MOL2NET, International Conference Series on Multidisciplinary Sciences
http://sciforum.net/conference/mol2net-03

Polyphenol extracts from Cocoa (*Theobroma cacao*) and Chuchuhuasi (*Maytenus macrocarpa*) as potential natural Amazonian antioxidants

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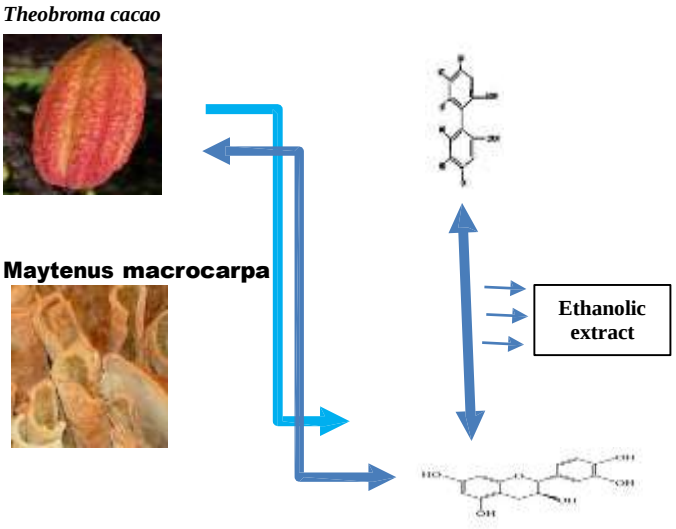
Graphical Abstract



Abstract.

The antioxidant activity, because of the presence and polyphenols chemical structure, has lead their interest in the promising valuable effects on health in foods and beverages with high content in polyphenols. Antioxidants protect the body from free radicals, which are highly reactive molecules that could damage it at the cellular level. This damage prompted by free radicals can increase the risk to the cancer development, cardiovascular diseases and other degenerative diseases. The present work aim is to achieve polyphenolic extracts from cocoa seeds (*Theobroma cacao*) and from Chuchuhuasi (*Maytenus macrocarpa*) cortex (bark) as potential natural Amazonian antioxidant source. The species were collected at the Research, Postgraduate and Amazonian Conservation Center, the botanist Dr. David Neill identified the specimens and they are to be found in the Ecuadorian Amazonian Herbarium (ECUAMZ). Polyphenolic activity was quantitatively determined in hydro alcoholic extracts by Folin Ciocalteau analytical method. Total polyphenolic concentration results based on gallic acid in the cocoa seeds (*Teobroma cacao*) extracts and chuchuhuasi (*Maytenus laevis)* cortex (bark) extracts were 24.44 and 19.90 $mg.mL^{-1}$, respectively. Thus, it was possible to conclude that the two Amazonian species under study provide relevant results in relation to the presence of total polyphenolic compounds, which allows the preliminary expectation of a promising antioxidant activity. This preliminary study allowed identifying, for the first time, new

	polyphenols sources in promising plant species of the Ecuadorian Amazon region.

Introduction

Ecuador is considered one of the most biodiverse countries on the planet. This biodiversity is not limited to the number of species per area but it also includes several natural environments or ecosystem types (Bravo, 2014).

The Amazon Region of Ecuador contains an important ecosystem variety; especially, its tropical rainforest is considered as one of the richest and one of the most complex habitats allover world for plants and animals (Matamoros, 2007). According to the book "Useful Plants of Ecuador", there are 5,172 useful plants in the country; this means that three out of ten species of plants growing in the country have some utility for people (De la Torre et al., 2008). From the species with edible uses, only 131 are cultivated (8%). The others are wild species or in the domestication process. From the total edible species, 80% are fruits or seeds, 12% are leaves and, on the other hand, 80% are consumed in raw form, 13% are prepared as drinks or juices, tea or aromatic waters or macerated with alcohol; 8% are used as sweet preserves and 5% are used as soups and stews.

In the Amazon forest there are ancestral plants with medicinal properties; cocoa is a tropical fruit, its crops are mostly found in the coasts and in the Amazonian region; it is a tree with small flowers that are observed in the branches and produce a cob that contains grains covered of some pulp rich in sugar, the grains have a high biological activity due to the occurrence of antioxidants like polyphenols that belong to the most extensive group of non-energetic substances existing in foods of plant origin. In recent years it has been shown that a diet rich in plant polyphenols can improve health and decrease the incidence of cardiovascular diseases (Quiñones et al., 2012).

Antioxidant activity, as a consequence of the polyphenolic content, has centered the interest on the promising beneficial effects on human health of foods and beverages rich in polyphenols (Scalbert et al., 2000). Antioxidants protect the living organisms from free radicals, which are highly reactive molecules that can damage the tissues at the cellular level. This damage inflicted by free radicals may increase the risk of developing cancer, cardiovascular diseases and degenerative diseases (Vinson et al., 1998).

The objective of the current work is to obtain polyphenolic rich extracts from cocoa (*Theobroma cacao*) seeds and Chuchuhuasi (*Maytenus macrocarpa*) bark that could be used as natural antioxidants.

Materials and Methods

Prior to the field operations, an extensive bibliographic search focused on recent publications concerned with the two the species under study was carried out. The search was using the following databases: Scopus, Scielo, PubMed and Scifinder. The scientific names for species under study were adopted as keywords. The articles found were detached into two groups: relevant (R) and non-relevant (NR) research, respectively. Studies focused on the bioactivity and the secondary metabolites characterization of the target species were identified in the first group (table 1). The articles considered to be irrelevant, although retaining their scientific value, were classified in this way because they address issues not related to phytochemistry, such as botany, genetics or conservation of the species under studied.

Table 1: Bibliographic research results

Species	Scopus		Scielo		PubMed		Scifinder	
	NR	R	NR	R	NR	R	NR	R
Teobroma cacao (cacao)	10	2	20	5	12	5	5	1
Maytenus laevis (chuchuhuasi)	2	0	3	0	5	0	0	0

The species (Table 2) were collected in the Amazon Region of Ecuador, especially at the Center for Research, Postgraduate and Amazonian Conservation (CIPCA), km 44 via Puyo-Tena and the Jartún Sacha Biological Station, which specimens were identified by the botanist specialist Dr. David Neill, and they rest in the Amazonian Herbarium of Ecuador (ECUAMZ).

Table 2: Botanical description of species under study

Common name	Scientific name	Botanical family	Collector	Nº collection	Origin
Cacao	*Teobroma cacao* L.	Malvaceae	D. Neill	18246	CIPCA Jartún Sacha Biological Station
Chuchuhuasi	*Maytenus macrocarpa* (Ruiz & Pav.) Briq.	Celastraceae	D. Neill	18244	

The plant material was washed with tap water and dried in a laboratory stove (Barnstead International, USA) with air recirculation at a temperature of 45 °C and further pulverized in a knife mill (Thomas Scientific, USA). Finally, it was sieved in order to guarantee a particle size less than 0.5 mm, considered suitable for subsequent extraction (Azwanida, 2015; Ph. Eur., 2017). The extracts obtained from the two

plants were made by means of a 9:1 ethanol: water mixture, with a ratio of 400 mL of solvent per 50 g of pulverized sample. Extractions were done in triplicate at 35 °C for 1 hour, the mixture was subsequently filtered on a Gooch filter and the crude extract obtained was concentrated with rotary evaporator (Büchi, Germany) at a temperature of 45 °C and a reduced pressure of 600 mmHg to 50 mL. For the implementation of the Folin-Ciocalteau test (Proestos and Varzakas, 2017, Yoshioka et al., 2017, Mansour et al., 2017; Apostolou et al., 2013), the previous standard calibration curve by successive dilutions from a concentrated solution (stock solution) of 1000 mg. L^{-1} gallic acid (reference standard) was made (table 3).

Table 3. Standard gallic acid curve preparation from the 1000 $mg.L^{-1}$ stock solution. Final volume: 10 mL (distilled water).

Components added	Gallic acid concentration of ($mg.L^{-1}$)				
	5	10	15	20	25
Gallic acid standard (µL)	50	100	150	200	250
Folin-Ciocalteu Reagent (µL)	500	500	500	500	500
Sodium carbonate solution 10% (µL)	500	500	500	500	500

For the sample preparation, 40 µL of each extract and 500 µL of Folin-Ciocalteau reagent were placed in a 10 mL volumetric flask, after shaking it was allowed to stand for 8 minutes protected from light; later, 500 µL of 10% sodium carbonate solution were added and the volumetric flask was flushed with distilled water to a volume of 10 mL. The resulting solution was homogenized by manually shaking and kept in the dark at room temperature for 2 hours. The absorbance values of prepared samples and standards were measured at 765 nm against the reagent blank. As reference, a sample of Chilean red wine Cabernet Sauvignon was analyzed.

Results and Discussion *(optional), no page limit*

The absorbance values recorded for the calibration curve are shown in table 4 and in figure 1. The total phenolic and polyphenolic antioxidants concentration results, based on gallic acid, in both Amazonian plants extracts analyzed, demonstrated that they could be an important source of polyphenols with antioxidant potential (tables 5 and 6).

Table 4. Calibration curve concentrations ($mg.L^{-1}$) and average absorbance values obtained.

Concentration $mg.L^{-1}$	5	10	15	20	25
Absorbance	0.297	0.747	1.193	1.497	1.657

The mathematical linear model obtained, as a result of the regression analysis, which allowed the further quantitative calculation was the following: C = (A + 0.0312) / 0.0778.

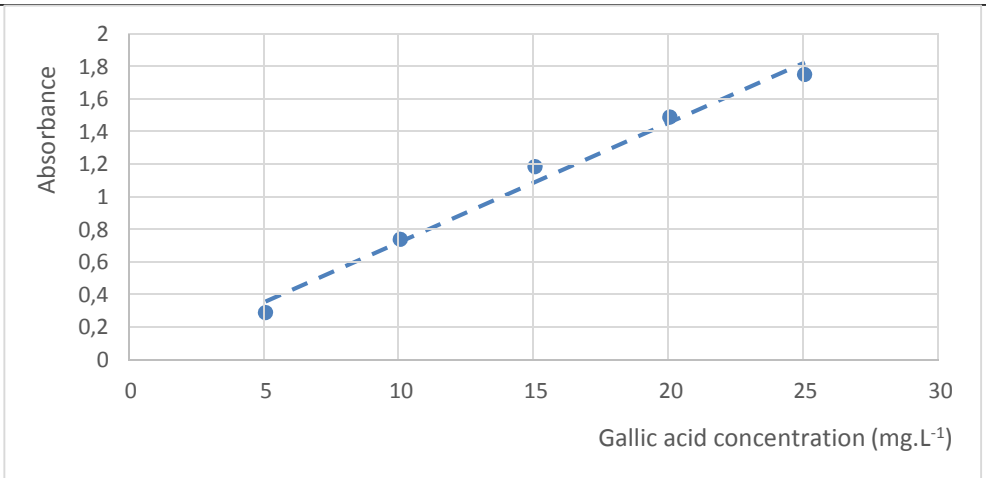


Figure 1. Gallic acid standard curve.

Table 5. Total polyphenols concentration based on gallic acid in the analyzed extracts.

Sample analyzed	Absorbance values				**Concentration**	**CV**
	A1	**A2**	**A3**	**Ā**	**(mg.mL^{-1})**	**(%)**
Teobroma cacao (cacao)	1.860	1.950	1.800	1.870	24.44	0.570
Maytenus laevis (Chuchuhuaso)	1.450	1.560	1.540	1.517	19.90	0.340
IIlex guayusa (Guayausa)	0.068	0.062	0.077	0.069	1.288	0.006
Chilean red wine	0.655	0.657	0.691	0.668	8.983	0.040

Table 6. Concentration (mg/100g of dry matter) for total phenolic and polyphenolic compounds in powdered solid samples.

Sample of pulverized solid analyzed	Concentration (mg/100g)
Teobroma cacao (cacao)	2443.70
Maytenus laevis (Chuchuhuasi)	1989.50
IIlex guayusa (Guayausa)	128.800
Chilean red wine	898.300

After analyzing the Amazonian species extracts under study, it is possible to encourage that, they provided relevant results in relation to the presence of phenolic and polyphenolic antioxidant compounds, which allows to carry out a preliminary statement about its promising antioxidant activity.

This research work supported an innovation element in the bibliographical research as it has been verified the lack of scientific information on the field.

In the application of the analytical method (Folin-Ciocalteu), an acceptable linearity for the calibration curve, with a correlation coefficient value of 0.9925 was obtained. In spite of the relatively complex process of obtaining the extracts, the precision of the polyphenol concentration results was adequate, with coefficients of variation in all cases lower than 5%.

Conclusions

The Amazonian species under study provided relevant results in relation to the phenolic and polyphenolic antioxidant compounds presence, which allows preliminary prediction of a promising antioxidant activity. There is an element of innovation in bibliographical research given by the lack of scientific information about this topic. On the other hand, the lack of phytochemical information about the species under study justifies new research work. This preliminary study allowed the identification, for the first time, of new sources of polyphenols in promising plant species of the Ecuadorian Amazon region.

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