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Effects of infusion conditions on *in vitro* antioxidant power of extracts obtained from nutraceutical tea products developed with *Osbeckia octandra* and black tea leaves

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Abstract: The present study was aimed at developing herbal tea bags using *Osbeckia octandra* leaves as the major ingredient and evaluating the effect of infusion conditions on their in-vitro antioxidant power of biologically active compounds extracted from the herbal tea bags. Five different tea bags (T_1-T_5) were prepared by incorporating dried *O. octandra* (leaves), *Camellia sinensis* (leaves), *Zingiber officinale* (rhizomes) and *Allium sativum* (bulbs) in different proportions. Each 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. Total phenolic (TP), total flavonoid (TF) contents, *in vitro* antioxidant activity by radical scavenging activity and ferric reducing antioxidant power of infused tea extracts were determined using spectroscopic methods. Results revealed that all extracts were rich with phytochemicals of phenolic compounds, flavonoids, tannins, terpenes, triterpenoids, phytosterols, saponins, alkaloids, amino acids and carbohydrates. Among the tea bags formulated, T_1 which contains *O. octandra* leaves as the major ingredient (87%) showed significantly high values of *in vitro* ferric reducing antioxidant power compared to that of other tea bags tested at both infusion conditions. The infusion temperature of 80 ºC and time of 7 minutes as optimized infusion conditions showed the highest values of *in vitro* antioxidant activities for formulated tea bag containing *O. octandra* leaves (T_1). Measures evaluated in this study were significantly different at both infusion conditions ($p<0.05$) for all the tea bags developed. Furthermore, the addition of *O. octandra* leaves to the black tea significantly improved TP and TF contents of tea products ($p<0.05$). Infusion temperature of 100 ºC and time of 3 minutes showed as the effective condition for extracting bioactive compounds from formulated black tea bags. Therefore, *O. octandra* incorporated to black tea have high content of bioactive compounds and should be shortly brewed with boiling water while tea bags containing only *O. octandra* should be brewed long at lower temperature.

Keywords: antioxidant; total flavonoid; total phenolic; *Osbeckia octandra* L. (DC.)

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INTRODUCTION

Nutraceuticals are regarded as food substances or part of food with many health and medical benefits which can prevent or treat a disease. They provide physiological benefits and protection against chronic diseases such as diabetes mellitus, cancer, cardiovascular diseases and neurological disorders. They also delay aging, improve health, increase life span, improve the quality of life and support body systems and functions. The term, "nutraceutical" is derived from nutrition and pharmaceuticals. They include herbal products, dietary supplements, special diets, processed foods and beverages which can be either nutrition or medicine. Due to their nutritional, safe and therapeutic effects; people have developed an interest towards nutraceutical consumption (Ansari *et al.*, 2013; Nasri *et al.*, 2014). Nutraceuticals can be classified broadly as potential nutraceuticals and established nutraceuticals. Based on the chemical composition; they can be classified as vitamin supplements, inorganic mineral supplements, digestive enzymes, probiotics, prebiotics, dietary fibre, cereals and grains, health drinks, antioxidants, phytochemicals and herbs as functional food. The food sources under the nutraceuticals can be classified as dietary fibre, probiotics, prebiotics, polyunsaturated fatty acids, antioxidant vitamins, polyphenols and spices (Ansari *et al.*, 2013). Herbal infusions currently have gained a great interest because of potential therapeutic effects and their presumed safety. For many centuries, herbal infusions have been widely exploited in traditional medicine and are now popular global beverages. These herbal beverages consumed by people for health purposes as daily beverages have natural bioactive compounds such as alkaloids, flavonoids, carotenoids, coumarins, terpenoids and polyacetylenes (Ravikumar, 2014; Chandrasekara and Shahidi, 2018).
Herbal tea products are rich in antioxidants which are the compounds that delay or inhibit the oxidation processes (Kumar *et al*, 2014; Chandrasekara and Shahidi, 2018).

Many different plant sources have gained a great interest to scientific research as a result of their naturally occurring antioxidants with lesser side effects (Ravikumar, 2014). Polyphenol based natural antioxidants have gained the considerable attention related to the radicals and oxidative stress, cancer and therapy. As safe sources of phenolic antioxidants; edible fruits, vegetables, medicinal plants and tea have been investigated for their antioxidant properties (Ravikumar, 2014; Banjarnahor and Artanti, 2015). Among the medicinal plants, *Osbeckia octandra* L. (DC.) (*Heen bovitiya*); known as Ayurveda bush tree is an endemic plant which is commonly used in Traditional and Ayurveda medicine system. Plant is well-known to have antioxidant, antidiabetic, anticancer, antimicrobial, anti-inflammatory, antimutagenic, anticlastogenic, antiaggregant, antispasmodic, antianaemic, analgesic, hepatoprotective, anesthetic and immunostimulant properties (Perera *et al.*, 2013). There is a growing interest in chemical compositions and biological activities of *Heen bovitiya* leaves and to assess their potential to be used in nutraceuticals and functional foods. *Camellia sinensis* (Tea) is determined to have antioxidant, antidiabetic, anticancer, lipid lowering, hepatoprotective, immunomodulatory, anti-inflammatory, chemoprotective, anesthetic, antiviral, antibacterial, antispasmodic and antigenotoxic properties (Kumar *et al.*, 2014). *Zingiber officinale* (Ginger) has antioxidant, antidiabetic, anti-inflammatory, antibacterial, antifungal, anticancer and antiarthritic properties (Bhatt *et al.*, 2013). *Allium sativum* (Garlic) is a medicinally important plant with antioxidant, antidiabetic, anticancer, anti-inflammatory, hepatoprotective, antibacterial, antifungal, antiviral, antiprotozoal and immunostimulant properties (Bayan *et al.*, 2014).

Therefore, this study aimed at developing different herbal tea products incorporating medicinally important herbs (Heen bovitiya, Ginger and Garlic) and evaluating the effect of infusion conditions including; infusion time and temperature for the bioactivity of the formulated tea products.

MATERIAL

The fresh leaves of matured *O. octandra* without any insect or microbial attacks were collected in three batches from Galle District, Southern Province, Sri Lanka with geographical coordinates including; 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. Dried powders of *C. sinensis* (black tea leaves), *Z. officinale* (rhizomes) and *A. sativum* (bulbs) were purchased from the local supermarket.

Chemicals including; 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 local agencies in Sri Lanka. Mayer's reagent, Wagner's reagent, Fehling's A, Fehling's B, Dragendroff's reagent, Ninhydrin reagent, Benedict's reagent were prepared by using the chemicals of routine laboratory work.

METHODS

Preparation of plant materials: Leaves were rinsed with distilled water to remove dirt and dust, and subjected for oven drying at the temperature of 50 ºC until a constant weight was obtained. It was powdered using the grinder (SISIL, Mixer, Grinder 550 W) and stored in the freezer (Haier Deep, Germany) below -40 ºC temperature for further use.

Preparation of herbal tea bags and extraction of biologically active compounds: Five different herbal tea bags were prepared by incorporating dry powders of *O. octandra* (leaves), *C. sinensis* (black tea leaves), *Z. officinale* (rhizome) and *A. sativum* (bulb) according to the compositions given in the Table 1 to a constant total weight of 1.50 g. Biologically active compounds from tea bags were extracted into distilled water (150.0 mL) by infusion method at two different conditions namely, 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 (Astill *et al.*, 2001; Niwat, 2019).

Qualitative analysis: Each tea infusion was subjected to preliminary phytochemical screening to screen for the phytoconstituents including; alkaloids, phenolic compounds, tannins, flavonoids, steroids, phytosterols, terpenoids, triterpenes, glycosides, saponins, carbohydrates, reducing sugars, proteins and amino acids available in the tea infusions according to the methods published (Visweswari *et al.*, 2013; Keo *et al.*, 2017).

Determination of effect of infusion conditions on TP, TF contents, DPPH and FRAP:

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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 (Hettihewa, 2014).

Table 1: Composition of herbal tea products developed

Type of Tea bag	*O. octandra*	*C. sinensis*	*Z. officinale*	*A. sativum*
T_1	87%	-	6.5%	6.5%
T_2	67%	26.5%	6.5%	-
T_3	-	50%	50%	
T_4	67%	20%	6.5%	6.5%
T_5	-	87%	6.5%	6.5%

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.

Ferric-reducing antioxidant power activity (FRAP assay): The FRAP assay was used to determine the electron donating potential 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.

Statistical analysis: All experimental measurements were carried out in triplicate and the results were expressed as average ± standard deviation. Data was analyzed using SPSS 25 software 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 was 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. T_1 tea bag showed significantly high *in vitro* antioxidant activities compared to other tea bags (T_4-T_5) at different infusion temperatures and times (Table 2 & 3). It was also found that TP, TF contents and *in vitro* antioxidant activity of black tea products were higher when infused at 100 ºC than at 90 and 80 ºC for a constant infusion time of 7 minutes (Table 2 & 3). The values were also higher for the black tea bags when infused for 3 minutes than for 5 and 7 minutes at a constant infusion temperature of 100 ºC. The addition of *O. octandra* leaves to black tea significantly improved TP, TF contents ($p<0.05$) at all the infusion conditions.

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Table 2: TP, TF contents, DPPH and FRAP values at different infusion temperatures for a 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/100g DW)	FRAP±SD[s] (mmol Fe^{2+}E/100g DW)
T1	100	1026.15±5.62[a]	300.73±0.31[a]	5.89±0.31[a]	14.44±0.12[a]
***HB: BT:ZO:AS**	90	1043.38±15.06[b]	320.40±3.13[b]	6.14±0.02[b]	15.40±0.38[b]
(87:0:6.5:6.5)	80	1126.37±22.69[c]	338.07±3.13[c]	6.40±0.09[c]	16.14±0.01[c]
T2	100	1446.28±17.10[d]	325.99±7.35[d]	5.16±0.03[d]	7.62±0.16[d]
HB: BT:ZO:AS	90	1399.48±15.98[e]	320.40±3.13[e]	5.14±0.00[e]	7.21±0.00[e]
(67:26.5:6.5:0)	80	1310.66±29.51[f]	309.35±3.13[f]	5.12±0.12[f]	7.08±0.01[f]
T3	100	1157.80±35.80[g]	333.65±3.13[g]	6.39±0.75[g]	9.54±0.00[g]
HB: BT:ZO:AS	90	1046.03±11.25[h]	331.44±3.13[h]	6.34±0.22[h]	9.35±0.01[h]
(67%:20%:6.5:6.5)	80	1045.24±21.37[i]	322.60±3.13[i]	5.87±0.02[i]	8.91±0.01[i]
T4	100	1382.30±0.99[j]	317.46±5.99[j]	5.04±0.05[j]	7.39±0.01[j]
***HB: BT:ZO:AS**	90	1365.12±1.36[k]	300.66±7.55[k]	4.89±0.06[k]	7.20±0.01[k]
(0:50:50:0)	80	1301.87±9.50[l]	298.91±4.13[c]	4.78±0.02[l]	7.05±0.02[l]
T5	100	1100.12±15.6[m]	314.22±7.30[m]	6.14±0.04[m]	9.50±0.02[m]
***HB: BT:ZO:AS**	90	1036.78±12.3[n]	306.91±6.52[n]	6.12±0.01[n]	9.28±0.04[n]
(0:87:6.5:6.5)	80	1024.22±6.98[o]	300.22±4.57[o]	5.83±0.00[o]	8.90±0.01[o]

*HB: Heen bovitiya: BT: Black tea: ZO: *Zingiber officinale*: AS: *Allium sativum*
[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/100g DW*
[s]*Ferric reducing activityis expressed as mmolFe^{2+}E/100g DW*
Results are expressed as mean±standard error. Means followed by the same letter with in the tea type in acolumn are not significantly different at p= 0.05

DISCUSSION

Among the tea bags (T_1-T_5) formulated, T_1 which contains *O. octandra* leaves as the major ingredient (87 %) showed significantly high values of *in vitro* antioxidant activities compared to all other tea bags tested (T_2-T_5) at different infusion temperatures and times (Table 2 & 3). The infusion temperature of 80 ºC and time of 7 minutes were found as the effective infusion conditions which showed the highest values of antioxidant activities for formulated tea bag with *O. octandra* leaves (T_1) (Table 2 & 3). Furthermore, the addition of *O. octandra* leaves to the black tea significantly improved TP, TF contents of tea products ($p<0.05$) at all the infusion conditions tested (Table 3). It was also found that TP, TF contents and *in vitro* antioxidant activity of black tea products were higher when infused at 100 ºC than at 90 and 80 ºC for a constant infusion time of 7 minutes (Table 2). The values were also higher for the black tea bags when infused for 3 minutes than for 5 and 7 minutes at a constant infusion temperature of 100 ºC (Table 3). Hence, it was observed that the infusion temperature of 100 ºC and time of 3 minutes as the effective extraction condition having highest values measured for formulated black tea bags.

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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/100g DW)	TFC±SD[q] (mg CAE/100g DW)	DPPH±SD[r] (mmol TE/100g DW)	FRAPC±SD[s] (mmol Fe^{2+}E/100g DW)
Sample T1	3	921.41±40.24[a]	308.90±0.63[a]	5.55±0.26[a]	14.47±0.06[a]
***HB: BT:ZO:AS**	5	962.51±5.62[b]	325.92±4.69[b]	5.75±0.44[b]	15.12±0.01[b]
(87:0:6.5:6.5)	7	1026.15±5.62[c]	335.86±3.13[c]	5.89±0.04[c]	16.14±0.01[c]
Sample T2	3	1641.70±17.26[d]	335.86±15.62[d]	5.62±0.11[d]	7.91±0.01[e]
HB: BT:ZO:AS	5	1464.98±10.74[e]	334.32±17.81[e]	5.21±0.26[e]	7.82±0.03[f]
(67:26.5:6.5:0)	7	1446.28±17.10[f]	325.99±7.35[f]	5.16±0.03[f]	7.62±0.16[g]
Sample T3	3	1246.09±0.56[g]	354.64±4.69[g]	6.96±0.46[g]	9.68±0.01[g]
HB: BT:ZO:AS	5	1215.47±25.87[h]	338.07±6.25[h]	6.58±0.00[h]	9.62±0.02[h]
(67%:20%:6.5:6.5)	7	1157.80±35.80[i]	310.78±16.72[i]	6.39±0.75[i]	9.54±0.00[i]
Sample T4	3	1210.19±5.62[j]	350.12±2.71[j]	6.42±0.01[j]	9.53±0.03[j]
***HB: BT:ZO:AS**	5	1199.26±6.98[k]	331.15±1.25[k]	6.29±5.89[k]	9.47±0.04[k]
(0:87:6.5:6.5)	7	1067.43±11.62[l]	300.11±3.69[l]	5.50±0.00[l]	9.41±0.01[l]
Sample T5	3	1596.12±2.39[m]	320.18±2.69[m]	4.81±0.33[m]	7.63±0.0[m]
***HB: BT:ZO:AS**	5	1412.26±6.97[n]	309.35±1.78[n]	4.79±0.00[n]	7.60±0.01[n]
(0:50:50:0)	7	1399.65±9.97[o]	300.22±6.97[o]	4.69±0.00[o]	7.39±0.00[o]

*HB: Heen bovitiya: BT: Black tea: ZO: *Zingiber officinale*: AS: *Allium sativum*
[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/100g DW*
[s]*Ferric reducing activityis expressed as mmolFe^{2+}E/100g DW*
Results are expressed as mean±standard error. Means followed by the same letter with in the tea type in acolumn are not significantly different at p= 0.05

Our research findings of the present study on herbal tea products of black tea incorporated with *O. octandra* leaves are in the concurrent with the study carried out by Astill *et al.*, in 2001 showing that the composition of the tea infusion is affected by duration of the infusion and the time of the infusion, and should be shorter around 3 minutes (Astill *et al.*, 2001). Another study carried out in Thailand in 2019 had confirmed that higher the infusion temperature; higher the bioactive compounds available and antioxidant properties. TP, TF contents, DPPH and FRAP values had increased with increasing the temperature of the infusion. Accordingly, the most effective infusion temperature was 100 ºC among 100 ºC, 90 ºC and 80 ºC (Niwat, 2019) which proved the present study findings.

Natural antioxidants are widely used and are more preferred than the synthetic antioxidants because natural antioxidants have less or no toxicity whereas synthetic antioxidants may have toxic or mutagenic effects (Sari *et al.*, 2018-Firuzi *et al.*, 2005). As

they reverse the oxidative damage of cellular and tissue lipids, proteins and nucleic acids caused by reactive oxygen species, they are able to reduce the risk of cancer and cardiovascular diseases. Use of herbal remedies for disorders of oxidative stress has been successful to some extent in alleviating pain and other symptoms in which naturally occurring phenolic compounds, flavonoids, vitamins and carotenoids are the major contributors to antioxidant properties in plants (Thaipong *et al*., 2006; Sari *et al*., 2018). Hence, these herbal tea products can be used to address pathological states related to oxidative stress.

CONCLUSION

Five different herbal tea bags were developed incorporating dried parts of medicinally important plants namely, *O. octandra* (leaves), *C. sinensis* (leaves), *Z. officinale* (rhizomes) and *A. sativum* (bulbs). All infusions showed high TP, TF contents which were exhibited through promising antioxidant levels while T_1 contains *O. octandra* leaves as the major ingredient (87%) showed significantly high values of antioxidant activities at both infusion conditions. Tea bags with the addition of *O. octandra* leaves showed high TP and TF contents compared to tea samples prepared without the addition of *O. octandra*. Therefore, it was concluded that formulated tea bags were rich in antioxidants and the addition of *O. octandra* has enhanced the antioxidant activity of the tea bags in this study. Hence, these tea products can be used as antioxidants rich herbal tea products to address the pathological states related to oxidative stress. Moreover, it was found that *O. octandra* incorporated to black tea should be shortly brewed with boiling while tea bags containing only *O. octandra* should be brewed long with boiling.

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DECLARATION OF CONFLICT OF INTEREST

We hereby declare that the study does not encompass any conflict of interest.

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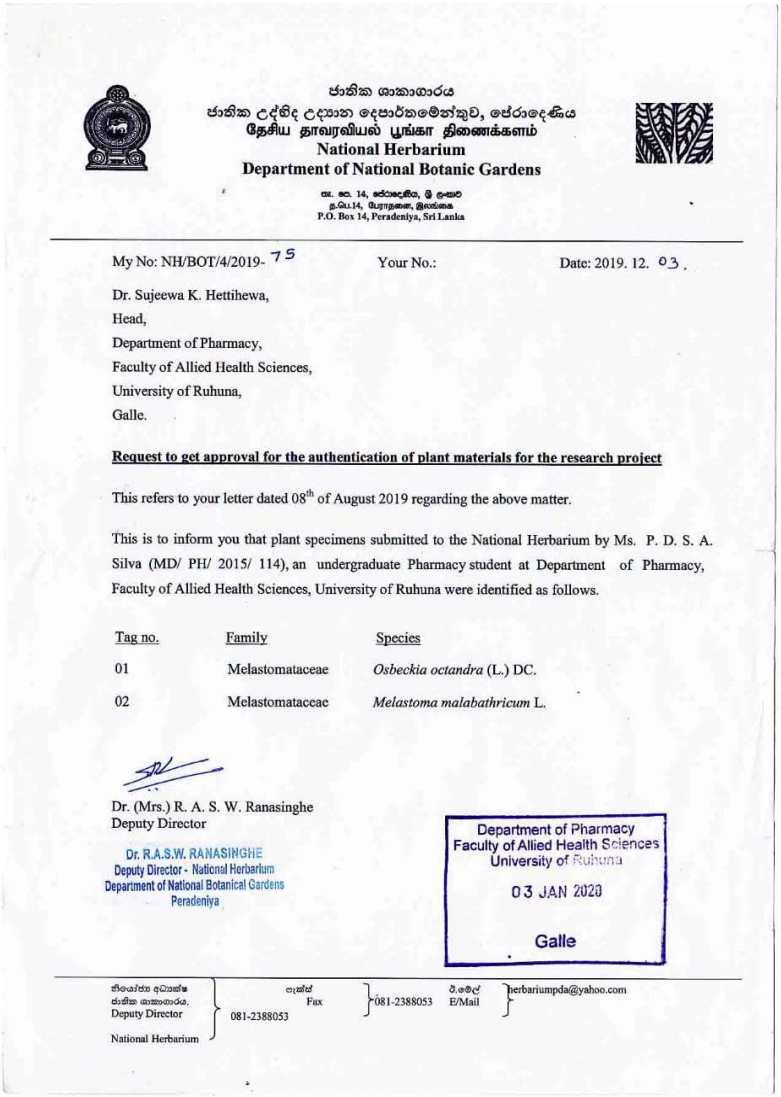

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ජාතික උද්භිද උද්‍යාන දෙපාර්තමේන්තුව, පේරාදෙණිය
தேசிய தாவரவியல் பூங்கா திணைக்களம்
National Herbarium
Department of National Botanic Gardens

ත. කැ. 14, පේරාදෙණිය, ශ්‍රී ලංකාව
த.பெ.14, பேராதனை, இலங்கை
P.O. Box 14, Peradeniya, Sri Lanka

My No: NH/BOT/4/2019- 75 Your No.: Date: 2019. 12. 03.

Dr. Sujeewa K. Hettihewa,
Head,
Department of Pharmacy,
Faculty of Allied Health Sciences,
University of Ruhuna,
Galle.

Request to get approval for the authentication of plant materials for the research project

This refers to your letter dated 08th of August 2019 regarding the above matter.

This is to inform you that plant specimens submitted to the National Herbarium by Ms. P. D. S. A. Silva (MD/ PH/ 2015/ 114), an undergraduate Pharmacy student at Department of Pharmacy, Faculty of Allied Health Sciences, University of Ruhuna were identified as follows.

Tag no.	Family	Species
01	Melastomataceae	*Osbeckia octandra* (L.) DC.
02	Melastomataceae	*Melastoma malabathricum* L.

Dr. (Mrs.) R. A. S. W. Ranasinghe
Deputy Director

Dr. R.A.S.W. RANASINGHE
Deputy Director - National Herbarium
Department of National Botanical Gardens
Peradeniya

Department of Pharmacy
Faculty of Allied Health Sciences
University of Ruhuna
03 JAN 2020
Galle

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ජාතික ශාකාගාරය,
Deputy Director
National Herbarium
ෆැක්ස් Fax 081-2388053
දු.ඇ.ලිපි E/Mail 081-2388053 herbariumpda@yahoo.com

Appendix: Letter of Authentication for the plant specimen; *O. octandra*