Antioxidant activity and total phenolics of plants used in traditional medicine in Ecuador

Adriana Jara[1]; Yeray Rodriguez[1]; Jorge Cornejo[1]; Maria Elena Cazar[3]; Margarita Gutierrez[1,2]*; Luis Astudillo[1,2]

[1]Laboratory of Organic Synthesis, Institute of Chemistry of Natural Resources, Universidad de Talca, Chile.

[2]Interdisciplinary Excellence Research Program on Healthy Aging, Universidad de Talca, Talca, Chile

[3]Laboratory of Biotechnology of Natural Products, Universidad del Azuay, Cuenca, Ecuador

ABSTRACT

The total content of phenolic compounds and antioxidant activity was evaluated six plants used in traditional medicine for Ecuadorian indigenous ethnicities. The species collected in this study were: *Potalia amara*, *Salvia corrugata* Vahl, *Ilex guayusa* Loes, *Scoparia dulcis*, *Monnina sp* and *Alternanthera porrigens.* The extracts were assessed for the method of bleaching o 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and the β-Carotene bleaching assay. The results revealed that amazonic plants are good antioxidant agent. The biological activity showed for extracts relationship with the total contents of phenolics and flavonoids.
Keywords: Antioxidant, phenols, flavonoids.

INTRODUCTION

Medicinal plants are organisms that are naturally endowed with chemical compounds (secondary metabolites) with properties of high therapeutic value, yielding advances in the development of synthetic drugs with physiological action beneficial to humans. The use of these plants has an ancient origin in different cultures around the world and their preparation is basically in the form of extracts and teas[1].

Medicinal plants that have a significant amount of phenolic compounds are of great interest since these compounds are attributed various activities; the most relevant is antioxidant activity, which is important in countering oxidative stress. Oxidative stress arises mainly as consequence of the overproduction of free radicals due to inbalance in production of antioxidants by the cells[2].

Natural products especially from plant sources have the ability to reduce oxidative stress by acting as antioxidants, therefore, in this work, the total content of phenolic compounds and antioxidant activity was evaluated six plants used in traditional medicine for Ecuadorian indigenous ethnicities.

MATERIALS AND METHODS

Preparation of extracts

The materials vegetal were collected in the provinces of the Ecuadorian highlands and Amazonia. The material were garbled and dried under sunlight. The dried material was powdered. The powdered materials were extracted for maceration with organic solvents of different polarity, filtered The solvents used for preparing the different extracts were ethanol (EtOH), ethyl acetate (EtOAc) and dichloromethane (DCM).

Determination of Total Phenolic

The total phenolic (TPC) of the extracts was determined according to the Folin-Ciocalteu method[3]. In brief, 20 μL of extracts (1%), were mixed with 1.58 mL of distilled water and 100 μL of Folin-Ciocalteu reagent, the reaction mixture was preincubated for 8 min and then 300 μL of sodium carbonate 20%, were added. The mixture was incubated for 2 h at room temperature and the absorbance was obtained in a spectrophotometer at a wavelength of 765 nm. TPC was expressed as gallic acid equivalents (GAE) in milligrams per g of extract.

Determination of flavonoids

The total flavonoids content (TFC) were determined spectrophotometrically using the method of Zhishen[4]. In brief, the extracts (100 μg/mL) were mixed with of distilled water and of sodium nitrate. After 6 min of incubation, Aluminum chloride 10% were added and allowed to incubate for another 6 min, after which, Sodium hydroxide 4% were added to the mixture. The mixture was incubated for another 15 min. The absorbance was obtained in a spectrophotometer at a wavelength of 510 nm. The standard curve of TFC was made

using quercetin standard solution. The results are reported as quercetina equivalents (QE) in milligrams per g of extract.

Free radical scavenging assay ($DPPH^{\cdot}$)

The antioxidant activity of the extracts was assessed by the DPPH• radical scavenging ability using the methodology of Brand-Williams[5]. A volume of 2 mL of a solution of DPPH • was mixed with 1 mL of the extract at various concentrations (10, 50 and 100 µg/mL), the mixtures were left to stand in the absence of light for 30 minutes. After this time the absorbance was read at 515 nm in a spectrophotometer. Quercetin was used as reference compounds and DMSO 2% as control. The free radical scavenging activity was calculated as percentage of DPPH decoloration using the following equation:

$$\% \text{ scavenging DPPH free radical} = 100 \times (1\text{-}AE/AD)$$

Where AE, is the absorbance of the solution after adding the extract and AD is the absorbance of the blank DPPH solution.

***β*-Carotene bleaching assay**

The *β*-Carotene bleaching assay was evaluated according to Miller[6]. A working solution was prepared by mixing *β*-carotene, linoleic acid, Tween-40 and hydrogen peroxide. 200 µL of the extracts (10, 50 and 100 µg/mL) were mixed with 5 mL of the working solution. The mixture was incubated at 50 °C and the absorbance was measured in a spectrophotometer at a wavelength of 470 nm, at 0 and 90 min, the initial lecture was considered as time zero. The antioxidant activity was calculated as:

$$AA\ (\%) = 100 - [(Abm\ 0s - Abm\ 90s\ /\ Abc\ 0s - Abc\ 90s) \times 100]$$

Where, Abm 0s and Abc 0s: Absorbance of samples and control at time zero, Abm 90s and Abc 90s: Absorbance of samples and control at the end of the incubation (90 min). The results were expressed as the percentage of bleaching inhibition at 90 min. Butylhydroxy toluene BHT were used as positive control.

RESULTS AND DISCUSSION

The following table shows the results of the antioxidant activity and the phenolic content and flavonoids obtained.

Tabla1. Amounts of total phenolics and flavonoids in crudes extracts. Antioxidant activity of crude extracts using the DPPH and β-carotene-linoleic acid bleaching assay.

Sample	Total Phenolics (GAE mg/g)	Total flavonoids (RE mg/g)	DPPH IC_{50}(µg/mL)	B- carotene IC_{50} (µg/mL)
Curarina EtOH	43.9±2.8	32.0±1.5	28.5±1.5	15.5±2.1
Curarina EtOAc	13.9±1.5	15.8±1.1	100.6±4.8	37.8±2.5
Zhute DCM	14.4±1.0	15.7±1.1	95.7±3.7	78.3±3.2
Zhute EtOAc	28.9±1.8	26.0±2.2	73.3±3.2	42.7±2.7
Guayusa EtOH	54.0±3.8	46.0±2.0	17.5±1.4	55.6±1.6
Guayusa EtOAc	36.0±2.2	20.0±1.8	52.7±4.3	85.7±3.7
S. dulcis EtOH	50±3.5	43.0±2.3	18.2±1.5	25.3±1.3
S. dulcis EtOAc	27.2±2.0	68.0±2.5	70.5±3.8	68.3±4.0
Iguila EtOH	57.0±4.3	48.0±2.4	15.3±2.3	23.3±1.8
Moradilla EtOH	14.0±0.7	48.0±2.5	87.5±2.8	93.7±2.2
Quercetine	-	-	5.8±0.4	6.7±1.2

According to the results, suggested that the content of phenolic compounds and flavonoids are directly related with the antioxidant activity of each extract tested. In general, the ethanolic extracts showed higher content of phenols and flavonoids, and likewise higher antioxidant activity. The chemistry of phenols has attracted continuing interest over the past two centuries. Compounds of this type are essential and have several applications in our daily lives. For example, phenols are, among others, an important class of antioxidants that inhibit the oxidative degradation of organic material including a large number of aerobic biological organisms. Flavonoids in their chemical structure contain a variable number of phenolic hydroxyl groups. Activity of flavonoids as antioxidants depends on the redox properties of the phenolic hydroxyl groups.

CONCLUSION

Our results suggested that ethanolic extracts of medicinal plants tested posses a promising antioxidant activity which is related with the total phenolic and flavonoids content.

REFERENCES

1. W., H. C., A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. *Anal Bioanal Chem* **2002,** *373*, 23-30.
2. Irshad Md; Zafaryab Md; Singh M; MM, R., Comparative analysis of theantioxidant activity of *Cassia fistula* extracts. *International Journal of Medicinal Chemistry* **2012,** *Article ID 157125*.
3. Singleton VL; A, J., Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *Am. J. Enol. Vitic* **1965,** *16*, 144-158.
4. Zhishen, J.; Mengcheng, T.; Jianming, W., The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* **1999,** *64* (4), 555-559.
5. Brand-Williams, W.; Cuvelier, M. E.; Berset, C., Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* **1995,** *28* (1), 25-30.
6. Miller, H. E., A simplified method for the evaluation of antioxidants. *J Am Oil Chem Soc* **1971,** *48* (2), 91-91.