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Effect of Different Drying Methods on Antioxidant Activity and Availability of Phytochemicals in Leaves of *Costus speciosus*, *Coccinia grandis* and *Gymnema sylvestre*

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***Abstract* - Drying is an important method for preserving phytochemicals in plant materials though the stability is altered with the type of drying method. This study aimed at evaluating the effectiveness of different drying methods on antioxidant activity and phytochemical availability in leaf ethanolic extracts of *Costus speciosus*, *Coccinia grandis*, and *Gymnema sylvestre*. Plant leaves of the selected species were subjected to shade drying at room temperature (25 ± 5°C), oven drying at 45 °C and freeze-drying methods. The extracts were obtained by a maceration method with 95% ethanol. Obtained ethanolic extracts were analyzed for antioxidant activity in terms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity, total reducing power and total antioxidant capacity. Phytochemical availability was determined qualitatively and quantitatively in the extracts. All the plant species and drying methods had a significant difference ($p<0.05$) for the tested antioxidant activities. Among the drying techniques, the freeze-drying method was more effective to preserve the antioxidant compounds and phytochemicals compared to oven drying and shade drying. The lowest antioxidant activity and phytochemical availability were shown by the leaves subjected to oven drying at 45 °C. The freeze-dried leaves of *C. speciosus* ethanolic extract demonstrated the best radical scavenging activity for DPPH and ABTS assays with IC_{50} values of 57.27 ± 2.08 µg/ml, 21.86± 3.55 µg/ml respectively. Furthermore, the highest content of total phenolics (82.88±2.31 mg GAE/g), tannins (9.58 ±2.71mg TAE/ g), terpenoids (45.10±3.46 %), and alkaloids (19.37±3.54%) were recorded in *C. speciosus* among all dried leaves from the selected species. It can be concluded that the freeze-dried leaves of all plant species showed a higher value of antioxidant activity and phytochemical availability and that can be potentially used for the production of pharmaceuticals and novel functional foods.**

Keywords: **Drying Techniques, Phytochemicals, Medicinal Plants, Antioxidant Activity, Bioactive Compounds**

I. INTRODUCTION

The use of medicinal plant materials is becoming popular in food and pharmaceutical applications all over the world due to their possible therapeutic properties [1]. The world health organization reported that 80% of the people on the globe primarily depend on traditional medicine [2]. Medicinal plant extracts contain a wide range of bioactive compounds. Thus, they can be used in a variety of functional food and pharmaceutical applications, such as the development of products with antioxidants, anti-diabetic, anti-inflammatory, anti-cancer, and anti-microbial properties [3]. Therefore, it is essential to screen the bioactive compounds in medicinal plants to explore novel pathways to develop innovative food products to deliver health benefits.

Medicinal plants are high in natural antioxidants and phytochemicals, which are able to account for suppressing or mitigating the negative effects of free radicals [4], [5]. However, only a limited amount of plant materials has been scientifically studied in terms of efficacy and mechanisms of free radical scavenging activity. The stability and composition of bioactive chemicals in plant materials can be altered by moisture level [6]. Therefore, drying is even more important for preserving phytochemicals in plant materials. Different drying methods named shade drying, oven drying, freeze-drying, and sun-drying have substantial effects on appearance, phytochemical efficiency, and volatile and heat-labile chemicals [7].

The leaves of three different plant species named *Costus speciosus*, *Coccinia grandis*, and *Gymnema sylvestre* were selected for this study based on their historical reputation in ayurvedic medicine for treating diabetes [5], [8], [9]. This study focused to compare the effect of shade drying, oven drying and freeze-drying on antioxidant activity and phytochemicals in leaves of three selected medicinal plants.

II. METHODOLOGY

A. Collection and Freeze-Drying the Plant Materials

The leaves of the *C. specious*, *C. grandis* and *G. sylvestre* were collected from the city of Badulla, Sri Lanka. The plant leaves were deposited and authenticated at the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka.

All plant leaves were cleaned in running water before being air-dried at room temperature (25 ± 5℃).

Three different drying techniques were applied for the selected leaves separately. Leaves were shade dried at normal room temperature (25± 5ºC), oven-dried at 45ºC using a laboratory oven (Memmert, Germany) and freeze-dried at −80 °C, 0.005 psi in a freeze dryer (Model No: LBFD-A11, Labtron, UK) until they reached a constant weight. After, the dried leaves were ground using a high-speed universal disintegrator (Biobase HSD 400A, China) into fine powder for extracting bioactive compounds.

B. Extraction of Plant Materials

Each ground leaf powder (*C. specious*, *C. grandis* and *G. sylvestre*) (5g) was separately placed in an Erlenmeyer flask with 50 ml of 95% ethanol and shaken in an orbital shaker at 120 rpm for 24 hours. The procedure was repeated twice, and the collected filtrates were filtered with the use of Whatman 1 filter paper. The excess solvents were evaporated using a rotary evaporator set to 40°C. The crude extracts were weighed and stored at -20°C for further analysis [10].

C. In-Vitro Antioxidant Assays

1. DPPH Radical Scavenging Activity: Free radical scavenging activity of the plant extracts was measured using DPPH as a free radical model according to the method described by a previous study [11]. Plant extracts with different concentrations (10 -100 µg/ml) were prepared. One milliliter of 0.1 M DPPH solution was combined with 1ml of plant extract of each concentration separately and incubated in the dark at room temperature (25 ± 2℃) for 30 minutes. Thereafter, the absorbance of the solutions was measured by using a 96-wells microplate reader (Thermo Scientific Multiskan Go) at 517 nm. Ascorbic acid and 100% methanol were used as the standard control respectively in this experiment. The capacity to scavenge the DPPH radical was stated as a percentage of inhibition and calculated as follows using the equation Eq. (1).

$$\% \text{ of DPPH Scavenging activity} = \frac{Ac - As}{Ac} * 100 \quad (1)$$

Where Ac = Absorbance control
As = Absorbance sample

2. ABTS+ Radical Scavenging Activity: The ABTS+ assay was executed based on the previously established protocol [12]. The ABTS+ stock solution was prepared by mixing equivalent volumes of 7 mM ABTS and 2.45 mM potassium persulfate, then incubated at room temperature (25 ± 2℃) in the dark for 12 hours. This stock solution was diluted with methanol until it obtained an absorbance of approximately 0.70+0.02 at 745 nm. The activity of free radical scavenging was determined by combining 100µl of plant extracts, ranging from 10-100 µg/ml, with 100µl of ABTS working solution separately. The absorbance was measured at 745nm using a 96-microplate reader (Thermo Scientific Multiskan Go) after 6 minutes at 30ºC. Ascorbic acid was kept as a standard and 100% methanol was employed as a control. The scavenging activity was estimated using the equation Eq. (2) as follows.

$$\% \text{ of ABTS scavenging activity} = \frac{Ac - As}{Ac} * 100 \quad (2)$$

Where Ac = Absorbance control
As = Absorbance sample

3. Total Reducing Power (TRP): The method described in [13] was used to investigate the TRP. Approximately 0.2 mL of extracts at various concentrations (10-100 g/ml), 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide (1 %) were combined together and incubated at 50°C for 20 minutes to reduce ferricyanide to ferrocyanide. The reaction was halted by adding 2.5 mL of 10% (w/v) trichloroacetic acid and centrifuged for 10 minutes at 1000 rpm. The centrifuged sample was added to the mixture of 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%). Finally, the absorbance was taken at 700 nm. The graph was plotted according to the absorbance versus sample concentration.

4. Total Antioxidant Capacity (TAC): The value of TAC was determined according to the method in [14]. Each extract (0.1 mL) with various concentrations (50-1000 µg/ml) was combined in a 1.5 mL volume eppendorf tube with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated for 90 minutes at 95°C in a thermal block. The absorbance of the aqueous solution was measured at 695 nm against a blank after cooling to room temperature. The standard is utilized as ascorbic acid. The percentage of TAC was calculated using the equation Eq. (3) as follows.

$$\%\text{TAC inhibition} = \frac{Ac - As}{Ac} * 100 \quad (3)$$

Where As = Absorbance of sample + reagent solution.
Ac = Absorbance of reagent solution + methanol.

D. Qualitative Analysis of Phytochemicals

The presence of tannins, terpenoids, phenols, saponins, flavonoids, alkaloids, steroids, coumarins, quinones, anthraquinones, and glycosides in the freeze-dried ethanolic extracts of *C. specious*, *C. grandis*, and *G. sylvestre* were determined using the standardized procedures according to the method in a previous study [15].

E. Quantitative Analysis of Phytochemicals

1. Determination of Total Phenolics: The total phenolic content (TPC) of the ethanolic extracts was evaluated based on the method of [16]. Ten times diluted freshly prepared Folin-Ciocalteu reagent 110 µL was assorted with 20 µL of each extract (1 mg/mL) separately. Then 70 µL of 10% sodium carbonate solution was incorporated and incubated for 30 minutes at room temperature (25±2℃). A 96-well

microplate reader was used to determine the absorbance at 765 nm (Thermo Scientific Multiskan Go). The standard curve was built with gallic acid. TPC was measured in milligrams of gallic acid equivalents (GAE) per gram of plant extract.

2. Determination of Total Flavonoids: To assess the total flavonoid content (TFC) of plant extracts, the reaction mixture was developed by combining the 25 µL of 1 mg/mL extract, 75 µL of methanol, and 100 µL of a 20% aluminum chloride. Then, it was incubated at room temperature (25±2ºC) for 10 minutes. A 96-well microplate reader was used to determine the absorbance at 415nm. The standard curve was constructed using quercetin. TFC was calculated as milligrams of quercetin (QCE) equivalents per gram of plant extract [16].

3. Determination of Tannins: The Folin-Ciocalteu method stated by a previous study [17] was followed to determine the tannin content. Briefly, 0.1 ml of the sample extract was combined with 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, and 1 ml of 35% sodium carbonate solution in a volumetric flask (10 ml) and diluted the solution mixed with distilled water up to the 10 ml mark. Then the mixture was thoroughly agitated and kept to settle at room temperature (25±2ºC) for 30 minutes. Tannic acid was used as the standard. The absorbance of the test samples and the reference solutions were measured at 700 nm. The tannin content of the dried sample was measured in milligrams of tannic acid (TAE) equivalents per gram of plant extract.

4. Determination of Saponins: The content of saponins in crude extracts was evaluated using the method described by a previous study [18]. Each sample (20 g) was placed together with 100 ml of 20% aqueous ethanol. The flask was then heated in a water bath at 55°C for four hours with constant stirring. After filtering the mixture, 200 mL of 20% ethanol was used to extract the residue. The combined extract was kept in a 90°C water bath until the volume was reduced up to 40 ml. The solution was poured into a 250 mL separating funnel and mixed with 20 mL diethyl ether.

After the separation, the aqueous layer was kept, and the ether layer was discarded. The purifying procedure was carried out twice more. Following that, 60 milliliters of n-butanol were incorporated into the aqueous layer. The 10 mL of 5% aqueous sodium chloride was used to rinse the mixed n-butanol extracts twice. The remaining solution was heated to 90 ℃ in a water bath. The obtained samples were dried in an oven (40 ℃) for two hours. Then the sample was weighed, and the saponins content was determined as a percentage using Eq. 4.

$$\% \text{ Saponins} = \frac{W2}{W1} \times 100 \quad (4)$$

Where W2 = Final weight of the sample
W1 = Initial weight of the extract

5. Determination of Alkaloids: The method described by a previous study [18] was referred to determine the alkaloid concentrations. The sample (5g) was weighed and mixed with 200 mL of 10% acetic acid in ethanol. The beaker was enclosed and set aside for four hours. After that, the extract was filtered and concentrated in a water bath at 90℃ to get one-quarter of its original volume. Concentrated ammonium hydroxide drops were added to the extract until it was completely precipitated. The solution mixture was allowed to stand until it settled. Then, it was filtered and rinsed with dilute ammonium hydroxide. The precipitated alkaloid was weighed once it had dried completely and the percentage was calculated using the equation (Eq. 5) as follows,

$$\% \text{ of alkaloids} = \frac{W2}{W1} \times 100 \quad (5)$$

Where W2 = Final weight of the sample
W1 = Initial weight of the extract

6. Determination of Terpenoids

The contents of terpenoids were analyzed using the method described by a previous study [19]. Each freeze-dried extract of 100 mg (Wi) was weighed separately and steeped for 24 hours in 9ml 100% ethanol. Following that, each extract was filtered, and the filtrates were re-extracted in a separating funnel with 10ml of petroleum ether. The petroleum ether extract was then added to the pre-weighed glass vial and kept at room temperature (25±2°C) until all of the petroleum ether evaporated. After the evaporation, the final weight of the glass vial was taken (W_f). The following Eq. 6 was used to compute the percentage of terpenoids.

$$\% \text{ of Terpenoids} = \frac{W_i - W_f}{W_i} * 100 \quad (6)$$

F. Statistical Analysis

All experiments were done in triplicate. MINITAB 16 software was used to analyze the data and the Tukey test was used to determine the significance of differences between crude extracts ($p<0.05$) using one-way ANOVA (Analysis of variance).

III. RESULTS AND DISCUSSION

Herbal drying is destined to inhibit microbial growth and slow enzymatic reactions that can affect the bioactive compound's stability and availability [20]. Therefore, the antioxidant activity of selected plant leaves of *C. speciosus*, *C. grandis*, *G. sylvestre* can be influenced by the application of different drying methods named shade drying, oven drying (45℃) and freeze-drying.

A. Antioxidant Properties of Leaf Extracts

The activity of the antioxidant assay can be changed according to the assay principle and experimental conditions. In addition to that, the crude extracts contain diverse

antioxidant compounds, and their collaborative effects can be altered the antioxidant activity [21]. As a result of that, it is difficult to adequately describe the antioxidant capacity of plant extracts using a single approach due to the complicated nature of their activity. Therefore, the antioxidant activity was assessed in this study utilizing four distinct methods: DPPH, ABTS, TRP, and TAP.

TABLE I ANTI-OXIDANT PROPERTIES OF *C. SPECIOUS, C. GRANDIS A*ND *G. SYLVESTRE* LEAVES AFTER APPLYING SHADE-DRYING, OVEN DRYING AND FREEZE-DRYING

Plant Species	Drying Technique	Anti-Oxidant Properties IC 50 (µg/ml)			
		DPPH	ABTS	TRP	TAP
C. specious	Shade drying	67.44 ±1.68^{g}	44.44 ±2.29^{g}	35.73 ±5.30^{cd}	641.58 ± 3.78^{f}
	Oven drying at 45 °C	102.56 ±2.01^{f}	84.40 ± 3.86^{f}	51.42±3.39^{b}	652.56±5.65^{f}
	Freeze- drying	57.27 ±2.08^{h}	21.86 ±3.55^{h}	28.46 ±1.80^{d}	527.96+1.06^{g}
C. grandis	Shade drying	136.82 ±1.67^{e}	112.49 ± 3.58^{d}	38.14±2.31^{c}	792.23±3.08^{e}
	Oven drying at 45 °C	175.29 ±2.89^{d}	166.06 ± 5.15^{b}	32.89± 1.68^{cd}	850.66±3.78^{d}
	Freeze- drying	106.22 ±5.69^{f}	96.90 ±4.79^{e}	16.84± 1.22e	784.70±3.53^{e}
G. sylvestre	Shade drying	238.99 ±3.74^{b}	172.36 ± 2.65^{b}	58.19 ±2.54^{ab}	1079.98±4.92^{b}
	Oven drying at 45 °C	288.06 ± 2.72^{a}	253.04 ±3.00^{a}	63.90 ±1.47^{a}	1113.38±4.61^{a}
	Freeze- drying	209.38 ±0.95^{c}	152.60 ± 1.46^{c}	32.43 ±4.28^{cd}	1024.12±5.26^{c}

All values are means of triplicate determination ± SD
The antioxidant activity of each assay was statistically analyzed separately for all plant species and drying methods
Different letters in the same column showed statistical difference according to the Tukey's test at $p<0.05$

All shade-dried, oven-dried and freeze-dried leaf extracts of *C. speciosus*, *C. grandis*, and *G. sylvestre* showed dose-dependent antioxidant activity for DPPH, ABTS, TRP and TAP assays. Among the drying techniques, the highest DPPH and ABTS radical scavenging activities were revealed in the freeze-dried leaves of *C. speciosus*, *C. grandis*, *G. sylvestre* ethanolic extracts and the lowest activities were shown by the oven-dried leaf ethanolic extracts of all selected plants. The ethanolic extract of freeze-dried *C. speciosus* obtained the maximum DPPH and ABTS free radical-scavenging activity with the lowest IC50 value of 57.27 ± 2.08 µg/ml and IC50 = 21.86±3.55 µg/ml respectively. The oven-dried *G. sylvestre* ethanolic extract had the lowest capacity for DPPH free radical-scavenging activity (IC50= 288.06 ± 2.72 µg/ml and ABTS free radical-scavenging activity (IC50 =253.04 ±3.00 µg/ml) (*Tab. 1*). The ascorbic acid was more effective compared to all plant extracts in both DPPH and ABTS antioxidant activity with IC50=19.53±2.98 µg/mL and IC50=10.85± 1.82 µg/mL respectively.

The literature revealed that freeze-drying is a more promising method for preserving the bioactive compounds which are responsible for the DPPH and ABTS radical scavenging activity. According to [22] the freeze-dried *Moringa stenoprtala* leaves extracted with the use of 70% ethanol showed higher DPPH and ABTS radical scavenging activity compared to oven-dried and shade-dried leaves. These results also agreed with [23] that freeze-drying is more effective to preserve the DPPH and ABTS radical scavenging activity than the sun drying, microwave drying and oven drying methods in Chinese cabbage (*Brassica rapa L.*)

The results of this study revealed that ABTS radicals demonstrated higher radical scavenging activity than the DPPH assay. This might be resulted due to the high solubility of ABTS reagent in both aqueous and organic solvents. Therein, it can react with both lipophilic and hydrophilic antioxidant species [24].

The reducing power of all shade-dried, oven-dried and freeze-dried *C. speciosus*, *C. grandis*, and *G. sylvestre* extracts increased with the increment of extract concentration. The results of TRP assay demonstrated the effectiveness of drying methods by decreasing the order of values i.e., freeze-drying > shade drying > oven drying for all three selected plants. The lowest IC50 value was obtained from freeze-dried leaves of *C. grandis* ethanolic extract having the highest reducing power with an IC50 value of 16.84 ± 1.22 µg/ml followed by the freeze-dried *C. speciosus* 28.46 ±1.80 µg/ml (Table I) extract. According to [25] stated that *Camellia sinensis (L*) freeze-dried leaves showed higher ferric reducing capacity than oven drying and superheated drying leaves. Moreover, TRP assay results showed the capability of extracts that can reduce Fe^{3+} ions due to the activity of reducing agents such as phenol groups as well as the amount or position of the hydroxyl molecules. Therefore, the antioxidant activity is enhanced with the increment of polyphenol concentration due to their redox characteristics [26]. The findings were consistent with DPPH and ABTS assays (Table I). The freeze-drying method had the maximum TAC compared to shade-drying and oven-drying methods. Among the plant species, ethanolic extract of freeze-dried leaves of *C. speciosus* showed the highest antioxidant capacity, with an IC50 of 527.96 ± 1.06 µg/mL, compared to 784.70 ± 3.53 µg/mL and 1024.12 ± 5.26 µg/mL for *C. grandis* and *G. sylvestre*, respectively. On the other hand, *C. speciosus* extract had a greater IC50 value for TAC nearly six times higher than conventional ascorbic acid (IC 50 = 148.99

± 1.70 µg/mL). TAC values give a direct estimation of reducing the capacity of antioxidants [27]. The variations in TAC values of selected plant leaves might be due to the effect of the drying method, drying time duration, extraction method, solvent, and concentration [28].

B. Qualitative and Quantitative Analysis of Phytochemicals in Selected Plant Extracts

The qualitative phytochemical screening test results were demonstrated in Table II Except for anthraquinones, the findings of the current qualitative analysis of phytochemicals indicated that all shade dried, oven-dried and freeze-dried leaf plant ethanolic extracts consisted of several bioactive compounds, including alkaloids, saponins, phenols, polyphenols, tannins, terpenoids, steroids, flavonoids, and phytosterols. Based on the results in Table II, freeze-dried and shade-dried leaf ethanolic extracts of *C. speciosus*, *C. grandis* and *G. sylvestre* were rich in phytochemicals than the oven-dried leaf extracts. Quinones, anthocyanins and phlobatanins were not detected in both *C. grandis* and *G. sylvestre* dried leaves. However, coumarins were detected only in freeze-dried leaves of both *C. grandis* and *G. sylvestre* ethanolic extracts. Cardiac glycosides were only present in *G. sylvestre* plant leaf extracts which were subjected to all three drying methods.

TABLE II PHYTOCHEMICALS PRESENT IN LEAVES OF C. SPECIOSUS, C. GRANDIS AND G. SYLVESTRE AFTER APPLYING SHADE- DRYING, OVEN DRYING AT 45°C AND FREEZE-DRYING

Plant Species									
Phytochemicals	***C. speciosus***			***C. grandis***			***G. sylvestre***		
	Shade drying	**Oven drying 45°C**	**Freeze drying**	**Shade drying**	**Oven drying 45°C**	**Freeze drying**	**Shade drying**	**Oven drying 45°C**	**Freeze drying**
Alkaloids	++	+	++	+	++	++	+	+	++
Saponins	+	+	+	+	+	+	+	+	+++
Phenols	+++	++	+++	++	++	++	++	+	++
Polyphenols	+++	++	+++	++	+	++	++	++	++
Tannins	+	+	++	+	+	++	++	++	+++
Quinones	+	-	++	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	+++	-	-	++
Terpenoids	+	+	+++	++	+	+	+	-	+
Steroids	+	+	++	+	+	++	+		++
Glycosides	+	-	-	+	-	++	++	++	+++
Cardiac glycosides	-	-	-	-	-	-	++	+	+++
Flavonoids	++	+	++	+++	+	+++	++	+	+++
Phlobatanins.	+	-	++	-	-	-	-	-	-
Anthocyanin	+	-	++	-	-	-	-	-	-
Phytosterols	+	-	+	++	-	-	+	+	+++
Heavily present: +++; slightly present: ++; present: +; absent: −									

The TPC, TFC, tannins, terpenoids, alkaloids and saponins contents were higher in freeze- dried leaves of *C. speciosus*, *C. grandis* and *G. sylvestre* compared to oven-dried and shade-dried leaves. Oven-dried leaves of every selected plant species showed the lowest number of phytochemicals. All the measured phytochemicals showed a significant difference ($p<0.05$) depending on the plant variety and drying methods. Among the plant species, (Table III) freeze-dried *C. speciosus* leaf ethanolic extract showed the highest TPC content (82.88 ± 2.31 mg GAE/g) followed by the freeze-dried *C. grandis* (37.71± 2.19 mg GAE/g) and freeze-dried *G. sylvestre* (22.02 ± 2.42 mg GAE/g). Apart from phenolic compounds, the content of terpenoids and alkaloids in freeze-dried leaves of *C. speciosus* ethanolic extract was considerably higher ($p<0.05$) than in freeze-dried leaves of *C. grandis* and *G. sylvestre* extracts.

The maximum amount of TFC was obtained in freeze-dried *C. grandis* (99.55 ± 2.38 mg QCE/g) leaf extract and the lowest TFC was recorded in oven-dried leaves of *G. sylvestre* (20.02 ±1.14 mg QCE/g). Furthermore, shade-dried *C. speciosus, C. grandis* and *G. sylvestre* leaf extracts contained a higher amount of TFC compared to oven-dried leaves of respective species.

Total tannin content was slightly varied in selected species after applying the three different drying methods and ranged between 0.40 mg TAE/g – 9.58 mg TAE/g. Freeze-dried *G. sylvestre* leaf extract had the highest saponins content (25.95 ± 1.83 percent). Next to that, saponins content was higher in freeze-dried *C. grandis* (13.77 ± 1.63 percent) and freeze-dried *C. speciosus* (13.03± 1.27 percent) respectively.

TABLE III QUANTIFICATION OF PHYTOCHEMICALS OF *C. SPECIOSUS*, *C. GRANDIS* AND *G. SYLVESTRE* AFTER APPLYING SHADE- DRYING, OVEN DRYING AT 45°C AND FREEZE-DRYING

Plant Species	Drying Method	Phytochemicals					
		TPC (mg GAE/g)	TFC (mg QCE/g)	Total tannins (mg TAE /g)	Terpenoids (%)	Alkaloids (%)	Saponins (%)
C. speciosus	Shade drying	52.40 ± 3.88^{b}	53.05± 3.37^{c}	4.52± 1.57^{abc}	23.00 ± 2.78^{b}	15.50± 2.24^{abc}	6.91± 1.91^{cd}
	Oven drying at 45 °C	11.33 ± 1.25^{f}	23.27± 2.58^{e}	1.44± 1.16^{bc}	20.01± 1.60^{b}	8.97± 1.19^{cde}	2.11± 1.34^{e}
	Freeze- drying	82.88± 2.31^{a}	57.66± 1.96^{c}	9.58 ± 2.71^{a}	45.10± 3.46^{a}	19.37± 3.54^{a}	13.03± 1.27^{b}
C. grandis	Shade drying	26.15± 1.93^{d}	92.81± 2.35^{a}	3.78± 4.05^{abc}	8.99± 1.34^{cd}	12.61± 2.69^{bcd}	6.92± 1.62^{cd}
	Oven drying at 45 °C	13.11± 2.16^{f}	34.80 ± 4.35^{d}	1.07± 1.01^{c}	6.00± 1.75^{cde}	8.11± 1.79^{de}	4.57± 0.59^{de}
	Freeze- drying	37.71± 2.19^{c}	99.55± 2.38^{a}	7.13± 2.66^{ab}	11.88± 3.11^{c}	16.31± 3.19^{ab}	13.77± 1.63^{b}
G. sylvestre	Shade drying	15.55± 3.37^{ef}	71.12± 3.01^{b}	1.12 ± 1.16^{c}	5.11± 1.25^{de}	17.23± 1.62^{ab}	10.30± 2.29^{bc}
	Oven drying at 45 °C	13.06± 1.89^{f}	20.02± 1.14^{e}	0.40± 0.52^{c}	1.25± 1.67^{e}	3.20± 1.08^{e}	9.79± 1.34^{bc}
	Freeze- drying	22.02± 2.42^{de}	74.49± 1.92^{b}	3.86± 0.55^{abc}	5.38± 1.30^{de}	17.79± 2.30^{ab}	25.95± 1.83^{a}

All values are means of triplicate determination ± SD
Phytochemicals were statistically analyzed separately for all plant species and drying methods
Different letters in the same column showed statistical difference according to Tukey's test at $p<0.05$

Previous studies reported that flavonoids and tannins were not present in sun-dried *C. grandis* leaf ethanolic extract [29]. In addition to that, [30] reported that the air-dried *G. sylvestre* leaf ethanolic extract was not contained terpenoids, steroids, coumarins and quinones. Moreover, the literature revealed that the air-dried *C. speciosus* and oven-dried *G. sylvestre* leaves preserved less quantity of phytochemicals such as flavonoids, phenols and terpenoids compared to the freeze-dried leaves *of C. speciosus* and *G. sylvestre* [31], [32]. Besides that, an increase in phenolic and flavonoid compounds might be the cause of an increment in the radical scavenging activity of selected plant extracts [11], [28]. This study also showed a significant ($p<0.05$) relationship between antioxidant activity and total phenolic and flavonoid contents. Therefore, the variation of antioxidant activity could be resulted due to an unequal distribution of antioxidant compounds such as polyphenols and flavonoids of selected plant extracts.

The differences in phytochemical availability and concentrations were observed in selected leaf extracts namely *C. speciosus*, *C. grandis* and *G. sylvestre* due to the weather, geographic location, variation in genetic makeup, extraction, and drying procedure [33]. Furthermore, the presence of phytochemicals can be altered by the activity of heat. The high temperature and long drying time may influence to degradation of the heat-sensitive phytochemicals. Result of that, antioxidant capacity may also be reduced in this study in oven-dried leaves compared to shade-dried and freeze-dried leaves.

Based on the results, freeze-drying has been shown to be an efficient method in the retention of plant bioactive compounds, owing to the fact that dehydration occurs at lower temperatures. During the freeze-drying process, plant tissue becomes more brittle due to the development of ice crystals within the tissue matrix. Result of that, the plant cell structure can be easily damaged and obtained a high amount of bioactive compounds in solvent extraction [34]. Despite the fact that it was an expensive method, freeze-drying is effective in retaining bioactive compounds in plant herbals.

IV. CONCLUSION

Freeze-dried leaves of *C. speciosus*, *C. grandis* and *G. sylvestre* were rich in natural antioxidants and phytochemicals compared to oven-dried and shade-dried leaves of respective plant species. Among the selected plant species, freeze-dried *C. speciosus* leaf extract had the highest antioxidant activity, and it contained a higher amount of phenolics, terpenoids, tannins and alkaloids than the freeze-dried leaves of *C. grandis* and *G. sylvestre*. Therefore, freeze-drying is a more promising method for drying plant materials in order to preserve bioactive compounds and it can be capable of being used to develop herbal drugs, dietary supplements, herbal drinks, and confectioneries. However, further research needs to be conducted to isolate, screen, and characterize particular phytochemicals that are responsible for antioxidant capabilities in order to apply them in innovative herbal-inspired functional foods and pharmaceuticals.

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