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### Assessment of Phytochemical Contents and Total Antioxidant Capacity of Five Medicinal Plants with Cosmetic Potential under Three Different Drying Methods

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**Abstract** Drying allows the quick conservation of medicinal properties of herbal materials. However, the instability of bioactive compounds in medicinal plants which exhibit potent antioxidant activity and wide range of pharmacological properties may indicate a sensitivity to different drying treatments. Therefore, the objective of the present study was to determine the effect of shade drying, solar drying and oven drying on bioactive ingredients of five cosmetic potential plant leaves. Leaves of Centella asiatica (L.) Urb., Senna alata (L.) Roxb., Justicia adhatoda L., Ocimum tenuiflorum L., Hibiscus rosa-sinensis L. were dried to a constant weight using shade drier at 30-35 °C, solar drier at 30-40 °C and oven at 40 °C. Aluminum chloride colorimetric assay, Folin- Ciocalteau method, and Phosphomolybdate assay were employed to analyse the total flavonoid content (TFC), total phenolic content (TPC) and total antioxidant capacity (TAC) of ethanolic extracts of leaves respectively. All assays were performed in triplicate. Data was analyzed using one -way ANOVA and Tukey's multiple comparison method. Results showed that significantly higher TFC, TPC and TAC of solar dried leaves of O. tenuiflorum (758.81±2.05 mg RE/100g DW, 3.54±0.71 mg GAE/100g DW and 22.56±0.38 mg AAE/100g DW respectively) and leaves of H. rosa-sinensis (89.72±1.38 mg RE/100g DW, 23.9±0.06 mg GAE/100g DW and 8.53±0.73 mg AAE/100g DW respectively). Solar dried C. asiatica and S. alata leaves showed high TFC and TAC while the TPC was high in oven dried leaves of C. asiatica and shade dried leaves of S. alata respectively. In contrast, J. adhatoda showed the maximum TFC in shade dried samples, the highest TPC in solar dried leaves and the maximum TAC in oven dried leaves. Moreover, there were no significant differences (p > 0.05) among drying methods in terms of antioxidant capacity and phenolic content of J. adhatoda. and antioxidant capacity of C. asiatica. Thus, it can be concluded that, solar drying of medicinal plant materials using solar drier would be an economical, efficient, and effective drying method for preserving bioactive compounds present in leaves of above-mentioned plants.

Keywords: antioxidant capacity, drying methods, flavonoid content, medicinal plants, phenolic content

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### 1. Introduction

Drying is considered as a critical factor for the post-harvest management of herbs [1]. The main purpose of drying is increasing the products shelf life, minimize packaging requirements and reducing the bulk weight of herbal materials [2]. Drying process increases the shelf life by slowing or stopping microorganisms' growth and

preventing certain biochemical reactions that might alter the organoleptic characteristics [3]. But, at the same time, it can give rise to other changes that affect the herb quality. Although some of the phytochemicals which may possess antioxidant activity and other health-promoting properties are more thermo stable, the drying of herbs is often accompanied with the loss of bioactive compounds [1]. Thus, it is paramount to reduce the exposure of herbs to excessive high temperatures and avoid contaminations during drying process. Improper drying of herbal materials

may negatively affect to the herbs quality while causing the major losses of their medicinal, culinary, visual, and nutraceutical properties. Under the right conditions, drying can produce a sufficiently shelf stable product without major losses in herb value [2,4]. Conventionally, low drying temperatures between 30 and 50°C are suggested to protect thermolabile bioactive compounds. However, the choice of drying method is determined by the sensitivity of the constituents to heat [5].

Incorporation of botanical extracts in cosmetic preparations has shown remarkable growth in recent years due to multifunctional effects of botanical extracts. Polyphenolic compounds which are known as the plant secondary metabolites, have been extensively investigated for their functional properties and used for various purposes in cosmetics such as free-radical scavenging, anti-inflammatory, anti-aging, sun protection, whiteners, and anti-microbial effects [6]. However, post-harvest practices directly influence on the biological compounds of botanical extracts. The effect is dependent on the drying method, storage conditions, type of chemical compounds, plant parts used and the type of plants [7].

Therefore, the aim of this present study was to determine the effect of three different drying methods on total flavonoid content, total phenolic content and total antioxidant capacity of *Centella asiatica* (L.) Urb., *Senna alata* (L.) Roxb., *Justicia adhatoda* L., *Ocimum tenuiflorum* L., *Hibiscus rosa-sinensis* L. leaves with potential use in cosmetics and determine the efficient drying method for economical usage.

### 2. Methodology

### 2.1. Collection of Plant Materials

Fresh leaves of *Centella asiatica* (L.) Urb. and *Ocimum tenuiflorum* L. were collected from Chilaw, North Western province, Sri Lanka while *Senna alata* (L.) Roxb., *Justicia adhatoda* L., and *Hibiscus rosa-sinensis* L. were collected from Dankotuwa, North Western province, Sri Lanka. The leaves were sorted, washed with running water and drained thoroughly on paper towels. Each sample was divided into 4 batches. One batch was used to measure the initial moisture content of each leaf sample whereas the remaining three batches were immediately dried using one of the drying methods tested. The moisture content of fresh leaves was determined using AOAC method 984.25. About 5 g of the leaves was dried at 103±2 °C until no change in mass was detected. Three replications were performed.

### 2.2. Drying of Herbal Materials

Drying of leaves was carried out by using three different methods.

#### 2.2.1. Shade Drying

Leaves were dried at ambient temperature ranged from 30-35 °C in a fabricated shade drier without exposure to solar light. Leaves were distributed uniformly on perforated trays and dried until the leaves reached 9-10% of moisture content.

#### 2.2.2. Oven Drying

The experiments were performed at 40°C in a laboratory oven (OF-22G, Jeio Tech, Korea). Leaves were distributed evenly on perforated stainless-steel trays and dried until the leaves reached 9-10% of moisture content.

#### 2.2.3. Solar Drying

The leaves were dried in a newly fabricated solar dryer equipped with forced air circulation system where inside temperature ranged from 30-40°C. Samples were placed uniformly on perforated trays and dried until the leaves reached 9-10% of moisture content.

### 2.3. Preparation of Crude Extracts

Dried samples were coarsely powdered using mechanical grinder. One gram of powdered sample from each was soaked in 20 ml of 80% ethanol (1W:20V) for 24 hours. The extracts were subsequently filtered through a filter paper (Whatman No. 01; Whatman Paper Ltd, Maidstone, UK) and concentrated under reduced pressure using a centrifugal evaporator (EYELA CVE-3000, Indonesia) to obtain the ethanol extracts. The prepared extracts were stored at 4°C until assayed within a week.

# 2.4. Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was measured according to a colorimetric assay method as described by Gunathilake et al. [9]. Briefly, 0.5 ml of ethanolic extracts of leaf solutions was added to 3 ml of distilled water. Then, 0.3 ml of 5% NaNO2 was added and stands for 5 min at room temperature (30 °C). About 0.3 ml of 10% AlCl<sub>3</sub> was added 5 min later and allows standing for another 6 min, and then 2 ml of 1 M NaOH was added and the solution was made up to 10 ml with distilled water and mixed. The absorbance was determined at 510 nm against blank using the spectrophotometer (GENESYS 10S UV-VIS). The blank was prepared providing similar conditions as mentioned above without the leaf extract. Rutin standard solutions were prepared by dissolving rutin in ethanol at the concentrations ranging from 50 to 250 mg/L. The standard curve of rutin, y = 0.0118x + 0.0126 $(R^2 = 0.981)$  was used to determine the TFC expressed as milligram of rutin equivalents (RE) per 100 g dry weight of leaves.

## 2.5. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using Folin-Ciocalteu assay as described by Gunathilake and Ranaweera [8]. About 0.5 ml of extract and 0.1 ml of Folin- Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 min in the dark. Then 2.5 ml 7.5% sodium carbonate was added to the mixture and further incubated for 2 hours in the dark at room temperature. Thereafter, the absorbance was measured at 760 nm against a blank using a spectrometer (GENESYS 10S UV-VIS). The blank was prepared providing similar conditions as mentioned above without

the leaf extract. Gallic acid standard solutions were prepared by dissolving gallic acid in ethanol at the concentrations ranging from 50 to 250 mg/ L. The standard curve of gallic acid,  $y=0.0906x+0.4804~(R^2=0.989)$  was used to determine the TPC expressed as milligram of gallic acid equivalents per 100 g dry weight of leaves.

# 2.6. Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacity (TAC) of the extracts was determined by phosphomolybdate method as described by Gunathilake and Ranaweera [8]. The tubes containing leaf extract (0.3 ml) and 3 ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm spectrophotometrically (GENESYS 10S UV-VIS) against a blank. The blank was prepared using reagent solution without the leaf extract and incubated under the same conditions. Ascorbic acid standard solutions were prepared by dissolving ascorbic acid in ethanol at

concentrations ranging from 50 to 250 mg/ L. The standard curve of ascorbic acid, y = 0.0657x + 0.1071 ( $R^2 = 0.995$ ) was used to determine the TAC expressed as milligram of ascorbic acid equivalents (AAE) per 100 g dry weight of leaves.

### 2.7. Statistical Analysis

The effect of drying methods on TAC, TFC and TPC of leaf materials were presented as mean ± standard deviation of minimum three replications. Mean values were compared using one - way ANOVA and Tukey's multiple comparison method. Statistical significance was set at 5%. The programmes used were Microsoft Excel 2016 and Minitab 17.

### 3. Results and Discussion

The moisture content of leaves of *C. asiatica, S.alata, J. adhatoda, O. tenuiflorum*, and *H. rosa-sinensis* was reported as 87.67±0.53%, 71.76±2.32%, 73.37±0.80%, 83.44±0.58% and 77.10±0.93% on fresh weight wet basis.

Table 1. Duration of different methods of drying

Plant name		Drying method		
	Shade drying	Solar drying	Oven drying	
Centella asiatica (L.) Urb.	7 Days	2 Days	5 hours	
Senna alata (L.) Roxb.	5 Days	1 Days	4 hours	
Justicia adhatoda L.	6 Days	1 Days	4 hours	
Ocimum tenuiflorum L.	6 Days	1.5 Days	5 hours	
Hibiscus rosa-sinensis L.	6 Days	1.5 Days	4.5 hours	

Table 2. TFC, TPC and TAC of Centella asiatica leaves under 03 different drying conditions

Parameter	Oven drying	Shade drying	Solar drying
TFC (mg RE /100g DW)	336.11±0.37 <sup>b</sup>	356.36±0.94 <sup>b</sup>	388.28±0.56 <sup>a</sup>
TPC (mg GAE /100g DW)	3.71±0.09 <sup>a</sup>	2.08±0.07 <sup>b</sup>	1.92±0.18 <sup>b</sup>
TAC (mg AAE/100g DW)	7.46±0.12 <sup>a</sup>	6.76±0.18 <sup>a</sup>	7.63±0.25 <sup>a</sup>

Means of each row which are not sharing the same superlative letter are significantly different (p < 0.05).

Table 3. TFC, TPC and TAC of Senna alata leaves under 03 different drying conditions

Parameter	Oven drying	Shade drying	Solar drying
TFC (mg RE /100g DW)	229.69±2.10°	277.04±2.45 <sup>b</sup>	329.13±1.58 <sup>a</sup>
TPC (mg GAE /100g DW)	2.27±0.18 <sup>b</sup>	5.44±0.16 <sup>a</sup>	4.37±0.14 <sup>a</sup>
TAC (mg AAE/100g DW)	32.11d±1.47 <sup>b</sup>	34.95±0.98 <sup>b</sup>	39.78±1.02 <sup>a</sup>

Means of each row which are not sharing the same superlative letter are significantly different (p < 0.05).

Table 4. TFC, TPC and TAC of Justicia adhatoda leaves under 03 different drying conditions

Parameter	Oven drying	Shade drying	Solar drying
TFC (mg RE /100g DW)	82.52±0.59 <sup>b</sup>	115.63±0.93 <sup>a</sup>	68.17±0.89°
TPC (mg GAE /100g DW)	1.63±0.03 <sup>a</sup>	1.22±0.05 <sup>a</sup>	1.69±0.16 <sup>a</sup>
TAC (mg AAE/100g DW)	15.11±1.31 <sup>a</sup>	10.64±1.67 <sup>a</sup>	14.53±1.27 <sup>a</sup>

Means of each row which are not sharing the same superlative letter are significantly different (p < 0.05).

Table 5. TFC, TPC and TAC of Ocimum tenuiflorum leaves under 03 different drying conditions

Parameter	Oven drying	Shade drying	Solar drying
TFC (mg RE /100g DW)	407.89±1.53°	568.14±1.62 <sup>b</sup>	758.81±2.05 <sup>a</sup>
TPC (mg GAE /100g DW)	3.01±0.29 <sup>a</sup>	1.32±0.65 <sup>b</sup>	3.54±0.71 <sup>a</sup>
TAC (mg AAE/100g DW)	11.38±0.11 <sup>b</sup>	$6.66\pm0.24^{c}$	22.56±0.38 <sup>a</sup>

Means of each row which are not sharing the same superlative letter are significantly different (p < 0.05).

Table 6. TFC, TPC and TAC of Hibiscus rosa-sinensis leaves under 03 different drying conditions

Parameter	Oven drying	Shade drying	Solar drying
TFC (mg RE/100g DW)	88.06±2.87 <sup>a</sup>	64.50±1.95°	89.72±1.38 <sup>b</sup>
TPC (mg GAE /100g DW)	1.88±0.07 <sup>ab</sup>	1.63±0.13 <sup>b</sup>	2.39±0.06 <sup>a</sup>
TAC (mg AAE/100g DW)	5.59±0.28 <sup>b</sup>	6.69±0.45 <sup>b</sup>	$8.53\pm0.73^{a}$

Means of each row which are not sharing the same superlative letter are significantly different (p < 0.05).

Tables from 2 to 6 present the data of TFC, TPC and TAC of C. asiatica, S.alata, J. adhatoda, O. tenuiflorum, and H. rosa-sinensis leaves dried under 03 different drying conditions; oven, shade and solar drying. Overall, results showed higher TFC, TPC and TAC of solar dried leaves of O. tenuiflorum and H. rosa-sinensis in comparison with oven drying and shade drying methods. Solar dried C. asiatica and S. alata leaves showed significantly higher flavonoid content and antioxidant capacity while the phenolic content was high in shade dried leaves of S. alata and oven dried leaves of C. asiatica respectively. In contrast, J. adhatoda showed the maximum flavonoid content in shade drying samples, the highest phenolic content in solar dried leaves and the maximum antioxidant capacity in oven dried leaves. Moreover, TFC of solar dried and TPC of oven dried C. asiatica leaves were significantly different (p < 0.05) from other drying methods while TAC of C. asiatica leaves dried under oven, shade and solar drying methods was not significantly different (p > 0.05). TFC of S. alata leaves dried under three different drying methods was significantly different whereas TPC of oven dried and TAC of solar dried S. alata leaves were significantly different from other drying methods. J. adhatoda leaves showed significant differences among all drying methods on TFC while all drying methods were non-significant on TPA and TAC of J. adhatoda leaves. TFC and TAC of O. tenuiflorum leaves dried under three different drying methods were significantly different whereas TPC of shade dried O. tenuiflorum leaves was significantly different from other drying methods. A significant difference was observed in TAC of solar dried H. rosa-sinensis leaves whilst TFC of H. rosa-sinensis leaves dried under all drying methods was significantly different. However, TPC of H. rosa-sinensis leaves dried under oven and solar drying methods as well as oven and shade drying methods were not significantly different.

In this study, leaves were dried in closed system using fabricated solar drier without exposing leaves to the direct sunlight. During the solar drying, the high variation of radiation, temperature and humidity generates unstable drying conditions [10] that would lead to the stress conditions of metabolically active plant materials [11]. From the perspective of plant physiology, freshly harvested plant materials, particularly the leaves, are physiologically active. As a result, water depletion during the drying process as well as other stress conditions will cause a series of dehydration-related physiological reactions that maintain the normal physiological function, particularly the increase in the levels of some secondary metabolites that are involved in defence mechanisms [12]. Thus, it may result the synthesis of several phenylpropanoid compounds (flavonoids, isoflavonoids, psoralens, coumarins, phenolic acids, lignin and suberin) [11] that could result high TFC, TPC and TAC. However, an increase in antioxidant

capacity of *J. adhatoda* and TPC of *C. asiatica* under oven drying method could be due to the release of bound phenolic compounds brought about by the breakdown of cellular constituents and the formation of new compounds with enhanced antioxidant potential [2].

TAC of C. asiatica, TPC, TAC of J. adhatoda, and O. tenuiflorum and TFC, TPC of H. rosa-sinensis leaves dried under shade exhibited significant low values compared with other drying methods. Reduction in bioactive compounds resulting from shade drying could be due to enzymatic degradation as the process was carried out at room temperature and took several days for samples to dry (Table 1). Thus, longer duration of oxygen exposure may result in increasing redox activity of plant materials and degradation of phenolic compounds [13]. Moreover, Chan et al. [14], stated that much of the antioxidant compounds are lost through enzymatic degradation by degradative enzymes such as lipoxygenase and polyphenol oxidase and/ or heat decomposition. On the contrary, Oduje and John [15] and Mudau and Ngezimana [16], report that shade drying method produces the best results in phytochemical and nutritional constituents.

Further, low values of TFC of C. asiatica and O. tenuiflorum, TFC, TPC, TAC of S. alata and TAC of H. rosa-sinensis oven dried leaves were observed. Depletion in TPC and antioxidant activity are often accompanied by loss of other bioactive properties [17]. The main mode of heat transfer for drying with oven is convection. Thus, phenolic compounds can be decomposed by thermal and oxidation degradation as phenolic compounds possess a hydroxyl group (-OH), which can be oxidized in the presence of oxygen [18]. Thus, loss in antioxidant properties of heat-treated samples have been attributed to thermal degradation of phenolic compounds, thermal degradation of phytochemicals and to loss of antioxidant enzyme activities [14]. Furthermore, Zainol et al. [19], highlighted that the loss of macromolecules like flavonoid during heat treatment might be due to the harsh drying conditions, particularly the temperature and duration used. Although many studies have reported loss in TPC and antioxidant activity of plant samples following thermal treatments, Bernard et al. [1], Chao et al. [12], Pham et al. [13] and Santana et al. [20], have emphasized that oven drying is the best method to ensure the quality of the raw materials in terms of phytochemical content and organoleptic properties as it can be attributed to the rapid inactivation of enzymes over a shorter duration.

#### 4. Conclusion

Solar drying of medicinal plant materials using solar dryer would be an economical, efficient and effective drying method for preserving bioactive compounds contained in leaves of C. asiatica, S.alata, J. adhatoda, O. tenuiflorum, and H. rosa-sinensis. However, optimizing the solar drying method is crucial to enhance the quality of the materials. Design of a solar dryer with control technique that enables continuous drying operation during sunny/ cloudy days and night hours is paramount. Further, issues generate due to unstable drying conditions such as high variation of radiation, temperature and humidity must also be addressed when developing a solar dryer to reduce the high variability in quality characteristics of the plant materials. As it is difficult to recommend a common method for drying herbal materials, it is important to conduct more research about the effect of drying methods on individual species to identify the optimum temperature and duration of drying. As some type of metabolites contained in plants are also impacted during the drying process, it is necessary to determine the best method of drying to maintain the antioxidant effects, total phenolic and total flavonoid content of individual species.

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