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Comparison of DPPH Free Radical Scavenging, Ferric Reducing Antioxidant Power (FRAP), and Total Phenolic Content of Two Meriania Species (Melastomataceae)

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Abstract

Searching for new antioxidants used in pharmaceuticals, cosmetics or agrochemicals have increased today. Many phenolic compounds have been reported as promising for this goal. Melastomataceae is rich in these compounds. Consequently, in this research the antioxidant power for Meriania nobilis and M. hernandoi (Melastomataceae) was compared. Both species were extracted in acetone 70%. The latest was liquid-liquid partitioned in ethyl acetate (Mn or Mh 2.1), n-butanol (Mn or Mh 2.2) and aqueous fractions (Mn or Mh 2.3). Antioxidant activity was measured by DPPH free radical scavenging method (FRS_{50}, $mg.L^{-1}$), ferric reducing antioxidant power assay (FRAP, mg FeSO4.7H2O (100 g)$^{-1}$ES) and total phenolic content (TPC, mg AG g^{-1}ES) by Folin-Ciocalteu. The present study revealed that Mh 2.1 and Mh 2.2 from M. hernandoi have a strong antioxidant power FRS_{50} = 5.71±0.085, FRAP = 53±1.5 and TPC = 224±7.1 and FRS_{50} = 4.22±0.001, FRAP = 65 mg±1.5, TPC = 240 ±8.6 respectively, as compared with positive control, quercetin DPPH FRS_{50} = 4.20±0.410, FRAP = 61±1.3. M. nobilis exhibited poor antioxidant power FRS_{50} = 286±1.5, FRAP = 2.01±0.079 and TPC = 26±1.5. This paper showed M. hernandoi as good source of antioxidant compounds with potential usage as cosmetic, pharmaceutical or agrochemical ingredients.

Keywords: Antioxidant power, DPPH free radical scavenging method (FRS), ferric reducing antioxidant power assay (FRAP), Estimation of total phenolic content (TPC), Meriania nobilis, Meriania hernandoi.

1 Introduction

Many studies have been published about phytochemicals extracted from plants with strong antioxidant activity. Compounds exhibiting this activity which are capable of neutralizing or eliminating free radicals and other reactive oxygen species (ROS) are generated in the body naturally (Tsao, Yang and Young 2003). An imbalance between oxidants and antioxidants in favor of oxidants develops the oxidative stress (Sies 1997). Oxidative stress is associated with an increased risk of some diseases such as diabetes, hypertension, obesity, dyslipidemia and inflammation (Hopps, Noto, Caimi and Averna,

2010). Many phenolic compounds have been reported as promising secondary metabolites for this goal with similar activities to vitamins. The genera belonging to the family of Melastomataceae have been reported as plants rich in phenolic compounds such as flavonoids (Mimura, Salatino and Salatino 2004, Rodrigues, Rinaldo, dos Santos and Villegas, 2007, Tarawneh, León, Ibrahim, Pettaway, McCurdy and Cutler, 2014), anthocyanins (Lowry, 1976), gallotannins, (Tan, Wong, Ling, Chuah and Kadir, 2012), hydrolyzable tannins, condensed tannins and ellagytanninos (Isaza 2007), having a range of biological activities: anti-inflammatory (Murugan and Parimelazhagan, 2013), antimicrobial (Nono, Barboni, Teponno, Quassinti, Bramucci, Vitali, Petrelli, Lupidi and Tapondjou, 2014), antitumor, antibacterial (Yoshida, Amakura and Yoshimura 2010) and antioxidant (Gordon, Schadow, Quijano and Marx, 2011) activities.

Meriania is a neotropical genus, with more than 50 species, distributed in the Antilles, and from southern Mexico to southeast Brazil. In Colombia are known around 36 species, including *Meriania nobilis* and *Meriania hernandoi*, used mainly as ornamental. Their biological activities and phytochemicals have not been previously reported; therefore this research evaluated and compared antioxidant activity by DPPH free radical scavenging method (FRS), ferric reducing antioxidant power assay (FRAP) and correlation with total phenolic content (TPC) estimated by Folin-Ciocalteu method for both species, *Meriania nobilis* and *Meriania hernandoi*.

2 Materials and Methods

2.1 Plants and materials

Meriania nobilis Triana (Voucher CUVC-51735) was collected at Caldas, Antioquia, Colombia, on May 2012 and *Meriania hernandoi* L. Uribe (Voucher CUVC-62003) at "Reserva Civil El refugio", in Dagua, Valle del Cauca, Colombia, on November 2013. Both species, in flowering stage, were identified in the herbarium "Luis Sigifredo Espinal Tascón" of the "Universidad del Valle".

2.2 Extraction

Dried and powdered leaves (500 g) of each species were successively extracted three times with hexane followed by acetone 70% in a Branson Scientific ultrasonic bath at room temperature for 15 minutes. The solvent was evaporated at low pressure in an IKA RV10 Control V rotary evaporator at 40 °C; the latest was liquid-liquid partitioned in ethyl acetate (Mn or Mh 2.1), n-butanol (Mn or Mh 2.2) and aqueous fractions (Mn or Mh 2.3).

2.3 DPPH Radical - Scavenging Method

DPPH free radical scavenging activity was determined in triplicate by the method of Sdiri, et al. (Sdiri, Bermejo, Aleza, Navarro and Salvador 2012) with slight modifications. Extracts (100 µL) at two-fold serial dilutions (0.5-512 $mg.L^{-1}$) were mixed with 100 µL of a 132 $mg.L^{-1}$ DPPH solution prepared in methanol. After 1 hour of reaction, the absorbance of the mixtures was read at 520 nm (Metertech, AccuReader M965+ microplate reader). Different concentrations (0.5-512 $mg.L^{3}$) of vitamin C (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:

$$\%FRS = \frac{A_C - A_S}{A_C} \times 100$$

Where Ac is the absorbance of DPPH radicals without sample or positive control and As is the absorbance 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 mg. L^{-1}.

2.4 Ferric Reducing Antioxidant Power Assay (FRAP)

Power reducing of Fe^{+3} to Fe^{+2} by an antioxidant was determined using the method reported by Benzie and Strain (Benzie and Strain 1996); this method was modified for the 96 well microplate reader. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (v/v/v). All standards and samples were prepared at 1024 mg.L^{-1} in water or methanol. The 140 μL reagent, the 30 μL standard ($FeSO_4.7H_2O$) or sample solutions and 10 μL water were added to the well and conveniently mixed. The absorbance readings were taken at 600 nm immediately after and 60 min after using a Metertech, AccuReader M965+ microplate reader. A standard calibration curve of Iron (III) sulphate penta hydrate ($FeSO_4.7H_2O$) was plotted. Results were expressed as mg $FeSO_4.7H_2O$ (100 $g)^{-1}$ dried extract.

2.5 Estimation of Total Phenolic Content (TPC)

Total phenolic content (TPC) were determined according to method of Sdiri, et al. (Sdiri, Bermejo, Aleza, Navarro and Salvador, 2012) with some modifications. An appropriately diluted sample (100 µL) was mixed with 50 µL of 20% v/v Folin - Ciocalteu 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 700 nm. A standard calibration curve of gallic acid (1-32 mgL^{-1}) was plotted. Results were expressed as gallic acid miligrams (mg AE) g^{-1} 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). FRS_{50} values were calculated using no nonlinear regression with one phase exponential decay calculation.

3 Results

The percentages of DPPH free radical scavenging (% FRS) for each extract from species *M. nobilis* and *M. hernandoi* were determined, as indicated in Figure 1; this figure shows a free radical scavenging activity of 90% for *M. hernandoi* extracts.

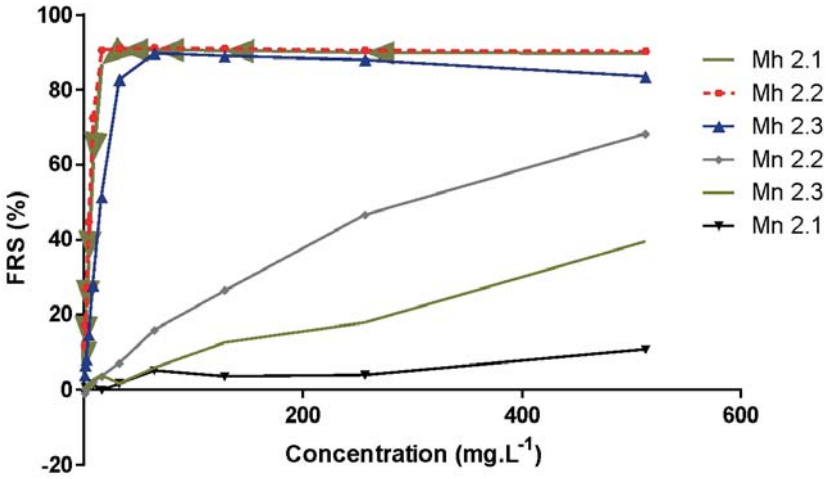

Figure 1. *Percentage of radical scavenging capacity for extracts of the species M. nobilis, M. hernandoi. Values are the average of three replicates*

Also the concentrations of samples and positive controls that scavenge 50% of the DPPH free radicals (FRS_{50}) were determined. The FRS_{50} values were ranging from the higher at 512 mg. L^{-1} (Mn 2.1) to the lowest at 4.22 mg. L^{-1} (Mh 2.2) as shown in the Figure 2. The extracts of *M. nobilis* showed a moderate radical scavenging activity, being Mn 2.2 the better with FRS_{50} equal to 287 ± 1.7 mg.L^{-1}; while *M. hernandoi* showed strong radical scavenging in vitro in all extracts, Mh 2.1 (5.71 mg. L^{-1} ± 0.085), Mh 2.2 (4.22 mg. L^{-1} ± 0.001) and Mh 2.3 (14.5 mg. L^{-1} ± 0.119), similar to the values obtained for quercetin (4.20 mg. L^{-1} ± W0.410) and vitamine C (5.34 mg. L^{-1}± 0.114) as the positive controls employed.

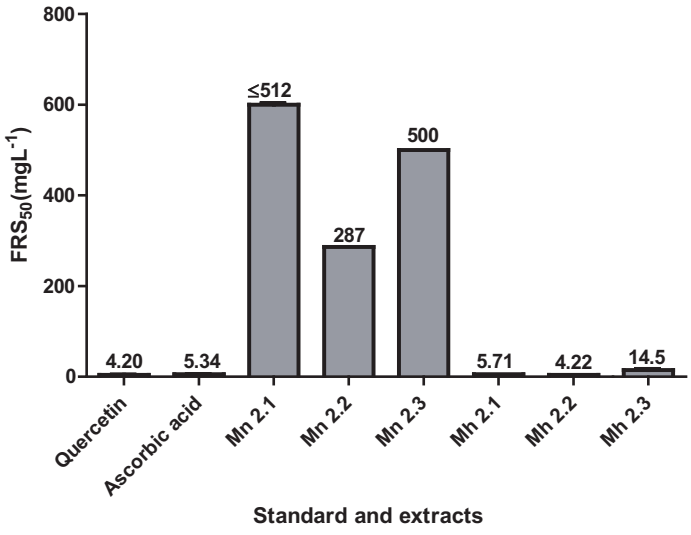

Figure 2. *Activities radical scavenging for extracts of the species M. nobilis, M. hernandoi and positive controls. Values are the average of three replicates.*

The antioxidant power was determined by the FRAP assay that measures the ability of the sample to reduce the Fe^{3+} in a solution of TPTZ to Fe^{2+}. The values obtained for the samples ranged from 65 mg $FeSO_4.7H_2O$ (100 g)$^{-1}$ES to 0.28 mg $FeSO_4.7H_2O$ (100 g)$^{-1}$ ES (Figure 3). The *M. hernandoi* extracts that showed strong antioxidant power were Mh 2.1 (53 mg $FeSO_4.7H_2O$ (100 g)$^{-1}$ES ±1.5) and Mh 2.2 (65 mg $FeSO_4.7H_2O$ (100 g)$^{-1}$ES ±1.5). These values were very similar and even the Mh2.2 extract was greater than that for quercetin 61 mg $FeSO_4.7H_2O$ (100 g)$^{-1}$ES ±1.3. Very low values of antioxidant power were obtained for *M. nobilis*, such as Mn 2.1 (0.28 mg $FeSO_4.7H_2O$ (100 g)$^{-1}$ES ± 0.009), Mn 2.2 (2.01 mg $FeSO_4.7H_2O$ (100 g)$^{-1}$ES ± 0.079), and Mn 2.3 (0.57 mg $FeSO_4.7H_2O$ (100 g)$^{-1}$ES ± 0.026).

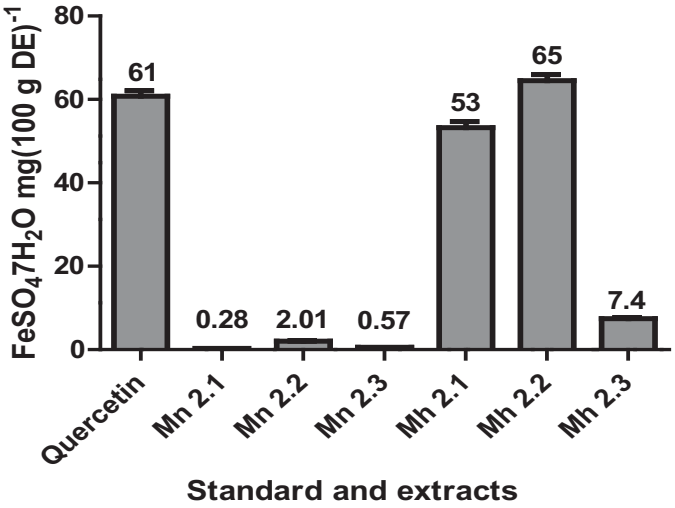

Figure 3. *The antioxidant power for extracts of the species M. nobilis, M. hernandoi and positive control. Values are the average of three replicates.*

Total phenolic content estimation by Folin - Ciocalteu method was in the range from 11.9 to 240 (mg AG) g^{-1}ES (Figure 4). The higher phenolic content was for *M. hernandoi*: Mh 2.1 (224 (mg AG) g^{-1}ES ± 7.1), Mh 2.2 (240 (mg AG) g^{-1}ES ± 8.6), and Mh 2.3 (120 (mg AG) g^{-1}ES ± 2.5).

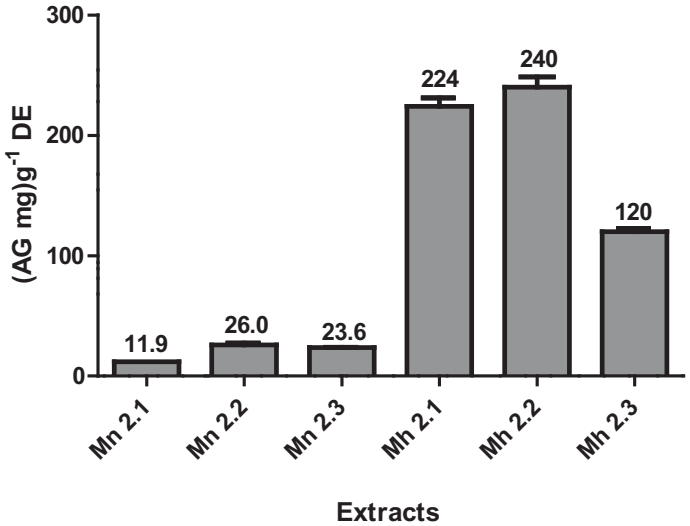

Figure 4. *Estimation of total phenolic content by Folin-Ciocalteu method of the species M. nobilis and M. hernandoi. Values are the average of four replicates.*

4 Discussion

The results obtained for % FRS showed that *M. Hernandoi* extracts possess a striking antiradical activity of 90%, while for the *M. nobilis* extracts the percentages were obtained from 10 to 60%. These results are also supported by the great correlations between DPPH FRS_{50} with FRAP (R^2 = 0.9974), DPPH FRS_{50} with TPC (R^2 = 0.901), and TPC with FRAP (R^2 = 0.9950) as shown in the Figure 5. FRAP values obtained for the two species confirmed the values found in FRS_{50} assay, showing to *M. hernandoi* extracts, specially Mh 2.1 and Mh 2.2 as good source of antioxidant compounds from natural origin with potential usage as cosmetic, pharmaceutical or agrochemical ingredients.

Therefore, extracts exhibiting strong radical scavenging capacity (FRS) and a high antioxidant power (FRAP) also have higher levels of total phenolic content (TPC), suggesting phenolic compounds as potential responsible for this activity. The values of FRS_{50} and FRAP for the two species behaved similarly. This was checked by determining the correlation coefficient for the two methods, which was: R^2 = 0.9974; (Y= (655-0.52) $e^{0.423X}$ - 0.52; 95% confidence intervals; 3 degrees of freedom), indicating a statistically significant correlation. Also the correlation coefficients were determined for the values obtained FRS_{50} and TPC and FRAP and TPC. The correlation coefficients obtained were: R^2 = 0.9010; (Y= (919-2.65) $e^{0.034X}$ – 2.65); R^2 = 0.9950; (Y= (8.26-2.36) $e^{0.423X}$ – 2.36); 95% confidence intervals; 3 degrees of freedom; respectively (Figure 5). A statistically significant correlation for the methods is observed.

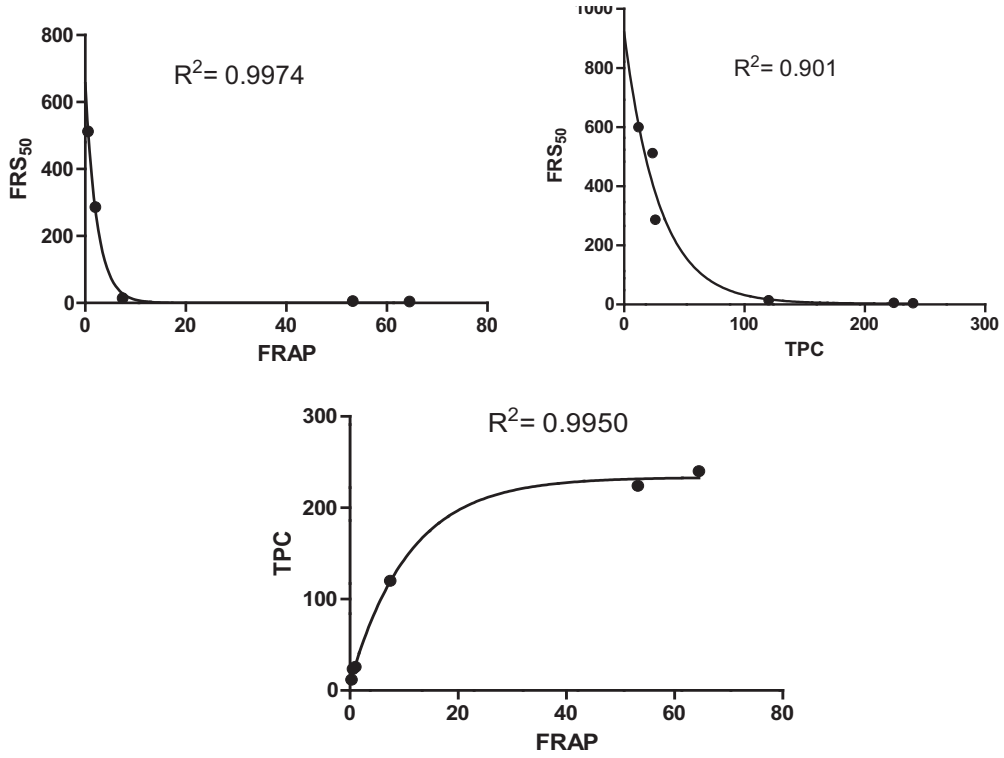

Figure 5. *Correlations between the methods used to determine antioxidant activity. FRS_{50}= DPPH free radical scavenging 50 , Efficient concentration that inhibits 50% of the DPPH radicals FRAP= Ferric Reducing Antioxidant Power, TPC= Total Phenolic Content.*

Many *in vitro* methods are used to determine the antioxidant activity, but they do not correlate well with each other (Tsao, Yang and Young, 2003); Although in this research methods with different mechanisms were used, they correlate really well, indicating that the main compounds responsible of both antioxidant activities, DPPH free radical scavenging and ferric reducing antioxidant power of *M. nobilis* and *M. hernandoi*, are phenolics.

5 Conclusions

This paper showed the species *M. hernandoi* as good source in antioxidant compounds from of natural origin with potential usage as cosmetic, pharmaceutical or agrochemical ingredients, due to activity found by the methods *in vitro* free radical DPPH scavenging, ferric reducing power antioxidant assay (FRAP) and total phenolic content. The extracts of *M. hernandoi* are promising for future studies to isolate and elucidate the metabolites that exhibit this activity. The specie *Meriania nobilis* showed a poor antioxidant activity, which agree to *in vitro* methods employed. The *in vitro* method employed correlated well which allowed determining the antioxidant power of the two species studied.

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