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# *In vitro* Antimicrobial Activity of Tropical Medicinal Plants used in Santo Domingo, Dominican Republic: Part 2

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#### ABSTRACT

Introduction: Medicinal plants of Dominican Republic are used for medicinal purposes, but still very little is known about these uses and very few evidence exist to support such uses. The objective of this study was to evaluate the antimicrobial activity of crude ethanolic extracts of 48 medicinal plants collected at regional or municipal markets in Dominican Republic for the treatment of several types of infections associated diseases. Methods: Crude ethanolic plant extracts were tested against five species of micro-organisms - E. coli, K. pneumoniae, S. aureus, P. aeruginosa and C. albicans using the disc diffusion method. For selected plants, antibacterial activity was also measured for seven (Gram positive and Gram negative) micro-organisms by the standard radial streak method in agar plates. MIC of selected plants was determined against B. subtilis by the broth microdilution method using MTT dye. Results and Discussion: Forty-three of the plants tested showed inhibitory activity against one or more of the micro-organisms at 50 µg of extract. Eleutherine bulbosa, Hymanea courbaril, Ocimum basilicum, Petiveria alliacea, Piper aduncum, Plantago major, Rosmarinus officinalis and Trichilia hirta were effective inhibiting growth of at least three out of the four bacteria strains

tested. Moderate MIC values were in the range of 140 µg/mL for *A. racemosus* to 2920 µg/mL for *Chamaesyce hyssopifolia*. A few extracts showed antifungal activity against *Candida albicans*. **Conclusion**: Several ethanolic crude extracts derived from plants used in traditional medicine in the Dominican Republic possess antimicrobial activity against a variety of the tested micro-organisms. Isolation and/or identification of potential biologically active compounds and elucidation of their mechanism(s) of action will require further study.

**Key words:** Ethnomedicine, Natural products, Antibacterial, Dominican Republic, Tropical medicinal plants.

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## INTRODUCTION

In recent years, there has been an increase in the incidence of new or re-emerging infectious diseases associated with significant increases in mortality. In many cases, this problem has been associated with an improper use of currently available antibiotics resulting in the generation of highly resistant micro-organisms. For these reasons, a continuous and urgent race to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action is currently in place. An increasing body of evidence shows that a large number of vascular plants produce hundreds to thousands of diverse chemical entities with significant biological activities.1 These metabolites have an important ecological role, such as, working as pollinator attractants or as chemical defenses against insects, herbivores and micro-organisms.<sup>2</sup> Moreover, these antimicrobial compounds produced by plants have been found to be active against plant and human pathogenic micro-organisms.<sup>3</sup> Estimates of the world-wide number of known species of higher plants are above 250,000.4 However, only 5-15% have been studied for any potential therapeutic value.5,6

In many countries, including the Dominican Republic, the traditional use of plants for medicinal purposes is a common practice. Many of the plants are used for their antimicrobial properties against organisms responsible for several pathophysiological conditions including cold, cough, bronchitis, diarrhea, skin lesions, gonorrhea, gastrointestinal and other urinary and respiratory infections. Although several authors have reported some of the most frequent medicinal uses of plants from the Caribbean, including the Dominican Republic, there is scarce scientific evidence to support the antibiotic properties of these traditional remedies.<sup>7-13</sup> In a previous report, our group presented evidence for antimicrobial activity of several plants commonly used with medicinal

purposes in the Dominican Republic.14

Although several studies have provided evidence for antibiotic activity in crude extracts from plants found in the Caribbean islands and South America,<sup>15-29</sup> only two of these studies were dedicated to the flora in the Hispaniola island (Dominican Republic and Haiti). For this reason, the main objective of this study is to search for medicinal plants, widely and commonly used by people of Dominican Republic, that show strong antimicrobial activities and therefore with the potential to serve as good candidates for the development of new antimicrobial agents and/or standardized phytomedicines.

In this report, results of our study for antimicrobial activity of 48 plants traditionally used in the treatment of several infectious or inflammatory diseases in Dominican Republic are presented. The plants were chosen based on their reported uses in an ethno-pharmacological survey of the principal markets of the Santo Domingo and San Cristobal metropolitan areas, in which 248 plants were initially identified as being used for the treatment of illnesses based on their proposed antimicrobial activity. The 48 plant species selected for this study, representing a total of 32 families, were evaluated for antimicrobial activity (by disk diffusion method) against several bacterial strains (S. aureus, E. coli, P. aeruginosa and K. pneumoniae) and a yeast strain (Candida albicans). Six of the extracts with the highest potential for antimicrobial activity, as determined by this initial screening, were selected for further analysis including antimicrobial activity potential by dilution agar plate method and evaluation of MIC against Bacilus subtilis. Our findings are consistent with the presence of antimicrobial activity in several ethanolic crude extracts of traditional medicinal plants of Dominican Republic.

#### **MATERIALS AND METHODS**

#### **Plant materials**

Plant materials used in this study were obtained from multiple medicinal plants dealers located at municipal markets in Santo Domingo, Dominican Republic. Each specimen was labeled with a number, collection date, location and intended medicinal use. Subsequently, species identification was conducted with the help of expert local taxonomists and voucher specimens were maintained in the National Botanical Garden of Dominican Republic.

#### Preparation of crude extracts

The plant materials were dried in the shade at room temperature for several days, pulverized in an electrical mill and stored until use. A 20 g-sample of the pulverized plant materials were macerated with 200 mL of 80% ethanol, extracted overnight and filtered and the remaining solid was exhaustively percolated three times using the same volume of fresh solvent. The filtrate and percolate were combined and evaporated under vacuum at 60°C until a thick residue was obtained. All extracts were immediately used or stored at room temperature until further use.

#### Antimicrobial susceptibility assay

All micro-organisms used in this study were obtained from American Type Culture Collection (ATTC). Three different methods for antimicrobial susceptibility were used in the present study: disk diffusion test, dilution agar test plate method and determination of minimal inhibitory concentration (MIC).

#### Disk diffusion method

For these experiments, four bacteria strains and one yeast strain were used: Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 13883), Staphylococcus aureus (ATCC 14990) and Pseudomonas aeruginosa (ATCC 27853) and the yeast strain Candida albicans (ATCC 60193). Micro-organisms were cultivated on nutrient agar followed by inoculation in Mueller-Hinton agar medium. Antibacterial activity of all plant extracts were tested using a modification of the method originally described by Bauer et al. 1996,<sup>30</sup> a method widely used for antibacterial susceptibility testing.26 A loop of bacteria from the agar-slant stock was cultured in nutrient broth overnight and spread with a sterile cotton swab onto petri dishes containing 20 ml of the suspension Mueller-Hinton agar. A sterile filter paper disc (6 mm in diameter) impregnated with the plant extracts (50 µg of solid extract), is then placed on the surface of the agar. Plant materials in the filter were allowed to diffuse for 5 min followed by incubation at 37°C for 24 hr. In this method, a positive antimicrobial effect of applied extracts can be detected as the formation of clearing growth zones around the discs. Following incubation, the diameter (recorded in millimeters, mm) of the inhibitory clear zones, if any, were recorded. Solvent that did not contain any plant extract served as negative control, while the standard antibiotics amikacin and norfloxacin (30 µg) were used as positive control agents. Measurements of inhibition activity were expressed in millimeters (mm) as averages of triplicate analyses, rounded to the nearest integer.

#### Dilution agar plate method

For selected plants, additional antibacterial activity was determined by an agar plate dilution method according to Mitscher *et al.* (1972).<sup>31</sup> For the screening, plates containing Muller-Hinton agar and 10 mg/mL of the extract were prepared and incubated at 37°C for 24 hr to confirm sterility of the medium. After 24 hr, standard ATCC bacterial strains (Gram positive: *Bacillus subtilis* (ATCC 6051), *Mycobacterium smegmatis* (ATCC 6071) and *Bacillus subtilis subsp Spizizenli* (ATCC 6633); Gram negative: *Shigella sp* (ATCC 23354), *Salmonella typhi* (ATCC 14028), *Pseudomona aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 8739) were inoculated as radial streaks in duplicate and incubated at 37°C for a further 24 hr. Gentamicin was used as positive control and results expressed as positive (no growth) or negative (growth).

#### Minimal inhibitory concentration (MIC) Determination

Minimal inhibitory concentration (MIC) against Bacillus subtilis was determined by the broth microdilution methods in 96 wells microplates. A 0.4 mg/mL on PBS solution of 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye was used for detecting viable bacteria.32 In this group of experiments, gentamicin (range:  $0.03-2 \ \mu g \ ml^{-1}$ ) was used as positive control while a PBS solution and broth were used as negative controls. All dilutions of crude extracts were cultured in agar media for sterility test. Sample concentration range was prepared from the stock solutions by two-fold dilutions in sterile broth. Six dilutions of the samples ranging from 32 to  $1024 \,\mu g \, ml^{-1}$  were tested. Inoculums of Bacillus subtilis prepared from fresh overnight cultures were adjusted to 0.5 McFarland standards, which equals to  $1-2 \times 10^8$  CFU ml<sup>-1</sup>. At the time of the experiment, the inoculums were further diluted, in 1:100 ratio, resulting in a 1-5× 105 CFU ml-1 final concentrations. A volume of 100  $\mu$ l of each sample was added to each well of a 96-well microplate (Sarstedt, Germany), followed by 100 µl of test strain suspensions. After 16 to 18 h of incubation at 37°C, 100 µl of MTT solution was added to each well and incubated for two more hours. Absorbance of the solutions was measured at 492 nm using an Epoch microplate spectrophotometer (Biotek Instruments, Vermont, USA). MIC values were obtained by determining the slope from the Absorbance-Extract Concentration curves.

## RESULTS

#### Antibacterial activity by plant species

Ethanolic extracts from 48 plant species belonging to 32 different families were investigated for their antimicrobial activity using the disk diffusion method. Determination of the activities (by inhibition zones) against three Gram-negative bacteria strains (*E. coli, K. pneumoniae, P. aeruginosa*), one Gram-positive bacterial strain (*S. aureus*) and one yeast strain (*C. albicans*) showed that 43 plant extracts exhibited an antimicrobial activity effect, with twelve extracts having high activities, against at least one of the five of the micro-organisms tested. Table 1 shows the botanical names, Voucher number, parts of the plants tested and results of the antibacterial screening experiments.

Antimicrobial activity of the plants studied differed in their values against the different micro-organisms tested. 69% (33 of 48) of the extracts exhibited antimicrobial activity against *S.s aureus* and 56% (27 of 48) against *K. pneumoniae*. Fewer extracts were active against *P. aeruginosa* (12 of 48) and *E. coli* (6 of 48), respectively. Most extracts exhibited a moderate to low activity against the micro-organism, with the exception of *Ocimum basilicum* aerial part extract and the *Trichilia hirta* leaf extract. Those extracts had high activity against *S. aureus* and *P. aeruginosa*, respectively. Only four extracts from *Caesalpinia coriaria, Eleutherina bulbosa, Xanthium strumarium* and *Zingiber cassumunar* showed significant inhibitory activity against the yeast *Candida albicans. Acalypha amanthacea, Agave antillarum, Ambrosia artemisifolia* and leafs of *Inga vera*, showed a lesser, more moderate activity against this yeast.

Of all the tested plants, eight extracts, namely *Gnaphalium domingensis*, *Hymanea courbaril*, *Ocimum basilicum*, *Petiveria alliacea*, *Piper aduncum*, *Plantago major*, *Rosmarinus officinalis* and *Trichilia hirta* showed a wide antimicrobial activity spectrum, inhibiting the growth of at least three out of the four tested bacteria. Two of these plant species showed the broadest spectrum of activity and were active against all four bacteria

Table 1: Antimicrobial activity of medicinal plants used in Santo Domingo, Dominican Republic. (Cont'd)							
Botanical Name (Voucher No.)	Family	Part Tested	SA	EC	PA	KN	CA
Acacia macracantha H.D. (SA01/P026)	Mimosaceae	bark	+	-	-	+	-
Achyranthes aspera L. (Du04/P063)	Amaranthaceae	Whole plant	-	-	-	-	-
Allophyllus racemosus ( SC002/047)	Sapindaceae	Leaf	++	+	+	-	-
Anredera vesicaria (leptostachys) (DU02/011)	Basellaceae	Rizhome	-	-	-	-	-
Aristolochia oblongata (SA01/P027)	Aristolochiaceae	Stem	+	-	-	-	-
Boerhavia scandens (L.) (DU03/P070)	Nyctaginaceae	Whole plant	-	-	-	-	-
Caesalpinia coriaria (Jacq.) willd. (AZ01/P030)	Caesalpiniaceae	Fruit	+	-	-	+	+
Capraria biflora (Du02/065)	Scrophulariaceae	Aerial Part	+	-	-	+	-
Cecropia schreberiana Miq. (AZ01/P029)	Cecropiaceae	Leaf	+	-	-	+	-
Chamaesyce hyssopifolia (DU02/085)	Euphorbiaceae	Whole Plant	++	-	++	-	-
Chenopodium ambrosioides (Me01/023)	Chenopodiaceae	Aerial Part	+	-	-	+	-
Cissus trifoliata (L) L. (DU02/020)	Vitaceae	Stem	-	-	-	+	-
Citharexylum Fruticosum L. (SA01/P137)	Verbenaceae	Aerial Part	-	-	-	-	-
Clusia rosea Jacq. (Sa01/p060)	Clusiaceae	Fruit	++	-	+	-	-
Costus speciosus (J. Koning) Smith (DU03/p048)	Costaceae	Aerial part	-	-	-	-	-
Cuphea parsoncia (L) R.BR. (ME04/012)	Lythraceae	Aerial Part	+	-	-	+	-
Dermorata odoratum (AZ01/P060)	Asteraceae	Aerial Part	-	-	-	+	-
Eleutherine bulbosa (mill.)Ur (SC001/025)	Iridaceae	Rizhome	++	++	-	-	++
Eupatorium aromatisans DC (HE01/021)	Asteraceae	Leafs	-	-	-	+	-
Gnaphalium domingense (ME01/048)	Asteraceae	Aerial Part	+	-	+	+	-

## Table 1: Antimicrobial activity of medicinal plants used in Santo Domingo, Dominican Republic. (Cont'd)

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Botanical Name (Voucher No.)	Family	Part Tested	SA	EC	PA	KN	CA
Hymenaea courbaril L (SA01/012)	Caesalpiniaceae	Bark Leaf	+ +	-	-+	-+	
Indigofera argentata (DO02/P103 )	Fabaceae	Whole plant	-	-	-	+	
Ipomea sp. (SC001/018)	Convolvulaceae	Stem/Leaf	+	-	-	-	-
Leonotis nepetifolia(L) R.Br (SA01/039)	Lamiaceae	Aerial Part	-	-	-	+	-
Marjorama hortensis (SA01/P118)	Lamiaceae	Aerial Part	+	-	-	-	-
Melocactus lamairei (monv) Miq. (AZ01/037)	Cactaceae	Root	-	-	-	+	-
Mentha x piperita (SA01/P120)	Lamiaceae	Aerial Part	+	-	-	-	-
Moringa oleifera (DU03/P044)	Moringaceae	Fruit	-	-	++	-	-
Ocimum basilicum L. (Me04/002)	Lamiaceae	Aerial Part	+++	-	+	+	-
Ocimum tenuiflorum L (Me04/003)	Lamiaceae	Aerial Part	+	-	-	+	-
Passiflora foetida (SA03/015)	Passifloraceae	Aerial Part	-	-	+	-	-
Pavonia spinifex (Du04/P007)	Malvaceae	Whole plant	+	-	-	+	-
Petiveria alliacea (L) (DU04/038)	Phytolaccaceae	Leaf/Root	++	-	++	+	-
Piper aduncum (AZ01/p035)	Piperaceae	Leaf	+	+	++	-	-
Pisonia aculeata (LM01/023)	Nyctaginaceae	Stem/Leaf	-	-	-	+	-
Plantago major (SC001/045)	Plantaginaceae	Whole plant	+	+	+	+	-
Rosmarinus officinalis L (HE02/062)	Lamiaceae	Aerial Part	++	+	+	++	-
Ruta chalepensis L (DU02/068).	Rutaceae	Aerial Part	+	-	-	+	-
Scoparia dulcis L (SA01/P020)	Scrophullariaceae	Aerial Part	-	-	-	+	-
Solanum torvum Sw. (AZ01/P032)	Solanaceae	Aerial Part	+	-	-	+	-

Botanical Name (Voucher No.)	Family	Part Tested	SA	EC	PA	KN	CA
Sonchus oleraceus L. (Me03/008)	Asteraceae	Whole plant	+	-	-	+	-
Stemodia maritima L (AZ01/003)	Scrophulariaceae	Whole plant	+	-	-	-	-
Tagetes erecta L (SA02/070)	Asteraceae	Aerial Part	+	-	-	+	-
Trichilia hirta (DU-2/033)	Meliaceae	Leaf	+	+	+++	-	-
Urena sinuata (Me01/017)	Malvaceae	Whole plant	+	-	-	++	-
Verbascum thapsus L. (SA01/033)	Scrophulariaceae	Aerial Part	+	-	-	-	-
Xanthium strumarium (Me01/016)	Asteraceae	Whole plant	+	-	-	-	++
Zingiber cassumunar (HBK) Baehn. (SC002/033)	Zingiberaceae	Rizhome	-	-	-	-	++
ANTIBIOTICS DISCS							
Amikacin			+++	+++	+++	+	ND
Norfloxacin			++	+++	++	+	ND

Table 1: Antimicrobial activity of medicinal plants used in Santo Domingo, Dominican Republic. (Cont'd)

 $\label{eq:Grading results: zone of inhibition 7 - 15 \ mm \ in \ diameter \ (+); zone \ of \ inhibition \ 16 - 20 \ mm \ in \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ (++); zone \ of \ inhibition \ 20 \ mm \ diameter \ diamete$ 

in diameter (+++). No activity (-). Standard antibiotics: amikacin, norfloxacin

Micro-organisms: SA: S. aureus; EC: E. coli; PA: P. aeruginosa; KP: K. pneumoniae; CA: C. albicans

(Plantago major and Rosmarinus officinalis. Hymanea courbaril). A marked difference in inhibitory activity between extracts from the bark (active only against S. aureus) and the leaf extract was observed for these plants. The last showed inhibitory activity against three micro-organisms (S. aureus, P. aeruginosa and K. pneumoniae). Six plant species showed activity against three bacteria, Gnaphalium domingensis, Hymanea courbaril, Ocimum basilicum, Petiveria alliacea, Piper aduncum and Trichilia hirta. Thirty-five plant species were active against one or two different bacteria. However, five plant species, Achyranthes aspera, Anredera vesicaria, Boerhavia scandens, Cytharexylum fruticosum, Costus especiosus and Zingiber cassumunar were found to be inactive against the tested bacteria. Nevertheless, Zingiber cassumunar inhibited the growth of the yeast Candida albicans. For comparison, we measured the inhibitory effects of the commercially available antibiotic drugs, amikacin and norfloxacin.

#### Antibacterial activity by family of plants

We observed notable differences between families of plants. The majority of the families (27 out of the 32 families of plants tested) showed some activity against one or more of the micro-organisms used (Figure 1). The *Lamiaceae* and *Asteraceae* families showed the major diversity with six members each (14% of all the plants with positive antibacterial activities, but 100% activity for plants in the each family with 6 out of 6 plants). The *Schrophulariaceae* family had four members (9% of the total and 100% inside the family with 4 out of 4 plants). We found that only four families of plants, *Amarantaceae*, *Basellaceae*, *Verbenaceae* and *Costaceae* did not have species active against any of the microbial strain used. Other families had more than one species with antibacterial activity included *Caesalpiniaceae* (2 of 2), *Malvaceae* (2

of 2) and *Piperaceae* (2 of 2). All remaining families showed only one member with activity against at least one strain of micro-organisms.

#### Dilution agar plate study

For the most promising plants (Allophyllus racemosus SW., Chamesyce hyssopifolia (L) J.K. Small, Eleutherine burbosa (Mill) Ulb, Ocimun basillicum, Plantago major and Trichilia hirta), ethanolic extracts were tested against three Gram positive (Bacilus subtilis, Mycobacterium smegmatis and Bacilus subtilis subsp Spizizenli) and four Gram negative (Shigella sp, Salmonella sp, Pseudomona aeruginosa and Escherichia coli) bactyeria, using the agar dilution plate method. Table 2 shows the results. All extracts inhibited the growth of the seven bacterial strains tested.

#### Minimal Inhibitory Concentration (MIC) Determination

For the same six (6) plants, the minimum inhibitory concentration of these extracts against *B. subtilis* was also determined using the broth microdilution methods. MIC values were from moderate to low ranging from 140 to 2920  $\mu$ g/mL compared to gentamicin (2  $\mu$ g/mL). MIC values obtained are shown in Table 3.

## DISCUSSION

This study evaluated the growth inhibitory activity of ethanolic extracts of 48 plants against selected bacterial strains using the disk diffusion method. As expected, antimicrobial activities for commercial antibiotics were higher than for the plants used in this study. Forty-three extracts demonstrated some amount of activity, inhibiting the growth of the tested micro-organisms. Only five plants did not show any antimicrobial activity against the micro-organisms studied. These plants were Achyranthes aspera, Anredera vesicaria, Boerhavia scandens,

Table 2: Results for antibacterial activity of selected medicinal plants used in Santo Domingo, Dominican Republic. Antibacterial activity was determined by the agar plate dilution method as described in Materials and Method section and results are expressed as N (no growth), P (partial growth) and G (growth). These results are the summary of two independent experiments in duplicate.

Plant Extracts (Final concentration)	B. subtilis (ATCC 6051)	M. smegmatis (ATCC 607)	<i>B. subtilis</i> sub sp <i>Spizizenli</i> (ATCC 6633)	Shigella sp.	Salmonella typhi (ATCC 14028)	P. aeruginosa (ATCC 27853)	E. coli (ATCC 8739)
Allophyllus racemosus (300 μg/mL)	Ν	Ν	Ν	Ν	Ν	Ν	Ν
Chamaesyce hyssopifolia (200 μg/mL)	Ν	Ν	Ν	Ν	Ν	Ν	Ν
Eleutherine bulbosa (200 μg/mL)	Ν	Ν	Ν	Ν	Ν	Ν	Ν
Ocimum basilicum (200 μg/mL)	Ν	Ν	Ν	Ν	Ν	Ν	Ν
Plantago major (650 μg/mL)	Ν	Ν	Ν	Ν	Ν	Ν	Ν
<i>Trichilia hirta</i> (200 μg/mL)	Ν	Ν	Ν	Ν	Ν	Ν	Ν

 Table 3: Minimal Inhibitory Concentration against B. Subtilis (ATCC 6051) of selected medicinal plants used in Santo Domingo, Dominican Republic.

Plant extract	MIC (µg/mL)
Allophyllus racemosus	140
Chamaesyce hyssopifolia	2920
Eleutherine bulbosa	409.6
Ocimun basillicum	285
Plantago major	1505
Trichilia hirta	317

**Standard:** Gentamicin 2 µg/mL

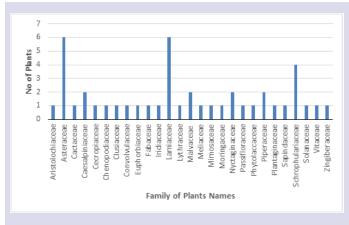


Figure 1: Distribution of antibacterial activity by plant families.

Cytharexylum fruticosum, Costus especiosus and Zingiber cassumunar. Nevertheless Zingiber cassumunar demonstrated a good activity against the yeast C. albicans. Antimicrobial activity is reported for the first time for the following plants: Allophyllus racemosus, Aristolochia oblongata, Cecropia schereberiana, Chamaesyce hyssopifolia, Cissus trifoliata, Dermorata odoratum, Gnaphalium domingensis, Melocactus lamairei, Pavonia spinifex, Pisonia culeata and Urena sinuata. For the following plants, data has been reported in the literature which are in agreement with our finding: Acacia macracantha,<sup>33</sup> Achyranthes aspera<sup>34</sup> Caesalpinia coriaria,<sup>35</sup> Capraria biflora,<sup>36</sup> Chenopodium ambrosioides,<sup>37</sup> Citharexylum fruticosum,<sup>15</sup> Clusia rosea,<sup>15</sup> Costus speciosus,<sup>38</sup> Cuphea parsoncia,<sup>39</sup> Eupatorium aromatizans,<sup>40</sup> Ipomea sp,<sup>41</sup> Leonotis nepetifolia,<sup>15,42-44</sup> Mentha piperita,<sup>45,46</sup> Moringa olifera,<sup>47</sup> Ocimum tenuiflorum,<sup>48</sup> Passiflora foetida,<sup>49,50</sup> Ruta chalepensis,<sup>51,52</sup> Scoparia dulcis,<sup>53</sup> Solanum torvum,<sup>54</sup> Sonchus oleraceus,<sup>55</sup> Stemodia maritima,<sup>56</sup> Tagetes erecta,<sup>57-59</sup> Verbascum Thapsus,<sup>60</sup> and Xanthium strumarium.<sup>61</sup>

Our results for the following plants: Clusia rosea, Eupatorium aromatisans, Mentha piperita, Moringa to previous reports. For example, we found that extract of the fruits of Clusia rosea to be active against S. aureus and P. aeruginosa, while Melendez and Capriles<sup>15</sup> did not find antibacterial activity for it. On the other hand, Cuesta-Rubio et al.62 reported minimal bactericide concentration of 1200 µg/mL against P. aeuruginosa for a polyisoprenylated benzophenone isolated from the propolis of Clusia rosea. On the other hand, Eupatorium aromatisans leaf extract showed only activity against K. pneumoniae, but Fiallo and Vasquez-Tineo<sup>40</sup> reported a significant activity of the hydroalcoholic extract against E. coli, P. aeruginosa, S. aureus and C. albicans. The extract of the aerial parts of Mentha piperita was only active against S. aureus, but Saeed and Tariq45 reported that the juice of the plant has good activity against several bacteria including E. coli, P. aeruginosa and K. pneumoniae. The extract of the fruit Moringa olifera, only showed a good inhibitory activity against P. aeruginosa. Caceres et al.47 reported that extracts from the leaf, as well as aqueous extract of the seeds inhibit the growth of P. aeruginosa and S. aureus. C. albicans was also inhibited. The aerial part extract of Passiflora foetida inhibited the growth of P. aeruginosa. Baby et al.50 reported a very good inhibiting activity of the methanolic extract of the roots against K. pneumoniae, P. aeruginosa and E. coli. The extract of the rhizome of Zingiber cassumunar showed good activity against C. albicans, but Kader et al.63 reported the minimal inhibitory concentration for different extracts of the plant. All three extracts evaluated (ethanol, ether and chloroform) showed moderate activity against E. coli and P. aeruginosa.

Antimicrobial activity of Aristolochia oblongata, Cecropia schreberiana, Chamaesyce hyssopifolia, Cissus trifoliata, Dermorata odoratum, Gnaphalium domingensis, Melocactus lamaire, Pavonia spinifex, Pisonia aculeata and Urena sinuata has not previously been reported in the literature. In our study the extract of the stem of Aristolochia oblongata, was moderately active against S. aureus. For Cecropia schreberiana extract of the leaf was active against S. aureus and K. pneumoniae. We also found that extract of the whole plant of Chamesy hyssopifolia was very active against S. aureus, P. aeruginosa, B. subtilis, M. smegmatis, B. subtilis subsp Spizizenli, Shigella sp and Salmonella sp. The MIC value for the extract against B. subtilis was measured at 2920 µg/ml. The extract of the stem of Cissus trifoliata, extract of the aerial part of Dermorata odoratum and extract of the roots of Melocactus lamaire showed moderate activity only against K. pneumoniae.

Of the other plants studied, *Gnaphalium domingensis* is a native plant that can be found in high altitudes and in the Caribbean.<sup>1</sup> The extract of the aerial parts of this plant showed inhibitory activity against *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. The whole plant extract of *Pavonia spinifex* has antimicrobial activity against *S. aureus* and *K. pneumoniae*. In the case of *Pisonia acuelata*, antifungal activity against several fungi had been evaluated, but only activity against *Pneumocystis carinii* was reported.<sup>64</sup> We found that extract of this plant to be active against *K. pneumoniae*. Finally, the whole plant extract of *Urena sinuata* showed a moderate activity against *S. aureus* and a very good activity against *K. pneumoniae*.

Of the forty-three plants with demonstrated antimicrobial activity, data corresponding to those with activity against at least three microorganisms are discussed below. These plants include *Allophyllus racemosus*, *Eleutherine bulbosa*, *Hymanea courbaril*, *Ocimum basilicum*, *Petiveria alliacea*, *Piper aduncum*, *Plantago major*, *Rosmarinus officinalis* and *Trichilia hirta*. For six of these plants, activity against additional micro-organisms and minimal inhibitory concentration was also evaluated. For instance, the antibacterial activity of the extract of the leaf of *Allophyllus racemosus* using the disc diffusion method showed it to be active against *S. aureus* and *E. coli* and *P. aeruginosa*. A study of the inhibitory effect of this extract using the diffusion agar method showed activity against *S. aureus* and *E. coli*, *P. aeruginosa*, *B. subtilis*, *M. smegmatis*, *B. subtilis* subsp *Spizizenli*, *Shigella* sp. and *Salmonella* sp. The MIC value for the extract against *B. subtilis* was 140 µg/ml.

The extract of the rhizome of *Eleutherine bulbosa* showed very good activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis*, *M. smegmatis*, *B. subtilis* subsp *Spizizenli*, *Shigella* sp and *Salmonella* sp. The MIC value obtained for the extract against *B. subtilis* was 409.6  $\mu$ g/mL. Padhi and Panda<sup>65</sup> reported moderate to high activity of the ethanolic extract of this plant against *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* and other microorganisms. The antibacterial activity of the plant may be attributed to the known presence of naphtoquinones<sup>66</sup> (elecanacine, eleutherine, eleutherine, eleutherine) which are recognized to exhibit antimicrobial, antifungal, antiviral and antiparasitic properties.<sup>67</sup>

According to the literature, for *H. courbaril* neither the bark or the seed extracts demonstrated antibacterial activity against several microorganisms evaluated, including *S. aureus.*<sup>68,69</sup> By contrast, our finding shows a moderate activity of the bark extract against this microorganism. Moreover, we found the leaf extract of the plant was active against *P. aeruginosa* and *K. pneumoniae*. In the case of *O. basilicum*, ethanolic extracts of had been reported to possess important antibacterial activity against several micro-organisms.<sup>48</sup> This finding is in agreement with our results, in which this extract was active against *S. aureus* and *K. pneumonia*, but inactive against *E. coli* and *P. aeruginosa*. Antibacterial activity for this extract was also observed against *B. subtilis*, *M. smegmatis*, *B. subtilis* subsp *spizizenli*, *Shigella* sp and *Salmonella* sp. The MIC value obtained for the extract against *B. subtilis* was 285 µg/mL. Another plant, *Petiveria alliacea*, root and leaf extract showed high activity against *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. It was previously reported that the ethanol extract of leafs showed good antibacterial activity against *S. aureus, E. coli, P. aeruginosa* and *C. albicans.*<sup>70</sup> In the case of *Piper aduncum* the leaf extract was evaluated. It presented moderate activity against *S. aureus* and *E. coli*, but a high activity against *P. aeruginosa*. No activity against *C. albicans* was observed. Okunade *et al.*<sup>71</sup> reported that the ethanolic extract of the leaves of *P. aduncum* demonstrated good antimicrobial activity against *S. aureus, B. subtilis, P. aeruginosa*, but not *C. albicans*. Kloucek *et al.*<sup>72</sup> measured the MIC values of the leaf ethanolic extract of the plant and found a very good activity against *S. aureus* (MIC value 1 mg/mL) and *B. subtilis* (MIC value of 2 mg/mL) but inactive against *E. coli* and *P. aeruginosa*.

The whole plant extract of Plantago major showed to be moderately active against all four bacteria tested but not C. albicans. It also was active against B. subtilis, M. smegmatis, B. subtilis subsp spizizenli, Shigella sp and Salmonella sp. The MIC value obtained against B. subtilis for the extract was 1505 µg/mL. Holetz et al.73 had reported a very low activity of the plant against the five micro-organisms tested in our study. For Rosmarinus officinalis the aerial part extract showed inhibitory activity against the four strains of bacteria but not against C. albicans. The higher activity was against S. aureus. Our results are in disagreement with those reports by Celitkas et al.74 They found a low activity of the methanolic extract against S. aureus, but not activity against the other micro-organisms. Our study on the activity of the leaf extract of Trichilia hirta showed a moderate activity against S. aureus and E. coli., but a very good one against P. aeruginosa. Not activity was observed against K. Pneumoniae or C. albicans. The extract also was effective against B. subtilis, M. smegmatis, B. subtilis subsp Spizizenli, Shigella sp and Salmonella sp. The MIC value obtained for the extract was 317 µg/mL. These results contradict the finding of Melendez and Capriles<sup>15</sup> that the plant had not activityagainst S. aureus and E. coli. Previously, Antoun et al.75 had reported the a 67% inhibition on the growth of Mycobacterium tuberculosis at a concentration of 100 µg/mL by the ethanolic extract of the plant.

## CONCLUSION

This study reports the antimicrobial activity of the ethanolic extracts of forty-eight (48) plants used regularly as traditional medicines of the Santo Domingo, Dominican Republic. Forty-three (43) plant extracts showed activity against one or more of the five micro-organisms tested. For the first time, here we report the antibacterial activity of Aristolochia oblongata, Cecropia schreberiana, Chamaesyce hyssopifolia, Cissus trifoliata, Dermorata odoratum, Gnaphalium domingensis, Melocactus lamaire, Pavonia spinifex, Pisonia aculeata and Urena sinuata. Eight plants, Eleutherine bulbosa, Hymanea courbaril, Ocimum basilicum, Petiveria alliacea, Piper aduncum, Plantago major, Rosmarinus officinalis and Trichilia hirta, showed a very wide spectrum of antibacterial activity, some of them with very promising MIC values. These findings support the apparent antimicrobial effects of plants used in the past by the people of this area. It also provides useful information that can lead to further pharmacological screening and phytochemical isolation studies, which should aim to characterize the specific, active compounds, to assist in the development of new antimicrobial drugs.

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#### PICTORIAL ABSTRACT





Dominican Republic

allophyllus racemosus

 Table 3: Minimal Inhibitory Concentration against B. Subtilis (ATCC 6051) of

 selected medicinal plants used in Santo Domingo, Dominican Republic.

Plant extract	MIC (µg/mL)			
Allophyllus racemosus	140			
Chamaesyce hyssopifolia	2920			
Eleutherine bulbosa	409.6			
Ocimun basillicum	285			
Plantago major	1505			
Trichilia hirta	317			

Standard: Gentamicin 2 µg/mL

#### **ABOUT AUTHORS**



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#### **SUMMARY**

- Methanolic extracts of forty-eight plants used in traditional medicine in Dominican Republic were evaluated for their antibacterial activity.
- Forty-three plants showed some type of activity against the bacteria evaluated and *Candida albicans*.
- Eight plants, Eleutherine bulbosa, Hymanea courbaril, Ocimum basilicum, Petiveria alliacea, Piper aduncum, Plantago major, Rosmarinus officinalis and Trichilia hirta, showed a very wide spectrum of antibacterial activity, some of them with very promising MIC values.



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