Original Article

Development of Herbal Green Tea Bags with *Osbeckia octandra* L. (DC.) (Heen bovitiya) and Evaluation of the Effect of Infusion Time and Temperature on Extracted Phytochemicals

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Abstract

Herbal tea or a tisane is a mixture of dried plant parts used for medicinal purposes. Present study was aimed to develop herbal green tea bags with *Osbeckia octandra* (*Heen bovitiya*) leaves predominantly and evaluate the effect of infusion time and temperature on their total phenolic (TP), total flavonoid (TF) contents and *in vitro* antioxidant activity. Six different tea bags (T_1 to T_6) were prepared by incorporating dried *O. octandra* (leaves), *Camellia sinensis* (leaves), *Zingiber officinale* (rhizomes), *Vanilla planifolia* (pods) and *Allium sativum* (bulbs) in different proportions. Each herbal tea bag was infused at a constant infusion temperature of 100 ºC for 3, 5 and 7 minutes and for a constant infusion time of 7 minutes at different temperatures; 100, 90 and 80 ºC. TP, TF contents, *in vitro* radical scavenging activity and ferric reducing antioxidant power of all tea extracts were determined by spectroscopic analysis. Data were analyzed with one sample t-test. All infusions were rich in phytochemicals; phenols, flavonoids, tannins, terpenes, triterpenoids, phytosterols, saponins, alkaloids, amino acids and carbohydrates. TP, TF contents and *in vitro* antioxidant activity of the formulated tea bags were significantly higher after the addition of *O. octandra* leaves and the values were different at different infusion conditions ($p<0.05$). At constant infusion time of 7 minutes, TP, TF contents and antioxidant activity of *O. octandra* incorporated green tea bags were higher when infused at 80 ºC compared to 90 ºC and 100 ºC. At constant infusion temperature of 100 ºC, the values were higher for the formulated green tea bags when infused for 7 minutes compared to 3 and 5 minutes. Hence, it was concluded that the formulated tea bags with *O. octandra* leaves were rich with antioxidants and that they should be brewed for a long time period (7 minutes) at a low temperature (80 ºC).

Keywords: ***antioxidant activity, phytochemicals, total flavonoid, total phenolic, Osbeckia octandra***

Introduction

Tea is the second most consumed drink worldwide which contains various types of phytochemicals such as; catechins, epicatechins, epigallocatechins, epigallocatechingallates, flavanol glycosides, saponins, phenolic acids, purine alkaloids, condensed tannins and proanthocyanidins [1]. It is rich with antioxidant and free radical scavenging properties [2]. *Camellia sinensis* (L.) Kuntze is a flowering plant which belongs to the family Theaceae, whose fresh leaves and leaf buds are used to produce tea. The green tea is prepared

by infusion of fresh tea leaves or unfermented fresh green shoots while black tea is prepared by the infusion of fermented tea leaves where tea leaves are oxidized with maceration and exposed to oxygen [3,4]. Green tea contains caffeine while black tea contains caffeine and theobromine [5]. There are many types of tea products such as; herbal tea, green tea, black tea, oolong tea and white tea. Herbal tea or a tisane is a mixture of dried leaves, flowers, fruits, nuts, seeds, barks, grasses, roots and other parts of a plant or other botanical elements which are used for medical purposes. Some herbal tea products are prepared by incorporating one or more herbal ingredients or extracts to tea leaves [6,7]. Chamomile tea, ginger tea, ginseng tea, cinnamon tea, peppermint tea, hibiscus tea are numerous types of herbal teas with their unique benefits. Herbal tea is brewed in the same way as normal tea (*Camellia sinensis*) [6].

There is a growing trend in the consumption of herbal tea due to their proven pharmacological properties such as; antioxidant, lipid lowering, anti-inflammatory, immune boosting activities. Antioxidants are the compounds that protect the cells from oxidative stress. There are many defense mechanisms in the body to limit the amount of available reactive oxidants and avoid the effects caused by them. In the presence of imbalance of oxidants and antioxidants of the body, there is a need of consumption of exogeneous antioxidants [8]. Natural antioxidants are more preferred than the synthetic antioxidants in which dietary antioxidants play a major role in the removal of free radicals formed in the body.

Osbeckia octandra L. (DC.) or *Melastoma octandra* belongs to the family Melastomataceae, is a rare, endemic plant which is widely used in Traditional and Ayurveda medicine systems due to the many pharmacological activities. It is commonly called as Ayurveda bush-tree, *Heen Bovitiya* in Sanskrit/ Sinhala, *Kathtoo mukhtohulai* in Tamil and Eight Stamen *Osbeckia* in English. Therefore, the present study was aimed to develop herbal tea bags enriched with antioxidants incorporating *Osbeckia octandra* (Heen bovitiya) leaves predominantly.

It has been reported that the composition of the tea infusion is affected by variety of tea and infusion time [3]. It had stated that green tea has a greater antioxidant capacity than black tea [4]. Since, the infusion conditions can change the composition and bioactivity of infusions; the effect of infusion time and temperature on total phenolic (TP), total flavonoid (TF) contents and *in vitro* antioxidant activity were evaluated in this study.

Materials and Method

Preparation of plant materials

Three batches of fresh leaves of mature *O. octandra* without any apparent insect or microbial attacks were collected from Galle District, Southern Province, Sri Lanka (geographical coordinates; latitude: 6.053519 and longitude: 80.220978) during the period from November 2019 to January 2020. Leaves were authenticated from National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. Dry powders of *Camellia sinensis* (green tea leaves), *Zingiber officinale* (rhizomes), *Vanilla planifolia* (pods) and *Allium sativum* (bulbs) were purchased from the local supermarket.

Chemicals

Folin-Ciocalteu phenol reagent, Catechin, Sodium carbonate, Sodium hydroxide, Sodium nitrite, Aluminium chloride, Gallic acid, 2-2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), Trolox, Sulphuric

acid, Nitric acid, Sodium hydroxide, Ferric chloride, Lead acetate, Ammonia, Benzene, Copper acetate, Chloroform, Acetic anhydride, Sodium carbonate, Ethanol, Sodium nitrite, Ferrus sulphate, Sodium phosphate, Starch, n-Hexane of analytical grade were purchased from Sigma Aldrich via local agencies in Sri Lanka. Mayer's reagent, Wagner's reagent, Fehling's A, Fehling's B, Dragendroff's reagent, Ninhydrin reagent and Benedict's reagent were prepared by using the chemicals of routine laboratory work.

Preparation of herbal tea bags and extraction of biologically active compounds

Methodology published in Astill *et* al., 2001 and Niwat, 2019 was followed with modifications. Six different herbal green tea bags were prepared by incorporating dry powders of *O. octandra* (leaves), *C. sinensis* (green tea leaves), *Vanilla planifolia* (pods), *Z. officinale* (rhizomes) and *A. sativum* (bulbs) according to the compositions given in the **Table 1** to a constant total weight of 1.50 g. Tea bags were infused in a constant volume of distilled water (150.0 mL) at two different conditions; at a constant infusion temperature of 100 ºC for infusion times; 3, 5 and 7 minutes and for a constant time of 7 minutes at different temperatures; 100, 90 and 80 ºC [3,6].

Table 1: Composition of formulated herbal green tea bags

Type of Tea bag	*O. octandra*	*C. sinensis*	*Z. officinale*	*A. sativum*	*V. planifolia*
T_1	67%	20%	6.5%	-	6.5%
T_2	-	87%	6.5%	-	6.5%
T_3	67%	20%	6.5%	6.5%	-
T_4	-	87%	6.5%	6.5%	-
T_5	67%	20%	-	6.5%	6.5%
T_6	-	87%	-	6.5%	6.5%

Qualitative analysis of phytochemical screening

Preliminary phytochemical screening was conducted to each tea infusion to screen for the available phytoconstituents; alkaloids, phenolic compounds, tannins, flavonoids, steroids, phytosterols, terpenoids, triterpenes, glycosides, saponins, carbohydrates, reducing sugars, proteins and amino acids following the published methodology in Keo *et al*., 2017 and Visweswari *et al*., 2013 [9,10].

Total phenolic and flavonoid contents

The Folin-Ciocalteu assay and aluminum chloride colorimetric methods of each tea infusion were performed to estimate the total phenol (TP), total flavonoid (TF) contents of extracts respectively as per the methodology published in Hettihewa, 2014 [11].

Radical Scavenging Activity (DPPH assay)

Radical scavenging activity of each extract was determined using DPPH assay according to the method described in Hettihewa, 2014 and the

results were expressed in mmol Trolox equivalents/ 100 g DW of the tea bag [11].

Ferric-reducing antioxidant power activity (FRAP assay)

The FRAP assay was used to determine the ferric reducing power of the extracts based on the assay described in Hettihewa, 2014 and the results were expressed in mmol Fe(II) equivalents/ 100 g DW of the tea bag [11].

Statistical Analysis

All experimental measurements were carried out in triplicate and the results were expressed as average ± standard deviation. Data was analyzed using SPSS software 25.0 version and one sample t-test was followed to determine the effect of infusion conditions on TP, TF contents and *in vitro* antioxidant activity. One-way ANOVA with Tukey post-hoc tests were conducted to compare the parameters of tea products analyzed. Multiple comparisons were conducted pair-wise and at p=0.05, the values were considered significantly different at 95 % level of confidence.

Results

Qualitative phytochemical analysis revealed that the phytoconstituents including; phenolic compounds, flavonoids, tannins, triterpenes, phytosterols, saponins, alkaloids, amino acids and carbohydrates were present in all tea infusions. The values of total phenolic, total flavonoid contents and antioxidant activities were higher for the developed herbal green tea bags when infused at 80 ºC than at 100 ºC and 90 ºC for a constant time of 7 minutes. The values were higher for the developed herbal green tea bags when infused for 7 minutes than for 3 and 5 minutes at a constant temperature of 100 ºC. TP, TF contents and *in vitro* antioxidant activity of tea products were significantly higher at p=0.05 in the developed green tea bags with *O. octandra* than green tea bags without *O. octandra* at all infusion temperatures and times (**Table 2 & 3**).

Table 2: TP, TF contents, DPPH and FRAP values at different infusion temperatures for constant infusion time (7 minutes)

Tea Bag with the composition %	Infusion Temperature (ºC)	TPC±SD[p] (mg CAE/100 g DW)	TFC±SD[q] (mg CAE/100 g DW)	DPPH±SD[r] (mmol TE/100 g DW)	FRAP±SD[s] (mmol Fe^{2+}E/100 g DW)
Sample T_1	100	1650.58±5.63[a]	371.14±4.03[a]	7.00±0.00[a]	20.63±0.01[a]
HB:GT:ZO:AS:VP	90	1710.24±5.63[b]	395.08±14.06	7.35±0.02[b]	20.84±0.02[b]
(67:20:6.5:0:6.5)	80	1929.00±5.63[c]	[b]	7.42±0.00[c]	21.20±0.06[c]
			406.57±3.13[c]		
Sample T_2	100	1612.32±2.36[d]	360.19±3.65[d]	7.38±0.19[d]	20.43±0.02[d]
HB:GT:ZO:AS:VP	90	1700.89±3.59[e]	378.96±9.81[e]	7.47±0.01[e]	20.65±0.04[e]
(0:87:6.5:0:6.5)	80	1899.25±4.61[f]	401.12±7.20[f]	7.51±0.00[f]	21.36±0.01[f]
Sample T_3	100	1960.28±23.79[g]	412.17±15.32[g]	7.41±0.00[g]	20.48±0.00[g]
HB:GT:ZO:AS:VP	90	1989.65±4.22[h]	423.14±10.94[h]	7.49±0.00[h]	20.74±0.03[h]
(67:20:6.5:6.5:0)	80	2032.41±5.63[i]	448.55±12.50[i]	7.52±0.00[i]	21.50±0.10[i]
Sample T_4	100	1888.56±26.11[j]	360.19±3.65[j]	7.38±0.19[j]	20.43±0.02[j]
HB:GT:ZO:AS:VP	90	1912.26±11.23[k]	378.96±9.81[k]	7.47±0.01[k]	20.65±0.04[k]
(0:87:6.5:6.5:0)	80	1979.61±12.26[l]	401.12±7.20[c]	7.51±0.00[l]	21.36±0.01[l]
Sample T_5	100	1762.10±3.69[m]	380.15±5.78[m]	7.04±2.15[m]	20.79±0.04[m]
HB:GT:ZO:AS:VP	90	1800.44±0.11[n]	398.33±0.56[n]	7.44±1.01[n]	20.99±0.56[n]
(67:20:0:6.5:6.5)	80	1990.20±4.41[o]	411.33±2.40[o]	7.69±0.06[o]	21.25±1.11[o]
Sample T_6	100	1712.09±1.22[m]	369.87±1.70[m]	7.00±0.50[m]	20.44±1.22[m]
HB:GT:ZO:AS:VP	90	1796.23±0.56[n]	373.56±4.77[n]	7.25±1.25[n]	20.65±0.14[n]
(0:87:0:6.5:6.5)	80	1892.44±1.14[o]	401.23±5.12[o]	7.55±0.80[o]	21.06±0.04[o]

HB: Heen bovitiya, GT: Green tea, ZO: *Zingiber officinale*, AS: *Allium sativum*, VP: *Vanilla planifolia*

[p]*Total phenolic content is expressed as mg GAE/100 g DW*
[q]*Total flavonoid content is expressed as mg CAE/100 g DW*
[r]*Radical scavenging activityis expressed as mmol TE/100 g DW*
[s]*Ferric reducing activityis expressed as mmolFe^{2+}E/100 g DW*
Results are expressed as mean ± standard error. Means followed by the same letter with in the tea type in a column are not significantly different at p= 0.05

Table 3: TP, TF contents, DPPH and FRAP values at different infusion time for a constant infusion temperature (100 ºC)

Tea Bag with the composition %	Infusion Time (minutes)	TPC±SD[p] (mg CAE/100 g DW)	TFC±SD[q] (mg CAE/100 g DW)	DPPH±SD[r] (mmol TE/100 g DW)	FRAP±SD[s] (mmol Fe^{2+}E/100 g DW)
Sample T_1	3	1463.65±12.36[g]	367.46±7.19[a]	6.66±0.17[g]	20.28±0.01[a]
HB:GT:ZO:AS:VP	5	1580.98±8.44[h]	370.11±4.69[b]	6.48±0.26[h]	20.38±0.01[b]
(67:20:6.5:0:6.5)	7	1650.58±5.63[i]	373.32±2.03[c]	7.00±0.00[i]	20.48±0.00[c]
Sample T_2	3	1598.74±6.35[d]	359.65±5.97[d]	7.20±0.00[d]	20.22±0.04[d]
HB:GT:ZO:AS:VP	5	1795.49±9.31[e]	362.45±3.69[e]	7.31±0.01[e]	20.29±0.02[e]
(0:87:6.5:0:6.5)	7	1843.12±6.69[f]	369.87±5.78[f]	7.35±0.01[f]	20.42±0.03[f]
Sample T_3	3	1672.46±2.81[a]	338.29±1.56[g]	7.35±0.15[a]	20.31±0.01[g]
HB:GT:ZO:AS:VP	5	1843.22±25.83[b]	374.09±0.31[h]	7.39±0.00[b]	20.53±0.05[h]
(67:20:6.5:6.5:0)	7	1960.28±23.79[c]	384.03±1.56[i]	7.41±0.00[c]	20.63±0.01[i]
Sample T_4	3	1412.55±8.39[j]	329.65±8.74[j]	6.41±0.01[j]	20.23±0.03[j]
HB:GT:ZO:AS:VP	5	1501.28±3.96[k]	356.89±7.14[k]	6.48±0.00[k]	20.40±0.03[k]
(0:87:6.5:6.5:0)	7	1599.12±3.69[l]	379.25±4.71[c]	6.91±0.02[l]	20.53±0.07[l]
Sample T_5	3	1489.23±1.09[m]	321.06±1.69[m]	7.06±0.17[m]	20.29±0.17[m]
HB:GT:ZO:AS:VP	5	1591.23±0.08[n]	372.12±0.03[n]	7.28±1.11[n]	20.49±0.04[n]
(67:20:0:6.5:6.5)	7	1888.32±1.42[o]	379.81±2.44[o]	7.22±1.04[o]	20.52±1.00[o]
Sample T_6	3	1399.29±0.17[p]	319.89±1.04[p]	6.99±0.17[p]	20.10±0.11[p]
HB:GT:ZO:AS:VP	5	1546.12±0.07[q]	365.84±0.11[q]	7.02±1.26[q]	20.28±1.11[q]
(0:87:0:6.5:6.5)	7	1720.02±0.40[r]	371.00±1.08[r]	7.14±0.06[r]	20.45±0.44[r]

HB: Heen bovitiya, GT: Green tea, ZO: Zingiber officinale, AS: Allium sativum, VP: Vanilla planifolia

[p]*Total phenolic content is expressed as mg GAE/100 g DW*
[q]*Total flavonoid content is expressed as mg CAE/100 g DW*
[r]*Radical scavenging activityis expressed as mmol TE/100 g DW*
[s]*Ferric reducing activityis expressed as mmolFe^{2+}E/100 g DW*

Results are expressed as mean±standard error. Means followed by the same letter with in the tea type in a column are not significantly different at p= 0.05

Discussion

Plants contain a variety of biologically active compounds called phytochemicals which can act as antioxidants by scavenging the free radicals, thus have a therapeutic potential against free radical associated disorders [12]. Medicinal plants are commonly rich in phenolic compounds; such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins which show antioxidant potential [13]. The formulated herbal green tea bags were rich with many phytoconstituents including; phenolic compounds, flavonoids, tannins, triterpenes, phytosterols, saponins, alkaloids, amino acids and carbohydrates. Tea is the most widely consumed beverage worldwide containing a variety of polyphenols [1] and therapeutic potential of tea can be enhanced by incorporating herbal ingredients [7]. All tea bags with the addition of *O. octandra* leaves (T_1, T_3, T_5) obtained higher values for TP, TF contents and *in vitro* antioxidant activity than that of the tea bags without *O. octandra* (T_2, T_4, T_6) at all infusion temperatures and times. The TP, TF contents and *in vitro* antioxidant activity were higher for all green tea bags when infused at 80 ºC than at 90 ºC and 100 ºC (**Table 1**). The TP, TF contents and *in vitro* antioxidant activity were higher for all green tea bags when infused for 7 minutes than for 3 and 5 minutes at a constant temperature of 100 ºC (**Table 2**). Green tea is prepared by infusing soaked dried green tea leaves in hot water (80-90 ºC) for 3-4 minutes. Catechins in tea act as antioxidants. It has been found that brewing of green tea at a temperature of 85 ºC for 3 minutes gives the maximum amount of catechins [14]. The precise infusion temperature is on par with the present study findings. TPC and antioxidant activity were found to be high when herbal tea was infused at 100 ºC for 5 minutes and 80 ºC for 10 minutes which is similar to the present study findings [15]. It had found that the highest antioxidant potential was obtained with prolonged cold steeping for green tea which is similar to the current study [16]. However, another study reported that the maximum antioxidant activity was obtained at 100 ºC for 9.5 minutes which is different from finding of the present study [17,18]. In addition, it has been found that *in vitro* antioxidant activity was correlated with the TPC and significantly high antioxidant activity was obtained at 80 ºC in par with the findings of the current study [19].

Conclusions

The green tea bags formulated with *O. octandra* leaves are rich with antioxidants and that they should be brewed for a long time period (7 minutes) at a low temperature (80 ºC) to achieve the maximum antioxidant activity.

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Conflict of Interests

No any potential conflict of interest exists in this publication.

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