academicJournals

Vol. 7(32), pp. 2358-2364, 25 August, 2013
DOI: 10.5897/JMPR2013.2597
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Evaluation of combinatory effects of *Anadenanthera colubrina*, *Libidibia ferrea* and *Pityrocarpa moniliformis* fruits extracts and erythromycin against *Staphylococcus aureus*

Luís Cláudio Nascimento da Silva[1,2], Rita de Cássia Mendonça de Miranda[3], Edelvio de Barros Gomes[4], Alexandre José Macedo[5], Janete Magali de Araújo[6], Regina Célia Bressan Queiroz de Figueiredo[7], Márcia Vanusa da Silva[2] and Maria Tereza dos Santos Correia[1]*

[1]Laboratório de Glicoproteínas, Departamento de Bioquímica, Universidade Federal de Pernambuco, Pernambuco, Brazil.
[2]Laboratório de Produtos Naturais, Departamento de Bioquímica, Universidade Federal de Pernambuco, Pernambuco, Brazil.
[3]Departamento de Engenharia de Alimentos, Centro de Ciências Exatas e Tecnologia, Universidade Federal de Sergipe. Sergipe, Brazil.
[4]Instituto Federal de Educação, Ciência e Tecnologia da Bahia, Bahia, Brazil.
[5] Universidade Federal do Rio Grande do Sul, Faculdade de Farmácia and Centro de Biotecnologia, Rio Grande do Sul, Brazil.
[6]Laboratório de Genética de Microrganismos, Departamento de Antibióticos, Universidade Federal de Pernambuco, Pernambuco, Brazil.
[7]Laboratório de Biologia Celular, Departamento de Bioquímica, Centro de Persquisa Aggeu Magalhães (FIOCRUZ/PE), Pernambuco, Brazil.

Accepted 23 August, 2013

Anadenanthera colubrina*, *Libidibia ferrea* and *Pityrocarpa moniliformis* are widely used in Caatinga and our group has demonstrated their biotechnology potential. This work evaluated the combined action of erythromycin with extracts obtained from fruits of *A. colubrina* (ACHE), *L. ferrea* (LFHE) and *P. moniliformis* (PMHE) against *Staphylococcus aureus.* The combinatory effects of hydroalcoholic fruit extracts were determined by the checkerboard method. The effects on bacterial DNA, membrane, protein and the major phenolic compounds were analyzed. PMHE demonstrated synergistic effects in six ratios (ΣFIC: 0.20 to 0.46), ACHE in five (ΣFIC: 0.18 to 0.33) and LFHE in four (ΣFIC: 0.20 to 0.47). An antagonistic effect was not found. The release of intracellular material was enhanced by about 30% in PMHE-treated cells, suggesting a contribution of membrane damage in its synergistic activity. DNA structural damage was observed when the bacteria were treated with LFHE and PMHE. The changes in protein release with all treatments suggest loss of cell viability and disturbances to metabolic pathways. Ultra fast liquid chromatographic (UFLC) analysis detected rutin (ACHE: 0.894 mg/g; PMHE: 0.177 mg/g), quercetin (ACHE: 0.353 mg/g; PMHE: 0.397 mg/g), gallic acid (LFHE: 0.855 mg/g) and a gallic acid derivative compound (LFHE: 0.866 mg/g). The extracts have great synergistic potential when combined with erythromycin, representing an alternative and sustainable use of natural resources from the Caatinga biome.

Key words: Antimicrobials, Caatinga biome, checkerboard method, ultra fast liquid chromatographic (UFLC) analysis.

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacteria responsible to cause a variety of human disease, usually marked by superficial skin and soft infections, but occasionally it can spread through the blood stream and lead to sepsis, bacteremia, endocarditis or pneumonia (Kim et al., 2012; Van Halvet al., 2012). The bacteremia caused by *S. aureus* has a large incidence rate and it provoke the death of 10 and 30% of these patients (Van Hal et al., 2012). The exceptional capacity of antibiotic resistance acquisition is another characteristic of *S. aureus*, which is a serious public concern considered the most important human pathogen in the 21st century (Tang and Stratton, 2010). Due to the global need to control resistant *S. aureus* strains, research on new sources of anti-*S. aureus* compounds has become crucial.

Some plants from the Caatinga biome (an exclusive Northeastern Brazilian ecosystem marked by a long dry season) have been widely used by local populations due to their supposed medicinal properties. In this region, people commonly treat diseases by means of drinking teas or infusions from plant extracts (Monteiro et al., 2006). A well-known example is *Anadenanthera colubrina* (Vell.) Brenan var. *cebil* (Griseb) Altschul (A) (Leguminosae - Mimosoideae) (*angico*), employed to treat cancer, influenza, anemia, diphtheria, gastritis and other inflammatory disorders (Monteiro et al., 2006), extracts of *Libidibia ferrea* Martius *ex* Tul. var*. ferrea* (Leguminosae - Caesalpinioideae) (*pau-ferro*) have been used to treat bronchitis, anemia, chronic headache, renal disorders, stress and fatigue (Nakamura et al., 2002). The genus *Pityrocarpa* has been reported as an antinociceptive and anti-inflammatory agent (Queiroz et al., 2010), and our group was the first to show the potential of *Pityrocarpa moniliformis* Benth (Leguminosae - Mimosoideae) (*angico-de-bezerro*) as an antioxidant and anti-biofilm agent (Silva et al., 2011; Trentin et al., 2011; Silva et al. 2012).

The value of plant as enhancer of antimicrobial action of some antibiotics has been known and it is denominated as synergism. The antimicrobial synergism occurs when two or more agents exert an inhibitory effect in combination that is greater than the additive effect of the individual antibiotic. Some studies have demonstrated that antimicrobial efficacy of diverse drugs can be improved by crude plant extracts (Jandú et al., 2013; Novy et al., 2013; Silva et al., 2013). The diminution of bacterial resistance and fewer side effects are related with the use of plant/drug products (Vuuren and Viljoen, 2011). The development of new methods for assessing the synergism potential of plant material permits the growth in this field and the perspective of the therapeutic utility of the formulations containing plant material and drug is very promising (Abreu et al., 2012).

Caantinga plants are valuable tool in the search of new bioactive compounds. In this work, the interaction between erythromycin and fruit extracts of *A. colubrina, L. ferrea* and *P. moniliformis* against *S. aureus*, the effects of these extracts on membrane, DNA and protein leakage and the identification of the predominant phenolic compounds in the extracts were presented.

MATERIALS AND METHODS

Plant and extraction preparation

The samples were collected during the rainy season in Catimbau National Park (Pernambuco, Brazil), a preservation area of the Caatinga biome. Botanical identification was made from Herbarium of Instituto de Pesquisa Agronômica de Pernambuco (IPA–PE), Brazil and voucher specimens were submitted in the herbarium (IPA 84.039 for *A. colubrina*; IPA 84,035 for *L. ferrea*; and IPA 84.048 for *P. moniliformis*).

Each fruit was dried at room temperature for 7 days, ground into a fine powder and used for extraction. The powder (5 g) was mixed with 50 ml ethanol:water (7:3) by agitation of 300 rpm for 15 h, then the extract was passed through filter paper Whatman No. 1, the powder residue was mixed again with 50 ml ethanol-water and the entire extraction process was repeated. The supernatants collected were mixed in a round bottom flask and concentrated at 45°C under vacuum in a rotary evaporator. The extracts were named as *A. colubrina* hydroalcoholic extract (ACHE), *L. ferrea* hydroalcoholic extract (LFHE) and *P. moniliformis* hydroalcoholic extract (PMHE).

Staphylococcus aureus strains

The standard UFPEDA strain of *S. aureus* (UFPEDA 02) used in this study, was purchased from the Culture Collection of the Department of Antibiotics, Federal University of Pernambuco, Brazil. This strain is maintained in nutrient agar (NA) and stored at 4°C.

Evaluation of synergetic effects of the extracts and erythromycin

Minimum inhibitory concentrations (MIC) of each extracts and the erythromycin were determined by microdilution method (CLSI, 2011). A twofold serial dilution of the sample was prepared in Mueller Hinton Broth (MHB) and 100 µl (approximately 1.5×10^8 CFU/ml) of bacteria suspension was added. The samples were incubated for 24 h at 37°C. Resazurin solution (0.01%) was used as an indicator by color change visualization: any color changes from purple to pink were recorded as bacterial growth. The lowest concentration at which no color change occurred was taken as the MIC.

After that, varying dilutions from stock solutions of extracts (50 mg/ml) and erythromycin (1 mg/ml) were prepared (final volume 20 µl) and antibacterial activity was tested by the checkerboard method (Chusri and Voravuthikunchai, 2009) in the same way as described for MIC determination. Interaction was assessed

*Corresponding author. Email: mtscorreia@gmail.com. Tel: +55 (81) 2126-8540. Fax: +55 (81) 2126-8576.

algebraically by determining the fractional inhibitory concentration indices (ΣFIC) according to the following equation:

$$\text{FIC index } (\Sigma FIC) = \frac{\text{MIC E/D}}{\text{MIC E}} + \frac{\text{MIC D/E}}{\text{MIC D}}$$

where MIC E and MIC D are the minimal inhibitory concentration of extract and erythromycin, respectively; MIC E/D is the minimal inhibitory concentration of extract in combination with erythromycin; MIC D/E is the minimal inhibitory concentration of erythromycin in combination with extract.

Data interpretation

After calculations, effects were characterized based on criteria define by Vuuren and Viljoen (2011).

If ΣFIC≤0.5, effect is synergistic (syn); if 0.5<ΣFIC≤1 effect is additive (add); if 1<ΣFIC<4 effect is noninteractive (non); if ΣFIC≥4 effect is antagonistic (ant).

Cell leakage assays

Bacterial strains were cultured in MHB media at 37°C for 18 h, cells were separated by centrifugation (10,000 g for 10 min at 4°C) and washed twice with PBS buffer (pH 7.4). The extracts (corresponding to 2 times the MIC) were added to the bacterial suspensions (10^9 CFU/ml). Suspensions were incubated at 37°C and afterwards, aliquots of 1.5 ml were withdrawn at different time-periods and centrifuged again. Supernatant (200 µl) was analyzed in a spectrophotometer at 260 nm (Chusri and Voravuthikunchai, 2009) and the protein content was determined (Lowry et al., 1951). Results were expressed as a proportion or percentage of the values at the initial time-period.

DNA damage assay

This assay was carried out to evaluate the involvement of DNA damage in cell death induced by the extracts. After cultivation of *S. aureus* (24 h at 37°C) in MHB medium, cultures of each treatment were centrifuged and DNA extracted using the AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen™) following the manufacturer's instructions. The DNA integrity was analyzed on 1.0% agarose gel electrophoresis (w/v in 1X TBE Buffer).

Determination of predominant phenolic compounds by ultra fast liquid chromatographic (UFLC) analysis

Investigations of phenolic content were performed by using a prominence ultra fast liquid chromatographic system (UFLC Shimadzu inc. Japan) which comprised a binary pump (model LC-20AD), diode array detector (model SPD 20A), auto-sampler (model SIL-20A HT), oven (model CTO-20A), controller (model CBM-20A) and degasser (model DGU-29A3). The LC Solution Software version 1.2 was used to control the auto-sampler, detector, data acquisition and run settings. An octadecil silane (C_{18}) reverse-phase column (Shimadzu inc., Japan, XR, ODS 50 × 3.0, 2.2 µm particle size) was employed for all chromatographic analysis.

Aliquots of powdered extracts (0.5 g) were diluted in methanol/water solution (20%, v/v) and sonicated (ultra-sonic bath) for 30 min. Afterwards, extracts were filtered and liquid fractions were directly loaded into solid phase extraction (SPE) cartridges (Strata C18-E cartridge Phenomenex™ Torrance – California, USA) in order to separate and concentrate target groups as phenolic acids and flavonoids. Cartridges were eluted with 2 ml of 1% trichloracetic acid followed by 2 ml of acetone, and 10 ml of methanol. Eluted fractions were concentrated under vacuum at 40°C until dry, and re-suspended in methanol.

Chromatographic runs were performed according to these settings: isocratic elution mode; flow rate of 0.6 ml/min; oven temperature of 40°C; dihydrogen-potassium phosphate (KH_2PO_4; 5 mmol) as solution A, and acetonitrile/water (12% v/v) as solution B. Prior to injection, samples (200 µl) were filtered with PTFE syringe 0.22 µm filters (Phenomenex, UK).

Phenolic compounds were identified by comparison of their retention times and their ultraviolet (UV) spectra obtained with the diode array detector (DAD, SPD-M20A), ranging from 210 to 310 nm. Gallic, vanillic, protocatechuic, chlorogenic, coumaric and ferulic acids, quecertin, and rutin were used as standard compounds (all purchased from Sigma-Aldrich), and the calibration graphs were constructed by plotting each chromatographic peak area from each standard, against their corresponding concentration. Concentrations ranged from 0.01 to 2 mg/ml. The resulting linear regression equation for each standard curve was used to quantify the compounds.

Statistical analysis

Each experiment was performed in triplicate and results are expressed as the mean ± standard deviation (SD). Statistical analysis was performed by the Student's *t*-test. Differences were considered significant at $p<0.05$.

RESULTS

In vitro effects of combinations between extracts and erythromycin were assayed by the checkerboard assay as depicted in Table 1. The combination of PMHE and erythromycin resulted in synergistic effects in six different erythromycin/extract ratios tested, while ACHE and LFHE had five and four ratios with synergistic effects, respectively. The antagonistic effect was not found. The best ΣFIC values were 0.18 for ACHE (ratio 7:3) and 0.20 for both PMHE (ratio 8:2) and LFHE (6:4).

The staphylococcal DNA integrity in the presence of the extracts was checked by electrophoresis analysis. DNA structural damage was observed when the bacteria were treated with LFHE and PMHE (Figure 1a). Additionally, in order to investigate possible membrane damage, the presence of released cell material (that absorbs at 260 nm wavelength) was checked (OD_{260} values). The OD_{260} values of supernatant only significantly increased when the cells were treated with PMHE after 60 min (Figure 1b). Alterations in protein leakage could be observed after treatment with all tested extracts. Significant differences between treated and control cells were found at 30 and 60 min of treatment ($p<0.05$) (Figure 1c).

The results of UFLC analysis showed that rutin (ACHE: 0.894±0.0 mg/g; PMHE: 0.177±0.0 mg/g) and quercetin (ACHE: 0.353±0.01 mg/g; PMHE: 0.397±0.04 mg/g) are the main phenolic compounds in ACHE and PMHE. In

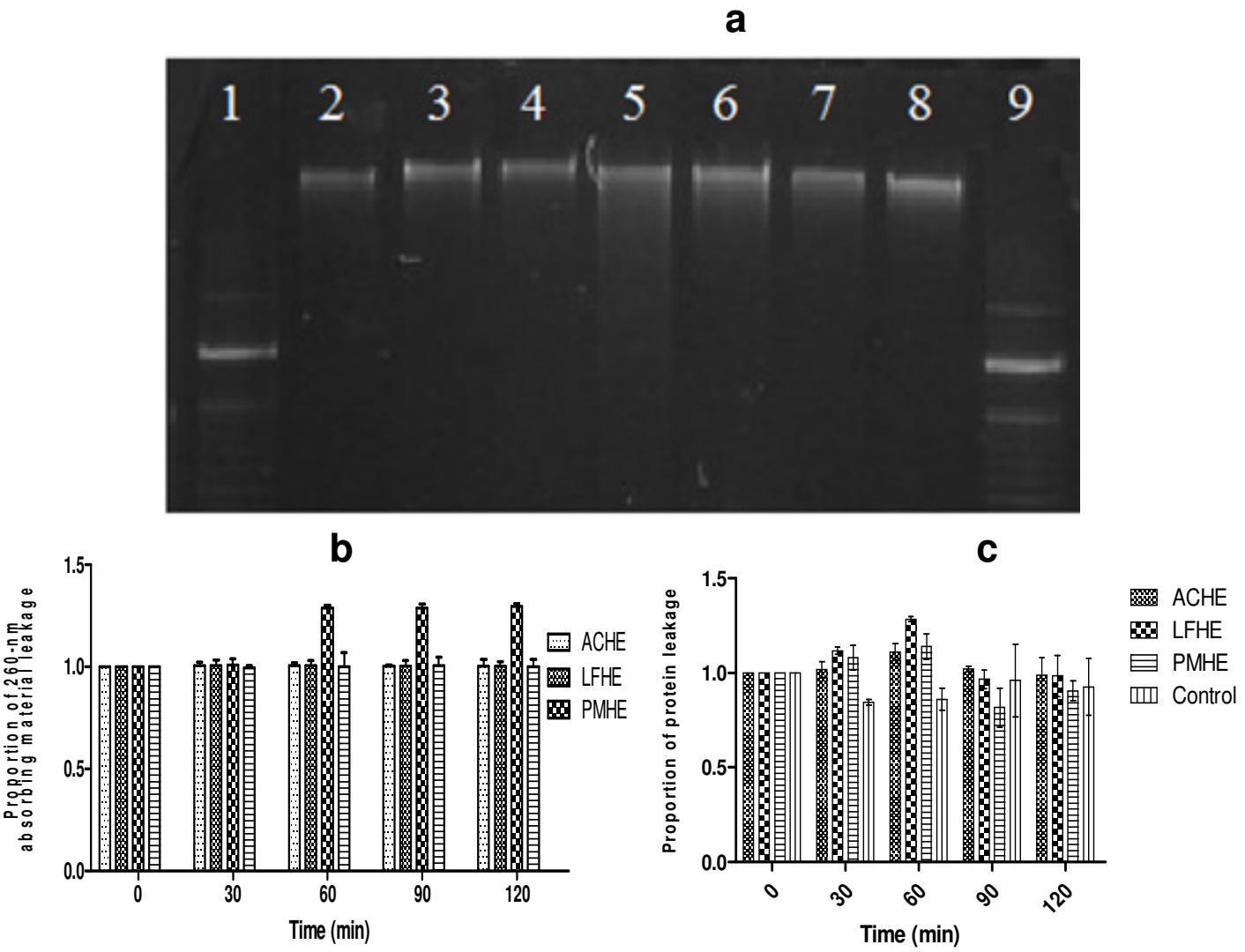

Figure 1. Analysis of DNA damage (a) and leakage of 260-nm-absorbing material (b) and proteins (c). (a) Lanes 1 and 9: DNA Ladder 100 bp; Lane 2: Positive control: bacteria growth in MHB with DMSO (10%) without extracts; Lane 3: ACHE at MIC; Lane 4: ACHE at 2X MIC; Lane 5: LFHE at MIC; Lane 6 LFHE at 2X MIC; Lane 7: PMHE at MIC; Lane 8: PMHE at 2X MIC. The MIC values are shown in Table 1

LFHE, gallic acid and one methylated gallate derivative compound, based on its spectral profile were identified (Table 2).

DISCUSSION

In this work, for the first time to our knowledge, the anti-*S. aureus* activity of the three plant extracts from the *Caatinga* biome in combination with erythromycin was been shown and their action on membrane, DNA and protein release was highlighted. Several research groups have already reported the antimicrobial activity of Brazilian plant extracts and their metabolites (Betoni et al., 2006; Almeida et al., 2012; Jand et al., 2013; Silva et al., 2013).

The extracts in the present study showed high synergistic effects with erythromycin in most of the combinations as shown by the checkerboard method. It was observed that the combined effects varied according to the ratio drug/extract tested. As an extract is composed of different molecules that react with each other, their interactions with a drug depend directly on the proportion used. The high synergism rate of protein synthesis inhibitors and plant-derived products has been demonstrated (Betoni et al., 2006; Adikwu et al., 2010). Erythromycin is a protein synthesis inhibitor, usually bacteriostatic, but can be bactericidal at high concentrations against very susceptible organisms, mainly aerobic Gram-positive cocci and bacilli bacteria (Adikwu et al., 2010). Synergistic interactions between natural products and antibiotics have been known due to

Table 1. Analysis of combinatory effects of extracts and erythromycin against *S. aureus*.

Plant	Ratio of combination Eryth:Extract	MIC* Plant	MIC* Eryth	FIC Plant	FIC Eryth	ΣFIC	Inference
Anadenanthera colubrina (ACHE)	10:0	-	3.91	-	-	-	-
	9:1	4.88	0.88	0.225	0.003	0.228	SYN
	8:2	9.77	0.78	0.199	0.006	0.206	SYN
	7:3	14.65	0.68	0.174	0.009	0.183	SYN
	6:4	39.06	1.17	0.299	0.025	0.324	SYN
	5:5	48.83	0.98	0.251	0.031	0.282	SYN
	4:6	234.38	3.13	0.801	0.150	0.951	ADD
	3:7	273.44	2.34	0.598	0.175	0.774	ADD
	2:8	625.00	3.13	0.801	0.401	1.201	NON
	1:9	2812.50	6.25	1.598	1.803	3.401	NON
	0:10	1560	-	-	-	-	-
Libidibia ferrea (LFHE)	10:0	-	3.91	-	-	-	-
	9:1	9.77	1.76	0.450	0.025	0.475	SYN
	8:2	9.77	0.78	0.199	0.025	0.224	SYN
	7:3	14.65	0.68	0.174	0.038	0.211	SYN
	6:4	19.53	0.59	0.151	0.050	0.201	SYN
	5:5	97.66	1.95	0.499	0.250	0.749	ADD
	4:6	234.38	3.13	0.801	0.600	1.401	NON
	3:7	136.72	1.17	0.299	0.350	0.649	ADD
	2:8	156.25	0.78	0.199	0.400	0.599	ADD
	1:9	351.56	0.78	0.199	0.900	1.099	NON
	0:10	390.63	-	-	-	-	-
Pityrocarpa moniliformis (PMHE)	10:0	-	3.91	-	-	-	-
	9:1	703.13	3.13	0.801	0.451	1.251	NON
	8:2	78.13	0.59	0.151	0.050	0.201	SYN
	7:3	273.44	3.13	0.801	0.175	0.976	ADD
	6:4	58.60	0.98	0.251	0.038	0.288	SYN
	5:5	48.83	1.17	0.299	0.031	0.331	SYN
	4:6	39.10	1.37	0.350	0.025	0.375	SYN
	3:7	29.30	1.56	0.399	0.019	0.418	SYN
	2:8	19.53	1.76	0.450	0.013	0.463	SYN
	1:9	9.77	1.95	0.499	0.006	0.505	ADD
	0:10	1560	-	-	-	-	-

*MIC is expressed in µg/ml. SYN: Synergistic effect (ΣFIC $\leq$ 0.5), ADD: Additive effect (0.5 < ΣFIC $\leq$ 1), NON: Noninteractive (1 < ΣFIC < 4).

their potential of enhancing therapy efficacy and are a parameter that should be taken in account in the investigation of plant extract action mechanisms (Vuuren and Vijoen, 2011; Abreu et al., 2012).

The increase of cell material (that absorbs 260 nm wavelength) on the supernatant of PMHE-treated cells indicates the leakage of nucleic acids and related compounds such as pyrimidines and purines (which have max UV light absorbance at this wavelength) as a result of bacterial cell wall and/or membrane damage (Chusri and Voravuthikunchai, 2009). DNA structural damage was observed when the bacteria were treated with LFHE and PMHE. The induction of DNA breaks is related to antimicrobial action of some anbiotics and it is attributed to the generation of reactive oxygen species (such as hydroxyl radical), inhibition of topoisomerases or direct compound–DNA interaction (Kohanski et al., 2010).

The highest number of ratios with synergetic effects in PMHE can be attributed to its capacity to cause membrane damage. In fact, in this extract the presence of saponins was detected (unpublished data), which contain a steroid or triterpenoid aglycone attached to one or more sugar chains. It is well known that saponins exhibit cell membrane permeabilizing activity and the

Table 2. UFLC investigation of plant extracts.

Compound	*Anadenanthera colubrina*			*Libidibia ferrea*			*P. moniliformis*		
	RT (min)	$\lambda_{máx}$	Concentration (mg/g)	RT (min)	$\lambda_{máx}$	Concentration (mg/g)	RT (min)	$\lambda_{máx}$	Concentration (mg/g)
Chlorogenic acid	-	-	-	-	-	-	-	-	-
Coumaric acid	-	-	-	-	-	-	-	-	-
Ferulic acid	-	-	-	-	-	-	-	-	-
Gallic acid	-	-	-	0.655	271	0.855±0.01	-	-	-
Gallic acid derivative	-	-	-	1.756	271	0.866±0.01	-	-	-
Protocatechuic acid	-	-	-	-	-	-	-	-	-
Quercetin	1.782	285	0.353±0.01	-	-	-	1.779	285	0.397±0.04
Rutin	1.420	275	0.894±0.00	-	-	-	1.426	275	0.177±0.0
Vanillic acid	-	-	-	-	-	-	-	-	-

antimicrobial activity of saponin-rich extracts has been reported (Hassan et al., 2010). These data also indicate that synergetic combinations between ACHE/LFHE are results of other cell targets.

Phenolic compounds have diverse health effect (as antibacterial, antifungal, antioxidant agents) and the liquid chromatographic fingerprint is very useful to detect these compounds in plant material (Kosar et al., 2011). A high level of polyphenols has been previously quantified in these plant extracts (Da Silva et al., 2011). The UFLC analysis showed that ACHE and PMHE are mostly composed of rutin and quercetin, while in LFHE, gallic acid and one of its derivative compounds were identified, which corroborated with the results found by Nakamura et al. (2002) that identified gallic acid and methyl gallate (a gallic acid derivative) as active constituents of *L. ferrea* fruits (*Caesalpinia ferrea*).

Conclusions

Our work demonstrated that *A. colubrina*, *L. ferrea* and *P. moniliformis* are promising sources of compounds against *S. aureus*, which have a high synergetic potential with erythromycin *in vitro*. The highest synergetic potential of *P. moniliformis* is related with its capacity to cause membrane damage. The isolation of bioactive compounds is the target for additional research of our group.

ACKNOWLEDGEMENTS

The authors wish to thank the Brazilian agencies (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco) for the financial support. Scott V. Heald, a native of the United States who is certified as an examiner by the University of Cambridge (England) to assess spoken English, is acknowledged for English review.

REFERENCES

Abreu AC, McBain AJ, Simões M (2012). Plants as sources of new antimicrobials and resistance-modifying agents. Nat. Prod. Rep. 29:1007-1021.

Adikwu M, Jackson C, Esimone C (2010). Evaluation of *in vitro* antimicrobial effect of combinations of erythromycin and *Euphorbia hirta* leaf extract against *Staphylococcus aureus*. Res. Pharm. Biotechnol. 2:22-24.

Almeida CFCBR, Cabral, DLV, Almeida CCBR, Amorim ELC, Araújo JM, Albuquerque UP (2012). Comparative study of the antimicrobial activity of native and exotic plants from the Caatinga and Atlantic Forest selected through an ethnobotanical survey. Pharm. Biol. 50:201–220.

Betoni JEC, Mantovani RP, Barbosa LN, Di Stasi LC, Fernandes Jr A (2006). Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem. Inst. Osw. Cruz. 101:387-390.

Chusri S, Voravuthikunchai SP (2009). Detailed studies on *Quercus infectoria* Olivier (nutgalls) as an alternative treatment for methicillin-resistant *Staphylococcus aureus* infections. J. Appl. Microbiol. 106:89-96.

Clinical and Laboratory Standards Institute (2011). Performance Standards for Antimicrobial Susceptibility Testing, M100-S21, Clinical and Laboratory Standards Institute, Wayne, Pa, USA, 21st edition.

Da Silva LCN, Silva-Júnior CA, Souza RM, Macedo AJ, Silva MV, Correia MTS (2011). Comparative analysis of the antioxidant and DNA protection capacities of *Anadenanthera colubrina, Libidibia ferrea* and *Pityrocarpa moniliformis* fruits. Food Chem. Toxicol. 49:2222-2228.

Hassan SM, Byrd JA, Cartwright AL, Bailey CA (2010). Hemolytic and antimicrobial activities differ among saponin-rich extracts from guar, quillaja, yucca, and soybean. Appl. Biochem. Biotechnol. 162:1008–1017.

Jandú JJB, Silva LCN, Pereira APC, Souza RM, Silva Júnior CA, Figueiredo RCBQ, Araújo JM, Correia MTS, Silva MV (2013). *Myracrodruon urundeuva* bark: An antimicrobial, antioxidant and non-cytotoxic agent. J. Med. Plan. Res. 7:413-441.

Kim HK, Thammavongsa V, Schneewind O, Missiakas D (2012). Recurrent infections and immune evasion strategies of *Staphylococcus aureus*. Curr. Opin. Microbiol.15:92-99.

Kohanski MA, Dwyer DJ, Collins JJ (2010). How antibiotics kill bacteria: From targets to networks. Nat. Rev. Microbiol. 8:423-435.

Kosar M, Göger F, Baser KHC (2011). *In vitro* antioxidant properties and phenolic composition of *Salvia halophila* Hedge from Turkey. Food Chem. 129:374-379.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Monteiro JM, Almeida CFCBR, Albuquerque UP, Lucena RFP, Florentino AT, Oliveira RL (2006). Use and traditional management of *Anadenanthera colubrina* (Vell.) Brenan in the semi-arid region of northeastern Brazil. J. Ethnobiol. Ethnomed. 2:1-6.

Nakamura ES, Kurosaki F, Arisawa M, Mukainaka T, Okuda M, Tokuda H, Nishino H, Pastore Jr F (2002). Cancer chemopreventive effects of constituents of *Caesalpinia ferrea* and related compounds. Cancer Lett. 177:119-124.

Novy P, Rondevaldova J, Kourimska L, Kokoska L (2013). Synergistic interactions of epigallocatechin gallate and oxytetracycline against various drug resistant *Staphylococcus aureus* strains *in vitro*. Phytomedicine 15:432-435.

Queiroz AC, Lira DP, Dias TLMF, Souza ET, Matta CBB, Aquino AB, Silva LHAC, Silva DJC, Mella LAC, Agra MF, Filho JMB, Araújo-Júnior JX, Santos BVO, Alexandre-Moreira MS (2010). The antinociceptive and anti-inflammatory activities of *Piptadenia stipulacea* Benth. (Fabaceae). J. Ethnopharmacol. 128:377-383.

Silva JFV, Silva LCN, Arruda IRS, Silva AG, Macedo AJ, Araújo JM, Correia MTS, Silva MV (2013). Antimicrobial activity of *Pityrocarpa monilifomis* leaves and its capacity to enhance the activity of four antibiotics against *Staphylococcus aureus* strains. J. Med. Plan. Res. 7:2067-2072.

Silva LCN, Sandes JM, Paiva MM, Araújo JM, Figueiredo RCBQ, Silva MV, Correia MTS (2012). Anti-*Staphylococcus aureus* action of three Caatinga fruits evaluated by electron microscopy. Nat. Prod. Res. pp. 1-5.

Tang YW, Stratton CW (2010). *Staphylococcus aureus*: an Old Pathogen with New Weapons. Clin. Lab. Med. 30:179-208.

Trentin DS, Giordani RB, Zimmer KR, Silva AG, Silva MS, Correia MTS, Baumvol IJR, Macedo AJ (2011). Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against *Staphylococcus epidermidis* planktonic and biofilm lifestyles. J. Ethnopharmacol. 137:327-335.

Van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB (2012). Predictors of mortality in *Staphylococcus aureus* Bacteremia. Clin. Micro. Rev. 25:362-386.

Van Hal SJ, Lodise TP, Paterson DL (2012). The clinical significance of vancomycin minimum inhibitory concentration in *Staphylococcus aureus* infections: A systematic review and meta-analysis. Clin. Inf. Dis. 54:755-771.

Vuuren S, Viljoen A (2011). Plant-based antimicrobial studies – Methods and approaches to study the interaction between natural products. Planta Med. 77:1168-1182.