International Journal of Sciences (ISSN 2305-3925) Nov-2012

Phytochemical Screening, Antioxidant and Antibacterial Activity of *Encholirium spectabile* (Bromeliaceae)

Clara R. R. Santana[1], Raimundo G. de Oliveira-Júnior[1], Camila de S. Araújo[1], Grasielly R. Souza[1], Sarah R. G. de Lima-Saraiva[1], Amanda L. Guimarães[1], Ana P. de Oliveira[1], José A. de Siqueira Filho[2], Alessandra G. M. Pacheco[3], Jackson R. G. da Silva Almeida[1]

[1] Núcleo de Estudos e Pesquisas de Plantas Medicinais, Universidade Federal do Vale do São Francisco, 56.304-205, Petrolina, Pernambuco, Brazil

[2] Centro de Referência para Recuperação de Áreas Degradadas da Caatinga (CRAD), 56.300-000, Petrolina, Pernambuco, Brazil

[3] Laboratório de Fitoquímica, Departamento de Saúde, Universidade Estadual de Feira de Santana, 44.036-900, Feira de Santana, Bahia, Brazil

Correspondence: Jackson R. G. da Silva Almeida, e-mail: jackson.guedes@univasf.edu.br

Abstract

The phytochemical screening, antioxidant and antibacterial activities of extracts from *Encholirium spectabile*, a species belonging to the Bromeliaceae family, were investigated. This species is known in Brazil as “macambira de flecha”. In this study, the phenolic and flavonoid contents were determined by the Folin-Ciocalteu and aluminum chloride methods, respectively. Antioxidant activities were evaluated by using DPPH radical scavenging and β-carotene-linoleic acid bleaching and compared with the reference compounds ascorbic acid, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The antibacterial effect was evaluated by the method of microdilution. Preliminary analysis demonstrated that

the extracts were found to be positive for the presence of flavonoids, tannins, lignans, monoterpenes, diterpenes, steroids and triterpenoids. The most significant total phenolic content was of 206.4 ± 29.14 mg of gallic acid equivalent/g for ethyl acetate extract (AcOEt), and 106.7 ± 3.35 for chloroform extract ($CHCl_3$). The total flavonoids content was of 279.3 ± 18.88 mg of catechin equivalent/g for AcOEt extract. The AcOEt extract presented the best antioxidant activity (IC_{50} 18.51 ± 2.90 µg/ml) for DPPH scavenging. BHA was the most effective antioxidant. The chloroform and ethyl acetate extracts showed activity against most of the microorganisms tested, especially *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Serratia marcescens*, *Shigela flexneri* and *Staphylococcus aureus*. Future investigations will be focused on isolation of chemical constituents.

Keywords: *Encholirium spectabile*, Bromeliaceae, antioxidant activity, antibacterial activity, medicinal plants.

1. Introduction

Nature is a rich source of biological and chemical diversity. The unique and complex structures of natural products cannot be obtained easily by chemical synthesis. A number of plants in the world have been used in traditional medicine as remedies (Barbosa-Filho et al. 2006).

Medicinal plants represent an important health and economic component of biodiversity and also conservation and sustainable use, according to Rhaman et al. (2004). Information on the traditional knowledge or ethnic groups of medicinal plants and their uses would represent a vital role in the discovery of novel products from plants as chemotherapeutic agents (Rocha et al., 2005).

The “Caatinga” biome consists of extensive semi-arid plains found mainly in Northeast region, from Piauí to North of Minas Gerais, with the exception of the State of Maranhão

which has no "caatinga". The plants in the surrounding area form an integral part of culture of these people and the information about plants is passed on from generation to generation (Agra et al., 2007).

The Bromeliaceae family is predominantly Neotropical and comprises 58 genera and approximately 3172 species (Luther, 2008). The phytochemistry of this family is characterized by the presence of flavonoids, triterpenoids, steroids, diterpenes, cinnamic acid derivatives, lignans, nitrogen compounds among others (Manetti et al., 2009).

Encholirium spectabile is popularly known in Brazilian Caatinga as “macambira de flecha” and “macambira de pedra” (Almeida et al., 2010). Previous study realized by our research group demonstrated that the ethanolic extract of *E. spectabile* has gastroprotective activity against gastric mucosal damage induced by ethanol, HCl/ethanol, ibuprofen, ischemia and reperfusion, which suggests that the extract may activate cytoprotective mechanisms that increase the release of prostaglandins (Carvalho et al., 2010).

Our research group have demonstrated that essential oils and crude plant extracts from species of the Caatinga biome are source of chemically defined molecules with potential antinociceptive (Sá et al., 2012; Almeida et al., 2011; Oliveira et al., 2009; Almeida et al., 2006a), antioxidant (Lima-Saraiva et al., 2012; Silva et al., 2012) and antimicrobial (Sá et al., 2011; Almeida et al., 2006b) activities.

The aim of this study was to investigate the chemical composition, antioxidant and antibacterial activities of extracts from *E. spectabile*.

2. Experiment and Materials

Plant material

The leaves of *Encholirium spectabile* Mart. ex Schult. f. were collected in the city of Petrolina (Coordinates: S 09°07'30"; W 40°26'00"), State of Pernambuco, Brazil, in January of 2010. The samples were identified by André Paviotti Fontana, a botanist from Centro de Recuperação de Áreas Degradadas da Caatinga (CRAD). A voucher specimen (6443) was deposited at the Herbarium Vale do São Francisco (HVASF) of the Federal University of San Francisco Valley.

Extraction

The leaves dried and pulverized (1196 g) were macerated with ethanol 95% at room temperature for 72 h. The solution was filtered and concentrated under reduced pressure in a rotatory evaporator oven at 50 °C, producing 64 g of crude ethanol extract (Es-EtOH). The Es-EtOH was suspended in a mixture of H_2O:MeOH (7:3) and extracted successively with hexane, chloroform ($CHCl_3$) and ethyl acetate (AcOEt) in crescent order of polarity to obtain the respective extracts.

Qualitative analysis of phytochemicals

The extracts were evaluated on thin layer plates of silica gel 60 F_{254} aluminum supports, applied with a micropipette and eluted in different solvent systems as described by Wagner and Bladt (1996), seeking to highlight the main groups of secondary metabolism (Table 1).

Table 1. Elution systems and revelators used to characterize the main secondary metabolites from the extracts of flowers of *Encholirium spectabile* by thin layer chromatography.

Phytochemicals	Elution systems	Revelators
Alkaloids	Toluene: ethyl acetate: diethylamine (70:20:10, v/v)	Dragendorff reagent
Anthracene derivatives	Ethyl acetate: methanol: water (100:13.5:10, v/v)	10% ethanolic KOH reagent
Coumarins	Toluene: ethyl ether (1:1 saturated with acetic acid 10%, v/v)	10% ethanolic KOH reagent
Flavonoids and tannins	Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26, v/v)	NEU reagent
Lignans	Chloroform: methanol: water (70:30:4, v/v)	Vanillin phosphoric reagent
Mono and diterpenes	Toluene: ethyl acetate (93:7, v/v)	Vanillin sulfuric reagent
Naphthoquinones	Toluene: formic acid (99:1, v/v)	10% ethanolic KOH reagent
Triterpenes and steroids	Toluene: chloroform: ethanol (40:40:10, v/v)	Lieberman-Burchard reagent

Total phenolic content

Total phenolic contents were assayed using the Folin-Ciocalteu reagent, it is based on the method reported by Slinkard and Singleton (1977). An aliquot (40 µl) of a suitable diluted EtOH, hexane, $CHCl_3$ and AcOEt extracts was added to 3.16 ml of distilled water and 200 µl of the Folin–Ciocalteu reagent, and mix well. The mixture was shaken and allowed to stand for 6 min, before adding 600 µl of sodium carbonate solution, and shake to mix. The solutions were left at 20 °C for 2 hours and the absorbance of each solution was determined at 765 nm against the blank and plot absorbance vs. concentration. Total phenolic contents of the extracts (three replicates per treatment) were expressed as mg gallic acid equivalents per gram

(mg GAE/g) through the calibration curve with gallic acid. The calibration curve range was 50–1000 mg/l (R^2 = 0.9938). All samples were performed in triplicates.

Determination of Total Flavonoid Content

Total flavonoid content was determined by using a colorimetric method described previously (Zhishen et al., 1999). Briefly, 0.30 ml of the EtOH, hexane, $CHCl_3$ and AcOEt extracts, or (+)-catechin standard solution were mixed with 1.50 ml of distilled water in a test tube followed by addition of 90 µl of a 5% $NaNO_2$ solution. After 6 min, 180 µl of a 10% $AlCl_3.6H_2O$ solution was added and allowed to stand for another 5 min before 0.6 ml of 1 M NaOH was added. The mixture was brought to 330 µl with distilled water and mixed well. The absorbance was measured immediately against the blank at 510 nm using a spectrophotometer (QUIMIS, Brazil) in comparison with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as mg of catechin equivalents per gram of extracts (mg CE/g) through the calibration curve with catechin (R^2 = 0.9948). The calibration curve range was 50-1000 mg/l.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazil (DPPH) assay (Mensor et al., 2001). Sample stock solutions (1.0 mg/ml) of extracts were diluted to final concentrations of 243, 81, 27, 9, 3 and 1 µg/ml, in ethanol. One ml of a 50 µg/ml DPPH ethanol solution was added to 2.5 mL of sample solutions of different concentrations, and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity (AA) using the following formula: AA% = [(absorbance of the control – absorbance of the sample)/ absorbance of the control] x 100. Ethanol (1.0 ml) plus plant extracts solutions (2.5 ml) were

used as a blank. DPPH solution (1.0 ml) plus ethanol (2.5 ml) was used as a negative control. The positive controls (ascorbic acid, BHA and BHT) were those using the standard solutions. Assays were carried out in triplicate. The IC_{50} values were calculated by linear regression using by GraphPad Prism 5.0 program.

β-Carotene Bleaching Test

The β-carotene bleaching method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion (Wannes et al., 2010). The rate of β-carotene bleaching can be slowed down in the presence of antioxidants. β-carotene (2 mg) was dissolved in 10 ml chloroform and to 2 ml of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40 ^{o}C and 100 ml of distilled water was added, then the emulsion was vigorously shaken during two minutes. Reference compounds (ascorbic acid, BHA and BHT) and sample extracts were prepared in ethanol. The emulsion (3.0 ml) was added to a tube containing 0.12 ml of solutions 1 mg/ml of reference compounds and sample extracts. The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50 ^{o}C for 120 min, when the absorbance was measured again. Ascorbic acid, BHA and BHT were used as positive control. In the negative control, the extracts were substituted with an equal volume of ethanol. The antioxidant activity (%) was evaluated in terms of the bleaching of the β-carotene using the following formula: % Antioxidant activity = $[1 - (A_0 – A_t) / (A_0^0 – A_t^0)]$ x 100; where A_0 is the initial absorbance and A_t is the final absorbance measured for the test sample, A_0^0 is the initial absorbance and A_t^0 is the final absorbance measured for the negative control (blank). The results are expressed as percentage of antioxidant activity (% AA). Tests were carried out in triplicate.

Microorganisms

The reference bacterial strains used in this study were obtained from National Institute of Quality Control in Health (INCQS/FIOCRUZ - Brazil). The microorganisms used were: *Bacillus cereus* (ATCC 11778), *Enterococcus faecalis* (ATCC 19433), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Salmonela enterica* (ATCC 10708), *Serratia marcescens* (ATCC 13880), *Shigella flexneri* (ATCC 12022) and *Staphylococcus aureus* (ATCC 25923).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antibacterial effect was evaluated by the method of microdilution (Santos et al., 2012) as recommended by The National Committee for Clinical Laboratory Standards (CLSI, 2003). Initially a stock solution of 25 mg/ml of extracts was prepared using an aqueous solution of 20% DMSO (v/v). It was transferred 200 µl of this dilution to the microplate containing 200 µl of Müller-Hinton broth. Then, serial dilutions were performed resulting in concentrations of 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.195 mg/ml. The inoculum containing 5 x 10^5 CFU ml^{-1} (0.5 in McFarland scale) was added to each well. It was reserved wells in microplate for sterility control of the broth, the bacterial growth and the action of antimicrobial reference (Gentamicin). For gentamicin was used an initial concentration of 1.6 mg/ml, which was diluted to concentrations of 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125 µg/ml. The microplates were incubated under conditions of aerobically for 18-24 h at 37 ° C when 10 µl of 2,3,5-triphenyl-tetrazolium (CTT) 2% were added to each well to detect the color change of the CTT (colorless) to red, reflecting the bacterial metabolism active. The MIC was defined as the lowest concentration of the extracts that visibly inhibited the bacterial growth.

To determine the MBC, aliquots of 10 µl were withdrawn from each well containing the extracts and transferred to Petri dishes containing agar Müller-Hinton. The plates were incubated for 24 h at 37 °C. The appearance of bacterial colony for a given concentration indicates that does not was able to kill 99.9% or more bacterial inoculum used. Assays were performed in triplicate. The density of the extracts was employed to convert µl/ml in mg/ml. The latter being used to express the MIC and MBC.

Statistical Analysis

All determinations were conducted in triplicates, and the data are expressed as mean ± SD. Values were considered significantly different at $p < 0.05$. The IC_{50} values were obtained by interpolation from linear regression analysis with 95% of confidence level. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%. Values are given as mean ± SD (n=3).

3. Results and Discussion

Preliminary analysis demonstrated that all extracts were positive for the presence of flavonoids and tannins, lignans, mono and diterpenes. The hexane, chloroform and ethyl acetate extracts also showed positive reaction for the presence of triterpenes and steroids. The $CHCl_3$ and AcOEt extracts were positive for the presence of anthracene derivatives. All extracts were negative for the presence of alkaloids, coumarins and naphtoquinones. The presence of compounds in the extracts ranged to low presence (+) to strong presence (+++). Some classes of secondary metabolites were not detected in extracts. These results are presented in Table 2.

Table 2. Phytochemical characterization of extracts from the leaves of *Encholirium spectabile*.

Phytochemicals	EtOH	Hexane	$CHCl_3$	AcOEt
Alkaloids	-	-	-	-
Anthracene derivatives	-	-	+	+
Coumarins	-	-	-	-
Flavonoids and tannins	++	+	+++	+++
Lignans	+	+++	++	+++
Mono and diterpenes	+	+++	++	+
Naphthoquinones	-	-	-	-
Triterpenes and steroids	-	++	+	+

(-) not detected; (+) low presence; (++) moderate presence; (+++) strong presence.

Table 3 summarizes the results from the quantitative determination of phenolic and flavonoids as well as the effect of extracts from *Encholirium spectabile*, ascorbic acid, BHA and BHT on the DPPH free radical scavenging and β-carotene-linoleic acid bleaching test.

The total phenolics content of the plant extracts was determined by the Folin-Ciocalteu method. This method for total phenol is useful in order to know the efficiency of extraction of phenolic in solvents. The most significant total phenolic content was of 206.4 ± 29.14 mg of gallic acid equivalent/g for AcOEt extract. The level of flavonoids, expressed in catechin equivalents (CEq) in mg/g of plant extract was of 279.3 ± 18.88 for the AcOEt extract.

The scavenging activity on DPPH free radical is a common method to evaluate the antioxidative activity of plant extracts. DPPH is a stable, organic free radical extensively used to evaluate scavenging activity of antioxidants because it is sensitive enough to detect active ingredients at low concentrations. In the DPPH assay, an antioxidant scavenges the free

radicals (Chen et al., 2011). When an antioxidant is mixed with any concentration of the free radical forming sample such as DPPH, it reduces the free radical formation which is detected by decrease in the absorbance of DPPH (Elayaraja et al., 2010). DPPH has a purple color which is reduced to yellow-colored diphenylpicrylhydrazine. DPPH is one of a few stable available organic nitrogen radicals and has a UV-vis absorption maximum at 515-518 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of color. The data showed that the AcOEt extract exhibited excellent free radical scavenging activity, with a value of IC_{50} of 18.51 ± 2.90 µg/ml. BHA was the most effective antioxidant, with a value of IC_{50} of 1.38 ± 0.64 µg/ml.

The antioxidant activity of extracts was also evaluated by the β-carotene/linoleate bleaching method. This method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. β-carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. The rate of the β-carotene bleaching can be slowed down in the presence of antioxidants (Kulisic et al., 2004). In this model, the extracts showed weak to moderate antioxidant activity, and the most active extract was the hexane extract with percentage of antioxidant activity of 64.85 ± 9.18, moderate activity. BHA was as effective as BHT, and much more effective than ascorbic acid. According to these results, it was concluded that plant extracts from *Encholirium spectabile* have potent antioxidant activity, achieved by scavenging abilities observed against DPPH. The existing data give new information for the antioxidant potential and polyphenolic content of plant species that have not been traditionally used as medicinal plant.

Table 3. Total phenolics (TP), total flavonoids (TF) and antioxidant activity of extracts from

the leaves of *Encholirium spectabile*.

Extract	TP (mg GAEq/g)	TF (mg CEq/g)	DPPH (IC_{50}, µg/ml)	β-carotene (% AA)
EtOH	64.38 ± 6.57	8.95 ± 8.16	47.06 ± 5.50	28.93 ± 8.08
Hexane	13.62 ± 2.03	---	> 243	64.85 ± 9.18
$CHCl_3$	106.7 ± 3.35	---	56.85 ± 8.88	35.15 ± 2.80
AcOEt	206.4 ± 29.14	279.3 ± 18.88	18.51 ± 2.90	33.76 ± 2.97
Ascorbic acid	---	---	2.36 ± 0.47	15.48± 5.53
BHA	---	---	1.38 ± 0.64	87.69 ± 1.44
BHT	---	---	13.10 ± 1.68	74.37 ± 6.26

Several studies have been conducted with products from plant secondary metabolism, aiming to find substances with antimicrobial activity that may serve as alternative therapeutics effective against infections caused by antibiotic-resistant microorganisms (Acosta et al., 2003). Resistance to antimicrobial agents in human and animal health is a serious problem which requires not only the study of new approaches for the treatment of bacterial infections but also research for the development of new pharmaceuticals (Lathers, 2002). The results for evaluation of the antibacterial activity for extracts of *E. spectabile* are shown in Tables 4-7, and are expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The antibacterial activity was evaluated against eight reference bacteria. The chloroform and ethyl acetate extracts showed activity against most of the microorganisms tested, especially *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Serratia marcescens*, *Shigela flexneri* and *Staphylococcus aureus*. This is the first study that shows that extracts of *E. spectabile* have antibacterial activity.

Table 4. Antibacterial activity of ethanolic extract (EtOH) from the leaves of *Encholirium spectabile*.

Antibacterial activity EtOH extract																
Microorganisms	**MIC (mg/ml)**								**MBC (mg/ml)**							
	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
Bacillus cereus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Enterococcus faecalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-
Klebsiella pneumoniae	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
Salmonella enterica	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-
Serratia marcescens	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
Shigela flexneri	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-
Staphylococcus aureus	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-

MIC: minimal inhibitory concentration. MBC: minimum bactericidal concentration. (+) absence of bacterial increase (n=3); (-) no effect.

Table 5. Antibacterial activity of hexane extract (Hexane) from the leaves of *Encholirium spectabile*.

Antibacterial activity Hexane extract																
Microorganisms	**MIC (mg/ml)**								**MBC (mg/ml)**							
	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
Bacillus cereus	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
Escherichia coli	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Klebsiella pneumoniae	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-
Salmonella enterica	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-
Serratia marcescens	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-
Shigela flexneri	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-
Staphylococcus aureus	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-

MIC: minimal inhibitory concentration. MBC: minimum bactericidal concentration. (+) absence of bacterial increase (n=3); (-) no effect.

Table 6. Antibacterial activity of chloroform extract ($CHCl_3$) from the leaves of *Encholirium spectabile*.

Microorganisms	Antibacterial activity $CHCl_3$ extract															
	MIC (mg/ml)								MBC (mg/ml)							
	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
Bacillus cereus	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
Escherichia coli	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-
Klebsiella pneumoniae	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-
Salmonella enterica	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-
Serratia marcescens	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-
Shigela flexneri	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-
Staphylococcus aureus	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-

MIC: minimal inhibitory concentration. MBC: minimum bactericidal concentration. (+) absence of bacterial increase (n=3); (-) no effect.

Table 7. Antibacterial activity of ethyl acetate extract (AcOEt) from the leaves of *Encholirium spectabile*.

Microorganisms	Antibacterial activity AcOEt extract															
	MIC (mg/ml)								MBC (mg/ml)							
	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
Bacillus cereus	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
Escherichia coli	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-
Klebsiella pneumoniae	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-
Salmonella enterica	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-
Serratia marcescens	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-
Shigela flexneri	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-
Staphylococcus aureus	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-

MIC: minimal inhibitory concentration. MBC: minimum bactericidal concentration. (+) absence of bacterial increase (n=3); (-) no effect.

4. Conclusion

In summary, the present study demonstrates that *Encholirium spectabile* contain phenolic compounds which can serve as natural sources of antioxidants and antimicrobial agents. The flavonoids present in the extracts could be responsible by antibacterial activity presented in this study. Further research will be conducted to reach the substance responsible for antioxidant and antimicrobial activities of extracts.

Acknowledgements

This work was supported by grants from Brazilian agencies CNPq (Process 476770/2010-6) and FACEPE (Process APQ-0542-4.03/10). The authors wish to express their thanks to Prof. Dr. José Alves de Siqueira Filho and André Paviotti Fontana of Centro de Referência para Recuperação de Áreas Degradadas (CRAD) for collection and botanical identification of the plant material.

References

Acosta, M., González, M., Araque, M., Velazco, E., Khourl, N., Rojas, L. & Usubillaga, A. (2003). Composición química de los aceites esenciales de *Ocimum basilicum* L. var *basilicum*, *O. basilicum* L. var *purpurenscens*, *O. gratissimum* L., y *O. tenuiflorum* L., y su efecto antimicrobiano sobre bacterias multirresistentes de origen nosocomial. *Revista Facultad de Farmacia*, *45*, 19-24.

Agra, M. F., Freitas, P. F. & Barbosa-Filho, J. M. (2007). Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. *Brazilian Journal of Pharmacognosy*, *17*, 114-140.

Almeida, J. R. G. S., Lima, J. T., Oliveira, H. R., Oliveira, M. R., Meira, P. R. M., Lúcio, A. S. S. C., Barbosa-Filho, J. M. & Quintans-Júnior, L. J. (2011). Antinociceptive activity of

discretamine isolated from *Duguetia moricandiana*. *Natural Product Research*, *25*, 1908-1915.

Almeida, C. F., Ramos, M. A., Amorim, E. L. & Albuquerque, U. P. (2010). A comparison of knowledge about medicinal plants for three rural communities in the semi-arid region of Northeast of Brazil. *Journal of Ethnopharmacology*, *127*, 674-684.

Almeida, J. R. G. S., Silva, M. S., Cunha, E. V. L., Athaíde-Filho, P. F., Diniz, M. F. F. M., Silva, M. G., Takemura, O. S. & Barbosa-Filho, J. M. (2006a). Chemical constituents and analgesic activity of *Conocliniopsis prasiifolia*. *Pharmaceutical Biology*, *44*, 76-78.

Almeida, J. R. G. S., Silva-Filho, R. N., Nunes, X. P., Dias, C. S., Pereira, F. O. & Lima, E. O. (2006b). Antimicrobial activity of the essential oil of *Bowdichia virgilioides* Kunt. *Brazilian Journal of Pharmacognosy*, *16*, 638-641.

Barbosa-Filho, J. M., Medeiros, K. C. P., Diniz, M. F. F. M., Batista, L. M., Athayde-Filho, P. F., Silva, M. S., Cunha, E. V. L., Almeida, J. R. G. S. & Quintans-Júnior, L. J. (2006). Natural products inhibitors of the enzyme acetylcholinesterase. *Brazilian Journal of Pharmacognosy*, *16*, 258-285.

Carvalho, K. I. M., Fernandes, H. B., Machado, F. D. F., Oliveira, I. S., Oliveira, F. A., Nunes, P. H. M., Lima, J. T., Almeida, J. R. G. S. & Oliveira, R. C. M. (2010). Antiulcer activity of ethanolic extract of *Encholirium spectabile* Mart. ex Schult and Schult f. (Bromeliaceae) in rodents. *Biological Research*, *43*, 459-465.

Clinical Laboratory Standards Institute (CLSI). Metodologia dos testes de sensibilidade a agentes antimicrobianos por diluição para bactérias de crescimento aeróbico: norma aprovada – 6ª ed., M7-A6, 2003, 23, 17.

Chen, Y., Huang, B., He, J., Han, L., Zhan, Y. & Wang, Y. (2011). *In vitro* and *in vivo* antioxidant effects of the ethanolic extract of *Swertia chirayita*. *Journal of Ethnopharmacology*, *136*, 309-315.

Elayaraja, A., Vijayalakshmi, M. & Devalarao, G. (2010). *In vitro* free radical scavenging activity of various root and rhizome extracts of *Acorus calamus* Linn. *International Journal of Pharma and Bio Sciences*, 1, 301-304.

Kulisic, T., Radonic, A., Katalinic, V. & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry, 85*, 633-640.

Lathers, C. M. (2002). Clinical Pharmacology of Antimicrobial Use in Humans and Animals. *The Journal of Clinical Pharmacology*, *42*, 587-600.

Lima-Saraiva, S. R. G., Guimarães, A. L., Oliveira, A. P., Saraiva, H. C. C., Oliveira-Júnior, R. G., Barros, V. R. P., Menezes, V. G., Oliveira, R. A., Silva, F. S., Lima, R. S., Matos, M. H. T., Amorim, E. L. C. & Almeida, J. R. G. S. (2012). Antioxidant activity and acute toxicity of *Neoglaziovia variegata* (Bromeliaceae). *African Journal of Biotechnology*, *11*, 13998-14006.

Luther, H. E. (2008). An alphabetical list of bromeliad binomials. Sarasota: The Bromeliad Society International.

Manetti, L. M., Delaporte, R. H. & Laverde-Júnior, A. (2009). Secondary metabolites from Bromeliaceae family. *Química Nova*, *32*, 1885-1897.

Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C., Coube, C. S. & Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, *15*, 127-130.

Oliveira, R. R. B., Góis, R. M. O., Siqueira, R. S., Almeida, J. R. G. S., Lima, J.T., Nunes, X. P., Oliveira, V. R., Siqueira, J. S. & Quintans-Júnior, L. J. (2009). Antinociceptive effect of the ethanolic extract of *Amburana cearensis* (Allemão) A.C. Sm., Fabaceae, in mice. *Brazilian Journal of Pharmacognosy*, *19*, 672-676.

Rahman, M. A., Mossa, J. S., Al-Said, M. S. & Al-Yahya, M. A. (2004). Medicinal plant diversity in the flora of Saudi Arabia 1: a report on seven plant families. *Fitoterapia 75*,

149-161.

Rocha, L. G., Almeida, J. R. G. S., Macedo, R. O. & Barbosa-Filho, J. M. (2005). A review of natural products with antileishmanial activity. *Phytomedicine 12*, 514-535.

Sá, P. G. S., Nunes, X. P., Lima, J. T., Siqueira-Filho, J. A., Fontana, A. P., Siqueira, J. S., Quintans-Júnior, L. J., Damasceno, P. K. F., Branco, C. R. C., Branco, A. & Almeida, J. R. G. S. (2012). Antinociceptive effect of ethanolic extract of *Selaginella convoluta* in mice. *BMC Complementary and Alternative Medicine*, *12*, 1-7.

Sá, M. C. A., Peixoto, R. M., Krewer, C. C., Almeida, J. R. G. S., Vargas, A. C. & Costa, M. M. (2011). Antimicrobial activity of caatinga biome ethanolic plant extracts against gram negative and positive bacteria. *Revista Brasileira de Ciência Veterinária*, *18*, 62-66.

Santos, T. G., Rebelo, R. A., Dalmarco, E. M., Guedes, A., Gasper, A. L., Cruz, A. B., Schimit, A. P., Cruz, R. C. B., Steindel, M. & Nunes, R. K. (2012). Chemical composition and antimicrobial activity of leaf essential oil from *Piper malacophyllum* (C. Presl.) C. DC. *Química Nova*, *35*, 477-481.

Silva, M. E. G. C., Guimarães, A. L., Oliveira, A. P., Araújo, C. S., Siqueira-Filho, J. A., Fontana, A. P., Damasceno, P. K. F., Branco, C. R. C., Branco, A. & Almeida, J. R. G. S. (2012). HPLC-DAD analysis and antioxidant activity of *Hymenaea martiana* Hayne (Fabaceae). *Journal of Chemical and Pharmaceutical Research*, *4*, 1160-1166.

Slinkard, K. & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, *28*, 49-55.

Wagner, H. & Bladt, S. (1996). Plant drug analysis: a thin layer chromatography atlas. Berlim Heidelberg: Springer Verlag., p. 384.

Wannes, W. A., Mhamdi, B., Sriti, J., Jemia, M. B., Ouchikh, O., Hamdaoui, G., Kchouk, M. E. & Marzouk, B. (2010). Antioxidant activities of the essential oil and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. *Food and Chemical*

Toxicology, *48*, 1362-1370.

Zhishen, J., Mengcheng, T. & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, *64*, 555-559.