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Effect of drying methods on physical and chemical properties of *Ilex guayusa* leaves

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Efecto de métodos de secado sobre las propiedades físicas y químicas de las hojas de *Ilex guayusa*

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ABSTRACT

Keywords:
Ilex guayusa
Caffeine
Air drying
Conventional oven
Solar drying

The influence of air-drying (AD), convection oven (CO) and solar drying (SOD) on the physical and chemical properties of *Ilex guayusa* leaves is discussed. Total ash (%), acid-insoluble ash (%), water-soluble ash (%), residual moisture (%) and caffeine content (%) were estimated. Additionally, alkaloids, flavonoids, reducing sugars, saponins, steroids, quinones, fats, phenols and tannins detection of dry leaves extracts were assessed. The results revealed that parameters of physical analysis were not affected by the drying techniques. The highest amount of secondary metabolites in ethanol and aqueous extracts were detected. In general, convection oven dried leaves showed the highest caffeine content (3.71%) and the lowest drying time (13 h) compared to other drying methods. The results revealed a fast method to dry *Ilex guayusa* leaves and indicate that this species possess a variety of bioactive compounds beneficial for health. Our results revealed an effective quick method to dry *Ilex guayusa* leaves without altering the content of beneficial bioactive components.

RESUMEN

Palabras clave:
Ilex guayusa
Cafeína
Secado al aire
Horno convencional
Secado solar

En este trabajo se discute la influencia del secado al aire (AD), el horno de convección (CO) y el secado solar (SOD) sobre las propiedades físicas y químicas de las hojas de *Ilex guayusa*. Se estimaron las cenizas totales (%), las cenizas insolubles en ácido (%), las cenizas solubles en agua (%), la humedad residual (%) y el contenido de cafeína (%). Además, se analizó la presencia los alcaloides, flavonoides, azúcares reductores, saponinas, esteroides, quinonas, grasas, fenoles y taninos en extractos de hojas secas. Los resultados revelaron que los parámetros del análisis físico no se vieron afectados por las técnicas de secado. La mayor cantidad de metabolitos secundarios se detectó en el extracto etanólico y acuoso. En general, las hojas secadas al horno de convección mostraron el mayor contenido de cafeína (3,71%) y el menor tiempo de secado (13 h) en comparación con los otros métodos de secado empleados. Los resultados obtenidos revelaron un método rápido para secar hojas de *Ilex guayusa* sin alterar el contenido de componentes bioactivos beneficiosos para la salud, estudios que no han sido reportados.

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Guayusa is the common name for *Ilex guayusa* which is a wild tree that belongs to the family Aquifoliaceae (Radice *et al.,* 2017). It is a holly species found in Colombia, Ecuador and Perú (Dueñas *et al.,* 2016). Decoctions of the leaves are consumed as a stimulating morning drink (Tene *et al.*, 2007). Amazonian Kichwa people prepare infusions every day to promote conviviality, for festivals and rituals (Overing *et al.,* 2000). On the other hand, mestizos and white populations in Ecuador consume the infusion as an additive to spirits (Dueñas *et al.,* 2016).

The main compounds reported in the leaves and tea of *I. guayusa* are caffeine, theobromine and polyphenols (Radice *et al*., 2017); chlorogenic acid, lutein and quercetin-3-O-hexose (Villacís-Chiriboga *et al*., 2017); phenolic mono- and dicaffeoylquinic acid derivatives (Pardau *et al.,* 2017). In addition, these authors report guayusa leaves have antioxidant and anti-inflammatory activity. Caffeine (1,3,7-trimethylxanthine) is an alkaloid of great interest because of the beneficial effects that brings to individuals. For instance, it increases alertness, improves concentration and exhibits antioxidant properties (Turnbull *et al.,* 2017; Metro *et al*., 2017). Thus, to quantify the content of caffeine in the leaves of *I. guayusa*, it is important to determinate the quality of the final product.

The Ecuadorian guayusa has a great demand in the international market for its good sensory attributes assigned to the preparations obtained from the leaves of the species. Ecuador exports almost 100% of the harvest which is reduced 75% to dry of the total fresh leaves collected (Proecuador, 2017)[1].

The producers commercialize aromatic and medicinal herbs by putting them through a drying processes. This is a complementary way of selling and inserting guayusa into new markets. The drying method is a critical step in the process because it extends the shelf life of herbal products (Figiel *et al*., 2016). This process inhibits microbial growth and prevents biochemical changes. Thus, it minimizes microbial contamination and possible modifications from the physical and chemical point of view and increases the shelf life of the product (García, 2014).

The most common methods are air-drying (AD), convection oven (CO) and solar drying (SOD). AD is very simple and economical, but it takes long periods to reach the adequate moisture content and may increase the probability of microbial spoilage (Kim *et al*., 2012). CO is considered as a cost – effective method but some compounds can get lost as a consequence of the high temperature (García, 2014). However, we have not found studies about the drying methods used to improve the stability of the leaves of *I. guayusa*.

The objective of this research was to determine the effect of air-drying, convection oven and solar drying on the physical and chemical properties of the leaves of *Ilex guayusa.*

MATERIALS AND METHODS

Plant materials and reagents

Fresh leaves of *I. guayusa* in vegetative phenological state were collected in Sucumbíos, Ecuador. A sample of the plant material was taken for botanical identification at National Herbarium of Ecuador, keeping an herbal witness (CIBE 020) at Centro de Investigaciones Biotecnológicas del Ecuador (Guayaquil – Ecuador). The plant material was previously washed and selected before drying process. After that, the dried material was grinded to homogeneous powder (800 µm) previous to physical and chemical analysis. All the chemicals were of analytical grade. The water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Drying methods

Air Drying (AD). Leaves of *Ilex guayusa* were air-dried at ambient temperature in a ventilated room without exposure to solar light. The highest temperature recorded was 31.83 °C and the lowest was 21.94 °C. The loss weight was measured every 24 h for 13 days until it reached a constant weight.

Convection Oven (CO). The experiments were performed at 46 °C in a convection oven (VWR Scientific Products, Atlanta, USA). Leaves were distributed uniformly on perforated stainless-steel trays (0.76 x 0.51 m). The time required to reach a constant weight of dried leaves was 13 h (Díaz-Maroto *et al.,* 2003).

Solar Drying (SOD). The plant material was dried in a handmade chamber solar dryer equipped with a forced

[1] https://www.proecuador.gob.ec/ficha-de-guayusa/

air circulation system through an average temperature of 33.54 °C. Samples were placed uniformly on perforated stainless-steel trays (0.50 x 0.50 m) into the system. The time required to reach a constant weight of dried leaves was 36 h.

Physical and chemical properties

Weight loss for fresh samples were obtained by the three different drying methods until constant mass was reached. Moisture was determined from sample weight loss after oven drying at 105 °C for 3 h (AOAC, 2005; method 930.15). Total ash, acid-insoluble ash and water-soluble ash were determinate for all samples (WHO, 1998).

Phytochemical analysis

Qualitative phytochemical analysis was performed using ethyl ether, ethanol and water solvents according to the procedure described by Miranda and Cuellar (2001). Briefly, 30 g of sample was weighted and 300 mL of solvent of increasing polarity was added, each one after 24 h of successive maceration extraction at temperature room. The presence or not of the following chemical compounds was determined: oils - fats; alkaloids; flavonoids; quinones; saponins; lactones; phenol and tannins; reducing sugars; mucilages; bitter principles; steroid and triterpenoid; aminoacids; resins.

Determination of caffeine

Grinded guayusa (500 mg) was added into 25 mL of distilled water at boiling temperature. Then, it remained without further heating for 10 min. The extracts passed through a filter paper (Whatman number 43), and then filtered through 0.22 µm disposable filters before analysis (Cai *et al*., 2004; Lima *et al*., 2004; Venditti *et al*., 2010). Five working solutions of standard caffeine were prepared in the range of 10 to 100 mg L^{-1} to determine the percentage of caffeine in aqueous solution. From the calibration curve, the correlation coefficient was r^2=0.998.

Chromatographic analysis was performed in a high-performance liquid chromatography (HPLC) with diode array detector system (Perkin Elmer, Norwalk, CA, USA) equipped with a C18 250 mm x 4 mm (5 µm). The mobile phase consisted of a mixture of acetonitrile and water (10:90, v/v) at a flow rate of 1.0 mL min^{-1}. Chromatograms were measured at 254 nm with a run time of 5 min (Rojo *et al*., 1999).

Statistical analysis

All assays were performed at least in triplicate. Analysis of variance (ANOVA) was employed to determinate significant difference between samples using the software Statgraphics Centurion XVI (Statistical Graphics Corporation, Rockville, USA).

RESULTS AND DISCUSSION

Physical and chemical properties

The observed values of total ash, ash insoluble in acid and soluble in water, showed no significant differences depending on the drying methods used (Table 1). This result was expected since mineral elements have a high stability. Further, minerals in the soil represent the ashes. The plant absorbs them through the roots. Hence, depending on the place of collection and the type of soil, ashes can vary, both in concentration and in qualitative composition.

The total content of ashes found (6.8%) was higher than that reported by Kapp *et al*. (2016) (4.9%) for guayusa leaves. It is high for the ranges established in the pharmacopoeias and the norms that determine a total index of ashes up to 5%. However, this value might relate to the concentration of minerals in the soil. As observed in the percentage of water-soluble ash, they are basically constituted by alkaline and alkaline-earth minerals. Furthermore, the percentage of ash insoluble in acid, representative of the presence of heavy metals, is within the established limits (Zhi-cen, 1980; WHO, 2011).

Moisture is the water content that remains inside the cell, once the extracellular water clears away by drying. Thus, moisture is of great importance because, in high amounts, it indicates a poor drying process and can cause deterioration of the material plant growth by microorganisms and biotransformation of secondary metabolites. The pharmacopoeias refer values of 8 and 14% depending on the vegetable organ studied.

The results of the physical analysis showed moisture values close to 8%, without significant differences between the different types of drying, which means that regardless of the method and the time used, a good drying of the plant material was achieved.

Another aspect analyzed was the concentration of caffeine (expressed in percentage). Hence, a small increase in concentration was observed in the CO method (3.71% ± 0.11). This may be related to a shorter drying time (13 h CO) compared to air drying (13 d AD) and solar drying (36 h SOD), avoiding the fermentation or degradation of the active ingredients (Christensen and Kaufmann, 1974; Reis *et al*., 2003). The content of caffeine found in guayusa is comparable to values reported for *Camellia sinensis* (Nishitani and Sagesaka, 2004) and higher than the content described for *Ilex paraguariensis* (1.8%) (Isolabella *et al*., 2010).

Table 1. Physical and chemical properties of *Ilex guayusa* leaves dried by different methods.

Parameter	Drying method		
	AD	CO	SOD
Total ash (%)	6.87 ± 0.12 a*	6.85 ± 0.11 a	6.86 ± 0.11 a
Acid-insoluble ash (%)	0.87 ± 0.07 a	0.86 ± 0.03 a	0.87 ± 0.06 a
Water-soluble ash (%)	3.10 ± 2.12 a	3.11 ± 2.12 a	3.09 ± 2.10 a
Moisture (residual) (%)	7.82 ± 0.41 a	7.33 ± 0.44 a	7.65 ± 0.08 a
Caffeine (%)	3.02 ± 0.07 a	3.71 ± 0.11 b	2.98 ± 0.15 a

*Significance letters in the same row indicate significant differences.

Phytochemical analysis

Phytochemical screening is an important test for the identification of a new source of therapeutic compounds from medicinal plants (Ambasta *et al*., 1986). In this study, the identification of metabolites of *Ilex guayusa* leaves dried by different methods were performed at ethyl ether (EEE), ethanol (EE) and aqueous extracts (AE). Plant constituents such as alkaloids, flavonoids, phenols, tannin, reducing sugars were identified at EE and AE. Meanwhile steroid, triterpenoid and quinones were only found at EE and saponins at AE (Table 2).

Table 2. Phytochemical evaluation of *Ilex guayusa* leaves dried by different methods.

Metabolite	Method	Extraction solvent								
		Ethyl ether (EEE)			Ethanol (EE)			Aqueous (AE)		
		SOD	AD	CO	SOD	AD	CO	SOD	AD	CO
Alkaloids	Dragendorff	-	-	-	-	-	-	+++	+++	+++
	Wagner	-	-	+	+	+	+++	++	++	
	Mayer	-	-	-	+	+	+	+	+	+
Flavonoids	Shinoda			-	-	-	+	+	+	
	Anthocyanins				-	-	-			
	Catechins				+	+	+			
Phenols and tannin	Ferric Chloride			+	+	+	+	+	+	
Reducing compounds	Fehling			+	+	+	+	+	+	
Mucilages	Mucilages							-	-	-
Bitter principles	Organoleptic							-	-	-
Saponins	Foam				-	-	-	+	+	+
Steroid and triterpenoid	Liberman Buchard	+	+	+	+	+	+			
Aminoacids	Ninhidrina					-				
Quinones	Borntranger				++	++	++			
Oils and fats	Sudan	+	+	+						
Lactones	Baljet	-	-	-	-	-	-			
Resins	Resins				-	-	-			

Air-drying (AD), convection oven (CO), solar drying (SOD). +: presence, –: absent

These results differ in the diversity of groups with those reported by Pacha (2012), which only indicated the presence of alkaloids and reducing compounds in the AE. However, they coincide in diversity with the work of Kothiyal *et al.* (2012), who report the presence in the genus of different pentacyclic triterpenoids derived from α and β-amyrins, saponins, flavonoids and purine alkaloids, among others. In addition, Villacís-Chiriboga *et al.* (2017) identified and quantified a total of 14 phenolic compounds in the leaves where chlorogenic acid and quercetin-3-O hexose were the major. Also, seven carotenoids were detected and lutein was the main component. The presence of alkaloids in the extracts could be explained by the caffeine content (purine alkaloid) of the leaves which has been reported by Verpoorte *et al.* (2007). Moreover, the different drying methods did not affect the presence of the compound in the different extracts.

CONCLUSIONS

The sample dried in a conventional oven showed a slight increase in the concentration of caffeine in comparison with the other drying methods (AD and SOD). Although, AD and SOD methods did not affect the caffeine concentration. The physical properties such as moisture, total ash, water soluble ash and acid insoluble ash, did not differ significantly for any method. The chemical groups found in all the samples were flavonoids, phenols, tannins, reducing compounds, saponins, triterpenes-steroids, quinones, fats and alkaloids.

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