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REGULAR ARTICLE

Antioxidant and antimicrobial capacity *Cecropia mutisiana* Mildbr. (Cecropiaceae) leave extracts

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ABSTRACT

Background: Given the lack of knowledge in *Cecropia mutisiana* Mildbr phytochemical and pharmacological properties, the objective of this work was to determine the leaves antioxidant and antimicrobial capacities, taking into account its wide use by Colombian communities. For this study *Rosmarinus officinalis* Govaerts extracts were used as experimental standards for comparisons, since there are no studies of *Cecropia mutisiana* Mildbr biological activities. **Methods:** Extraction was performed by dried leaves obtaining extracts of different polarity (petroleum ether, ethyl acetate, dichloromethane, and ethanol). To identify *Cecropia mutisiana* Mildbr functional groups and main compounds preliminar phytochemical analysis was run. Likewise, antioxidant capacity for both plants was determined by colorimetric assays; followed by phenol quantification correlation by Folin Ciocalteu reagent. Last, its antimicrobial capacity was evaluated by the Kirby-Bauer test. **Results:** *Cecropia mutisiana* Mildbr ethanol extract had the highest antioxidant capacity expressed as IC_{50} (165.47 ± 3.0 ppm), as well as the dichloromethane *Rosmarinus officinalis* Govaerts extract (272.63 ± 4.9 ppm), without any correlation with total phenols. Additionally, antimicrobial activity was observed for *Cecropia mutisiana* Mildbr in the ethyl acetate extract and for *Rosmarinus officinalis* in the ethanol extract. **Conclusion**: Regarding both plant comparison *Cecropia mutisiana* Mildbr ethanol extract had the highest antioxidant capability, whereas *Rosmarinus officinalis* Govaerts ethanol extract had the greatest antimicrobial activity.

Keywords: Antioxidant; Antimicrobial; DPPH; ABTS; ORAC; *Cecropia mutisiana*; *Rosmarinus officinalis*

INTRODUCTION

Ample vegetation species diversity in the Neotropic makes it indispensable to describe, study and characterize plants with high phytotherapeutic potential and possible industrial use, where its ethnobotany knowledge is fundamental for its identification and classification as a promissory species (Bernal et al., 2011). In Colombia approximately 2,404 plant species with ethnobotanical reports are used, of which 1,656 are cultivated in the country. Despite this fact few have been scientifically studied regarding their phytochemical, toxicological, and pharmacognostic characteristics (Bernal et al., 2011). Therefore, it is necessary to perform studies to validate the etnobotanical knowledge elucidating their active compounds, biosynthesis pathways, and pharmacological activities that define their phytotherapeutic and industrial potential for traditional medical use.

Cecropia mutisiana Mildbr., is a Colombian species (Berg et al., 2005; Bernal et al., 2011) with Vademecum medicinal plant etnobotanical registration, classified as phytotherapeutic and pharmacological promissory (Minprotección, 2008; Manosalva-Moreno, 2011). This plant is known etnobotanically for its effectiveness against nosocomial diseases, hypertension, as a cardiac tonic, and nervous system depressant (Sequeda-Castañeda et al., 2015). However, pharmacological and chemical determinations are somewhat unspecific. Zambrano-Ospina described *Cecropia mutisiana* Mildbr aqueous leaf extract use as an antianxiety and anticonvulsant treatment (Zambrano-Ospina, 2000). In addition, Ahumada performed a chromatographic analysis identifying flavonoid type compounds, tannis, coumarins, steroids, and terpene lactones (Ahumada, 2006). None the less, currently no scientific registry has documented

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this Colombian plant antimicrobial and/or antioxidant capacity to shed light on its pharmacological and phytotherapeutic use. Due to its Vademecum indexing as a medicinal plant that can be employed for traditional medicinal use, it is therefore important to study *Cecropia mutisiana* Mildbr. Hence, studies validating this understanding at the experimental level are essential (Bernal et al., 2011; Manosalva-Moreno, 2011).

Given *Cecropia mutisiana* Mildbr antioxidant and antimicrobial activities have not been addressed, we proposed to use *Rosmarinus officinalis* Govaerts, a widely studied plant as a comparison standard with known phytochemical and pharmacognostic characteristics (Borras-Linares et al., 2014; Hay et al., 2015; Abkhoo and Jahani, 2016; Habtemariam, 2016; Moore et al., 2016).

MATERIALS AND METHODS

Extract preparation

Cecropia mutisiana plant material was purchased in Mogambo Environmental Trail in the Municipality of Viotá (Cundinamarca, Colombia) and *Rosmarinus officinalis* in the Marketplace Municipality of Chía (Cundinamarca, Colombia). Plants without mechanical (trauma, damage, and defoliations), biological (leaf damage caused by herbivores) or microbiological (phytopathogen signs or symptoms) lesions were purchased. *Cecropia mutisiana* was identified by taxonomic classification in National Herbarium of Universidad Nacional de Colombia, Bogotá campus, under voucher number: COL 575453.

Leaves were separated and dried at 20°C. Metabolic compounds were obtained by solvent extraction with increasing polarity using petroleum ether (PE), ethyl acetate (EtOAc), dichloromethane (CH_2Cl_2), and ethanol (EtOH) as the solvent with the maximum polarity, shaking at 100 rpm for seven days for all solvents. Extracts were filtered and then concentrated by rotary-evaporation at 40°C to prevent damaging thermolabile compounds, and finally the excess solvent was removed by drying under extraction hood for six hours.(Rodríguez-Rojo et al., 2012).

Phytochemical assays for each *Rosmarinus officinalis* and *Cecropia mutisiana* extracts were performed to qualitatively identify compounds and associate them with their biological activity identifying main compounds through Liebermann-Burchard (Steroids and sterols), Salkowski (Terpenes), Baljet (Terpenes and sterols), ferric hydroxamate (Sesquiterpene lactones), Shinoda (Flavonoids and phenolics), ferric chloride (Flavonoids and phenolics), anthrone (Flavonoid glycosides), Dragendroff (Alkaloids), and froth (saponins) tests (Tiwari et al., 2011; Dos-Santos et al., 2014).

Antioxidant capacity characterization

Antioxidant capacity was characterized by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH); 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Oxygen Radical Absorbance Capacity (ORAC). As antioxidant comparison standards, ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and gallic acid were used. A statistical correlation was carried-out to evaluate an association between antioxidant capacity and total phenolic content.

DPPH method

For DPPH assay 2,2-diphenyl-1-picrylhydrazyl radical chromogen was used according to Asadujjaman (Asadujjaman et al., 2013). Percentage free radical scavenging was determined by sample concentration inhibiting 50% of radical production (IC_{50}) with a 1:39 µL, sample: radical ratio. Spectrophotometric UV-VIS (Cary 100 CONC-Varian instruments) delta of absorbance was determined with kinetics every two minutes at 515 nm until stabilization tendency was observed, indicating maximum analyte-radical reaction capacity (Karadag et al., 2009; Asadujjaman et al., 2013). Ascorbic acid and trolox were used as standards. Plant and control half maximal inhibitory capacity (IC_{50}) were determined nine times (n=9) to have statistical representativeness.

ABTS method

ABTS methodology was performed with 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) chromogen with radical production by potassium persulphate (2.5 mM $K_2O_8S_2$) addition. Results are presented as $IC_{50,}$ since they correspond to total extract measurement at different polarities. Spectrophotometric UV-Vis at 734 nm absorbance change was determined (Cary 100 CONC-Varian instruments) with the same sample: radical ratio as for DPPH every three minutes until a stabilization tendency was observed indicating a maximum reaction analyte/radical capacity (Nilsson et al., 2005; Karadag et al., 2009). Ascorbic acid and trolox were used as standards; plant and control half maximal inhibitory capacity (IC_{50}) were established nine times (n=9) to have statistical representativeness.

ORAC method

Oxygen Radical Absorbance Capacity (ORAC) was determined by using 2,2'-azobis(2-aminido-propane) dihydrochloride (AAPH) and a sodium fluorescent salt such as fluorescein by which the antioxidant protective capacity was evidenced (IP_{50}) against radical attack, by determining a fluorometric delta difference between 485 nm excitation and 520 nm emission (FLUOstar Optima BMG Labtech). 96 well plates containing fluorescein blanks in phosphate buffer, ascorbic acid and trolox positive controls and extracts to be tested in a 1:11.5 µL sample: radical ratio

were employed. Obtained data was graphed as third order adjusted polynomial curves using polynomial orthogonal test to ensure all data met such distribution. Area under the curve (AUC) was used as a comparable measurement between the extracts and controls to which statistical analysis was performed (Dudonné et al., 2009; Karadag et al., 2009; Armstrong3, 2010).

Total phenolic content (Folin-Ciocalteu reagent)

For this method a gallic acid standard curve was used at the following concentrations 30, 60, 90, 120, and 150 ppm (r^2 = 0.9998; p = 0.000). 250 µL of 1N Folin-Ciocalteu reagent + 250µL of 20% Na_2CO_3 + 2mL distilled water at a ratio 1:25 sample: mix ratio were employed (Cicco et al., 2009). Sample was analyzed at 760 nm and results were expressed as mg of gallic acid equivalent per g of extract (at different polarities).

Antimicrobial capacity determination

Staphylococcus aureus CMPUJ 080*, Bacillus cereus* CMPUJ 251*, Salmonella* sp. CMPUJ 302 and *Pseudomonas aeruginosa* CMPUJ 055 bacterial strains obtained from Pontificia Universidad Javeriana Microorganism Collection Bogotá Campus (CMPUJ Certification: National collection registry No. 148, WFFC and WDMC No.857) were used. A 25% glycerol bank was established and kept at -80°C, from which all antimicrobial assays were carried.

Antimicrobial capacity determination for four *Rosmarinus officinalis* and *Cecropia mutisiana* extracts was verified using the Kirby-Bauer test with modifications according to Clinical Laboratory Standard Institute (M02-A12 document) (Klancnik et al., 2009; CLSI, 2015). Each assay was performed nine times in time (n=9). Muller-Hinton medium (pH 7.2-7.4) was used adjusting the inoculum to the 0.5 McFarland nephelometer standard ($1x10^8$ cells/mL) for *Staphylococcus aureus* CMPUJ 080, *Bacillus cereus* CMPJU 251, *Salmonella* sp. CMPUJ 302, and *Pseudomonas aeruginosa* CMPUJ 055.

An initial antimicrobial susceptibility screening was performed – antibiogram employing vancomycin, streptomycin, gentamycin, and chloramphenicol to establish a positive control. As a negative control, paper disks were loaded with dimethyl sulfoxide (DMSO) and 90% ethanol (EtOH) (1:1 ratio) and analytical-reagent grade extraction solvents (PE, EtOAc, CH_2Cl_2 and EtOH). (Rojas et al., 2006; Klancnik et al., 2009).

For treatments paper discs with 10 µL for each extract and control at established concentrations were applied to each Petri dish. All Petri dishes were kept between 2 - 4°C for 12 h to overnight (O/N) to allow for proper diffusion, followed by 37°C 24 h incubation. Inhibition halo was verified at 24 h and 48 h after incubation. 1,000, 100 and 10 ppm were established as extract treatment concentrations for each plant species extract. Microorganism sensibility was determined by inhibition halo caused by the antibacterial, determining minimum inhibitory concentration (MIC) against the microorganism (Bonev et al., 2008). Likewise, inhibition diameter relative percentage was determined according to Rojas formula (Rojas et al., 2006). Since it is important to specify plant extract concentration for each of the treatments performed, as the weight that inhibits organism growth, inoculated concentrations are expressed as the net quantity applied 10 µL on the disk with the real extract quantity, which was 10 mg, 1 mg and 0.1 mg for each treatment (1)

$$\frac{X\ mg}{L} \times \frac{1L}{10^6 \mu L} \times \frac{10^3 \mu g}{1mg} \times 10\ \mu L = X\ \mu g\ extract \quad (1)$$

Statistical analysis

Antioxidant level response comparison for *Rosmarinus officinalis* and *Cecropia mutisiana* was performed by a completely randomized design. First, Normality distribution was assessed by the Kolmogorov-Smirnof and Shapiro-Wilk test. Additionally, variance homogeneity was determined by the Levenne test. Comparison among groups was established by ANOVA with HSD Tukey *post hoc* tests to identify antioxidant capacity significant differences among groups. Transformations were performed when required (square root, natural logarithm, base 10 logarithm and reciprocal). P value < 0.05 was considered as significant. Data not following a normal distribution were assessed by Kruskal-Wallis statistical analysis to determine significant differences among group means followed by mean comparison *post hoc* tests. SPSS Statistics 20 (IBM, Armonk, New York USA) and SigmaPlot V11 (Systat Software Inc, London UK) were used. Statistical correlation was determined by Pearson correlation to establish an association between IC_{50} values per plant extract obtained by the ABTS method and phenolic content for obtained concentrations defined as mg of gallic acid/kg extract (ppm). Completely randomized design was performed for *Rosmarinus officinalis* and *Cecropia mutisiana* antimicrobial response applying the same statistical tests previously described for antioxidant level comparison.

RESULTS AND DISCUSSION

Extract characterization

Four extracts of different polarities were obtained for each of the plants from 200 g dried *Rosmarinus officinalis* and 1,000 g *Cecropia mutisiana* yields are detailed in Table 1.

Extraction yield for each polarity in *Rosmarinus officinalis* ranged between 1.0 % and 2.8 % extract per g of dried

plant material. In comparison to Rodríguez-Rojo results yields were low (Rodríguez-Rojo et al., 2012). Moreover, *Cecropia mutisiana* yields ranged between 1.2% and 1.9%. Showing similarities among extract percent yield, given the extraction methodology for each one.

Preliminary phytochemical analysis

The tests identified for both plants triterpenes, terpenoids, sesquiterpene lactones, flavonoids and phenols and flavonoids glycosides (Table 2). Liebermann-Bouchard test identified triterpenes are derived from squalene cyclization that in some cases can be found in a free form or glycosylated (anthrone test) (Sanabria-Galindo, 1999). For all *Cecropia mutisiana* extracts these compounds were identified, as well as for *Rosmarinus officinalis* medium polarity extracts. Oliveira et al., established these types of compounds have pharmacological properties such as antimicrobial, hypocholesterolemic, anti-inflammatory and cytotoxic against cancer cell lines (Oliveira et al., 2005).

Taking into account no compound identification studies have been performed for *Cecropia mutisiana* a correlation with other species of the same genus could be established, where terpenes and glycosides have been identified (Table 3).

Salkowski and Shinoda tests confirmed sterol presence, flavonoids, and derivatives of these (flavanols, isoflavones, flavanes, among others) for both plants. According to Uchoa et al., for Cecropia species sterols and flavonoids are secondary metabolites that are not involved in the plant's development and growth (Uchoa et al., 2009). This type of compound has been investigated in other Cecropia species (Table 4).

Presence of phenols was observed, as well as alkaloid type compounds, saponins, sesquiterpene lactones, lactones, and coumarins. Studies in other species have demonstrated these compounds, mainly chlorogenic acid in *Cecropia glaziovii, Cecropia obtusifolia, Cecropia pachystachya*, and *Cecropia peltata* (Andrade-Cetto and Wiedenfeld, 2001; Lacaille et al., 2001; Herrera-Arellano et al., 2004; Andrade-Cetto et al., 2007; Rocha et al., 2007a; Nicasio-Torres et al., 2009; Andrade-Cetto and Vázquez, 2010; Aragão et al., 2010; Arend et al., 2011; Mora Izquierdo et al., 2011; Nicasio-Torres et al., 2011; Petronilho et al., 2012; Beringhs et al., 2013; Cruz et al., 2013). In addition, for *Cecropia glaziovii* caffeic acid (Arend et al., 2011; Beringhs et al., 2013); protocathechuic acid in *Cecropia glaziovii* (Lacaille et al., 2001); alkaloids in *Cecropia pachystachya, Cecropia glaziovii,* and *Cecropia peltata* (King and Haddock, 1959; Consolini and Migliori, 2005; Ninahuaman et al., 2007). Furthermore, other phenolic compounds in *Cecropia obtusifolia* (Guerrero et al., 2010), in addition to saponins in *Cecropia pachystachya* (Consolini and Miglori, 2005), have been specified.

Given the lack of studies for primary or secondary metabolites and evaluation of the closest phylogenetic species must be perfomed. Therefore identification and comparison of all compounds present in species of the Cecropia genus are valid as an approximation potentia *Cecropia mutisiana* activities. Rocha et al. (2007) described typical chemical constituents such as catechins, different classes of flavonoids and procyanidins, terpenes, triterpenoids and other compounds for this genus, thus, suggesting possible promising pharmacological activities for *Cecropia mutisiana* an approximation of possible activities (Rocha et al., 2002; Rocha et al., 2007a; Rocha et al., 2007b).

Antioxidant capacity characterization

Currently no studies have reported antioxidant capacity for *Cecropia mutisiana,* and are scarce for other species of

Table 1: Extract yield/plant species

Extract	Percent yield (%Y)*	
	Rosmarinus officinalis	*Cecropia mutisiana*
Petroleum ether	2.8±0.8	1.9±0.6
Ethyl acetate	1.0±0.2	1.4±0.4
Dichloromethane	2.6±0.8	1.5±0.5
Ethanol	1.7±0.5	1.2±0.3
Total yield	8.1±1.3	6.0±0.9

*n=3

Table 2: Preliminary phytochemical analysis for *Rosmarinus officinalis* and *Cecropia mutisiana* extracts

Metabolite (test)	Extract							
	Rosmarinus officinalis				*Cecropia mutisiana*			
	PE	EtOAc	CH_2Cl_2	EtOH	PE	EtOAc	CH_2Cl_2	EtOH
Steroids and sterols (Liebermann-Burchard)	-	+	+	-	+	+	+	+
Terpenoids (Salkowski)	+	-	-	-	+	+	+	+
Terpenoids and sterols (Baljet)	-	+	+	+	+	+	+	+
Sesquiterpene lactone (Ferric hydroxamate)	+	+	+	+	+	+	+	+
Flavonoids and phenols (Shinoda)	-	-	-	-	-	-	+	-
Flavonoids and phenols (Ferric chloride)	+	+	+	+	-	+	+	+
Flavonoid glycosides or terpene (Anthrone)	-	+	+	+	+	+	+	+
Alkaloids (Dragendroff)	+	+	-	-	-	+	+	-
Saponins (Froth tests)	-	-	-	-	+	-	-	-

PE: Petroleum ether extract, EtOAc: Ethyl acetate extract, CH_2Cl_2: Dichloromethane extract, EtOH: Ethanol extract

Table 3: Cecropia genus terpene and glycosidic compounds

Plant	Described compound	Reference
Cecropia catharinensis	Tormentic acid, euscapic acid, pomolic acid, ursolic acid, oleanolic acid, maslinic acid, 2-*O*-acetyl-tormentic acid, 2α-acetoxy-3β-19α-dihydroxy-11α-12α-epoxi-ursan-28-13β-olide, 3β-acetoxy-2α,19α-dihydroxy-11α,12α-epoxi-ursan-28-13β-olide	Machado et al. (2008), Li et al. (2013)
Cecropia glaziovii	Other terpenes and triterpenes	Ninahuaman et al. (2007), Sarris et al. (2013)
Cecropia lyratiloba	Tormentic acid, isoarjunolic acid, euscaphic acid, 3-acetyl tormentic acid	Oliveira et al. (2005), Li et al. (2013)
Cecropia obtusifolia	Other terpenes and triterpenes	Morton (1981)
Cecropia pathystachya	Tormentic acid, pomolic acid, α-amyrin, other terpenes and triterpenes	Hikawczuk et al. (1998), Consolini and Migliori (2005), Schinella et al. (2008), Teixeiria-Uchoa et al. (2010), Li et al. (2013)
Cecropia peltata	Glycans	Marshall and Rickson (1973)
Cecropia schreberiana	Tormentic acid, ursolic acid, pomolic acid, α-amyrin	Schinella et al. (2008), Li et al. (2013)

Table 4: Cecropia genus compounds of sterol, flavonoids, and sterol/flavonoid derivatives

Plant	Described compound	Reference
Cecropia catharinensis	Isoorientin, orientin, isovitexin, vitexin	Machado et al. (2008)
Cecropia glaziovii	Isoorientin, orientin, isovitexin, vitexin, catechin, epicatechin, procyanidine B_2, B_3, B_5 and C_1, other flavonoids and sterols.	Lacaille-Dubois et al. (2001), Rocha et al. (2002), Rocha et al. (2007), Lima-Landman et al. (2007), Delarcina et al. (2007), Silva et al. (2010), Petronilho et al. (2012), Beringhs et al. (2012), Sarris et al. (2013), Costa et al., (2014)
Cecropia lyratiloba	Isoorientin, orientin, isovitexin, apigenin 6-*C*-galactosyl-6"-*O*-β-galactopyranoside.	Oliveira et al. (2003)
Cecropia obtusifolia	Isoorientin, orientin, isovitexin, vitexin, other flavonoids and sterols.	Andrade-Cetto and Wiedenfeld (2001), Herrera-Arellano et al. (2004), Nicasio-torres et al. (2009), Andrade-Cetto and Cardenas-Vazquez (2010), Aragao et al. (2010), Guerrero et al. (2010), Nicasio-Torres et al. (2012)
Cecropia pachystachya	Isoorientin, orientin, isovitexin, catechin, epicatechin, procyanidine B_2, B_3, B_5 and C_1, isoquercetin, β-sitosterol, luteolin, sitosterol, other flavonoids and sterols	Consolini and Migliori (2005), Teixeiria-Uchoa et al. (2010), Aragao et al. (2010), Mello-Cruz et al. (2013), Oliveira-Aragao et al. (2013)
Cecropia peltata	Isoorientin, other flavonoids and sterols	Pardo-Concepción et al. (2000), Andrade-Cetto et al. (2007), Nicasio-Torres et al. (2009), Andrade-Cetto and Cardenas-Vazquez (2010), Aragao et al. (2010), Mora-Izquierdo et al. (2011), Ospina-Chávez et al. (2013)
Cecropia schreberiana	Isoorientin, orientin, vitexin, catechin, epicatechin, cinchonain 1a and 1b	Li et al. (2013)

this same genus (Aragão et al., 2010; Mora Izquierdo et al., 2011; Petronilho et al., 2012). Therefore, for this study a comparison with *Rosmarinus officinalis*, a plant broadly known for its antioxidant activities was proposed to establish as a biological referral. Summary of antioxidant capacities for both plants by different test are summarized in Table 5.

Significant differences (ANOVA) were observed when comparing between DPHH, ABTS, and ORAC for each *Rosmarinus officinalis* extraction method. DPPH ($F = 3790.49$, $p < 0.001$), ABTS ($F = 3044.86$, $p < 0.01$), and ORAC ($F = 3582.36$, $p < 0.01$). It was evidenced dichloromethane extract had the highest antioxidant capacity for this plant (Tukey $p < 0.01$). Similarly, significant differences were also observed for each method in *Cecropia mutisiana* extracts: DPPH ($F = 93588.817$, $p < 0.01$), ABTS ($F = 84358.817$, $p < 0.01$), and ORAC ($F = 90327.01$, $p < 0.01$). Ethanol extraction had the highest antioxidant and protective capacity (ORAC) for this plant (Tukey $p < 0.01$).

Analysis of standardized IC_{50} values for both plants evidenced *Rosmarinus officinalis* dichloromethane extract and *Cecropia mutisiana* ethanol extract had the greates antioxidant capacity (IC_{50}) against DPPH, ABTS, and AAPH free radicals, comparable to trolox and ascorbic acid control IC_{50} values.

To establish if extract antioxidant capacity was associated with total phenolic content, these compounds were quantified for both plants (Table 6). No significant correlation for *Rosmarinus officinalis* and *Cecropia mutisiana* extracts was found between total phenol content and IC_{50} concentration ($r = -0.815$, $p = 0.185$) and ($r = -0.580$, $p = 0.420$), respectively.

Table 5: Antioxidant capacity determination by DPPH, ABTS and ORAC – IC_{50} or IP_{50} [ppm]

Method	Extract								Control	
	EP		CH_2Cl_2		AcOEt		EtOH			
	I	II	I	II	I	II	I	II	A	B
DPPH (IC_{50})	1813±68	5578±6	558±9*	14597±90	718±38	3843±54	3505±168	631±11*	163±8	124±9*
ABTS (IC_{50})	1303±25	2544±21	439±12*	2333±8	481±14	414±16	2083±168	253±2*	134±7	96±10*
ORAC (IP_{50})	915±17	1695±31	273±5*	2040±37	273±5	2075±37	1279±23	165±3*	88±2*	107±2

I: *Rosmarinus officinalis*, II: *Cecropia mutisiana*, Control A: Trolox, Control B: Ascorbic acid. (*) Extracts with highest antioxidant capacity (p<0.05)

Table 6: Total phenolic content/plant extract

Total phenols/plant (mg GA/g Ext)*		
Extract	*Rosmarinus officinalis*	*Cecropia mutisiana*
PE	27.3±1.4	24.6±1.5
EtOAc	135.1±6.4	60.6±2.9
CH_2Cl_2	239.8±10.3	35.8±1.7
EtOH	28.9±1.6	169.6±6.9

*Total phenolic content was established by Folin-Ciocalteu and is expressed as mg gallic acid per g plant extract (n=3). PE: Petroleum ether, EtOAc: Ethyl acetate, CH_2Cl_2: Dichloromethane, EtOH: Ethanol

Petronilho et al., (2012) performed *in vitro* and *in vivo* antioxidant capacity assays from *Cecropia glaziovii* hydroethanolic crude extracts through lipid peroxidation enzyme activity quantification. Their findings revealed a significant activity at low concentrations (2 ppm). In contrast, the minimum activity obtained in our study was in ethanol extract (253.2 ppm). An approximation can be therefore established to the type of antioxidant present in the plant species as a possible "scavenger", interrupting lipid peroxidation through iron chelation, directly influencing lipid solubility and preventing ROS generation. IC_{50} antioxidant capacity quantification defines the concentration required to obtain 50% inhibition/sequestration of the free radical to attain its chemical stabilization, as determined by DPPH and ABTS tests.

The highest IC_{50} observed were for high polarity ethanol extract, suggesting presence of phenolic and flavonoid compounds, phytochemically characterized for *Cecropia mutisiana* including chlorogenic acid, orientin, isoorientin, isovitexin reported by other authors (Nicasio-Torres et al., 2009; Aragão et al., 2010; Petronilho et al., 2012; Cruz et al., 2013). Aragao et al. (2010) performed *Cecropia pachystachya* antioxidant capacity for the methanol extract using the DPPH test establishing an IC_{50} of 10.8 ± 0.7 ppm. Moreover, Mora-Izquierdo et al. (2011) characterized *Cecropia peltata* antioxidant capacity in function of chlorogenic acid (CGA) standard by the ABTS methodology finding a stabilization capacity at 13.8 ± 2.2 mg CGA/g dry weight (13,780 ppm) for the methanol extract. For *Cecropia mutisiana* ethanol extracts the following IC_{50} were established for DPPH (630 ± 11.1 ppm) and ABTS (253.2 ± 2.0 ppm). Demonstrating the nature of the antioxidant molecule is of high polarity, typical behavior of previously described polyphenols and flavonoids (Aragão et al., 2010; Mora Izquierdo et al., 2011; Petronilho et al., 2012).

ORAC methodology allows a more thorough approximation of the antioxidant type, its nature and possible mechanism of action for the species in question. Additionally, a positive correlation between the protective capacity determined by ORAC and the antioxidant inhibitory capacity (ABTS and DPHH) was evidenced ($r = 0.968$, $r = 0.949$, $p < 0.01$). Demonstrating the presence of plant antioxidant compounds particularly of polar nature, acting as free radical "scavengers". In addition, they can act as quenchers *in vitro* sequestering lipid ROS production. Given its antioxidant activity this bivalent behavior could be due to complex interaction between majority and minority compounds present in *Cecropia mutisiana* extracts. For *Cecropia mutisiana* they have not been totally identified, in contrast to other species where majority compounds responsible for antioxidant capacity have been described, such as glycosylated flavonoids.

In comparison to other Cecropia species a greater antioxidant capacity was observed for *Cecropia mutisiana* compared with *Cecropia peltata*, and to lesser extent when compared with *Cecropia pachystachya,* due to environmental conditions to which the Colombian species is submitted in comparison to other species in the Neo-tropic, such as light exposure and nitrate supply or different quantities and types of compounds between the methanolic and ethanolic extracts. Mora-Izquierdo et al. (2011) have established the aforementioned factors are fundamental for natural antioxidant production, since high photosynthesis conditions and reduction of available nitrate increase ROS production, and with them the production of defensive compounds, such as antioxidant molecules based on carbon compounds.

Different authors have studied *Rosmarinus officinalis* antioxidant capacity characterizing plant extracts with findings evidencing medium polarity extract result in the best IC_{50} values, specifically for acetone and dichloromethane extractions (Yesil-Celiktas et al., 2007), given the tripenic nature of carnosic acid (CA), the main molecule responsible for the antioxidant capacity. Rodríguez-Rojo et al. (2012) established a *Rosmarinus officinalis* IC_{50} value determined by the DPPH scavenging assay between 69 and 45 ppm from a bioassay guided fractionation extraction. Moreover, Chang et al. (2008),

obtained with a supercritical fluid extraction an IC_{50} of 5 mg/mL, representing 5,000 ppm with extraction yields higher than solvent extraction, yet a marked decrease in antioxidant capacity (Chang et al., 2008). Likewise, Jordán et al. (2013b), compared different locations for Rosemary extractions in the Mediterranean finding on average an IC_{50} of 565.9 ppm for DPPH and 533.9 ppm for ABTS assays (Jordán et al., 2013b). Both values are comparable to our findings, where the best IC_{50} value was 558.3 ± 8.6 ppm observed with a DPPH assay from a CH_2Cl_2 extract. In addition to an IC_{50} value of 439.1 ± 11.9 ppm with an ABTS test.

Cecropia mutisiana and *Rosmarinus officinalis* antioxidant activity of each obtained plant extract was determined by using DPPH and ABTS radical tests. A significant IC_{50} value difference ($p < 0.05$) was observed for *Cecropia mutisiana* ethanol and EtOAc extracts for their antioxidant capacity determined by ABTS in comparison with *Rosmarinus officinalis*. For *Rosmarinus officinalis* DPPH antioxidant capacity from the dichloromethane extract was significantly higher compared ($p < 0.05$) with EtOAc or EtOH extracts from the same plant. Additionally, for *Cecropia mutisiana* ethanol extract ABTS antioxidant activity was also significantly higher compared to other extracts ($p < 0.05$). These results are likely due to the types of molecules that are known. Some authors have established CA (antioxidant molecule in *Rosmarinus officinalis*) acts as a proton donor and "scavenger" of free radicals (Masuda et al., 2001; Yesil-Celiktas et al., 2007; Rodríguez-Rojo et al., 2012). Thus, Karadag et al. (2009) described for DPPH and ABTS test results similar *in vitro* behavior. DPPH identifies antioxidant capacity with proton/electron donor capacity, and ABTS determines molecules of donating and or quenching capacity. *Rosmarinus officinalis*, DPPH and ABTS results for this study had appreciable IC_{50} differences, yet of low magnitude. This finding is supported by the lack of correlation between antioxidant capacity and total phenol quantification, given CA triterpenic nature.

In contrast, antioxidant capacity molecule or molecules for *Cecropia mutisiana* were different. For other *Cecropia* species chlorogenic acid (phenol compound) and/or flavonoids such as orientin, isoorientin, and isovetexin were described by Aragao et al. (2010), Mora-Izquierdo et al. (2011) and Petronilho et al. (2012) as the molecules responsible for antioxidant capacity. In this regard, our data evidenced a greater antioxidant capacity through the ABTS methodology compared with DPPH test. *Cecropia mutisiana* antioxidant activity could be the result of a possible proton donor or radical "scavenging-quenching" compound, as described by Karadag et al. (2009). This, in part supported by *Cecropia mutisiana* lack of correlation between antioxidant capacity and total phenol quantification (Folin Ciocalteu reagent). Thus, it could be inferred *Cecropia mutisiana* antioxidant capacity could be mostly accounted by flavonoid type of compounds with chain-blocking activity. Last, given antioxidant capacities attained stem from different solvent extraction at distinct polarities direct comparisons cannot be established. However, *Rosmarinus officinalis* data grants an approximation to the nature and possible compound mechanisms of antioxidant capacity action in Cecropia.

Gold standard trolox and ascorbic acid antioxidant capabilities were significantly higher compared with both plant extracts ($p < 0.05$). A better IC_{50} was observed for ascorbic acid in both DPPH and ABTS assays; most likely due to the molecule's purity and proton/electron donor mechanism and latter radical inactivation and destruction.

Antimicrobial capacity determination

To determine *Rosmarinus officinalis* and *Cecropia mutisiana* extract antimicrobial properties and Minimal Inhibitory Concentration (MIC) 10 μg, 1 μg or 0.1 μg extract/disc was used. Data is summarized in Table 7.

No significant differences were observed for *Rosmarinus officinalis* percentage of relative MIC among treatments ($p = 0.395$), thus responses at the inhibition level among extracts were not different. Likewise, no significant differences were observed for *Cecropia mutisiana* ($p = 0.601$). In addition, no significant differences were attained for comparisons between both plants (ANOVA, $p = 0.660$). Despite no statistically significant differences *Rosmarinus officinalis* ethanol extract and *Cecropia mutisiana* dichloromethane extract were capable of inhibiting a greater number of microorganisms at the lowest concentrations (10 ppm and 100 ppm) respectively. Furthermore, *Rosmarinus officinalis* ethanol extract had the highest antimicrobial activity.

At present no studies have addressed antimicrobial activity for members of the Cecropia species. *Cecropia mutisiana* extracts were capable of inhibiting Gram positive and Gram negative bacteria, within a gamut of distinctive compounds and routes of action, likely due to variations in extract polarity. Even though this study was a first attempt to characterize *Cecropia mutisiana* antioxidant and antimicrobial properties in comparison to a widely studied plant *Rosamarinus officinalis* future studies should also include other species such as *Cecropia pachystachya*, *Cecropia glaziovii,* and *Cecropia peltata* against *Leishmania* spp., and *Plasmodium falciparum* parasites (Uchoa et al., 2009; Cruz et al., 2013). In addition, comparison studies could include their antiviral properties, as case in point herpes (Silva et al., 2010), pathogenic bacteria: β hemolytic *Streptococcus*, *Escherichia coli*, and *Candida albicans* yeast (Rojas et al., 2006).

Table 7: Relative percentage inhibition of the minimum inhibitory concentrations (in bold number)*

Microorganism	µg extract/disk	*Rosmarinus officinalis* Govaerts				*Cecropia mutisiana* Mildbr			
		PE	EtOAc	CH_2Cl_2	EtOH	PE	EtOAc	CH_2Cl_2	EtOH
Pseudomonas aureuginosa	10	-	5.8 ± 3.2	-	10.1 ± 7.5	-	16.6 ± 4.8	10.5 ± 8.9	-
	1.0	-	-	-	-	-	-	5.4 ± 4.1	-
	0.1	2.2 ± 0.4	-	-	-	-	-	-	-
Staphylococcus aureus	10	1.6 ± 0.6	-	-	5.2 ± 2.3	-	-	-	17.6 ± 6.6
	1.0	-	-	-	-	-	-	-	9.8 ± 5.3
	0.1	-	-	-	-	-	-	-	-
Salmonella sp.	10	-	22.4 ± 4.8	16.0 ± 4.1	22.4 ± 6.9	2.7 ± 0.8	28.5 ± 4.5	22.4 ± 2.7	11.7 ± 5.3
	1.0	-	10.8 ± 3.0	9.6 ± 4.6	9.7 ± 2.0	-	16.0 ± 5.4	12.8 ± 3.0	8.0 ± 1.8
	0.1	-	-	-	4.3 ± 2.9	-	6.9 ± 4.7	5.3 ± 3.0	-
Bacillus cereus	10	13.0 ± 3.9	12.4 ± 4.2	4.5 ± 2.6	15.6 ± 6.2	16.1 ± 4.9	6.3 ± 1.3	5.6 ± 2.3	-
	1.0	6.8 ± 1.1	-	-	7.4 ± 2.5	7.4 ± 3.5	-	-	-
	0.1	-	-	-	1.9 ± 0.6	-	-	-	-

*Positive control, gentamycin (100±2%). PE: Petroleum ether, EtOAc: Ethyl acetate, CH_2Cl_2: Dichloromethane, EtOH: Ethanol

Rojas et al. (2006) described for *Cecropia peltata* an important antimicrobial activity against *Staphylococcus aureus* and *Bacillus cereus*, mainly in their ethanol extract, with greater than 78% inhibition for both bacteria. In this study *Cecropia mutisiana* had a 9.8% inhibition against *Staphylococcus aureus,* and was not capable of inhibiting *Bacillus cereus,* with a MIC > 1,000 ppm. These results could be due to differences in plant variability. Moreover, such contrasting results could also be attributed to the microbial strains utilized in this study (*Staphylococcus aureus* CMPUJ 080, *Bacillus cereus* CMPJU 251, *Salmonella* sp. CMPUJ 302, and *Pseudomonas aeruginosa* CMPUJ 055).

Rosmarinus officinalis and *Cecropia mutisiana* inhibition percentage comparison for each extract, as previously described, was not statistically significant. Never the less, biologically differences in percentage magnitude, as well as the number of microorganisms sensitive to the extracts were observed. The highest antimicrobial activity was for *Rosmarinus officinalis* ethanol extract followed by *Cecropia mutisiana* EtOAc extract.

At present, there are no conclusive Cecropia genus antimicrobial molecule studies. It has been described they are achieved through flavonoids and steroids (Rojas et al., 2006; Uchoa et al., 2009; Silva et al., 2010; Cruz et al., 2013). In contrast, phenolic compounds and terpenes have been specified as the main antimicrobial molecules for *Rosmarinus officinalis* (Celiktas et al., 2007; Klancnik et al., 2009; Jordán et al., 2013a; Zampini et al., 2013; Gemeda et al., 2015). This in part could account for *Rosmarinus officinalis* superior activity in comparison with *Cecropia mutisiana.* Polyphenols are more soluble in lipids and have better membrane permeability in comparison with flavonoids (Yi et al., 2010). The extract can penetrate bacteria more feasibly, thus having a direct antimicrobial effect (Varela and Ibañez, 2009).

CONCLUSIONS

Cecropia mutisiana Mildbr ethanol extract presented the best antioxidant capacity, as determined by DPPH and ABTS IC_{50} values. Additionally, dichloromethane extract for *Rosmarinus officinalis* Govaerts had the leading antioxidant activity. Furthermore, regarding antimicrobial activity *Cecropia mutisiana* Mildb EtOAc extract had the greatest antimicrobial capacity. For *Rosmarinus officinalis* Govaerts the ethanol extract was responsible for the highest microorganism growth inhibition. When comparing both plants *Cecropia mutisiana* Mildbr ethanol extract had the highest antioxidant capacity, while *Rosmarinus officinalis* Govaerts presented the highest antimicrobial activity.

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Author's contributions

All authors contributed substantially to the writing and revising of the manuscript. LGSC and AEOA designed the work, acquired, analyzed, and interpreted data. AEOA and JPCC obtained *Cecropia mutisiana* and *Rosmarinus officinalis* extracts and fractions in different solvents, and statistical analysis. AEOA, CACZ, and LGSC (corresponding author) develop and standardized antioxidant methods.

AEOA, JPCC, MXRB, JRC, and LGSC standardized microbiological methods and preliminar phytochemical analysis.

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