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Determination of the antioxidant and antifungal activities of twelve plants belonging to the Colombian coffee region

Oscar M. Mosquera[I], Yeny J. Cortes[I], Anyela M. Ríos[I], Jaime Niño[I]

Abstract

The aims of this study were determinate the antioxidant and antifungal activities of twelve plants belonging to the Colombian Coffee Region (CCR). A phytochemical characterization was performed to the hexane, dichloromethane and hexane-isopropanol (3:1) extracts. Also the antioxidant activities through Diphenylpicrylhydrazyl (DPPH$^{\bullet}$) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS$^{\bullet+}$) methods were assayed; In addiction, the total phenolic content and the activities against the fungus *Fusarium oxysporum*, *Fusarium solani* and *Mycosphaerella fijiensis* Morelet were also evaluated. In this study 10 hexane-isopropanol (3:1) and 6 dichloromethane extracts as well as 1 hexane extract displayed antioxidant capacity superior to 25% through the DPPH$^{\bullet}$ assay, standing out the hexane extract of *Piper umbellatum* (Piperaceae) with an activity of 44.8%. The extracts that showed the most important antifungal activities were the hexane-isopropanol (3:1) extract of *Mikania lloensis* (Asteracea) that inhibited 44.8% the growth of the fungus *Fusarium oxysporum* and the hexane extract of *Piper pessaresanum* (Piperaceae) that showed 59% of growth inhibition against *Fusarium solani*. Further more the *Piper pessaresanum* dichloromethane and hexane-isopropanol (3:1) extracts and the dichloromethane extract of *Alchornea coelophylla* (Euphorbiaceae) gave 100% of inhibition against the fungi *Mycosphaerella fijiensis* Morelet. This study is a contribution to the bioprospecting to the Colombian flora.

Key words: Asteraceae, ABTS$^{\bullet+}$, DPPH$^{\bullet}$, Euphorbiaceae, *Fusarium oxysporum*, *Fusarium solani*, *Mycosphaerella fijiensis* Morelet, Piperaceae, Phytochemical characterization.

[I] Grupo of Biotecnología-Productos Naturales, Escuela de Tecnología Química, Universidad Tecnológica de Pereira, Pereira, Colombia. *Address correspondence to:* Oscar M. Mosquera, Grupo of Biotecnología-Productos Naturales, Escuela de Tecnología Química, Universidad Tecnológica de Pereira, Carrera 27 No 10-02 Barrio Los Álamos, Pereira, Colombia; Tel: 3137243; Fax: (+57) 6 3213206; E-mail: omosquer@utp.edu.co

1. INTRODUCTION

Plants are recognized by biosynthesize secondary metabolites (SM) with different purposes, among them can be mentioned the ones those related to growth regulation, intra and inter-specific interactions, the defense against different types of abiotic stress, predators, pests and pathogens. Between the most studied SM are: terpenes, steroids, coumarins, tannins, flavonoids and alkaloids, due to their known biological activities such anti-herbivory, antioxidant, antimicrobial, antifungal and protection against the radiation UV-B; that is why several plant species have been widely used in traditional medicine for the control of different diseases and as a source of new drugs (Mazid et al., 2011; Yadav & Agarwala, 2011).

Due to the presence of bioactive phytocompounds, the pharmacological industry uses medicinal plants as one of the bases for the development a great number of synthetic medicines that were produced in the 19th century (Djeridane et al., 2006; D'Souza, 2014). In addition, the finding of new antioxidant and antimicrobial compounds were valuable for the food processing and cosmetics industries (Škrovánková et al., 2012). Chromatographic fractions and isolated compounds from leaves, stems or roots have been studied in order to discover novel SM (Kanegusuku et al., 2002).

Colombia is a country that possess a great number of jungles and natural protected zones located along the national territory with endemic plants, animals and microorganisms, which might provide biological active compounds that could be used as new drugs or biocide agents. Especially the protected zones located at Colombian Coffee Region (CCR) would allow to take advantage in a rational and sustainable way of the resources based on the conservation, as well as the expansion and the sustainability of the above mentioned zones and the generation of economic and social benefits to the involved communities, with the added value that could be obtained from these resources (Duarte & Velho, 2009).

For the potential utilization of biodiversity it is necessary to study the natural resources that will allow detecting those species with biological important activities. Thus, in the development of this study, two different types of bioassays were performed: antioxidant and antifungal with extracts of twelve plants belonging to the botanical families Asteraceae, Euphorbiaceae, Piperaceae and Rubiaceae collected in the CCR, to determine the species that possess the highest biological activities above mentioned.

2. METODOLOGÍA

2.1 Materials and Methods

Biological materials and reagents

The strains used for this work were: *Fusarium oxysporum*, *Fusarium solani* and *Mycosphaerella fijiensis* Morelet (obteined from infected bananas leaves). The solvents: hexane, dichloromethane, isopropanol, methanol, ethanol, *n*-butanol, ethyl acetate Mallinckrodt® (Phillipsburg, NJ, USA) were used; agar bacteriological (Agar No.1) and potato dextrose agar were Oxoid® (Hampshire, England); Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], DPPH$^{\bullet}$ (Diphenylpicrylhydrazyl), ABTS$^{\bullet+}$ [2,2'-Azino-bis(3-

ethylbenzothiazoline-6-sulfonic acid) diammonium salt], gallic acid (3,4,5-Trimethoxybenzoic acid) were Sigma-Aldrich® (Saint Louis, MO, USA) and TLC Silica gel F_{254} 0.2 mm Merck® (Darmstadt, Germany).

Plant materials and obtention of extracts

The aerial parts of twelve plants were collected in the Reserve Zones located on the CCR: Regional Natural Park Ucumarí (4° 43' 22'' N; 75° 33' 90'' W) and La Nona Park (4° 56' 25'' N; 75° 44' 28'' W). The plant collected materials were classified by the taxonomist Fransisco Javier Roldán and a voucher (FJR) of each specie was deposited in the Herbarium of the Universidad de Antioquia (HUA, Medellín, Colombia). To obtain the different extracts, each dry ground plant material was submitted three times to passive maceration extraction for 48h using hexane (HEX), dichloromethane (DCM) and hexane-isopropanol (HEX-iPrOH) (3:1) successively (Niño et al., 2006); each type of extract was concentrated in a rotary evaporator Heidolph Laborota 4000 (Bayern, Germany) at 50 °C and were stored at -10 °C up to its utilization for the accomplishment of the different tests.

Table 1. Plants used in this study

FAMILY	SPECIES	Voucher Number (FJR)	Percentage of extract obtained		
			HEX[a]	DCM[b]	HEX-iPrOH (3:1)[c]
Asteraceae	*Clibadium asperum*	4045	0.50	0.76	0.52
	Mikania lloensis	3977	1.25	1.28	0.80
Euphorbiaceae	*Acalypha diversifolia*	3967	1.33	1.24	0.94
	Alchornea coelophylla	3969	0.88	1.15	1.23
	Hyeronima antioquensis	3905	0.88	1.00	0.84
	Mabea montana	3912	1.98	2.03	2.543
Piperaceae	*Piper crassinervium*	4021	1.79	6.30	2.71
	Piper eriopodon	4007	1.20	2.06	1.32
	Piper pesaresanum	3996	1.91	5.15	1.41
	Piper umbellatum	4012	4.80	5.53	2.13
Rubiaceae	*Palicourea petiolaris*	3995	0.88	0.75	1.29
	Rubiacea	3973	1.32	2.92	4.93

[a]HEX: hexane; [b]DCM: dichloromethane and [c]HEX-iPrOH (3:1): hexane-isopropanol.

Phytochemical characterization

The extracts were phytochemically characterized through thin layer chromatography (TLC) based in the methodology described by Wagner and Bladt (1996). The aim of this characterization was made a screaning of the main metabolites in the different types of extracts. For the elution of the HEX, DCM and HEX-iPrOH (3:1) extracts the following solvents systems: *n*-HEX-EtOAc-MeOH (67:30:3), *n*-HEX-EtOAc-MeOH (68:30:2 and *n*-HEX-EtOAc-*n*-BuOH (66:30:4), were used as a mobile phases, respectively.

Scavenging capacity of diphenyl-picrylhydrazyl (DPPH•) radical

This activity was evaluated by following the methodology proposed by Brand-Williams et al., (1995); in brief for each extracts solution 0.25 mL at a concentration of 1000 mg/L were taken and allowed to react with 1.0 mL of the DPPH• solution at 20 mg/L during 30 minutes in the darkness; then, the absorbance (A_{sample}) of each reactive mixture was measured at 517 nm in aspectrophotometer V-VIS light Xs Schott (ALÈX Cedex France). As a positive control, hydroquinone at 1000 mg/L was used, for the negative control the solvents mixture to dissolve the respective extracts was used ($A_{Control\ (-)}$) and as blank for every extract was combined the solution of extract with methanol. All measurements were performed by triplicate with two repetitions tested at different days. The antioxidant capacity was calculated as follows:

$$\%AA = \frac{A_{Control(-)} - A_{Sample}}{A_{Control(-)}} \times 100$$

The results also were reported in Trolox equivalents (µM/g extract), calculated from a calibration curve of Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] at 1, 2, 4, 6, 12 and 32 µM (Wetwitayaklung et al., 2006).

Scavenging capacity of ABTS•+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation

To measure the antioxidant activity of the extracts with the radical cation ABTS•+ the methodology described by Re et al., (1999) was followed. The radical was produced through a reaction between ABTS (7 mM) and potassium persulfate (2.45 mM) diluted with distilled water and kept in darkness for 16 hours. When the radical ABTS•+ was formed, it was diluted with absolute ethanol to obtain a solution with an absorbance ranging from 0.700 ±0.02 at 734 nm; then to perform the assay, 1470 µL of this solution was thoroughly mixed with 30 µL of each sample. As positive control hydroquinone (1000 mg/L) was used, for the negative control the solvent system in which was dissolved the extracts ($A_{control\ (-)}$) was used and as blank for every extract sample a mixture of ethanol-water proportional to the solution used to dilute the radical in ethanol was added. The absorbance was measure lapsed 30 minutes at 734 nm (A_{sample}) and the inhibitory activity was calculated in the same way as for the of DPPH• method. The results were reported in Trolox's equivalents (µM/g extract); the concentrations of the trolox calibration curve were 1, 5, 10, 20, 40 and 80 µM.

Determination of the total phenolic compounds content.

For this determination the spectrophotometric method of Folin-Ciocalteu (Singleton & Rossi, 1965; Cardeño et al., 2007) was followed. In brief, 3 mL of distilled water were added 50 µL of the extract sample at 1000 mg/L; then 250 µL of the reagent of Folin-Ciocalteu diluted (1:1 with water) were added and after 1 minute 750 µL of a solution of a 20% Na_2CO_3 were added; finally, the working solution was made up to 5 mL with distilled water and homogenized. This solution was incubated during 30 minutes in the darkness and after this period, the absorbance was measured in a spectrophotometer UV-VIS light Xs Schott® (ALÈX Cedex France) at 760 nm. To determine the phenolics content a calibration curve was developed with Gallic acid (0, 25, 50, 100, 200, 400 and 800 µg/mL) and the results expressed as mg of gallic acid/ 100 mg of extract (GAE).

In vitro* determination of the antifungal activity of the plant extracts against *Fusarium oxysporum* and *Fusarium solani.

The evaluation of the antifungal activity of HEX, DCM and HEX-iPrOH (3:1) extracts of twelve plants against *Fusarium oxysporum* and *Fusarium solani* fungi, was performed through the agar dilution procedure and the radial growth test in plates (Marquez et al., 2007), using Potato Dextrose Agar (PDA) culture medium. The evaluated concentrations of every extract were 429, 857, 1714 and 3429 mg/L, using as negative control DMSO in the same proportion to which it was used in the solution evaluated at higher concentration (The maximum quantity of DMSO tolerated by the fungi was 30 mL/L in PDA); additionally, an absolute control composed by PDA was used, to check if the DMSO concentration used in the extract solutions not affect the growth of the fungi used in this work. As positive control ketoconazole at 1000 mg/L was used; then 48 hours the radial growth (Diameter) of each fungus was measured with a vernier caliper and the percentage of inhibition was calculated using the following ecuation:

$$\%\ Inhibition = \frac{Diameter_{\ Control(-)} - Diameter_{\ Extract}}{Diameter_{\ Control(-)}} \times 100$$

Three replies were performed for every solution evaluated in the bioassay, with two repetitions at different days to verify the reproducibility of the method.

***In vitro* determination of the fungicidal activity of plant extracts against *Mycosphaerella fijiensis* Morelet.**

The *in vitro* determination of the activity against Black Sigatoka of the HEX, DCM and HEX-*i*-PrOH (3:1) extracts of twelve plants was evaluated on the sexual phase of *Mycosphaerella fijiensis* by the method of germinative tube elongation of the ascospores (Du Pont, 1983), using infected bananas leaves with the fungus in the stage 5 or 6 of the disease (Belalcázar, 2011). Initially, on Kraft paper discs of 11 cm of diameter were staple nine chunks of approximately 2 cm^2 of infected leaves of banana and they were wrapped in moistened paper towels, later they were incubated for 48 hours at room temperature in plastic bags with hermetic seal. Once finished the incubation period, the discs were submerged in water by 5 minutes. Then, the discharge of ascospores was performed during 4 - 6 hours on bacteriological agar supplemented at 20% with each solution of the extracts evaluated at 1000 mg/L in Petris dishes. Once finished the unload, the material was incubated at 26 °C for 48 hours and the reading of 150 ascosporas in two Petri dishes was performed, distributed in three visual fields of 50 ascospores each and the percentage of ascosporas with: normal germination (NG), short germination (SG), deformed germination (DG) and not germinated (NG) was calculated.

For the *in vitro* activity of the above mentioned extracts on the asexual phase of *M. fijiensis* were applied the method radial growth of mycelium (Peláez et al., 2006); for which were needed the isolation of *M. fijiensis* by means of monosporic culture, incubated in PDA in wedge during 14 - 20 days under constant light. With every isolated strain, a solution of microorganism was prepared in sterilized distilled water and 100 µL were sowed on the surface of a PDA Petri dishes supplemented with each extract being evaluated at 1000 mg/L. The Petri dishes were incubated at 26 ºC for 20 days and the radical growth of 5 colonies of *F. fijiensis* per each Petri dish

were measured at 7, 9, 12, 15 and 20 days of incubation. Both types of test against *M. fijiensis* were performed by triplicate whit two repetitions; in addiction, as positive control Benlate® at 1 mg/L and as negative control the dilution solvent of the extracts were used. The HEX and HEX-iPrOH (3:1) extracts were dissolved in EtOH:DMSO (9:1) and those of dichloromethane in EtOH absolute.

The results of the elongation test of the germinative tube of the ascospores (sexual phase) and of the mycelium radial growth (asexual phase) of *M. fijiensis* were analyzed by the statistical method of conglomerates and the of Kruskal-Wallis (p = 0,5) not parametric analysis, respectively; using the software Infostat (2007d.3).

3. RESULTS AND DISCUSSION

Phytochemical characterization

The results of the phytochemical characterization demonstrated low presence of alkaloids in all extract types of the studied species, with the exception of *Piper pesaresanum* (FJR-3996) showed presence of this type of metabolite, these results are similar to the obteined by authors like Perazzo et al., 2013 and Liu et al., 2015; the main phytocompounds found in all the evaluated extracts were sterols, steroids, terpenes, saponins and flavonoids; among the types of flavonoids the most abundant were aurones, catequins, flavones and chalcones while the less abundant were flavonols, flavonones and isoflavones; none of the HEX extracts evaluated showed presence of coumarins and anthraquinones, as it can be shown in the table 2. These results contrast with different reports of the phytochemical composition for each family on this research (Mughal et al, 2010; Parmar et al, 1997; Ravipati et al, 2014; Seebaluck et al, 2015).

Table 2. Phytochemical characterization of twelve plants collected in the Colombian Coffee Region (CCR)

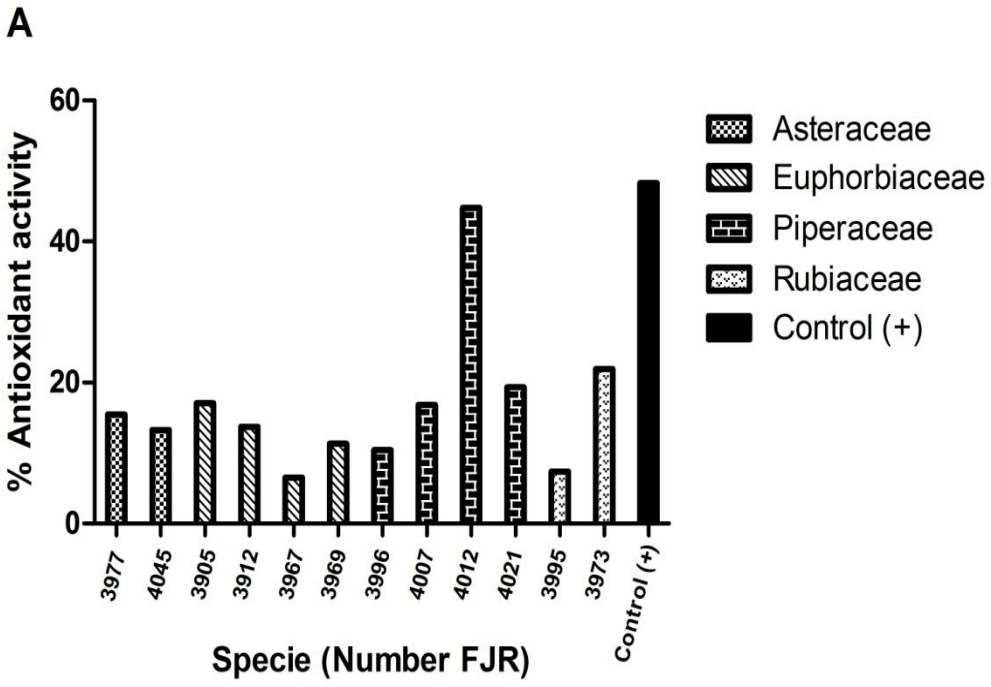
Species			*Mikania lloensis*	*Clibadium asperum*	*Hyeronima antioquensis*	*Mabea Montana*	*Acalypha diversifolia*	*Alchornea coelophylla*	*Piper pesaresanum*	*Piper eriopodon*	*Piper umbellatum*	*Piper crassinervium*	*Rubiacea*	*Palicourea petiolaris*
Secondary metabolites tested	Alkaloid	HEX	-*	+ǂ	-	-	+	-	+	-	-	-	-	-
		DCM	-	-	-	-	-	-	+	-	-	-	-	-
		HEX-iPrOH (3:1)	-	-	+	-	-	-	+	-	-	-	-	-
	Phenols and tannins	HEX	+	+	+	+	+	+	+	+	+	+	+	+
		DCM	+	+	+	+	+	+	+	+	+	+	+	+
		HEX-iPrOH (3:1)	+	+	+	+	+	+	+	+	+	-	+	+
	Sterols, steroids, terpenes	HEX	+	+	+	+	+	+	+	+	+	+	+	+
		DCM	+	+	+	+	+	+	+	+	+	+	+	+
		HEX-iPrOH (3:1)	+	+	+	+	+	+	+	+	+	+	+	+
	Steroidal saponins	HEX	+	+	+	+	+	+	+	+	+	+	+	+
		DCM	+	+	+	+	+	+	+	+	+	+	+	+
		HEX-iPrOH (3:1)	+	+	+	+	+	+	+	+	+	-	+	+
	Flavonoids	HEX	+	+	+	+	+	+	+	+	+	+	+	+
		DCM	+	+	+	+	+	+	+	+	+	+	+	+
		HEX-iPrOH (3:1)	+	+	+	+	+	+	+	+	+	+	+	+
	Lactones and Sesquiterpene lactones	HEX	-	-	-	-	-	-	-	-	-	-	-	-
		DCM	-	-	-	-	-	-	-	-	-	-	-	-
		HEX-iPrOH (3:1)	-	-	-	-	-	-	-	-	-	-	-	-
	Coumarins	HEX	-	-	-	-	-	-	-	-	-	-	-	-
		DCM	-	-	+	+	-	-	-	-	+	-	+	+
		HEX-iPrOH (3:1)	+	-	+	+	-	+	-	+	+	+	+	+
	Anthraquinones	HEX	-	-	-	-	-	-	-	-	-	-	-	-
		DCM	+	+	-	+	+	+	+	+	+	+	+	+
		HEX-iPrOH (3:1)	+	+	+	+	+	+	+	+	+	+	+	+

*(-): Absence of phytocompound ǂ(+): Presence of phytocompound

[a]HEX: Hexanes, [b]DCM: Dichloromethane and [c]HEX-iPrOH Hexanes-isopropanol

Antioxidant Activity

In the determination of antioxidant activity (%AA), through the free radical sequestration method were considered as actives those extracts that showed antioxidant activity ≥ 25 % , which corresponds to half the activity value presented by the positive control (hidroquinone at 1000 mg/L) (Gaviria et al., 2014). In figure 1 the extracts that displayed significant activity were: in the HEX extracts only one, on the other hand, for the twelve evaluated dichloromethane plant extracts only six of them and finally, ten of twelve HEX-iPrOH (3:1) extracts showed activity higher to 25%. In general, the antioxidant capacity of the extracts according to its polarity was in the following order: HEX-iPrOH (3:1) › DCM › HEX. These results could be explained by the presence of secondary metabolites as coumarins and anthraquinones in the extracts of DCM and HEX-iPrOH (3:1) (Borges et al, 2013; Zhang et al, 2005).



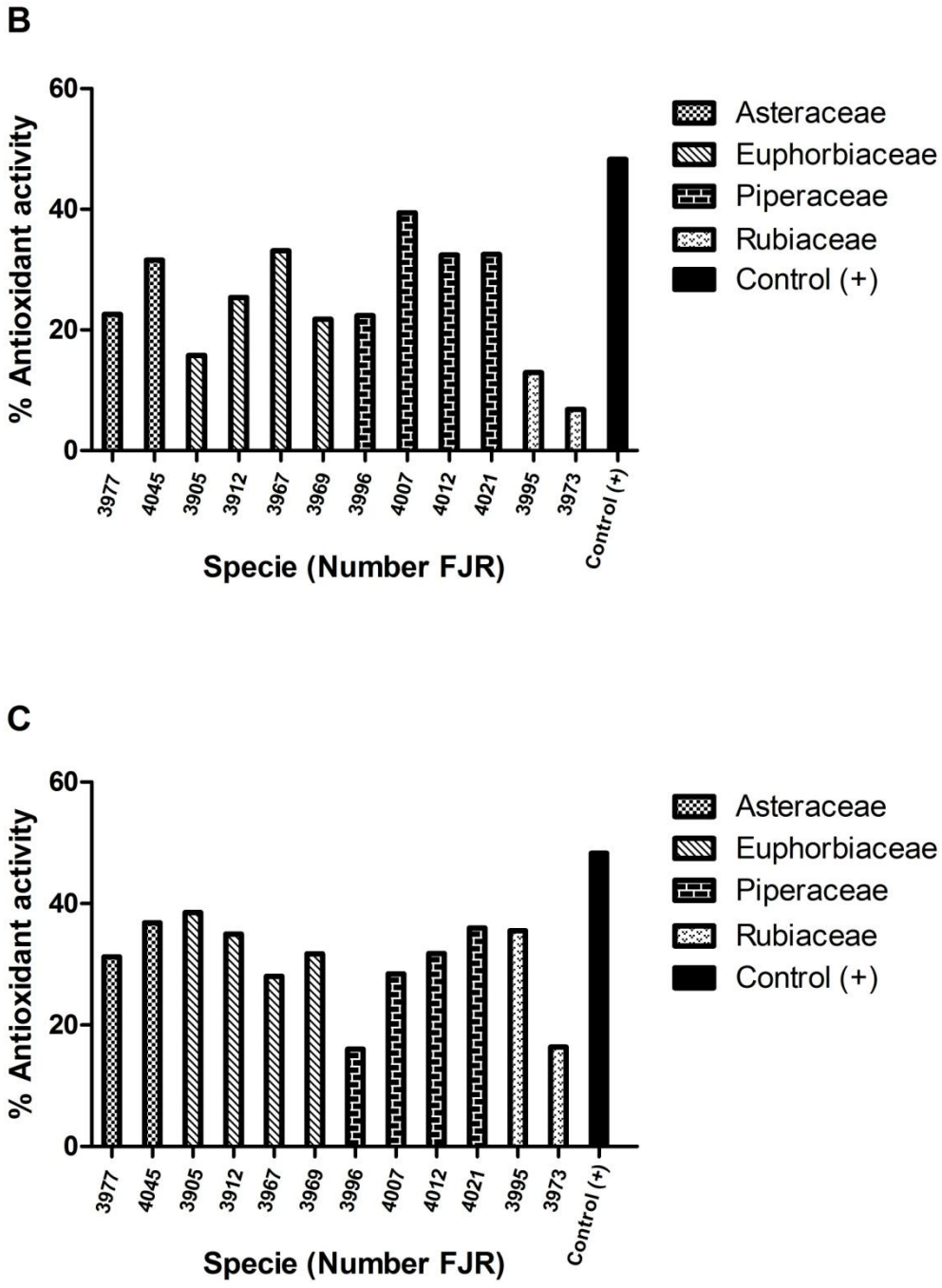


Figure 1. Percentage of antioxidant activity through the DPPH• radical method in (A) hexane (B) dichloromethane and (C) hexane-isopropanol (3:1) extracts

Of the twelve evaluated species the highest antioxidant activity in the three types of extracts was displayed by *Piper umbellatum* (FJR-4012), standing out its HEX extract with 44.8 % of activity. However, significant differences were not demonstrated in the antioxidant capacity in the four families studied. These results agree with the obtained for Swapna et al., (2012) and Zarai et al., (2013), whom reported important antioxidant activities for the species belonging to the *Piper* genus like *Piper betel* L. and *Piper nigrum*.

On the other hand, through the phytochemical characterization was detected the presence of common phytocompounds in the species that presented good antioxidant activity, such as: phenols, tannins, sterols, steroids, terpens, saponins and flavonoids, which have been reported as phytocompounds with high radicals capture capacity in researches developed by Alabri et al., (2014), Akharaiyi, (2011) and Koşar et al., (2011).

For the evaluation of the antioxidant activity by the $ABTS^{\bullet+}$ assay, in table 3 it is possible to observe that the obtained results by this method were higher than those displayed through $DPPH^{\bullet}$ assay, indicating the good sensibility of the first assay compared to the last one, it was possibly by the type of metabolites detected in the extracts. This determination was reaffirmed by studies developed by Floegel et al., (2011).

Table 3. Antioxidant activity expressed in trolox equivalents of the most active plants for the $DPPH^{\bullet}$ and $ABTS^{\cdot+}$ methods

SPECIES	DPPH· (µmol trolox/g extract)*			ABTS·+ (µmol trolox/g extract)*		
	HEX	DCM	HEX-iPrOH (3:1)	HEX	DCM	HEX-iPrOH (3:1)
Clibadium asperum	ND	19.93	23.38	ND	109.00	169.30
Mikania lloensis	ND	ND	19.70	ND	ND	349.08
Acalypha diversifolia	ND	20.94	17.64	ND	140.03	902.93
Alchornea coelophylla	ND	ND	20.04	ND	ND	914.76
Hyeronima antioquensis	ND	ND	24.46	ND	ND	341.19
Mabea montana	ND	15.89	22.14	ND	168.88	469.07
Piper crassinervium	ND	20.54	22.81	ND	525.89	690.82
Piper eriopodon	ND	25.06	17.89	ND	193.00	295.04
Piper pesaresanum	ND	ND	ND	ND	ND	ND
Piper umbellatum	28.53	20.48	20.07	519.44	347.83	370.92
Palicourea petiolaris	ND	ND	22.53	ND	ND	152.74
Rubiacea	ND	ND	ND	ND	ND	ND
Control (+) Hydroquinone 1000 mg/L	30.84			917.76		

*ND: No detected

Determination of total phenolic content

The content of total phenols expressed mg gallic acid /100 mg extract (GAE) of the species that presented activity with DPPH• is shown in the table 4. From this information was calculated the correlation between the quantity of this metabolite in the extract with its antioxidant capacity (Magalhães et al., 2008).

Table 4. Total phenols content expressed as equivalents of Gallic acid of the active extracts

SPECIES	Total phenols* (mg gallic acid/100 mg extract)		
	HEX	DCM	HEX iPrOH
Clibadium asperum	ND	1.77	2.82
Mikania lloensis	ND	ND	5.57
Acalypha diversifolia	ND	9.47	14.57
Alchornea coelophylla	ND	ND	28.42
Hyeronima antioquensis	ND	ND	8.57
Mabea montana	ND	0.92	9.45
Piper crassinervium	ND	8.77	9.92
Piper eriopodon	ND	1.77	0.23
Piper pesaresanum	ND	ND	ND
Piper umbellatum	1.97	2.55	4.25
Palicourea petiolaris	ND	ND	3.77
Rubiacea	----	----	----

*ND: No detected

These results point out that some of the species with high percentages of antioxidant activity presented low values of GAE, while others with moderate activity showed higher levels; this is the case of the HEX extract of *Piper umbellatum* that showed the major percentage of antioxidant activity (44.8%) and displayed a content of 1.97 GAE, whereas in the HEX- iPrOH (3:1) extract of *Acalypha diversifolia* there was 14.57 GAE, in spite of the fact that its antioxidant activity was 28%. Due to these results it is possible to infer that the content of total phenols of the evaluated species does not correlate directly with the antioxidant activity that they present; the correlation coefficient of Pearson between the total phenols content in the extracts of DCM and HEX-iPrOH (3:1) with the antioxidant activity for DPPH• method were 0.1419 and -0.1362 respectively. This result was similar to the presented one for Hassimotto et al., (2005) indicating that the antioxidant activity can be owed to the presence of another type of compounds such as flavonoids, saponins or alkaloids (Kim et al., 2012; Arun & Brindha, 2012).

In vitro* fungicidal activity against *Fusarium oxysp*orum and *Fusarium solani.

In the determination of the antifungal activity of the three types of crude extracts one of the twelve species studied at concentrations of 429, 857, 1714 y 3429 mg/L against *Fusarium oxysporum* and *Fusarium solani* fungi displayed moderate activity; but the highest percentages of inhibition were observed at 3429 mg/L. The most

affected fungus by the plants extracts tested was *F. oxysporum*, because the majority of the plant extracts evaluated inhibited its radial growth; the HEX-iPrOH (3:1) extracts showed the highest activity, standing out the specie *Mikania Iloensis* (FJR-3977) with a percentage of inhibition of 44.8%. The species from the Piperaceae family were the most actives since all the plant extracts assayed inhibited the growth of *F. oxysporum*, where *P. pesaresanum* (FJR-3996) exhibited an activity range between 34.6 – 42.3% as is showed in the figure 2A.

In case of *F. solani*, the most active extracts were those of the Piperaceae family, been the HEX and DCM extracts from the *Piper pesaresanum* (FJR-3996) that exhibited 59% and 38% of antifungal activity respectively. The extracts of HEX-iPrOH (3:1) of *Hyeronima antioquensis* (FJR-3905) and *Mabea montana* (FJR-3912) both of the Euphorbiaceae family were the least active against this fungus; while all the extracts of the species assayed from the families Asteraceae and Rubiaceae presented moderate to weak activities, with values lower than 23% (figure 2B).

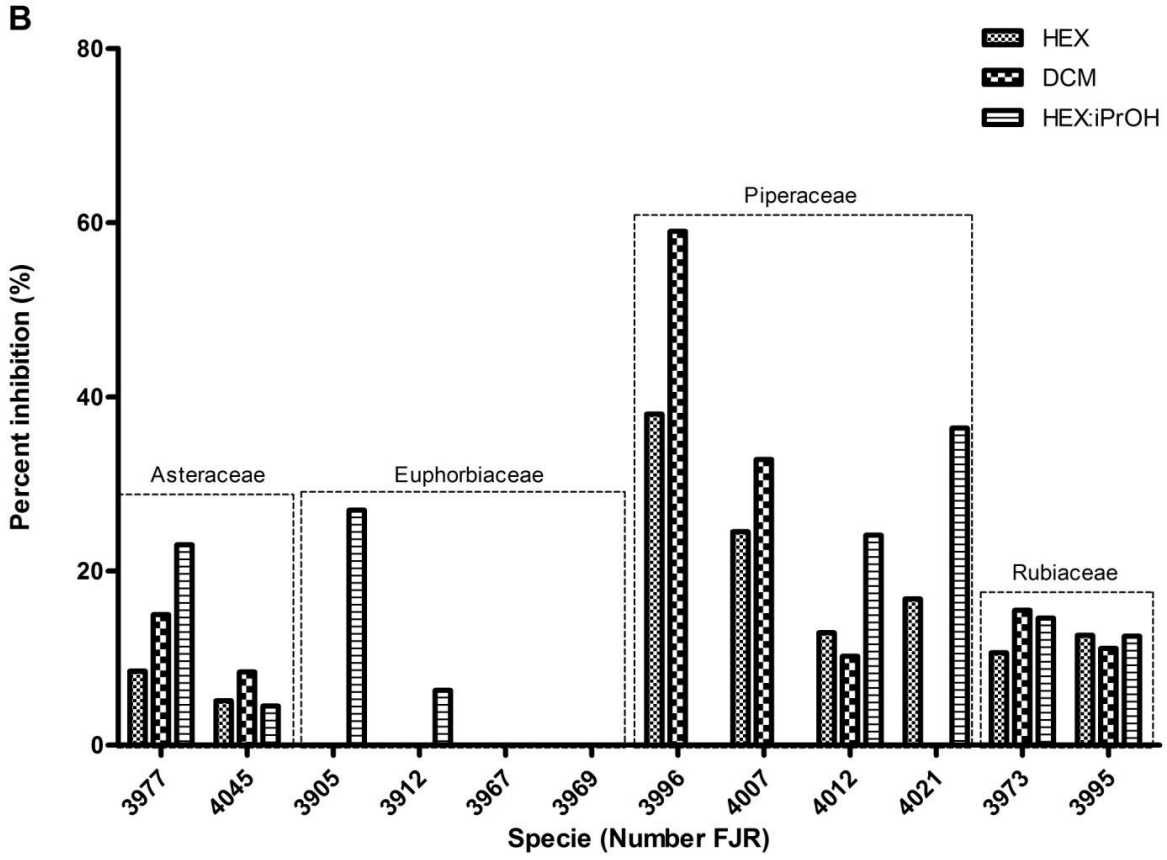
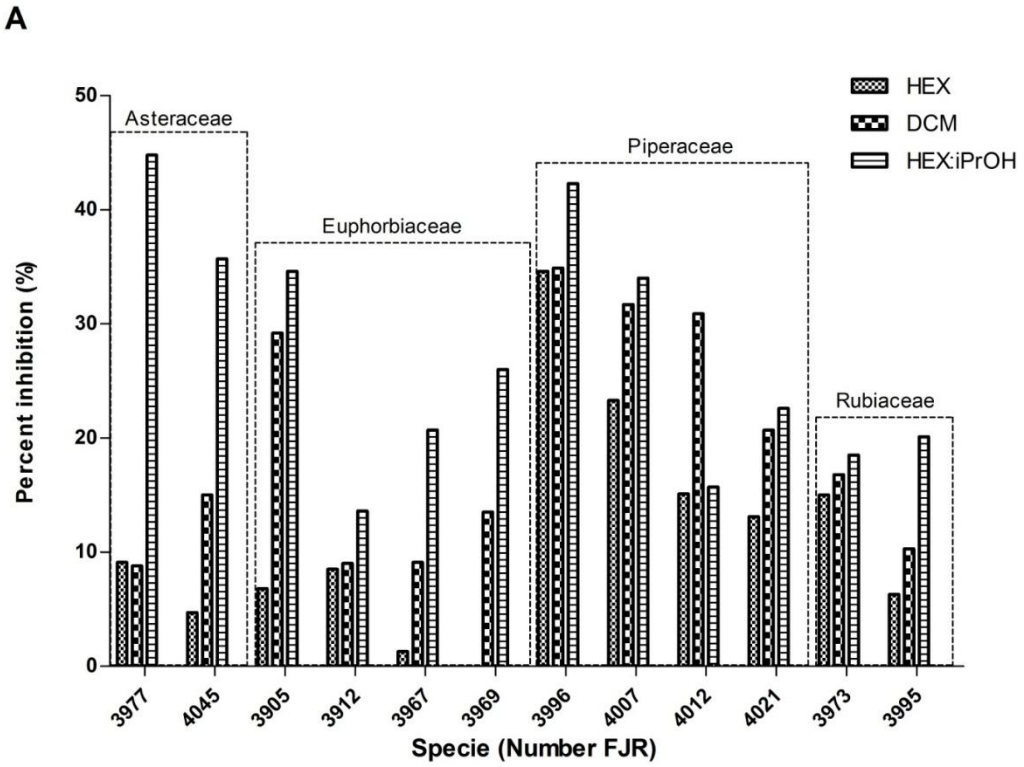


Figure 2. Percentage of inhibition of the extracts evaluated to 3429 mg/L of: A) *Fusarium oxysporum*; B) *Fusarium solani*

***In vitro* fungicidal activity against *Mycosphaerella fijiensis* Morelet.**

The extracts with the highest antifungal activity against the sexual phase of *M. fijensis* were the DCM and HEX-iPrOH (3:1) extracts from *Piper pesaresanum* (FJR-3996) as well as the DCM extract of *Alchornea coelophylla* (FJR-3969), these extracts inhibited ascospores germination in 100 % followed by the DCM extract of *Hyeronima antioquensis* (FJR-3905) and the HEX extract of *P. pesaresanum*, with an activity of 80.5 and 39.5%, respectively. On the other hand, the DCM extracts from *Piper crassinervium* (FJR-4021), *Mabea montana* (FJR-3912) and *Acalypha diversifolia* (FJR-3967) affected the *M. fijiensis* ascospores growth in 82.5, 69.5 and 54.5%, respectively as it is shown in Figure 3.

By means of the non parametric statistical analysis of Kruskal-Wallis (p = 0,5) through the statistical software Infostat (2007d.3) the results obtained in the radial growth test showed that the HEX and HEX-iPrOH (3:1) extracts were the most active against the asexual phase of the fungus. It was determined that the HEX-iPrOH (3:1) extracts of *Mikania lloensis* (FJR-3977) and *Clibadium asperum* (FJR-4045) presented 100% of activity against the asexual phase of *M. fijiensis*, followed by the HEX extracts of *Acalypha diversifolia* (3967), *Hyeronima antioquensis* (3905) and *Piper crassinervium* (4021) and finally the DCM extract of *Alchornea coelophylla* (3969), since the above mentioned extracts presented an inhibitory effect superior to 50% of the radial growth of the fungus without any statistical difference between them and the positive control (Benlate 1 mg/L).



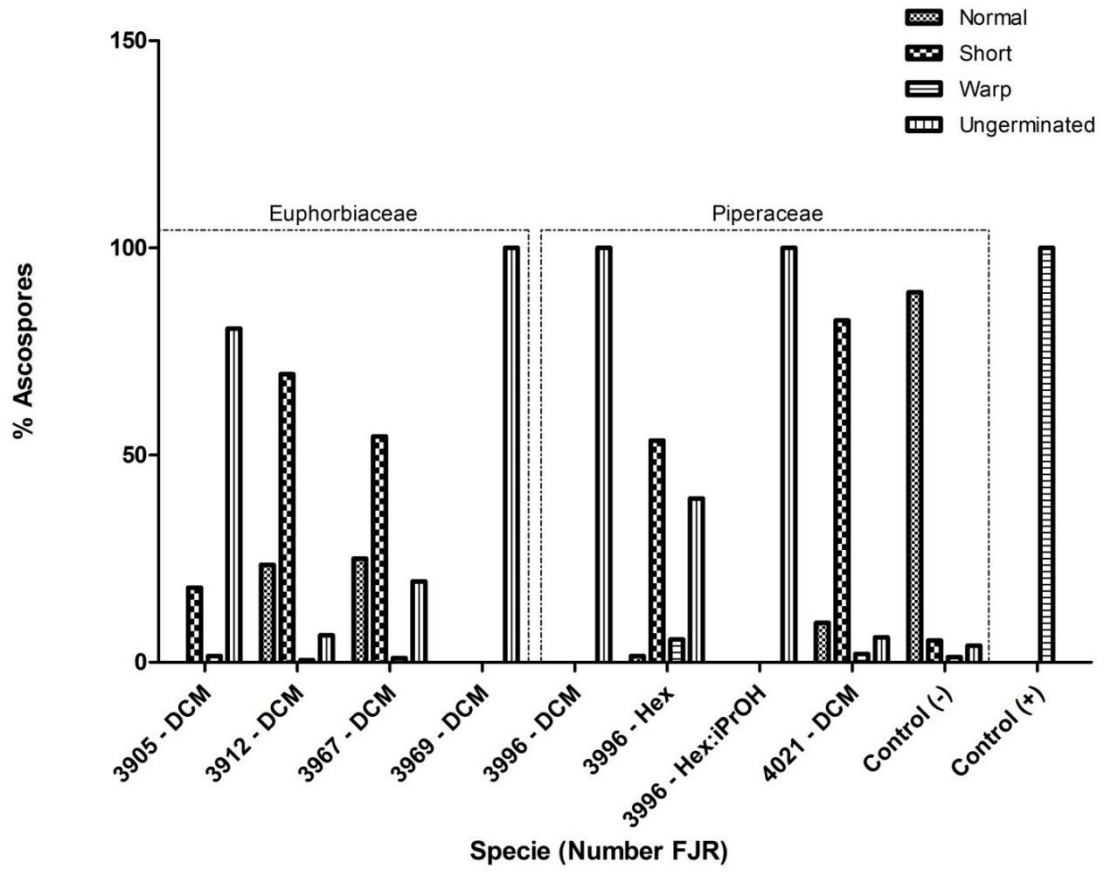


Figura 3. Active extracts at 1000 mg/L against of *Mycosphaerella fijiensis* Morelet sexual phase through the elongation of the germinative tube method

In previous investigations was determined that both species belonging to the Asteraceae family *Lepidaploa lehmannii* and *Critoniella acuminate* as well *Alchornea coelophylla, Acalypha diversifolia, A. macrostachya, Hyeronima antioquensis and Phyllantus sp* associated to the Euphorbiaceae family as *Piper pesaresanum* and *Piper crassinervium* from to the Piperaceae family and *Palicourea guianensis* Aubl related to the Rubiaceae family, displayed activity against *M. fijiensis* (Mosquera et al., 2009). Whereas Riveros & Arciniegas, (2003), reported that *Piper hispidum* and *P. peltatum* (Piperaceae family) disables the growth and development of the ascospores and colonies of *M. fijiensis*; demonstrating in this way, the potential anti-Black Sigatoka activity of species of the Asteraceae, Euphorbiaceae, Piperaceae and Rubiaceae families.

In general, all the species that showed activity against *F. oxysporum*, *F. solani* and *M. fijiensis*, contain phytochemical compounds as phenols, tannins, triterpenes, sterols, steroids, steroidal saponins and flavonoids; some of them differ in the content of coumarins, antraquinones and another type of flavonoids; in the case associated to the species of the Piperaceae family that contain autones or aurones, chalcones, flavones, flavonols and catechins, besides coumarins and antrones or anthranols that could be responsible for their activity

against *M. fijiensis*. Among the evaluated species the only one that presented alkaloids was *Piper pessaresanum* (FJR-3996) in all of its extracts. These results agree with those reported by Palacios et al., (2009) who detected alkaloids, amides, pyrones, dihydrochalcones, flavonoids, phenylpropanoids, lignans, neolignans, tannins, saponins, coumarins belonging to the Piperaceae family to the same way flavonolic compounds like chalcones, dihydrochalcones, flavones and flavanones of the inflorescences of *Piper hispidium* Kunth (Plazas et al., 2008).

In different species extracts with anti-Black Sigatoka (anti-BS) activity, were identified secondary metabolites as alkaloids, steroids, phenols, tannins, polyphenols, coumarines, quinones, saponins, triterpens and flavonoids; in addition, it was found compounds like oligosaccharides, flavonoids, terpenoids and acetylenic acid, that promote or induce the phytoalexins production, that help to attack diseases from fungi origin in plants, for example increasing the resistance to Black Sigatoka (*Mycosphaerella fijiensis* Morelet) in banana (Echeverri et al., 2006).

4. CONCLUSIONS

The relation observed between the phenolic content and the antioxidant activity did not showed to be directly related to the evaluated extracts, for that reason it was assumed that this activity is originated for another type of compounds like flavonoids, terpens, saponines, among others.

The family that showed the highest number of species with antioxidant and antifungal activities was the Piperaceae followed by Asteraceae and Euphorbiaceae families. For the antioxidant activity the most active species was *Piper umbellatum* (Piperaceae), whereas against the fungi *Fusarium oxysporum* and *Fusarium solani* they were *Mikania Iloensis* (Asteraceae) and *Piper pessaresanum* (Piperaceae), respectively. Against the sexual phase of the fungus *Mycosphaerella fijiensis* Morelet the extracts that displayed total inhibition were *Piper pesaresanum* (Piperaceae) and *Alchornea coelophylla* (Euphorbiaceae); while, against the asexual phase they were *Mikania Iloensis* (Asteraceae) and *Clibadium asperum (*Asteraceae) which inhibited in 100 % the fungus growth.

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Conflict of interest

The author(s) declare that they have no conflicts of interest.

5. REFERENCES

Akharaiyi FC. (2011). Antibacterial, phytochemical and antioxidant activities of *Datura metel*. *International Journal of Pharmacology Technology Research*, 3, 478–483.

Alabri THA., Musalami AHSA, Hossain MA, Weli AM, Al-Riyami Q. (2014). Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. *Journal of King Saud University – Science*, 26, 237-243.

Arun KP, Brindha P. (2012). Studies on antioxidant and antiarthritic potentials of *Jatropha tanjorensis* Ellis and Saroja. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4, 136-138.

Belalcázar S, Merchán VM, Mayorga M, et al. Plagas y enfermedades del plátano. *Boletín de Sanidad Vegetal (ICA)*. [Online]. Available at: http://www.corpoica.org.co/SitioWeb/publicaciones/verpublicacion.asp?id_publicacion=842. Accessed on 26 October 2014.

Borges G, Da Rocha D, Medina A, Von Poser G, Lamuela R.M, Eifler V. L, Garcia S.C. (2012). The Antioxidant Activity of Coumarins and Flavonoids. *Mini-Reviews in Medicinal* Chemistry, 13(3), 1-17.

Brand - Williams W, Cuvelier M, Berset C. (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. *Lebensmittel-Wissenschaft und-Technologie*, 28, 25-30.

Cardeño A, Molina M, Miranda I, Tafurt G, Martínez J, Stashenko E. (2007). Actividad antioxidante y contenido total de fenoles de los extractos etanólicos de *Salvia aratocensis, Salvia Sochensis, Bidens reptons y Montanoa ovalifolia*. *Scientia et Technica*, 33, 205-207.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. (2006). Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97, 654–660.

D'Souza DH. (2014). Phytocompounds for the control of human enteric viruses. *Current Opinion in Virology*, 4, 44–49.

Duarte O, Velho L. (2009). Capacidades científicas y tecnológicas de Colombia para adelantar prácticas de bioprospección. *Rev. iberoam. cienc. tecnol. Soc,* 4(12), 55-68.

Du Pont. (1983). Black and yellow sigatokas: Improved identification and management techniques. Florida. U.S: Du Pont Latin America, Coral Gables.

Echeverri F, Torres F, Pineda R, Zuleta S. (2006). Inducción de fitoalexinas en hojas de banano con varios agroquímicos. XVII Reunión Internacional. Brasil: Acorbat, Joinville, 683- 686.

Floegel A, Kim D-O, Chung S-J, Koo SI, Chun OK. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, 24, 1043–1048.

Gaviria A, Correa CE, Mosquera OM, Niño J, Correa YM. (2015). Evaluación de las actividades antioxidante y antitopoisomerasa de extractos de plantas de la ecorregión cafetera colombiana. *Revista Facultad de Ciencias Básicas*, 11(1), 86-101.

Hassimotto NMA, Genovese MI, Lajolo FM. (2005). Antioxidant activity of dietary fruits, vegetables and commercial frozen fruit pulps. *J. Agric. Food Chem,* 53, 2928-2935.

Kanegusuku M, Benassi JC, Pedrosa RC, Yunes RA, Filho VC, Maia AA, Souza MM, Monache FD, Niero R. (2002). Cytotoxic, hypoglycemic activity and phytochemical analysis of *Rubus imperialis* (Rosaceae). *Z. Naturforsch*, 57, 272-276.

Kim YA, Kong C-S, Lee JI, Kim H, Park HY, Lee H-S, Lee C, Seo Y. (2012). Evaluation of novel antioxidant triterpenoid saponins from the halophyte *Salicornia herbacea*. *Bioorganic & Medicinal Chemistry Letters*, 22, 4318–4322.

Koşar M, Göger F, Başer KHC. (2011). *In vitro* antioxidant properties and phenolic composition of Salvia halophila Hedge from Turkey. *Food Chemistry*, 129, 374–379.

Liu H-L, Luo R, Chen X-Q, Ba Y-Y, Zheng L, Guo W-W, Wu X. (2015). Identification and simultaneous quantification of five alkaloids in *Piper longum* L. by HPLC–ESI-MSn and UFLC–ESI-MS/MS and their application to *Piper nigrum* L. *Food Chemistry*, 177, 191–196.

Magalhães L, Segundo M, Reis S, Lima J. (2008). Methodological aspects about *in vitro* evaluation of antioxidant properties. *Analytica Chimica Acta*, 613, 1-19.

Marquez RT, De la Rosa C, Mercado A. (2007). Actividad antifúngica del extracto total en etanol de la hojas de *Pedilanthus tithymaloides* L Poit (Ultimorrial). *Scientia et Technica*, 33, 155–159.

Mazid M, Khan TA, Mohammad F. (2011). Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine*, 3, 232-249.

Mosquera OM, Echeverry LM, Niño J. (2009). Evaluación de la actividad antifúngica de extractos vegetales sobre el hongo *Mycosphaerella fijiensis* Morelet. *Scientia et Technica*, 41, 232–236.

Mughal T, Mamona A, Saddiuqe Z, Qureshi S, Mehboob S. (2010). Phytochemical and pharmacognostical evaluation of Euphorbiaceae species from Lahore region, Pakistan. *J App Pharm*, 3(2), 79-85.

Niño J, Narváez DM, Mosquera OM, Correa YM. (2006). Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and two Rubiaceae plants from colombian biodiversity. *Brazilian Journal of Microbiology*, 37, 566-570.

Palacios ZGF, Delgado GE, Moreno MC, Kato MJ, Rojas C. (2009). Actividad antifúngica *in vitro* de extractos crudos de *Piper tuberculatum*. *Revista Peruana de Biología*, 16, 209–214.

Parmar V.S, Jain S.C, Bisht K.S, Jain R, Taneja P, Jha A, Tyagi O.D, Prasad A.K, Wengel J, Olsen C.E, Boll P.M. (1997). Phytochemistry of the genus *Piper*. *Phytochemistry*, 46(4), 591-673.

Peláez JE, Vásquez LE, Díaz TJ, Castañeda DA, Rodríguez E, Arango RE. (2006). Use of microtitle plate dilution assay to measure activity of antifungal compounds against *Mycosphaerella fijiensis* Morelet. *Rev. Fac. Nal. Agr. Medellín*, 59, 3425-3433.

Perazzo FF, Rodrigues IV, Maistro EL, Souza SM, Nanaykkara NPD, Bastos JK, Carvalho JCT, de Souza GHB. (2013). Anti-inflammatory and analgesic evaluation of hydroalcoholic extract and fractions from seeds of *Piper cubeba* L. (Piperaceae). *Pharmacognosy Journal*, 5, 13-16.

Plazas E, Cuca L, Delgado W. (2008). Flavonoides aislados de las inflorescencias de *Piper hispidium* Kunth (Piperaceae) y derivados acetilados. *Revista Colombiana de Química*, 37, 135-144.

Ravipati A.S, Reddy N, Koyyalamudi S.R. (2014). Biologically active compounds from the genus *Uncaria* (Rubiaceae). *Studies in Natural Products Chemistry*, 43, 381-408.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. (1999). Antioxidant activity applying and improved ABTS radical cation decolorization assay. *Free Radical Biol. Med*, 26, 1231-1237.

Riveros AS, Arciniegas AM. (2003). Productos naturales como biofungicidas e inductores de resistencia para el manejo de la Sigatoka Negra. In: Galileo Rivas & Franklin Rosales, ed. *Actas del Taller: Manejo convencional y alternativo de la Sigatoka Negra, nematodos y otras plagas asociadas al cultivo de Musáceas en los trópicos*. Ecuador: Guayaquil, 31-32.

Seebaluck R, Gurib-Fakim A, Mahomoodally F. (2015). Medicinal plants from the genus Acalypha (Euphorbiaceae)–A review of their ethnopharmacology and phytochemistry. *Journal of Ethnopharmacology*, 159, 137-157.

Singleton V, Rossi J. (1965). Colorimetry of total phenolics with phosphomolybdic – phosphotungstic acid reagents. *American Journal of Enology and Viticulture* , 16, 144-58.

Škrovánková S, Mišurcová L, Machů L. (2012). Chapter Three – Antioxidant Activity and Protecting Health Effects of Common Medicinal Plants. *Advances in food and nutrition research*, 67, 75-139.

Swapna NL, Ammani K, Prasad Saripalli HKR. (2012). Antioxidant activity of Mokkathotapapada leaves of Piper betel L. Cv. Kapoori. *Free Radicals and Antioxidants*, 2, 68-72.

Wagner H, Bladt S. (1996). *Plant drug analysis: thin layer chromatography*. Berlin, Germany: Springer.

Wetwitayaklung P, Phaechamud T, Limmatvapirat C, Keokitichai S. (2006). The study of antioxidant capacity in various parts of *Areca catechu* L. *Naresuan University Journal*, 14, 1-14.

Yadav R.N.S, Agarwala M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3, 10-14.

Zhang X, Thuong PT, Jin W, Su ND, Sok DE, Bae K, Kang S.S. (2005). Antioxidant Activity of Anthraquinones and Flavonoids from Flower of *Reynoutria sachalinensis*. *Archives of Pharmacal Research*, 28(1), 22-27.

Zarai Z, Boujelbene E, Ben Salem N, Gargouri Y, Sayari A. (2013). Antioxidant and antimicrobial activities of various solvent extracts, piperine and piperic acid from *Piper nigrum*. *LWT - Food Science and Technology*, 50, 634-641.

Referencia	Fecha de recepción	Fecha de aprobación
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