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Antimicrobial activity of several Brazilian medicinal plants against phytopathogenic bacteria

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What is currently raised as a new approach in the management of plant diseases is the development and formulation of plant based biopesticides. The objective of present study is to evaluate the antibacterial activity of aqueous extracts of twelve species belonging to seven families collected from the Northeast of Brazil against four economically important phytopathogenic bacteria. Antibacterial activities of the aqueous extracts were studied by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Twelve aqueous extracts of twelve species were evaluated. Only three extracts were not active against *Ralstonia solanacearum* and other three extracts were not active against *Xanthomonas campestris* pv. *campestris*. *Anadenanthera colubrina* var. *cebil*, *Croton pedicellatus* and *Eugenia brejoensis* presented a broad spectrum of the inhibitory effect (MIC 3.12 to 12.5 mg/mL). According to these results, we conclude that the flora in the northeast of Brazil can be regarded as a rich source of plants with antibacterial activity. Therefore, further screening of other plant species, identifying active fractions or metabolites and *in vivo* application of active extracts are warranted.

Key words: Caatinga, Atlantic Forest, antibacterial activity, aqueous extract, anti-phytopathogenic activity.

INTRODUCTION

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and disease preventing properties (Kavitha and Satish, 2013). The use of plant compounds to treat infection is an ancient practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases (Gangoue-pieboji et al., 2006). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. In recent years, attention has been given to natural systems of treatment for protection and management against pathogens. Already for some decades there has been an increasing interest in plant uses and in the detection of their antibacterial activity (Harvey, 2008). Some phytopatogenic bacteria were reported to be severe phytopathogens, causing damage to carrot, potato, tomato, leafy greens, onion, green pepper, squash and other cucurbits. Furthermore, these phytopathogens cause disease in any plant tissue they invade (Ahameethunisa and Hopper, 2010).

Pesticides have been universally considered for long as the most efficient solution to control crop diseases. However, synthetic pesticides may enter the food chain and the resistance developed by plant pathogens has rendered some of them ineffective. This has highlighted the need for the use of alternatives compounds that are environmentally friendly and safe to humans.

There has been a growing interest in the research of the possible use of the plant-derived natural pesticides such as plant extracts, which can be relatively ecofriendly for disease control in agriculture (Choi et al., 2008). Besides, the plants or plant extracts have long been recognized to provide a potential source of chemical compounds or more commonly products known as phytochemicals with potent antifungal efficacy (Choi et al., 2008). Research focused on plant-derived fungicides and their possible applications in agriculture are being intensified as these are having enormous potential to inspire and influence modern agrochemical research (Duke, 1990).

Plant secondary metabolites, such as essential oils and plant extracts are known to possess insecticidal, antifungal, acaricidal, antibacterial and cytotoxic activities (Tepe et al., 2004). Therefore, they have been intensively screened and applied in pharmacology, pharmaceutical botany, medical and clinical microbiology, plant pathology and food preservation (Daferera et al., 2000). Some plant extracts (Davidson et al., 1989) and essential oils (Bakkali et al., 2008) show activity against a wide range of bacteria.

Biopesticides have been suggested as an effective substitute for chemicals (Kapoor, 2001). Reports are available on the use of several plant by-products, which possess antimicrobial properties, on several pathogenic bacteria and fungi (Bylka et al., 2004; Kilani, 2006). Here, we evaluate the potential of several plant extracts for antibacterial activity against important phytopathogenic bacteria.

Brazil is a country rich in biodiversity. To date, 44,813 species are recognized for Brazilian flora: 4,594 algae, 32,391 angiosperms, 1,529 bryophytes, 5,028 fungi, 30 gymnosperms and 1,241 ferns and lycophytes. Brazil is a country rich in biodiversity. To date, 46,096 species are recognized for Brazilian flora: 4,747 algae, 32,817 angiosperms, 1,525 bryophytes, 5,711 fungi, 30 gymnosperms and 1,266 ferns and lycophytes (Brazilian Flora, 2016). The aim of this work was to investigate the antibacterial activity of twelve species belonging to seven botanical families, collected at Parque Nacional do Catimbau (PNC) and Reserva de Floresta Urbana Mata de Camaçari (RFUMC), located in the Northeastern Brazil (State of Pernambuco, Brazil) against phytopathogenic bacteria.

MATERIALS AND METHODS

Collection of plant material

Twelve species of plant, belonging to seven families, were collected from the various phytophysiognomy at the PNC and RFUMC in Northeastern Brazil (Pernambuco, Brazil) (Table 1). The species were collected based on their popular use by the local community, giving priority to species that had reproductive organs to facilitate their identification. As a part of a wider screening program, plants were randomly collected to increase the chance of finding plants with bioactive extracts. The plants were identified at the Herbarium from Instituto Agronômico de Pernambuco (IPA) and the scientific names were checked in the International Plant Names Index (http://www.ipni.org/ipni/plantnamesearchpage.do) and Brazilian Flora Checklist (http://floradobrasil.jbrj.gov.br/jabot/listaBrasil/ConsultaPublicaUC/ConsultaPublicaUC.do). Each collected plant sample was oven dried at 45°C with forced air for 72 h. The dry materials were ground to a fine powder.

Preparation of aqueous plant extracts

Extracts were prepared from dried plants' parts according to methods described by Azmir et al. (2013). The powdered plant materials were extracted at room temperature using water by maceration successively. Aqueous extraction was achieved by adding 100 ml distilled water to 10 g of plant powder and boiled for 72 h. The extract was then lyophilized. A sample of extract at 100 mg/mL was bioassayed, as described in bioassay section.

Test microorganisms

Plant pathogenic bacteria such as *Acidovorax citrulli, Pectobacterium carotovorum* subsp. *carotovorum, Ralstonia solanacearum* and *Xanthomonas campestris* pv. *campestris* were sampled from the culture collection of Departmento de Agronomia, Universidade Federal Rural de Pernambuco, Brazil. All the tested bacterial species were maintained on nutrient agar media.

Determination of minimum inhibitory and minimum bactericidal concentrations

Micro-dilution susceptibility assay was performed using the Clinical and Laboratory Standard Institute (CLSI) method for the

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Table 1. Plants employed in this study and their ethnobotanical information.

Family/Species	Voucher	Common name	Distribution	Traditional uses of plants
Anacardiaceae				
Anacardium humile A.St.-Hil.	IPA 84049	Caju do sertão	Atlantic Forest, Caatinga and Cerrado	The fruit in nature are used as food against anemia and as tonic. The juice of pseudo fruit is indicated against anemia and diabetes (Agra et al., 2007, 2008). The external use against burnings and ulcers (Agra et al. 2007, 2008). The decoction of stem-bark is used as bath against vaginal and external ulcers. The internal use is indicated against diarrheas (Agra et al., 2007, 2008). The topical use of resin is indicated against warts coughs and wounds (Agra et al., 2007, 2008)
Apocynaceae				
Allamanda blanchetii A.DC.*	IPA 84112	Quatro-patacas-roxa, leiteiro	Caatinga	The latex is used as laxative, emetic, cathartic and vermifuge. One teaspoon of the latex in a cup of water. It is drunk after meals. It is referred to as poisonous (Agra et al., 2007, 2008)
Aspidosperma pyrifolium Mart.*	IPA 85734	Pereiro	Caatinga and Cerrado	The stem-bark is used against inflammations of urinary tract. A decoction of a teaspoon in a cup of water. It is used as tea until the symptoms disappear. The same recipe as above. It is used in baths. Entire plant is referred as poisonous (Agra et al., 2007, 2008)
Burseraceae				
Commiphora leptophloes (Mart.) J.B.Gillett	IPA 84037	Umburana	Caatinga and Cerrado	The stem-bark is used in treatment of grippes, coughs, bronchitis, treat urinary and liver diseases (Agra et al., 2007, 2008). A decoction of a handful in a liter of water and made with sugar as syrup. A spoonful is drunk 5-6 times a day. The external use against ulcers in washes or baths against vaginal ulcers (Agra et al., 2007, 2008)
Fabaceae				
Anadenanthera colubrina var. cebil (Griseb.) Altschul*	IPA 84039	Angico, angico de caroço	Caatinga and Cerrado	The stem-bark is used against coughs, whooping coughs and bronchitis. A maceration of a handful in a liter of wine or “cachaça”. It is drunk until three times a day until the symptoms disappear (Agra et al., 2007, 2008). The fruits are used as narcotic and poison. In maceration or infusion of a handful in a liter of water. It drunk before sleep (Agra et al., 2007, 2008)
Stryphnodendron pulcherrimum (Willd.) Hochr.	IPA 85968	Barbatimão	Amazonic Forest and Atlantic Forest	The decoction is used against inflammations of uterus and for wash external ulcers (Oral communication)
Crotalaria holosericea Nees & Mart.*		-	Caatinga	Used in agriculture as a green manure. In the dry period is given to cattle as food (Oral communication)
Euphrobiaceae				
Croton pedicellatus Kunth.	IPA 85734	Alecrim	Caatinga and Cerrado	A decoction of leaves is used as antiseptic against dermatitis (Oral communication)
Jatropha mutabilis (Pohl)Baill.Δ	IPA 84054	Pinhão-bravo, pinhão manso	Caatinga and Cerrado	The latex is used to treat snake bites (Agra et al., 2007, 2008)

Table 1. Contd.

Myrtaceae				
Algrizea minor Sobral et al.*	IPA 84346	Murta	Caatinga and Cerrado	Used in folk by practitioners the infusion of the fleshy leaves for the treatment of pain and fever (Oral communication)
Eugenia brejoensis Mazine *	IPA 84033	Cutia	Atlantic Forest	Used in folk by practitioners the infusion of the fleshy leaves for the treatment of pain and fever (Oral communication)
Turneraceae				
Turnera cearensis Urb.*	IPA 85143	-	Atlantic Forest and Caatinga	Against amenorrhea and dysmenorrheal. A decoction of a handful in a liter of water (Oral communication)

* = Endemic specie of Brazilian flora. Δ = Unknown endemism.

determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (CLSI, 2009). Bacteria were cultured overnight at 30°C. The test samples of the extracts were dissolved in 10% DMSO. Dilutions were prepared in a 96-well microtiter plates to get final concentrations ranging from 0 to 50 mg/ml. Finally, 20 µl of inoculum (10^6 to 10^{-7} CFU/ml) was inoculated onto the microplates and the tests were performed in a volume of 200 µl. Plates were incubated at 30°C for 24 h. The standard reference drug, chloramphenicol, was used as a positive control for the tested plant pathogenic bacteria. The lowest concentrations of tested samples, which did not show any visual growth after macroscopic evaluation, were determined as MICs, which were expressed in mg/mL. Using the results of the MIC assay, the concentrations showing complete absence of visual growth of bacteria were identified; 50 µl of each culture broth was transferred to the agar plates and incubated for the specified time and temperature as mentioned above. The complete absence of growth on the agar surface in the lowest concentration of sample was defined as the MBC. Each assay in this experiment was replicated three times.

RESULTS AND DISCUSSION

The minimum inhibitory concentrations (MICs) of 12 aqueous extracts obtained by microdilution technique against the four phytophathogenic bacteria are shown in Table 2. The antibacterial activity of the aqueous extracts showed varying magnitudes. All four bacterial strains tested were sensitive to all aqueous extracts, with the MIC values ranging from 3.12 to 25 mg/mL. The MIC values of *Anadenanthera colubrina* var. *cebil*, *Croton pedicellatus* and *Eugenia brejoensis* ranged from 3.12 to 12.5 mg/mL (Table 2). The comparison of MICs and MBCs values allows a better evaluation of antibacterial effect of bioactive compounds. According to Biyiti et al. (2004), a substance is bactericidal when the ratio MBC/MIC ≤ 2, and bacteriostatic if the ratio MBC/MIC > 2. Based on these data, the 12 extracts exert bactericidal effects against all bacteria evaluated.

Phytopathogenic bacterial infections are of great concern in agricultural practices, given that some strains are responsible for severe sickness and losses in appropriate climatic conditions. *Acidovorax*, *Pectobacterium*, *Ralstonia* and *Xanthomonas* are among the main phytopathogenic bacterial genera. Plants and plant products have shown to be useful candidates for prevention and control of phytopathogenic bacteria. Several studies have shown that the crude extracts and purified components of plants possess inhibitory activity against plant pathogenic bacteria (Iwu et al., 1999; Mohana and Raveesha, 2006; Kumaran and Karunakaran, 2007; Parekh and Chanda, 2007).

Plant extracts are rich in many phytocompounds which are the cause of their bioactivities. The mechanism of action of many antimicrobials is complex and may not be the consequence of their action on a single target. In addition, the phenomenon of membrane bleeding has been observed with several antimicrobial agents (Epand et al., 2008). For example, phenolic compounds make their actions through different mechanism, which includes membrane disruption, proteins binding, inhibition of proteins synthesis, enzyme inhibition, production of cell wall complexes, formation of disulfide bridges and intercalation with cell wall and/or DNA, among others (Bozdogan and Appelbaum, 2004). In the same manner, the antimicrobial action of alkaloids could be throughout intercalation with cell wall and/or DNA constituents; while, terpenoids display their action through membrane disruption mechanisms (Cowan, 1999).

The antimicrobial activity evaluated in this work could be attributed to the presence of different phytocompounds in variable amounts in plant extracts. The assayed antimicrobial activity from the plant species depends on the botanical species, age, part of the plant studied as well as the solvent used for the extraction procedures (Mahida and Mohan, 2006).

Table 2. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of aqueous extracts of medicinal plants of Caatinga against the growth of plant pathogenic bacteria.

Species	Phytopathogenic bacteria											
	Acidovorax citrulli			*Pectobacterium carotovorum* subsp. *carotovorum*			*Ralstonia solanacearum*			*Xanthomonas campestris* pv.*campestris*		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
Algrizea minor	25	50	2	25	50	2	12.5	25	2	-	-	-
Allamanda blanchetii	12.5	12.5	1	12.5	25	2	-	-	-	6.25	6.25	1
Anacardium humile	12.5	12.5	1	12.5	12.5	1	-	-	-	-	-	-
Anadenanthera colubrina var. *cebil*	6.25	12.5	2	12.5	12.5	1	3.12	3.12	1	6.25	6.25	1
Aspidosperma pyrifolium	12.5	12.5		25	25	1	12.5	12.5	1	25	25	1
Commiphora leptophloes	12.5	25	2	25	25	1	12.5	12.5	1	-	-	-
Crotaria holosericea	12.5	12.5	1	12.5	25	2	6.25	12.5	2	12.5	25	2
Croton corchoropsis	12.5	12.5	1	25	25	1	3.12	6.25	2	12.5	12.5	1
Eugenia brejoensis	12.5	25	2	12.5	25	2	3.12	6.25	2	6.25	6.25	1
Jatropha mutabilis	12.5	12.5	1	12.5	25	2	-	-	-	12.5	25	2
Stryphnodendron pulcherrimum	12.5	25	2	25	25	1	6.25	6.25	1	6.25	6.25	1
Turnera cearencis	12.5	12.5	1	12.5	12.5	1	6.25	12.5	2	12.5	25	2
Chloramphenicol	0.39	0.39	1	0.39	0.39	1	0.19	0.39	2	0.39	0.39	1

Results indicate the presence of antibacterial compounds in aqueous plant extracts, which was in agreement with the results reported by authors who tested the aqueous plant extracts on different plant pathogens (Bahraminejad et al., 2011, 2012). The broad spectra of the inhibitory effect of *A. colubrina* var. *cebil* indicated that the extract of this species is potent antibacterial plants with possible potential for the control of different bacterial diseases in plants. Therefore, more research on the activity of this plant against the other plant pathogenic bacteria and fungi would be of great value.

All plants used in this study have not been tested before as inhibitor of phytopathogenic bacteria. Therefore, this is a new report. The results of the present investigation are successful in identifying the antibacterial activity of selected medicinal plants which will help in further identifying the nature of the bioactive principle and its solubility, isolation and characterization of the active principle responsible for the activity.

Field existences of antibiotic resistant phytopathogenic bacteria are increasing in recent years. The World Health Organization (WHO) banned many agriculturally important pesticides due to wide range of toxicity against non-target organisms including humans which are known to cause pollution problem (Barnard et al., 1997). Some of the developing countries are still using these pesticides despite their harmful effects. Exploitation of naturally available chemicals from plants, which retard the reproduction of undesirable microorganism, would be a more realistic and ecological method for plant protection and it will have a prominent role in the development of future commercial pesticides (Verma and Dubey, 1998; Gottlieb et al., 2002). Many reports of antibacterial activity of plants extract against human pathogens and their pharmaceutical application are available (Cowan, 1999; Gibbons, 2005), but not much has been done on the antibacterial activity of plants extract against plant pathogens (Satish et al., 1999). This is mainly due to lack of information on the screening/evaluation of diverse plants for their antibacterial potential.

Considering the rich diversity of Brazilian flora, it is expected that screening and scientific evaluation of plant extracts for their antimicrobial activity may provide new antimicrobial substances; hence in the present investigation the antibacterial investigation of all pants has been demonstrated for the first time against phytopathogenic bacteria. Thus the present study reveals that *A. colubrina* var. *cebil*, *C. pedicellatus* and *E. brejoensis* is a potential candidate plant that could be successfully exploited for management of the diseases caused by different phytopathogens which are known to cause many diseases in wide variety of crops, causing considerable losses in yield and quality in an eco-friendly way.

These results and the encouraging percentage of plants with antibacterial activity (85% in this research) confirmed that plant extracts originated from Northeast in from Brazil can be used directly to develop new and effective classes of natural bactericide to control severe bacterial diseases. These findings persuaded us to continue screening more plant species.

Conflict of Interests

The authors have not declared any conflict of interests.

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