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Research Letter

In vitro antimicrobial activity screening of tropical medicinal plants used in Santo Domingo, Dominican Republic. Part I.

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ABSTRACT: **Introduction:** Very little is known on the uses of medicinal plants in the Caribbean island of Dominican Republic. There is a lack of scientific data supporting medicinal plant uses. The objective of the present study was to evaluate the antimicrobial activity of crude extracts of 50 medicinal plants collected from vendors at regional or municipal markets and that are used in the treatment of several types of infections in Dominican Republic. **Methods:** Ethanolic plant extracts were tested against five microorganisms species - *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomona aeruginosa*, and *Candida albicans* using the disc diffusion method. Extracts were inoculated onto discs and placed on agar plates spread with microorganisms using sterile swabs. Following 24 hours of incubation at 37°C, the plates were analyzed and the zones of inhibition measured. **Results and Discussion:** Fourty-four of the plants tested showed inhibitory activity against one or more of the microorganisms at a 35 µg/mL extract concentration. Plants exhibiting the widest antimicrobial activity were *Inga vera*, *Ruellia tuberos*a, *Calophyllum calaba*, and *Merremia dissecta*, and were effective on at least three out the four of the bacteria tested. The test antibiotics Amikacin and Norfloxacin showed strong inhibitory activities against all bacteria but only low activity against *Klebsiella pneumonia*. Very few extracts showed antifungal activity against *Candida albicans*, however higher activity was found using *Cardiospermum halicacabum* extract. **Conclusions:** Several ethanolic extracts derived from plants used in traditional medicine in Dominican Republic possess significant antimicrobial activity against a variety of the tested microorganisms, although the mechanism(s) of action require further study.

KEYWORDS: Ethnomedicine, natural products, antibacterial, antimicrobial, Dominican Republic, tropical medicinal plants

INTRODUCTION

The use of plants for medicinal purposes is a common practice in many countries. The Dominican Republic is no exception, where many residents living on the island acquire plants in markets and botanical stores. Many of the plants are used for their antimicrobial properties against several pathophysiological conditions such as cold, cough, bronchitis, diarrhea, skin lesions, gonorrhea, gastrointestinal, and urinary and respiratory infections. Although several authors have reported the most frequent uses of plants in the Caribbean, including the Dominican Republic, there is a lack of credible scientific evidence to support the antibiotic properties of these traditional remedies.[1–7]

Due to the resistance that pathogenic microorganisms generate towards antibiotic drugs, researchers are screening of extracts of medicinal plants in the search for biologically active compounds isolated from specific plant species currently used in traditional medicine.[8] Antimicrobials isolated from plants represent a very wide source of medicines, and thus an in-depth exploration of antimicrobials from plant origins is needed. Antimicrobials from plant extracts are effective in the treatment of infectious diseases without many of the side effects often associated with synthetically-derived antimicrobials.[9]

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Recently, a number of studies have been conducted in order to provide evidence for the antibiotic properties of plants from Caribbean (Puerto Rico,[10] Cuba,[13,15,16] Trinidad and Tobago,[11] and Jamaica[20,21]), from Central (Guatemala,[14] Colombia,[24] and Mexico[17,18]), and South America (Argentina[12] and Peru[22,23]). Only one of these studies were dedicated to the flora in the Hispaniola island (Dominican Republic and Haiti).[19] In this study, the susceptibility or resistance of the bacteria *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* toward four plants commonly used by the habitants of Palavé Batey, in the Dominican Republic was evaluated. Plants *Bunchosia glandulosa*, *Malphigia punicifoli* and *Chamaesyce sp*. were not effective in front of the tested bacteria. However, the *E. coli* was sensitive to *Spermacoce assurgens*.

In a recent ethno-pharmacological survey performed by our group of the principal markets of the metropolitan area (Santo Domingo and San Cristóbal), 248 plants were identified as being used in the treatment of illnesses for their apparent antimicrobial activity. These plants, as well as others mentioned in the study, were selected for evaluation of the antimicrobial activity of their ethanolic extracts against four bacterial strains (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomona aeruginosa)* and one yeast strain (*Candida albicans*).

MATERIALS AND METHODS

Plant materials

The plants used in this study were obtained from several plants dealers located at municipal markets in Santo Domingo, Dominican Republic. Each plant specimen was labeled with a number, collection date, location and their intended medicinal use. Each specimens was identified while voucher specimens were maintained in the National Botanical Garden (Dr Rafael M. Moscoso Botanical Gardens), Santo Domingo, Dominican Republic.

Preparation of crude extracts

The plant materials were dried in the shade at room temperature for several days, pulverized by an electrical mill and stored on bags until use. The dried plant materials (20 g) were each macerated with 200 mL of 80% ethanol 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.

Microorganisms tested

Escherichia coli ATCC 25922, *Klebsiella neumoniae* ATCC 13883*, Staphylococcus aureus* ATCC 14990*,* and *Pseudomonas aeruginosa* ATCC 27853 were used in this study. The yeast strain was *Candida albicans* ATCC 60193. Microorganisms were cultivated on a nutrient agar followed by inoculation in Mueller-Hinton agar medium.

Antimicrobial assay

Antibacterial activity was tested using a modification of the method originally described by Bauer *et al*.[25] This method is 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 plates containing 20 ml of the suspension Mueller Hinton agar. Sterile filter paper disc (6 mm in diameter) impregnated with the plant extract containing 50 μg of solid extract were placed in the cultured plates and incubated at 37°C for 24 h to generate the formation of clearing zones around the discs. Following incubation, the diameter (recorded in millimeters) of the inhibitory clear zones, if any, were recorded. Solvent that did not contain any plant extract served as negative control. The standard antibiotics amikacin and norfloxacin (30 μg), were used positive control agents. Measurements of activity in millimeters were expressed as means of triplicate analyses, rounded to the nearest integer.

RESULTS

A total of 57 ethanolic extracts from 50 different plant species belonging to 34 families were investigated. Determination of the activities (inhibition zones) against one Gram-positive bacterial strain (*S. aureus*), three Gram-negative bacteria strains (*E. coli, K. pneumoniae, P. aeruginosa*), and one yeast strain (*C. albicans*) indicated that 44 plant extracts exhibited an antimicrobial activity effect against at least one of the five of the microorganisms tested. Table 1 shows the botanical names, parts of the plants tested, and the results of the antibacterial screening experiments.

Although the plants differed in their activities against the microorganisms, 51% (29 of 57) of the extracts exhibited antimicrobial activity against *Staphylococcus aureus* and 52% (30 of 57) against *Klebsiella neumoniae*. Fewer extracts were active against *Pseudomona aeruginosa* (14 of 57) and *Escherichia coli* (5 of 57), respectively. Three extracts, those from *Cardiospermum halicacabum, Cestrum diurnum, Solamum americanum*, and *Yucca aloifolia*, showed significant inhibitory activity against *Candida albicans*. *Acalypha amanthacea,*

Table 1: Antimicrobial activity of medicinal plants used in Santo Domingo, Dominican Republic

Botanical Name (Voucher No.)	Family	Part Tested	SA	EC	PA	KN	CA
Acalypha amanthacea subsp.. wilkesiana (Muell. Arg.) Fosberg (DU02/P052)	Euphorbiaceae	Aerial Part	++	–	–	++	+
Agave antillarum Descourt. (HE02/031)	Agavaceae	Leafs	–	–	–	–	+
Ambrosia artemisifolia Willd. (ME01/072)	Asteraceae	Aerial Part	–	–	–	–	+
Bunchosia glandulosa (Cav.) L.C.Rich. (SA01/P037)	Malpighiaceae	Leaf	–	–	–	+	–
Calophyllum calaba L (DU03/P038)	Clusiaceae	Bark	++	–	+	+	–
		Leaf	++	–	++	+	–
Cardiospermum halicacabum, L. (ME02/P024)	Sapindaceae	Aerial Part	–	–	–	–	+++
Cassia fistula L. (AZ01/011)	Caesalpiniaceae	Fruit	+	–	–	+	–
		Leaf	+	–	–	+	–
Cassia javanica L. (AZ01/029)	Caesalpiniaceae	Fruit	+	–	–	+	–
Cestrum diurnum L. (SA01/116)	Solanaceae	Leaf	–	–	–	–	++
Chiococca alba (L.) Hitchc. (DU02/P006)	Rubiaceae	Stem	+	–	++	–	–
Cissus verticillata (L.) Nicols.& Jarvis (ME01/031)	Vitaceae	Stem	+	–	+	–	–
Cleome viscosa L. (LM01/113)	Capparaceae	Aerial Part	–	+	–	+	–
Cupania Americana L. (SA01/P088)	Sapindaceae	Whole plant	+	–	–	–	–
Ehretia tinifolia L. (PM/009)	Boraginaceae	Stem	–	+	++	–	–
Entada gigas (L.) Fawc. & Rendl (DU04/P106)	Mimosaceae	Fruit	–	–	–	+	–
Hamelia patens Jacq. (DU03/052)	Rubiaceae	Leaf	+	–	–	+	–
Inga vera Willd. (ME01/009)	Mimosaceae	Bark	++	+	+	++	–
		Leaf	+	–	–	++	+
Jatropha curcas L. (DU02/100)	Euphorbiaceae	Leaf	–	–	+++	–	–
Lippia nodiflora (L.) Michx. (SA01/P129)	Verbenaceae	Aerial Part	+	–	–	+	–
Macfadyena unguis–cati (L.) A. Gentry (DU02/034)	Bignoniaceae	Stem	+	–	–	+	–
		Leaf	+	–	–	–	–

(Continued)

Table 1: (Continued)

Botanical Name (Voucher No.)	Family	Part Tested	SA	EC	PA	KN	CA
Mentha x spicata L. (ME02/P033)	Lamiaceae	Aerial Part	++	–	–	ND	–
Merremia dissecta (Jacq.) Hall.f. (ME04//027)	Convolvulaceae	Aerial Part	+	+	+	+	–
Ocimum gratissimum L. (DU04/047)	Lamiaceae	Aerial Part	–	–	+	+	–
Parthenium hysterophorus L.	Asteraceae	Stem	++	–	+	+	–
(DU01/131)		Leaf	+	–	–	+	–
Pelargonium X hortorum L.H. Bailey (SA01/P073)	Geraniaceae	Whole plant	+	–	–	+	–
Pimenta racemosa. var. grisea (Kiaersk.) Fosb (DU02/066)	Myrtaceae	Leaf	+	–	+++	+	–
Piper marginatum Jacq. (AZ01/P049)	Piperaceae	Aerial part	+	–	–	+	–
Plectranthus amboinicus (Lour.) Spreng. (DU03/088)	Lamiaceae	Leaf	+	–	–	–	–
Ruellia tuberosa L. (SC002/027)	Acanthaceae	Root	+	+	+	+	–
Satureja hortensis L. (SA01/P065)	Lamiaceae	Aerial Part	+	–	–	+	–
Senna occidentalis (L.) Link (SC002/P011)	Caesalpiniaceae	Aerial Part	+	–	–	+	–
Solanum americanum Mill. (DU02/112)	Solanaceae	Whole plant	–	–	+++	–	++
Spermacoce assurgens R. & P. (ME01/033)	Rubiaceae	Aerial Part	–	–	–	+	–
Tabebuia berterii (DC.) Britton (AZ01/040)	Bignoniaceae	Stem	–	–	–	+	–
Tournefortia hirsutissima L.	Boraginaceae	Stem	++	–	–	+++	–
(DU02/P009)		Leaf	++	–	–	++	–
Turnera diffusa Willd. (BA01/061)	Turneraceae	Leaf	–	–	++	+	–
Urera baccifera (L.) Gaud.	Urticaceae	Stem	–	ND	–	–	–
(M03/019)		Leaf	–	ND	–	–	–
Wallenia laurifolia (Jacq.) Sw. (DU03/058)	Myrsinaceae	Leaf	+	–	–	+	–
Yucca aloifolia L. (SC002/P023)	Agavaceae	Stem	–	–	–	–	++
ANTIBIOTICS DISCS							
Amikacin			+++	+++	+++	+	ND
Norfloxacin			++	+++	++	+	ND

Grading results: zone of inhibition 7 – 15 mm in diameter (+); zone of inhibition 16 – 20 mm in diameter (++); zone of inhibition > 20 mm in diameter (+++). No activity (–). Standard antibiotics: amikacin, norfloxacin

Microorganisms: ***SA, S. aureus; EC, E. coli; PA, P. aeruginosa; KN, K. neumoniae; CA, C. albicans.***

Agave antillarum, Ambrosia artemisifolia, and leafs of *Inga vera,* showed a lesser, more moderate activity against this yeast.

Of all the tested plants, only *Calophyllum cabala, Inga vera, Merremia dissecta,* and *Ruellia tuberosa* showed broad spectrum antimicrobial activity, inhibiting the growth of at least three out of the four tested bacteria. Three of these plant species showed the broadest spectrum of activity, and were active towards four bacteria - *Inga vera, Merremia dissecta,* and *ruellia tuberosa*. *Inga vera* also showed a marked inhibitory difference between extracts that were obtained from the bark (four microorganisms) versus the leaf (three microorganisms). Three plant species showed activity against three bacteria; *Calophyllum cabala, Pathenium hysterophorus* and *Pimenta racemosa* against *S. aureus, P. Aeruginosa*, and *K. pneumoniae*. Twenty seven plant species were active against one or two different bacteria. However, twelve plant species, *Agave antillarum, Ambrosia artemisifolia, Argemone mexicana, Arundo donax, Cestrum diurnum, Commelina elegans, Foeniculum vulgare, Guaiacum officinale, Hymenocallis caribaea, Martynia annua, Mentha aquatica, Rhynchosia pyramidalis, Thespesia populnea, Tillandsia usneoides,* and *Yucca aloifolia* were found to be inactive against the tested bacteria, although *Agave antillarum, Ambrosia artemisifolia, Cestrum diurnum,* and *Yucca aloifolia* inhibited the growth of the yeast *Candida albicans*. The maximum inhibitory zones, a shown in Table 1 as +++, were observed using extracts obtained from: *Jatropha curcas* against *Pseudomonas aeruginosa* (28 mm); *Pimenta racemosa* against *Pseudomonas aeruginosa* (27 mm); *Solanum americanum* against *Pseudomonas aeruginosa* (34 mm), and *Tournefortia hirsutissima* against *Klebsiella neumoniae* (21 mm). All findings were compared with the effects of the commercially available antibiotic drugs, amikacin and norfloxacin.

In total, 23 of the 34 families of plants tested showed some activity against one or more of the microorganisms used. The most abundant was the *Lamiaceae* family with activity towards five individual strains, followed by *Caesalpiniaceae* and *Rubiaceae* with three strains each. Eleven families, *Amaryllidacea, Apiaceae, Bromeliaceae, Commelinaceae, Fabaceae, Malvaceae, Myrtaceae, Papaveraceae, Poaceae, Urticaceae* and *Zygophyllaceae* were not found to be active against any microbial strain. Families with the most numbers of individuals with activity were: *Lamiaceae* (4 of 5), *Caesalpiniacea* (3 of 3), *Rubiaceae* (3 of 3), *Agavaceae* (2 of 2), *Asteraceae* (2 of 3), *Bignoniaceae* (2 of 2), *Boraginaceae* (2 of 2), *Euphorbiaceae* (2 of 2), *Mimosaceae* (2 of 2), *Sapindaceae* (2 of 2), and *Solanaceae* (2 of 2). All remaining families showed activity towards one strain. Interestingly, individuals of the *Agavaceae* family did not show any activity against any of the bacterial microorganisms tested, but were active against *Candida albicans* only.

DISCUSSION

In this paper the antibacterial activity of plants used for medicinal purposes in the Dominican Republic was evaluated and reported. From the plants studied, antimicrobial activity has been reported previously for *Acalypha wilkesiana, Cassia fistula, Chioccoca alba, Cissus sicyoides, Cleome viscose, Hamelia patens, Jatropha curcas, Lippia nodiflora, Mentha spicata, Ocimum gratissimum*, *Parthenium hysterophorus*, *Pelargonium Hortorum, Pimenta racemosa*, *Piper marginatum, Plectranthus amboinicus, Satureja hortensis L., Solanum americanum, Spermacoce Assurgens, Tornefortia hurtisisima*. However, Melendez and Capriles (2006) have shown that *Chiococca alba* does not possess activity against *Staphylococcus aereus* and *Bacillus subtilis*.[10] Antimicrobial activity of *Bunchosia glandulosa*, *cassia javanica*, *Cupania americana*, *Ehretia tinifolia, Entada gigas*, *Macfadyena unguis-cati*, *Tabebuia berberii*, *Wallenia laurifolia,* and *Yucca aloifolia* has not previously been reported in the literature.

In general, antimicrobial activities measured for commercial antibiotics were higher than for the plants studied, except for *Klebsiela pneumonia*. Plants exerting higher activity have been already reported: *Inga vera*,[27] Merremia dissecta,[28] *Ruellia tuberosa* (antiviral).[30,31] Previous studies report that methanolic extracts from branches of *Inga vera* did not show significant inhibitory activity against *S. aureus, P. aeruginosa* and *K. pneumoniae* among other bacteria, as indicated by the *in vitro* minimal inhibitory concentration (MIC) values.[27] This is in contrast with our results that indicated that *Inga vera* bark has a very broad activity against the mentioned organisms. The difference in activity could be explained in terms of the concentration of extract and by the part of the plant used in each study. Extracts of the plant *Merremia dissecta* have been found to be antimicrobial,[28] possibly due to the presence of a variety of alkaloids and cyanogenic glycosides,[29] known to possess antimicrobial activity, contained within the plant, although this was not discussed. Methanol and ethylacetate extracts of *Ruellia tuberosa* exhibit high rates of antibacterial activity against *S. aureus* and *P. aeruginosa*,[30,31] which is in agreement with our findings. The chemical constituents of *Calophyllum cabala* have been previously determined,[32,33] but its antibacterial activity is reported here for the first time.

CONCLUSIONS

This study reports on the antimicrobial activity of fifty plants that are used regularly as traditional medicines of the Santo Domingo, Dominican Republic. Forty-four plants showed activity against one or more of the microorganisms tested. This supports 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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