea cultivars. Values (means±SD) with different superscript are significantly different (*P*<0.05).

Tea plant	Mean Rank				
	Colour	Aroma	Taste	Strength	Overall acceptability
Wild tea	$6.15±0.59^{A}$	$6.35±0.67^{A}$	$5.20±0.77^{A}$	$5.60±0.99^{A}$	$5.90±0.79^{A}$
TRI2025-Free growing	$4.65±0.99^{B}$	$4.45±0.83^{B}$	$5.10±0.72^{AB}$	$4.70±0.98^{B}$	$4.55±1.05^{B}$
TRI 4049	$5.00±0.97^{B}$	$4.05±0.76^{BC}$	$4.60±0.99^{BC}$	$4.50±0.83^{B}$	$4.85±0.93^{B}$
TRI 2023 Ratnapura	$2.85±0.49^{D}$	$3.95±0.76^{C}$	$3.45±0.69^{D}$	$3.85±0.67^{C}$	$3.35±0.49^{D}$
TRI 2023 Erathna	$3.95±0.89^{C}$	$3.80±0.77^{C}$	$4.20±0.83^{C}$	$3.90±0.64^{C}$	$3.90±0.55^{C}$

Table 3: Sum of rank of organoleptic properties of made tea of different tea plants.

Tea plant	Color	Aroma	Taste	Strength	Overall acceptability
Wild tea	122.5	126.5	103.5	111.5	117.5
TRI 2025 (Free growing)	92.5	88.5	101.5	94.0	91.0
TRI 4049	100.0	81.0	92.0	90.0	97.0
TRI 2023 (Rathnapura)	56.5	78.5	69.0	76.5	67.0
TRI 2023 (Erathna)	78.5	75.5	84.0	78.0	77.5

Colour intensity of black tea is one of the quality characteristics that determines market demand. The different ratios of theaflevins (TFs) and thearubigins (TRs) constitute the different shade of well-processed black tea. There is a variance of theaflavins depending on which polyphenol it is made from. It is formed by the conjugation of 2 to 3 flavonoids and is mediated by the oxidation enzyme which produces the substances that show bright yellow colour (Hojo, 2013). Liu *et al.* (1990) pointed out that the pheophytins/TR ratio value could be used to reflect the color of black tea. The higher the ratio value, the more black bloom the made tea has. The variation of level of chemical contents could be due to genetic make-up of cultivars and factory tea processing conditions as well as climatic conditions (Ahmed *et al.*, 2019; Alasalwa *et al.*, 2012; Chaturvedula and Prakash, 2011). Surrounding environment of wild tea was a mixture of plants and no any management has been practiced. This natural environment condition would lead to development of consumer acceptable colour. As the manufacturing process of all tea cultivars used in the experiment was exactly similar, there could be no any effect of processing for these differences.

Aroma is one of the critical characteristics of tea quality which can determine acceptance or rejection of tea before it is tasted. Amino acid degradation involves in the biogenesis of the tea aroma (Balentine *et al.,* 1997). The differences in aroma of various teas are caused by the manufacturing process (Chaturvedula and Prakash, 2011); however, in our experiment the manufacturing process was uniform for all cultivars and hence the differences were due to cultivar used. As per previous findings, more than 630 compounds are responsible for tea aroma (Hara *et al.*, 1995; Shimoda *et al.*, 1995; Chaturvedula and Prakash, 2011). These could vary from cultivar to cultivar as genetic codes for different compounds from vary plant to plant. Many researchers worked on identifying the key compounds for the aroma of tea (Takei *et al.*, 1976; Yamaguchi *et al.*, 1981; Yamanishi, 1978) but no particular compounds have been recognized as responsible for the full tea aroma. It is generally believed that the characteristics of various kinds of tea consist of a balance of very complicated mixtures of aroma compounds in tea. The significant ($P<0.05$) high favorable aroma in wild tea than commercial and abandoned tea showed that the zero management practices and the surrounding crop environment could lead to the differences. Further, second best favorable aroma content in TRI 2025, which was 10 y abandoned land, proved this concept as it also under zero management for 10 y. As the wild tea contained significantly ($P<0.05$) higher polyphenole content (Table1) over the others it partly leads to favorable taste (Alasalwar *et al.*, 2012). Strength and overall acceptability were also significantly ($P<0.05$) high in wild tea and therefor, in the export market this wild tea would be a highly attractive commodity once introduced.

In the natural forest, mineral nutrients can be rapidly immobilized by sequestration with organic compounds. In top soils that include the bulk of the soil N and P are present as organic compounds. Microbial biomass considered a multifunctional role as a product of assimilation or immobilization of mineral nutrients and a product of enzymatic re-mineralization of organic N. Also, it is strongly correlated with alkylic C of the soil (Kirk and Olk, 2000). This diffrence in forest soil than commercial land could be a one reason which leads to favourable qualty characters of wild tea in antioxidant properties and consumer preference as evaluated by organoleptic qualities.

CONCLUSION

Physicochemical and organoleptic properties of wild tea are superior to selected free growing and commercially cultivated tea.

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