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TOTAL PHENOLICS ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL PROFILE OF SOME PLANTS FROM THE YOTOCO NATIONAL PROTECTED FOREST. VALLE DEL CAUCA, COLOMBIA

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Received: November 15, 2013 Accepted: December 17, 2013

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Abstract

Determining the antioxidant activity and the total phenolic content are routine procedures in most natural product laboratories; however, when dealing with large number of samples, it is necessary to employ methods that allow a quick, easy and economical screening. The aim of this study is to determine the antioxidant activity and the total phenolic content of plant species as criteria for the selection of promising species. To reach this aim, we used twenty species from Yotoco National Protected Forest in Valle del Cauca, Colombia. The species *Clidemia tococoidea* and *Miconia aeruginosa*, showed the highest total phenolic content together with the best antioxidant activity in terms of DPPH radical scavenging activity. The excellent correlation (R^2=*0.9610*) shown between these parameters, demonstrated the utility of the process used as a method for primary screening and selection of promising species for phytochemical analysis at a preparative scale for this two assays.

Keywords: antioxidant activity, phenols, DPPH, Folin-Ciocalteau.

1 Introduction

Antioxidants may inhibit or retard biomolecules oxidation, through inhibition of the initiation and propagation steps of reactive oxygen species (ROS) mediated chain reactions [1], whose overproduction can lead to immuno-pathological phenomena related to oxidative stress. ROS are also involved in the generation of a wide variety of health disorders or conditions including inflammation [2, 3], cancer [3], atherosclerosis [4] and degenerative diseases [5, 6].

Nowadays, there is great interest in natural antioxidants, especially from plant sources, as they are seen as potentially therapeutic agents for diseases caused by ROS and useful as nutraceuticals, due to its positive impact on human health [7, 8]. These properties have been attributed to different types of phenolic compounds which have a growing interest in

the food industry. In agriculture, species with high content of phenolic compounds have been shown to possess antimicrobial [9], cytotoxic [10] and the insecticide properties [11].

In this research, we use the advantages of techniques based on absorbance reading in microplates, for quick, simple and inexpensive primary screening of twenty plant species from Reserva Nacional Forestal Bosque de Yotoco, requiring only an incubator and conventional microplate reader. The use of a microplate-based method requires small amounts of sample and reagents and it allows simultaneous measurement of multiple replicates to calculate the effective concentration of samples, which is required to scavenge 50% of DPPH free radicals (FRS_{50}).

2 Materials and methods

2.1 Plant materials

The species were collected in August 2010 at "Reserva Forestal Nacional de Yotoco" (Table 1), located at the municipality of Yotoco, Valle del Cauca, Colombia. Those species were identified at the collection site by Professor Eugenio Escobar, Botanist of "Universidad Nacional de Colombia", Palmira, Colombia [12]. Voucher specimens were prepared and deposited at the Luis Sigifredo Espinal Tascón Herbarium CUVC at Universidad del Valle, and their identity was confirmed by Dr. Philip A. Silverstone-Sopkin.

Table 1. *List of plant species from Yotoco National Protected Forest.*

Species	Abbrev.	Species	Abbrev.
Flacourteaceae		Piperaceae	
Banara glauca (Kunth) Benth	*Bg*	*Piper aequale* Vahl	*Pa*
Casearia megacarpa Sw.	*Cm*	*Piper imperials* (Mic.) DC.	*Pi*
Gesneriaceae		*Piper augustum* Rudge	*Pau*
Besleria solanoides Kunth	*Bs*	*Piper setosum* Trel.	*Ps*
Melastomataceae		Rubiaceae	
Clidemia tococoidea (DC.) Gleason	*Ct*	*Palicourea thyrsiflora* (Ruiz-Pav) DC.	*Pt*
Miconia acuminifera Triana	*Ma*	*Psychotria compta* Standl.	*Pc*
Miconia aeruginosa Naudin	*Mae*	*Psychotria hazeni* Standl.	*Ph*
Miconia prasina (Swartz) DC.	*Mp*	*Psychotria longirostris* (Rusby) Standl.	*Pl*
Erythroxylaceae		*Psychotria macrophylla* Ruiz-Pav.	*Pm*
Erythroxylum citrifolium A. St-Hill	*Ec*	Siparunaceae	
Lacistemataceae		*Siparuna aspera* (Ruiz-Pav.) A.D.C.	*Sa*
Lacistema aggregatum (P.J. Bergius) Rusby	*La*	*Siparuna gigantotepala* S.S. Renner&Hausner	Sg

2.2 Extraction

Dried and powdered aerial parts (5 g) of each species were successively extracted three times with chloroform and acetone-water (70:30) in a Branson Scientific ultrasonic bath at room temperature for 15 minutes. The extracts were decanted and filtered through Whatman filter paper No. 1. The solvent was evaporated at low pressure in an IKA RV10 Control V rotary evaporator at 40 °C and the water extracts were dried in a lab with a freeze-dryer. The extraction yield was measured and the result was expressed as a percentage (%).

2.3 Phytochemical screening

Screening of chemical constituents was carried out by using test tube chemical methods. Extracts were analyzed for the presence of alkaloids, saponins steroids, tannins, flavonoids and phenolics according to standard methods [13].

2.4 Antioxidant activity assays

2.4.1 Qualitative DPPH radical scavenging assay using thing layer chromatography

Qualitative screening for antioxidant activity was done using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) (Sigma-Aldrich) radical assay. A thin-layer chromatoplate of each extract on silica gel 60 F_{254} (10 x 20 cm, Merck) was developed with hexane-acetone (8:2) and *n*-butanol-acetic acid-water (7:2:1) as mobile phase for the chloroform and aqueous extracts respectively. DPPH radical test was performed directly on thin-layer chromatography (TLC) plates by spraying with DPPH methanol solution (0.2% w/v).

2.4.2 Quantitative DPPH radical-scavenging assay

DPPH free radical scavenging activity was determined in triplicate by the method of Sdiri, *et al*. [14], with slight modifications. Extracts (100 µL) at two-fold serial dilutions (0.5-512 mgL^{-1}) were mixed with 100 µL of a 132 mgL^{-1} DPPH solution prepared in methanol. After 1 hour of reaction, the absorbency of the mixtures was read at 520 nm (Metertech, AccuReader M965+ microplate reader). Different concentrations (0.5-512 mgL^{-1}) of ascorbic acid and quercetin (Sigma-Aldrich) were used as positive controls and run in parallel. The results were expressed as a percentage of radical scavenging activity (%FRS) according to the equation:

$$\%FRS = [(A_c - A_s)/A_c]x100$$

where A_c is the absorbency of DPPH radicals without sample or positive control and A_s is the absorbency of DPPH radicals with sample or positive control. Efficient concentration of samples and positive controls that inhibits 50% of the DPPH radicals (FRS_{50}) was calculated and expressed as mgL^{-1}.

2.5 Estimation of total phenolics (TP)

Total phenolic content (TP) of aqueous extracts were determined according to method of Sdiri, *et al*. [14] with some modifications. An appropriately diluted sample (100 µL) was mixed with 50 µL of 20% v/v Folin-Ciocalteau reagent (Sigma-Aldrich).

Then, 50 µL of sodium carbonate solution (1,6% p/v) was added to the mixture. The mixture was incubated for 1 hour at 60 °C and then was allowed to stand at room temperature for 15 min. The absorbance was read at 650 nm. A standard calibration curve of gallic acid (1-32 mgL^{-1}) was plotted. Results were expressed as gallic acid equivalents (GAE)/g dried extract.

2.6 Statistical analysis

Results were given as the mean±SD for at least three replicates for each sample. Statistical analysis was performed using GraphPad Prism 5® (GraphPad Software Inc., San Diego, California, USA). FRS_{50} were calculated using nonlinear regression with one phase exponential decay calculation.

3 Results and discussion

3.1 Extraction yields and phytochemical profile

In order to determine the antioxidant potential and phenolic content of twenty plant species from Yotoco National Protected Forest, for which extractions were performed with chloroform and acetone-water (70:30). *S. gigantotepala* showed the highest overall yield, followed by *C. tococoidea, E. citrifolium, P. setosum, P. augustum, P. compta* and other species (Fig. 1).

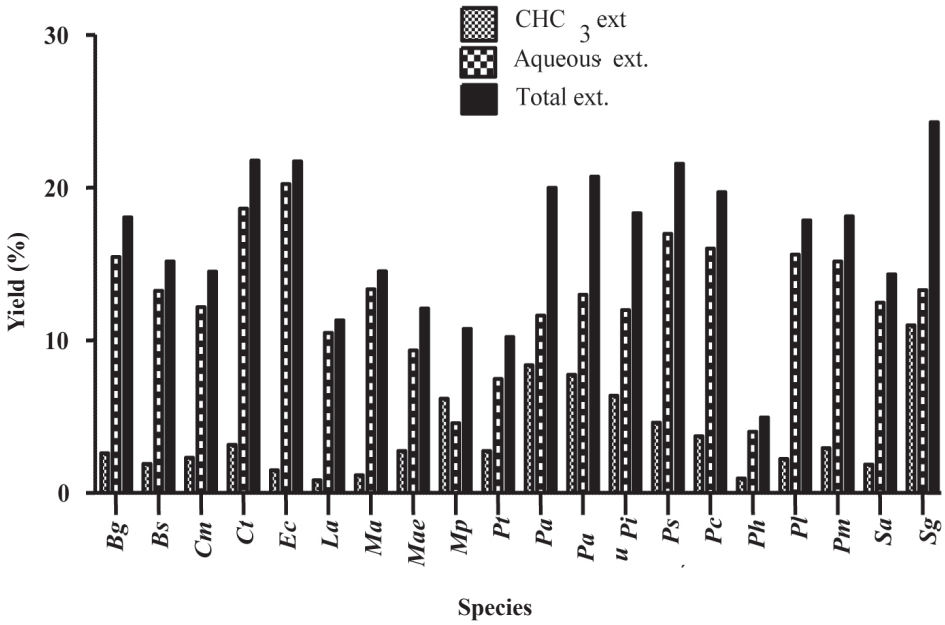

Figure 1. *Extraction process yields (for species complete names, see table 1).*

The results of the phytochemical screening indicated the presence of terpenes, steroids and phenols in all samples; however, the specific assays for alkaloids, flavonoids, soaps, hidrolyzable and condensed tannins showed more restricted distribution at specific level (Table 2).

Table 2. *Phytochemical screening of plant species from "Reserva Nacional Forestal Bosque de Yotoco".*

Species	Terpenoids and steroids	Soaps	Phenols	Flavonoids	Condensed tannins	Hydrolysable tannins	Alcaloids
Bg	++	++	++	-	++	-	-
Bs	+	-	+	-	-	-	-
Cm	+	+	++	-	-	-	-
Ct	+	++	++	++	-	++	-
Ec	+	-	++	-	++	-	+
La	+	-	++	-	-	-	-
Ma	+	++	++	-	++	-	-
Mae	+	++	++	-	-	-	-
Mp	+	-	++	-	-	-	-
Pt	++	-	+	+	-	-	+
Pa	++	++	++	-	-	-	++
Pau	+	-	+	-	-	-	-
Pi	++	-	++	-	+	+	-
Ps	++	++	++	-	++	-	-
Pc	++	++	+	++	+	-	++
Ph	++	-	+	-	-	-	-
Pl	++	++	++	++	++	-	-
Pm	++	-	++	+	+	-	+
Sa	+	-	+	-	-	-	-
Sg	++	++	++	++	++	+	-

- No appreciable evidence; + weak evidence; ++ strong evidence

3.2 Estimation of the antioxidant potential and total phenolics

TLC analysis showed that most of aqueous extracts possessed potent antioxidant activity, in contrast with low activity exhibited by the chloroform extracts. The ability of the extracts to scavenge DPPH radicals was also investigated at various concentrations to determine the FRS_{50} values. As shown in Figure 2, the extracts possessed substantial dose-dependent antioxidant activity. Some species, such as *C. tococoidea* and *M. aeruginosa* exhibited similar behavior to quercetin (Figure 2). In others species as *B. glauca*, almost complete DPPH radical scavenging was observed at concentrations higher than 50 mgL^{-1}. This activity was comparable to that of ascorbic acid and quercetin, which were used as control antioxidants [15]. Interestingly, *P. longirostris* has a higher activity than *S. gigantotepala* at concentrations less than 50 mgL^{-1}. However, at concentrations higher than 50 mgL^{-1} the activity of *S. gigantotepala* is greater.

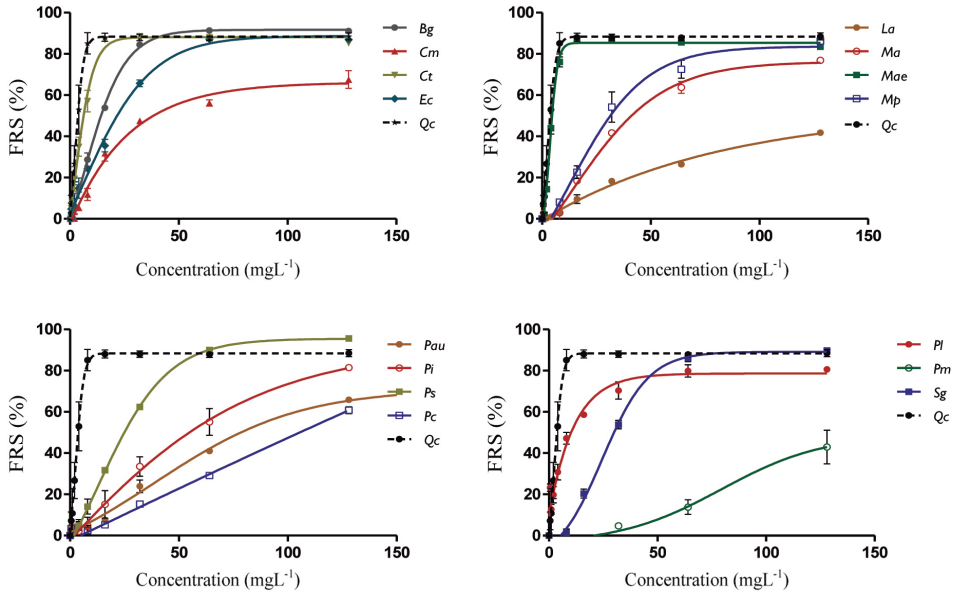

Figure 2. *DPPH free radical scavenging activity (FRS).*

In DPPH assay, the FRS_{50} values range from 4.4 mgL^{-1} (*M. aeruginosa* aqueous extract) to 486 mgL^{-1} (*B. solanoides* aqueous extract) (Figure 3). As it is known, the lower the FRS_{50} value the higher the antioxidant activity of plant extract. Also, the FRS_{50} of aqueous extracts from *C. tococoidea* (6.0 mgL^{-1}), *P. longirostris* (9.0 mgL^{-1}), *B. glauca* (14.2 mgL^{-1}), *E. citrifollium* (20.9 mgL^{-1}), *P. setosum* (24.0 mgL^{-1}) and *S. gigantotepala* (31.5 mgL^{-1}) are considered to have a strong free radical scavenging activity.

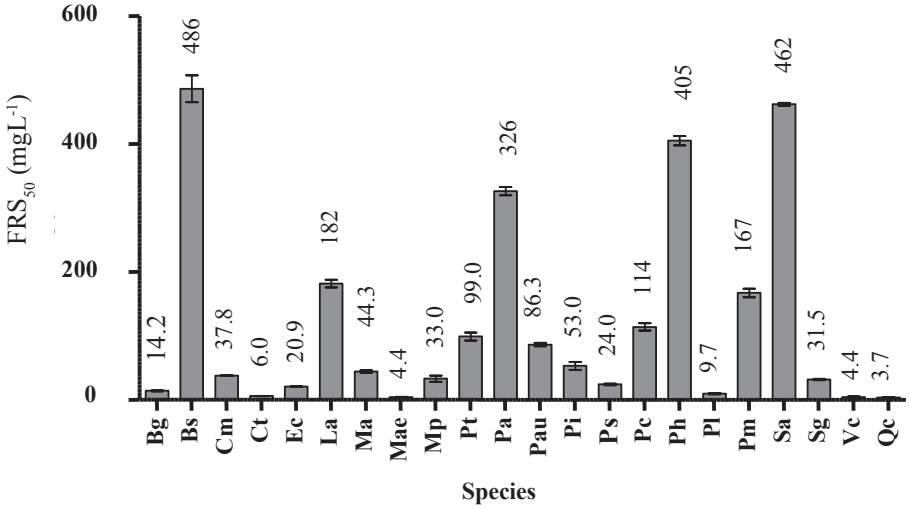

Figure 3. *FRS_{50} of 20 species studied after 1 hour of reaction.*

Folin-Ciocalteau total phenolics ranged from 34 to 480 mg GAEg^{-1} DE. In average, the species showed a high phenolic content, particularly *M. aeruginosa*, *P. setosum* and *C. tococoidea* (Figure 4). The comparison between FRS_{50}) and TP showed that there was a statistically significant strong correlation (R^2=0.9610; $Y=(891.9+6.957)e^{(0,01668X)}+6.957$; *95% confidence intervals; 15 degrees of freedom*) (Figure 5), suggesting, that the phenolic compounds present in the extracts could be responsible for the observed DPPH radical scavenging activity, since they can readily donate hydrogen atom to the radical, and even more, if they bear catechol moieties [16].

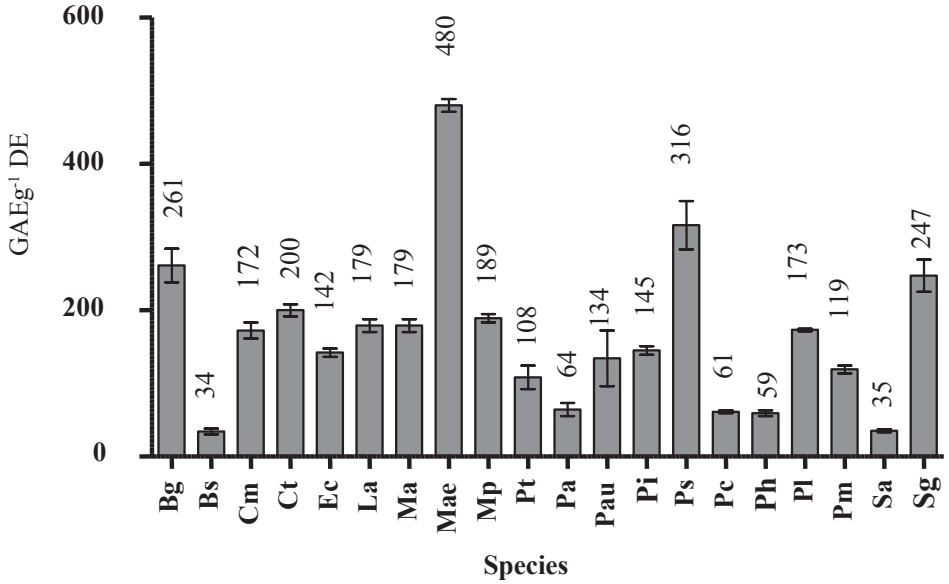

Figura 4. *Folin-Ciocalteau estimated total fenolics (TP) of the 20 species studied.*

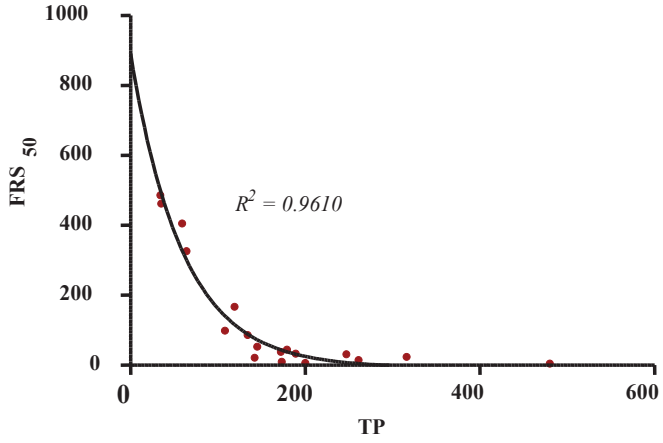

Figure 5. *Correlation between DPPH fre radical scavenging (FRS_{50}) and total phenolics (TP) stimated by Folin Ciocalteau method.*

4 Conclutions

This study shows that the species *B. glauca, C. tococoidea, E. citrifolium*, *M. aeruginosa, P. setosum, P. longirostris,* and *S. gigantotepala*, significantly scavenged DPPH free radicals *in vitro* (Table 3). These suggest that the extracts of these species could be used as a natural antioxidants source to limit free radical damage. Therefore, it is worth to isolate the active secondary metabolites from these extracts and to do further research on the potential effectiveness of the plant extracts in preventing oxidative stress-mediated diseases in humans, animals and plants.

Table 3. *Promising species selected according to preliminary screening base on DPPH free radical scavenging activity and total phenolics.*

Specie	FRS FRS_{50} (mgL^{-1})	TP ($AGEg^{-1}$ DE)
Banara glauca (Flacourteaceae)	14.2±0.7	261±23
Clidemia tococoidea (Melastomataceae)	**6.0±0.3**	**596±8.4**
Erythroxylum citrifolium (Erythrpxylaceae)	20.9±0.5	200±6
Miconia aeruginosa (Melastomatacea)	**4.4±0.2**	**480±8.9**
Piper setosum (Piperaceae)	24±1.3	316±33
Psychotria longirostris (Rubiaceae)	10±1.0	173±2.0
Siparuna gigantotepala (Siparunaceae)	32±1.0	247±22

Acknowledgements

Authors thanks to Universidad del Valle (CI7852) and Universidad Nacional de Colombia in Palmira for logistical support. Also thanks the financial support of this research by COLCIENCIAS through of the grant No. CT-557-2011.

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