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Phenolic and Flavonoid Contents and Antioxidant Capacity of Selected Green and Black Tea (*Camellia sinensis* L.) Blends with Medicinal Herbs

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ABSTRACT

Aqueous extracts of eight herbal tea blends (*viz. ranawara*+Gunpowder, *beli-mal*+Gunpowder, *kowakka*+Gunpowder, *polpala*+Gunpowder, *gotukola*+BOP, *karapincha*+BOP, *iramusu*+BOP and *koththamalli*+BOP) and methanolic extracts of eight medicinal herbs (*viz. ranawara, beli-mal, koththamalli, iramusu, polpala, kowakka, gotukola,* and *karapincha*) were analyzed for total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC). TAC was determined by ferric reducing antioxidant power (FRAP) assay. Colourimetric methods were used to quantify total phenolics (Folin-ciocalteu method) and total flavonoids. Among methanolic extracts of medicinal herbs, *ranawara* showed significantly the highest TPC (111.9±4.1 mg GAE/ g DW) and TAC (143.7±1.8 mg TE/ g DW). Order of TAC was *ranawara* > *iramusu* > *karapincha* > *beli-mal* > *gotukola* > *polpala* > *koththamalli* > *kowakka*. Significantly the highest TFC (176.4±12.5 mg RE/ g DW) was recorded in methanolic extracts of *iramusu*. Higher TACs were observed in green tea herbal blends than black tea herbal blends. *Beli-mal*+Gunpowder blend showed the highest TAC (80.3±0.1 mg TE/ g DW) and TPC (34.6±0.1 mg GAE/ g DW) when compared to other selected herbal tea blends. The positive and significant correlations existed between total antioxidant capacity and phenolic compounds (TPC, R^2 = 0.90, p<0.001 and TFC, R^2 = 0.53, p<0.001), revealing that phenolic compounds were the dominant antioxidant components in medicinal herbs and herbal tea blends. Herbal tea blend, a widely consumed polyphenolic beverage play a significant role as a naturally occurring antioxidant substitute and hence contribute to human health.

KEYWORDS: Antioxidant capacity, Flavonoids, Herbal tea blends, Medicinal herbs, Phenolic compounds

INTRODUCTION

The main dietary antioxidants are vitamins C and E, carotenoids, terpenes, and polyphenols, including flavonoids (Benzie, 2003). Phenolics are secondary plant metabolites mainly found in the medicinal herbs and teas (Lin *et al.*, 2003).

Tea (*Camellia sinensis* L.), the second most commonly consumed beverage in the world after water, is rich in flavonoids (Sarkar and Bhaduri, 2001). Tea is produced predominantly in three forms namely black, green and oolong teas (Reeves *et al.*, 1987). Green tea, which is known as unfermented tea contains mainly tea catechins and black tea, which contains mainly thearubigins (TR) and theaflavins (TF) due to fully fermented step, during manufacturing.

Medicinal herbs are normally used as a treatment for different ill conditions. Traditionally, some medicinal herbs such as *beli-mal, ranawara, iramusu, koththamalli* were widely used as beverages before introduction of tea to Sri Lanka. Several studies have shown that these medicinal herbs contain lot of phenolic compounds (Kahkonen *et al.*, 1999) and antioxidant properties (Aaby *et al.*, 2004).

Recently, tea blend with medicinal herbs is popularizing as a value added product among tea consumers. Tea is blended with *ranawara, beli-mal, iramusu, koththamalli, karapincha, gotukola, kowakka, polpala etc.* Though lot of researches on bioactive compound and antioxidant capacity of black and green tea, lack of studies are available on bioactive compounds and antioxidant capacity of their blends with medicinal herbs. Therefore, the objective of this study was to find antioxidant capacity and bioactive compounds of popular tea blends with medicinal herbs produced in Sri Lanka.

MATERIALS AND METHODS

Location

The experiment was conducted in the laboratory of Department of Plantation Management, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) from January to May 2016.

Materials

Commonly used processed medicinal herbs (*i.e. ranawara, beli-mal, koththamalli, iramusu, polpala, kowakka, gotukola* and *karapincha*), four types of popular green tea

herbal blends (*i.e. ranawara*+Gunpowder, *beli-mal*+Gunpowder, *polpala*+Gunpowder and *kowakka*+Gunpowder) and four types of popular black tea herbal blends (*i.e. iramusu*+BOP, *koththamalli*+BOP, *gotukola*+BOP and *karapincha*+BOP,) were obtained from Marah Trading (Pvt.) Ltd., Kelaniya, Sri Lanka (Table 1). BOP and Gunpowder grades were used as black tea and green tea to manufacture these blends respectively. Tea bags containing 2 g of respective tea herbal blends were received from the Marah Trading (Pvt.) Ltd.

Chemicals and Reagents

Folin-Ciocaltue reagent, Gallic acid, Rutin, 2, 4, 6-Tripyridyl-s-Triazine (TPTZ), Ferric chloride ($FeCl_3$) and 6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2- carboxilic acid (Trolox) were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo). All other chemicals used were of analytical grade.

Methanolic Extraction of Medicinal Herbs

The medicinal herbs were ground in a coffee grinder to a fine powder. Ground sample was weighed (0.1 g) into a 15 mL centrifuge tube containing 5 mL of 80% methanol and the samples were homogenized for 1 min at 80 rpm by using the homogenizer (Witeg, 0400189139t002, German). Then homogenate was vortexed for 15 min and placed in a water bath at 60 °C for 40 min and vortex procedure was repeated in 10 min intervals. After centrifugation at 4,000 rpm for 5 min, the supernatant was removed and extraction was repeated one more time. Supernatants were pooled and stored at -20 °C until analysis.

Brewing of Tea Herbal Blends with Boiling Water

Tea bags containing 2 g of tea herbal blends were used for brewing. The tea bag was brewed for 5 min with 25 mL of boiling mineral water. Then the liquor was strained and stored at -4 °C until analysis.

Quantification of Phenolics

The total phenolic contents of samples were determined using a modified Folin-Ciocaltue method as described by Abeysinghe *et al.* (2007). Briefly, 4 mL of distilled water and 0.5 mL of sample extract were added to a 15 mL centrifuge tubes. Then 0.2 N folin-ciocaltue reagent (0.5 mL) was added to the mixture and allowed to react for 3 min. One milliliter of saturated sodium carbonate solution was mixed and samples were incubated in a water bath for 2 h at 30 °C. The absorbance was measured at 760 nm using UV visible spectrophotometer (Shimadzu, UV Mini 1240, Japan). Gallic acid was used as the standard and TPC in one gram of dried sample was calculated and expressed as milligram of Gallic acid equivalent (GAE).

Quantification of Flavonoids

Total flavonoid content (TFC) was determined by a colourimetric method described by Liu *et al.* (2002) with slight modifications. Briefly, 0.5 mL of the plant extract was diluted with 3.5 mL of distilled water. Then 0.3 mL of a 5% $NaNO_2$ solution was added to the mixture. After 6 min, 0.3 mL of a 10% $Al(NO_3)_3.6H_2O$ solution was added, and the mixture was allowed to stand for another 6 min. Two milliliter of 2 M NaOH was added, and the total was made up to 8 mL with distilled water. The solution was well mixed, and the absorbance was measured immediately at 510 nm using UV visible spectrophotometer (Shimadzu, UV Mini 1240, Japan). Rutin was used as the standard and TFC in one gram of dried sample was calculated and expressed as mg of Rutin equivalent (RE).

Quantification of Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) was determined using ferric reducing antioxidant power (FRAP) assay as described by Benzie and Stain (1996). Briefly, sample extract (100 µL) was mixed with 900 µL of freshly prepared FRAP reagent of pH 3.6 containing 2.5 mL of 10 mmol/L, 2,4,6-Tripyridyl-s-Triazine (TPTZ), 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 300 mmol/L acetate buffer. Absorbance of the reaction was measured at 593 nm using the spectrophotometer (Shimadzu, UV Mini 1240, Japan) after incubating for 4 min.

Table 1. Commonly used medicinal herbs and tea blends with medicinal herbs

Common name	Botanical name	Part used	Tea blends with medicinal herbs
Ranawara	*Cassia auriculata*	Flower	*Ranawara* + Gunpowder
Beli-mal	*Aegle marmelos*	Flower	*Beli-mal* + Gunpowder
Koththamalli	*Coriandrum sativum*	Pod	*Koththamalli* + BOP
Iramusu	*Hemidesmus indicus*	Whole plant	*Iramusu* + BOP
Polpala	*Aerva lanata*	Whole plant	*Polpala* + Gunpowder
Kowakka	*Coccinia grandis*	Leaves	*Kowakka* + Gunpowder
Gotukola	*Centella asiatica*	Whole plant	*Gotukola* + BOP
Karapincha	*Murraya koenigii*	leaves	*Karapincha* + BOP

BOP-Broken Orange Pekoe

Trolox was used as the standard and TAC in one gram of dried sample material was calculated and expressed as mg of Trolox equivalent (TE).

Statistical Analysis

Statistical comparison of mean values was performed by general linear model (GLM) of ANONA followed by a Turkey Multiple Range Test and presented means±SD with 95% confidential level (SAS, Version 9). Correlations between various parameters also were investigated.

RESULTS AND DISCUSSION

Phytochemicals and Antioxidant Capacity of Medicinal Herbs

There was a significant variation in content of phenolics in methanolic extracts of all selected medicinal herbs (Figure 1). The highest total phenolic content was observed in *ranawara* (111.9±4.1 mg GAE/g DW) whereas the lowest total phenolic content was recorded in *koththamalli* (1.2±0.3 mg GAE/g DW). Significantly, higher total phenolics were recorded in *ranawara*, *iramusu*, *karapincha* and *beli-mal* than other selected medicinal herbs.

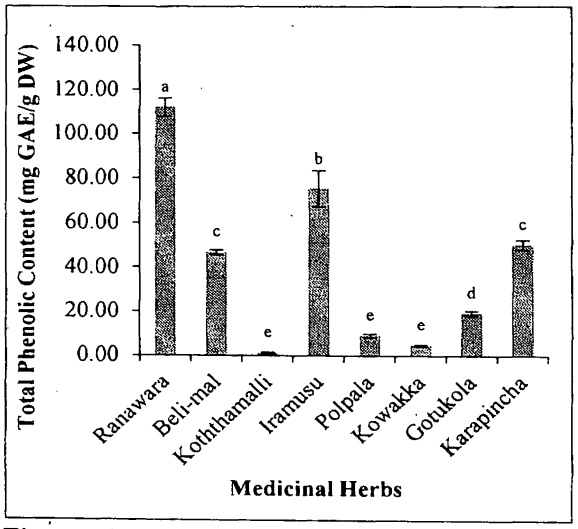

Figure 1. Total phenolic content of eight selected medicinal herbs. *Means with the same letter are not significantly different at p<0.05. GAE-Gallic acid equivalent; DW- Dry weight*

The total flavonoid contents varied significantly among selected herbs (Figure 2). Significantly higher total flavonoid content was observed in *iramusu* (176.4±12.5 mg RE/g DW) than other selected herbs. Same as phenolics, *ranawara*, *iramusu*, *karapincha* and *beli-mal* had significantly higher total flavonoids than other selected medicinal herbs. The lowest total flavonoid content was reported in *polpala* (7.1±1.4 mg RE/g DW).

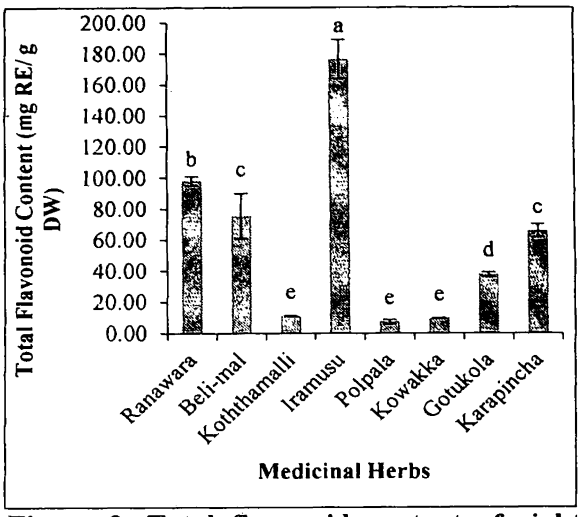

Figure 2. Total flavonoid content of eight selected medicinal herbs. *Means with the same letter are not significantly different at p<0.05. RE-Rutin equivalent; DW- Dry weight*

The total antioxidant capacities, measured by FRAP method, varied widely in methonolic extracts of medicinal herb materials and ranged from 5.8±0.1 to 143.7±1.8 mg TE/g DW (Figure 3). *Ranawara* (143.7±1.8 mg TE/g DW), *iramusu* (113.1±4.8 mg TE/g DW), *karapincha* (83.5±5.3 mg TE/g DW) and *beli-mal* (60.3±0.4 mg TE/g DW) had significantly high levels of antioxidant capacity whereas *gotukola* (28.1±3.0 mg TE/g DW), *polpala* (20.0±1.4 mg TE/g DW), *koththamalli* (17.2±0.7 mg TE/g DW) and *kowakka* (5.8±0.1 mg TE/g DW) antioxidant capacities were quite low (Figure 3).

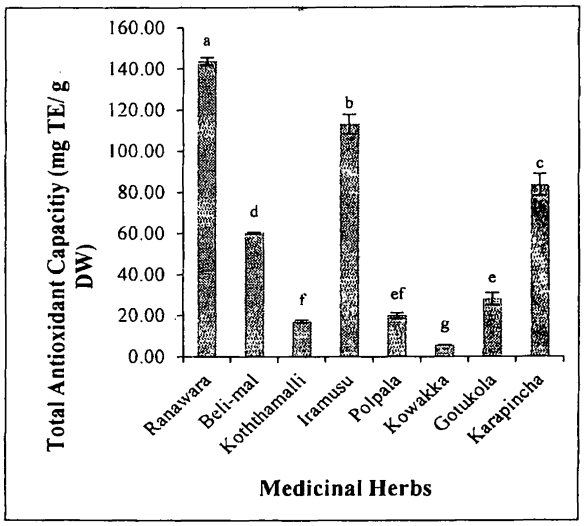

Figure 3. Total antioxidant capacity of eight selected medicinal herbs *Means with the same letter are not significantly different at p<0.05. TE-Trolox equivalent; DW- Dry weight*

Green Tea Blends with Medicinal Herbs

Ranawara, *beli-mal*, *kowakka* and *polpala* are commonly used to produce green tea blends with medicinal herbs.

Total phenolic content, total flavonoid content and total antioxidant capacity significantly varied in tested green tea herbal blends (Table 2). *Beli-mal*+Gunpowder had the highest total Phenolic content (34.6±0.1 mg GAE /g DW), followed by *ranawara* +Gunpowder (32.1±0.6 mg GAE /g DW), *polpala*+Gunpowder (21.8±0.3 mg GAE /g DW) and then *kowakka*+Gunpowder (14.6±0.1 mg GAE /g DW).

The total flavonoid content among selected green tea herbal blends ranged from 7.95 to 9.5 mg RE /g DW (Table 2). Highest level of flavonoids was found in *beli-mal*+Gunpowder blend, while the lowest was in *kowakka* + Gunpowder blend.

Variation of total antioxidant capacities of selected green tea herbal blends similar to total phenolics and total flavonoids (Table 2). The highest total antioxidant capacity was observed in *beli-mal*+Gunpowder blend (80.3±0.1 mg TE/g DW) whereas the lowest was recorded in *kowakka*+Gunpowder blend (48.9±0.1 mg TE/g DW).

Black Tea Blends with Medicinal Herbs

Famous black tea herbal blends *iramusu* +BOP, *karapincha*+BOP, *gotukola*+BOP and *koththamalli*+BOP were used for this study.

The total phenolic contents varied significantly among selected black tea herbal blends (Table 3). *Iramusu*+BOP blend had higher total phenolics (25.3±0.6 mg GAE/ g DW) than other selected herbs. The lowest phenolic content was observed in *koththamalli*+BOP blend (15.6±0.1mg GAE/ g DW).

There was a significant variation in total flavonoid contents of selected black tea herbal blends (Table 3). The highest total flavonoid content was recorded in *iramusu*+BOP blend (11.7±0.4 mg RE/ g DW), followed by *gotukola*+BOP (10.2±0.4 mg RE/ g DW), *karapincha* + BOP (8.7±0.3mg RE/ g DW) and then *koththamalli*+BOP (7.5±0.1 mg RE/ g DW).

Total antioxidant capacities of selected black tea herbal blends ranged from 26.9 to 41.8 mg TE/g DW (Table 3). The results showed that the antioxidant capacity values of black tea herbal blends were comparatively lower than green tea herbal blends. The highest antioxidant capacity among black tea herbal blends was observed in *iramusu*+BOP (41.8 ± 0.3 mg TE/ g DW) whereas the lowest was in *koththamalli*+BOP (26.9 ± 0.1 mg TE/ g DW). The variation pattern of antioxidant capacity was similar to variation pattern of total phenolics among selected black tea herbal blends. Cai *et al.* (2004) who studied 112 plant species used in Chinese medicine showed that plants with a higher content of total polyphenols had a higher antioxidant activity.

Correlations between TAC and Phytochemicals

Total antioxidant capacity and bioactive compounds (phenolics and flavonoids) showed significant positive correlations. A strong significant correlation was observed between total antioxidant capacity and total phenolic content (R^2= 0.90, $p<0.001$) (Figure 4). The total flavonoids also showed a satisfactory positive correlation (R^2 = 0.53, $p<0.001$) with total antioxidant capacity. Wojdylo *et al.* (2007) has also demonstrated a linear correlation between the content of total phenolic compounds and their antioxidant capacity in medicinal herbs.

These results also prove the importance of phenolic compounds in the antioxidant behavior of tea herbal blend extracts and also that they contribute significantly to the total antioxidant capacity.

Table 2.Total phenolics, total flavonoids and total antioxidant capacity of selected green tea herbal blends

Green tea herbal blend	TPC (mg GAE /g DW)	TFC (mg RE /g DW)	TAC (mg TE/g DW)
Beli-mal + Gunpowder	34.6 ± 0.1^a	9.5± 0.1^a	80.3 ± 0.1^a
Ranawara + Gunpowder	32.1 ± 0.6^a	8.8 ± 0.2^a	65.8 ± 0.4^b
Polpala + Gunpowder	21.8 ± 0.3^b	8.2 ± 0.1^{ab}	56.5 ± 0.2^c
Kowakka + Gunpowder	14.6 ± 0.1^c	7.95 ± 0.1^b	48.9 ± 0.1^d

Means with the same letters in a column are not significantly different at 0.05 confidence level. GAE- Gallic acid equivalent, RE- Rutin equivalent, TE- Trolox equivalent

Table 3.Total phenolics, total flavonoids and total antioxidant capacity of selected black tea herbal blends

Black tea herbal blend	TPC (mg GAE /g DW)	TFC (mg RE /g DW)	TAC (mg TE/g DW)
Iramusu + BOP	25.3 ± 0.6^a	11.7 ± 0.4^a	41.8 ± 0.3^a
Karapincha + BOP	20.4 ± 0.6^b	8.7 ± 0.3^c	41.2 ± 0.1^a
Gotukola + BOP	17.7 ± 0.7^c	10.2 ± 0.4^b	31.1 ± 0.4^b
Koththamalli + BOP	15.6 ± 0.1^d	7.5 ± 0.1^c	26.9 ± 0.1^c

Means with the same letters in a column are not significantly different at 0.05 confidence level. GAE- Gallic acid equivalent, RE- Rutin equivalent, TE- Trolox equivalent

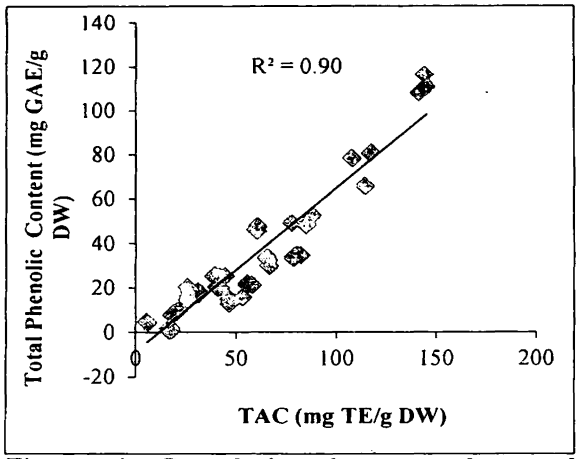

Figure 4. Correlation between the total phenolic contents and total antioxidant capacities. *TAC- Total antioxidant Capacity, DW- Dry weight, GAE- Gallic acid equivalent and TE- Trolox equivalent*

CONCLUSIONS

Methanolic extracts of *ranawara*, *iramusu*, *beli-mal* and *karapincha* had significantly higher TAC, TPC and TFC than other selected medicinal herbs. Higher TAC was observed in green tea herbal blends than black tea herbal blends. *Beli-mal*+Gunpowder blend showed the highest TAC and TPC when compared to other selected tea herbal blends. The positive and significant correlations exist between total antioxidant capacity and phenolic compounds (TPC and TFC), revealing that phenolic compounds are the dominant antioxidant components in medicinal herbs and herbal tea blends. Herbal tea blend, a widely consumed polyphenolic beverage play a significant role as a naturally occurring antioxidant substitute and hence contribute to human health.

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