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Full Length Research Paper

Syagrus coronata seed oils have antimicrobial action against multidrug-resistant *Staphylococcus aureus*

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Syagrus coronata **(Mart.) Becc. (Arecaceae) is a native Brazilian palm (*ouricuri*) and despite the use of its derived products by traditional communities, few scientific reports have been published regarding its biomedical activity. This study investigates the chemical composition and anti-*Staphylococcus aureus* effects of both manufactured oil (SCO) and essential oil (SCEO) from *S. coronata* seeds. SCO was provided by rural inhabitants, while SCEO was obtained by hydrodistillation. Chemical characterization was performed by gas chromatography-mass spectrometry (GC/MS). *In vitro* antimicrobial activity was determined against 17 *S. aureus* strains, including multidrug-resistant strains. Eleven compounds were detected in the SCEO, octanoic (28.61%) and dodecanoic acids (22.97%) were the major constituents. On the other hand, nineteen fatty acids (FA) were identified in the SCO, the major ones were dodecanoic acid (41.58%) and 9-octadecenoic acid (23.81%). Both oils showed strong activity against all tested strains. Most strains (68.75%) were sensitive to SCEO at minimum inhibitory concentrations (MIC) between 0.002 and 0.01 µL/mL; and minimum bactericidal concentrations (MBC) ranging from 0.002 to 0.312 µL/mL. SCO inhibited the growth of 52.94% of strains with MIC between 0.16 and 0.625 µL/mL. MBC values for SCO were between 0.16 and 5 µL/mL; however, 47.05% of isolates were killed by 2.5 µL/mL of SCO. These results encourage further research into the toxicological and pharmacological aspects of SCO and SCEO. Such work would likely support their use in the development of new antimicrobial agents for the pharmaceutical, food and cosmetic industries.**

Key words: Caatinga, essential oil, anti-*Staphylococcus aureus*, natural products.

INTRODUCTION

Bacteria, with their increasing drug resistance and their capacity to spread around the world, have become the most complex threats to a global public health system that is increasingly in need of effective antimicrobial treatments (Gould et al., 2012). Among human and animal pathogens, *Staphylococcus aureus* is of particular concern due to its ability to express a variety of virulence factors that facilitate cell adhesion, immune evasion, host

cell damage, and provoke symptoms of disease (Du Toit et al., 2014). Furthermore, *S. aureus* strains have developed increased resistance to antimicrobial agents. In fact, methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA) have been found to be the major cause of hospital-acquired infections (Davis et al., 2013).

The use of plants or their derived products (extracts, oils, infusions, etc.) to treat infections is an age-old practice in many parts of the world, especially in developing countries such as Brazil, where folk medicine is widely used for a variety of diseases (Nascimento et al., 2013). These plant materials apparently have less toxicity compared to synthetic drugs, which make them attractive candidates for drug development. Brazil is the fifth-largest country in the world and is characterized by a huge biological and cultural diversity. Amongst Brazilian biomes, one in particular stands out for being exclusively Brazilian: the Caatinga, which occupies a large portion of the Brazilian Northeast. The Caatinga is marked by an accentuated dryness (rainfall is usually less than 900 mm/year) and is, therefore, considered a semi-arid region. It supports a great diversity of plant species (Albuquerque et al., 2012). As a result of the environmental conditions to which they are exposed, Caatinga plants have developed interesting chemical features, some of which have been described as excellent weapons against microorganisms (Castelo Branco Rangel de Almeida et al., 2012; Oliveira et al., 2012; Da Silva et al., 2013).

Paradoxically, the Caatinga ecosystem harbors many under-utilized plant species with biotechnological and economic potential. *Syagrus coronata* (Mart.) Becc. (Areaceae), a palm species native to the Brazilian semi-arid and cerrado regions, is a good example of this situation. This species is popularly known as *licuri* or *ouricuri* and its derived products have played a vital role in the diet and subsistence economy of traditional communities of the Brazilian Northeast region. Nevertheless, few scientific reports have been published regarding the biomedical activity of *S. coronata*. Recent studies have demonstrated that crude extracts or fractions of this plant have anti-*Leishmania amazonensis* (Rodrigues et al., 2011), antimicrobial (Hughes et al., 2013), and antioxidant (Belviso et al., 2013) activities. Specifically, oils from *S. coronata* have been evaluated for use as biodiesel (Teixeira da Silva de La Salles et al., 2010) and topical emulsion (Leal et al., 2013).

This study provides the chemical characterization and reports the anti-*S. aureus* activity of two oils from seeds of *S. coronata*. The first seed oil is a commercial available and is extracted by traditional rural inhabitants of Catimbau National Park, a national park of Brazil for Caatinga preservation. The second is an essential oil extracted in our laboratory. This is the first report of the chemical profile of an essential oil from *S. coronata* and antimicrobial activity of both materials.

MATERIALS AND METHODS

Plant

Samples of fruits were collected at Catimbau National Park (Pernambuco, Brazilian Northeast) in mature fruit stage, during the month of March 2013. The identification of this material was made by Dr. Alexandre Gomes da Silva, and a voucher specimen (IPA 86950) was deposited at the Agronomic Institute of Pernambuco (IPA/PE). The seeds were removed from mature fruits and dried (at 33°C) in an open area with active ventilation until constant weight was attained (three weeks). Lastly, the seeds were ground using a household grinder.

Extraction and analysis of the essential oil from *S. coronata* seeds (SCEO)

Samples of *S. coronata* seeds (250 g) were submitted to hydrodistillation for 4 h, in a Clevenger-type apparatus. The oils were dried over anhydrous Na_2SO_4. The oils were stored at 4°C until further analysis. All experiments were done in triplicate and results were expressed in terms of dry mass. The main constituents were analyzed by GC/MS, which were performed in the EI mode on a Hewlett Packard-6890 GC system with a fused capillary column (30 m × 0.25 mm × 0.25 µm, HP-5MS, Crossbond 5% phenyl/95% dimethylpolysiloxane) directly coupled to a Hewlett Packard 5973 selective mass detector. The mass spectrometer was operated at 70 eV. The constituents of the essential oils were identified by comparison of their mass spectral pattern and retention indices (RI) with those reported in the literature (Adams, 2009).

S. coronata seed oil (SCO) and its fatty acid composition

The commercial oil from seeds of *S. coronata* was kindly provided by traditional rural inhabitants of Catimbau National Park in March 2013. Fatty acid methylation was performed by the saponification and esterification procedure described by Metcalfe et al. (1966). Dosage of methyl esters was achieved using gas chromatography coupled with mass spectrometry (GC/MS). A GC/MS/QP 2010 Shimadzu instrument was used, equipped with a capillary column of type HP5 MS, 30 mm long by 250 µm internal diameter; the thickness of the film was 0.250 µm. The temperature of the injector was 250°C. Helium was used as the carrier gas at a flow rate of 0.8 mL/min, the injection mode was Split 50:1 and the temperature

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program was set at 150 to -240°C (5°C/min).

Isolation, identification and resistance profile of *S. aureus* isolates

Sixteen *S. aureus* strains were isolated from samples processed in the microbiology laboratories of referral health care institutions in Recife (Pernambuco, Brazil) between September and December 2012. The isolates were cultured on sheep blood agar and the phenotypic identification of *S. aureus* was based on colony morphology, Gram stain, positive plasma coagulase reaction (slide and tube test) and growth in mannitol salt agar (positive colonies changed the medium color from red to yellow).

The antibiotic-susceptibility profile of isolates was performed using a disc diffusion assay on Müeller-Hinton agar (MHA) according to the recommendations of CLSI (2011). In brief, each *S. aureus* isolate was grown overnight on Mueller-Hinton agar at 37°C and the colonies were suspended in sterile saline water equivalent to 0.5 McFarland standard. The suspension (100 µL) was spread over a medium plate and an antibiotic disk was applied aseptically onto the surface. Afterwards, the plates were incubated at 37°C for a period of 24 h. The antibiotics used were erythromycin, clindamycin, oxacillin, penicillin, linezolid, tetracycline, vancomycin, chloramphenicol and gentamicin. The multiple antibiotic resistance (MAR) index was calculated as previously described by Krumperman (1983) using the formula MAR = x/y, where "x" is the number of antibiotics to which the isolate demonstrated resistance and "y" is the total number of antibiotics tested.

Determination of minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The antimicrobial activity was determined using broth microdilution assay against all sixteen *S. aureus* strains identified in this work and a standard *S. aureus* strain (UFPEDA 02), which was provided by the Culture Collection from Department of Antibiotics, Federal University of Pernambuco (UFPEDA). Solutions of both oils used in the antimicrobial assays were obtained according to the following procedure: 400 µL of the SCEO or SCO were mixed with 40 µL of Tween 80 and 5 mL of sterile water (q. s. f.) in a sterile tube and shaken using a vortex (Fanem). After 5 min, solutions with a final concentration of 80 µL/mL were obtained from both samples, SCEO or SCO.

MIC was determined by the microdilution method (CLSI, 2011). Twofold serial dilutions of each solution containing SCEO or SCO (40 to 0.002 µL/mL) were prepared in Müeller-Hinton broth (MHB) and 10 µL of bacterial suspension (approximately 1.5×10^8 CFU/ml) were 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. Afterwards, cultures were seeded in MHA and incubated for 24 h at 37°C to determine the minimum bactericidal concentration (MBC), which corresponds to the minimum concentration of the sample that eliminated the bacteria.

Statistical analysis

All tests were performed in triplicate. Statistical analysis was performed using the Student's t-test. Differences were considered significant at $p<0.05$. The correlation indices were calculated using the Pearson coefficient (ρ).

RESULTS

Chemical composition of SCEO

The GC/MS analysis of SCEO is shown in Table 1. A total of 11 compounds were detected in the SCEO, of which the major constituents were octanoic acid (28.61%) and dodecanoic acid (22.97%), followed by hexanoic acid (17.9%) and decanoic acid (14.04%). Thus, fatty acids are the predominant component of the oils, as they accounted for 86.87% of the total SCEO (including tetradecanoic acid and methyl octanoate). α-Cubebene was the major sesquiterpene detected (9.16%), followed by Δ-cadinene and ɣ-cadinene (2.08 and 1.36%, respectively). Finally, α-humulene and 1H-cycloprop[e]azulene were found as minor components of the SCEO (<1%).

Fatty acid composition of SCO

The fatty acid composition of *S. coronata* seed oil is shown in Table 2. In total, 19 fatty acids were identified in this oil, which correspond to 99.84% of the total. A predominance of saturated fatty acids was observed (72.3%), while unsaturated fatty acids represented 27.49% of total fatty acid content (23.9% for monounsaturated, and 3.6% for polyunsaturated fatty acids). The most represented fatty acids were dodecanoic acid (41.58%) and 9-octadecenoic acid (23.81%), followed by tetradecanoic acid (9.68%), hexadecanoic acid (7.19%) and octanoic acid (5.32%). All fatty acids with odd numbers of carbon atoms (C7, C9, C11, C13 and C15) were found in trace concentrations. It is noteworthy that medium-chain fatty acids accounted for 51.44% of total fatty acid content, namely hexanoic, heptanoic, octadecanoic, nonanoic, decanoic, undecanoic and dodecanoic acids. The levels of saturated fatty acids were approximately three times higher than unsaturated fatty acids.

Antibiotic susceptibility of *S. aureus* strains

The tested *S. aureus* strains had their antibiotic-susceptibility profile analyzed. All of them were susceptible to vancomycin and linezolid, and susceptible or intermediately susceptible to chloramphenicol (Table 3). On the other hand, all strains were resistant to penicillin-G. Higher resistance was seen against erythromycin (62.5% of the strains), clindamycin (50%), oxacillin (43.75%), tetracycline and gentamicin (31.25% for both). Oxacillin resistance is a marker characterizing MRSA/ORSA strains, and we used this to classify the clinical isolates of *S. aureus* used in this study as resistant (MRSA) or sensitive (MSSA) to methicillin, as

Table 1. Chemical composition of essential oil from *S. coronata* seeds.

Compounds[a]	Retention Indices		EO (%)
	Caculated[b]	Literature[c]	
Hexanoic acid	975	974	17.9
Methyl octanoate	991	988	1.25
Octanoic acid	1003	1002	28.61
Decanoic acid	1008	1004	14.04
1H-Cycloprop[e]azulene	1024	1022	0.23
α-Humulene	1049	1044	0.3
ɣ-Cadinene	1100	1095	1.36
Δ-Cadinene	1177	1174	2.08
Dodecanoic acid	1190	1186	22.97
Tetradecanoic acid	1337	1335	2.1
α-Cubebene	1444	1444	9.16
Total	-	-	100

[a]Calculated on DB-5MS column; [b] According to Adams (2009).

Table 2. Fatty acid composition of *S. coronata* seed oil.

Fatty acid	Commun name	Lipid numbers	% of the total fatty acids
Saturated fatty acids:			72.35
Hexanoic acid	Caproic Acid	C6:0	Tr
Heptanoic acid	Enanthic acid	C7:0	Tr
Octadecanoic acid	Stearic acid	C8:0	5.32
Nonanoic acid	Pelargonic acid	C9:0	Tr
Decanoic acid	Capric acid	C10:0	4.54
Undecanoic acid	Undecylic acid	C11:0	Tr
Dodecanoic acid	Lauric acid	C12:0	41.58
Tridecanoic acid	Tridecylic acid	C13:0	Tr
Tetradecanoic acid	Myristic acid	C14:0	9.68
Pentadecanoic acid	Pentadecylic acid	C15:0	Tr
Hexadecanoic acid	Palmitic acid	C16:0	7.19
Heptadecanoic acid	Margaric acid	C17:0	Tr
Octadecanoic acid	Stearic acid	C18:0	3.54
Eicosanoic acid	Arachidic acid	C20:0	0.21
Docosanoic acid	Behenic acid	C22:0	0.22
Tetracosanoic acid	Lignoceric acid	C24:0	0.07
Monounsaturated fatty acids			23.90
9-octadecenoic acid	Oleic acid	C18:1	23.81
11-eicosenoic acid	Gondoic acid	C20:1	0.09
Polyunsaturated fatty acids			
9,12-octadecadienoic acid	Linoleic acid	C18:2	3.59

Tr: Trace concentrations.

shown in Table 3. Six strains (37.5%) could be classified as MRSA. These isolates showed the highest MAR indices (0.44-0.67) and the following resistance patterns: penicillin G-clindamycin-erythromycin-gentamicin-tetracycline-oxacillin (6.25%; MAR index: 0.67), penicillin G-clindamycin-erythromycin-gentamicin-oxacillin (12.5%;

Table 3. Antibiotic-resistance profile and clinical source of *S. aureus* strains.

Strain	Source	Ery	Clin	Oxa	Pen	Lin	Tetra	Van	Chlor	Gen	MAR INDEX
MSSA 1	Oropharynx	R	S	S	R	S	S	S	S	S	0.22
MSSA 2	Eye discharge	S	S	S	R	S	I	S	S	S	0.11
MRSA 3	Blood	R	R	R	R	S	S	S	S	S	0.44
MRSA 4	Wound secretion	R	R	R	R	S	S	S	S	S	0.44
MSSA 5	Oropharynx	S	S	S	R	S	I	S	S	S	0.11
MSSA 6	Blood	S	S	S	R	S	I	S	S	S	0.11
MSSA 7	Wound secretion	I	S	S	R	S	S	S	S	S	0.11
MSSA 8	Wound secretion	S	S	S	R	S	S	S	S	S	0.11
MRSA 9	Blood	R	R	R	R	S	S	S	I	R	0.56
MSSA 10	Blood	R	R	S	R	S	R	S	S	S	0.44
MRSA 11	Blood	R	R	R	R	S	S	S	S	S	0.44
MSSA 12	Wound secretion	R	S	S	R	S	S	S	S	S	0.22
MSSA 13	Blood	R	S	S	R	S	S	S	S	S	0.22
MSSA 14	Blood	S	S	S	R	S	S	S	S	S	0.11
MRSA 15	Blood	R	R	R	R	S	I	S	I	R	0.56
MRSA 16	Blood	R	R	R	R	S	R	S	I	R	0.67

Ery: Erythromycin, Clin: clindamycin; Oxa: oxacillin; Pen: penicillin; Lin: linezolid; Tetra: tetracycline; Van: vancomycin; Chlor: chloramphenicol; Gen: gentamicin. R: resistant; S: sensitive; I: intermediate (CLSI, 2011). MRSA: Methicillin-resistant *S. aureus* strain; MSSA: Methicillin-sensitive *S. aureus* strain.

MAR index: 0.56), penicillin G-clindamycin-erythromycin-oxacillin (18.75%; MAR index: 0.44). These strains are also considered multidrug-resistant according to Magiorakos et al. (2012). Among the MSSA strains, the resistance profile was the following: penicillin G-clindamycin-erythromycin-tetracycline (6.25%; MAR index: 0.44 - multidrug-resistant) and penicillin G-erythromycin (18.75%; MAR index: 0.22), other isolates were only resistant to penicillin G (37.5%).

Antimicrobial activity of SCEO

The essential oil from *S. coronata* seeds showed very strong activity against the standard *S. aureus* strain (UFPEDA 02) and also against both MRSA and MSSA strains (Table 4). The values of MIC ranged from 0.002 µL/mL to 0.08 µL/mL. The growth of the *S. aureus* UFPEDA 02 was inhibited by 0.002 µL/mL of SCEO. Among the clinical isolates, the majority (68.75%) were sensitive to concentrations between 0.002 and 0.01 µL/mL. Regarding the MBC values, a variation of 0.002 to 0.312 µL/mL was observed, as well as a strong correlation between MIC and MBC values (ρ= 0.89). The MBC/MIC ratios ranged from 1 to 4, thus SCEO is a bactericidal agent (Pankey and Sabath, 2004). Finally, a weak correlation was observed between the MAR indexes and MIC (ρ = 0.17) or MBC (ρ = 0.01) values, indicating that there is no relationship between the SCEO efficacy and the multidrug-resistance profile of *S. aureus* strains. A weak correlation between SCEO and chloramphenicol was also detected (ρ values of -0.18 and -0.19 for MIC and MBC, respectively), revealing that SCEO was effective against *S. aureus* strains less sensitive to the drug's action.

Antimicrobial activity of SCO

The oil obtained from *S. coronata* also showed a strong anti-*S. aureus* activity (Table 4). The SCO, at a concentration of 0.16 µL/mL, inhibited the growth of 41.18% of the strains (including UFPEDA 02). The remaining strains were sensitive to oil at 0.625 µL/mL (11.76%), 1.25 µL/mL (23.53%) and 2.5 µL/mL (23.53%). The MBC values of SCO ranged from 0.16 to µL/mL; however, 47.05% of isolates were killed by 2.5 µL/mL of SCO. Both bactericidal and bacteriostatic effects were observed for SCO (MBC/MIC ratios ranged from 1 to 16), but bactericidal action was more prominent (for 81.25% of strains). The MIC and MBC values were strongly related (ρ = 0.78), while these values were moderately correlated with MAR indices of clinical isolates (ρ = 0.49 for MIC/MAR correlation and ρ = 0.56 for MBC/MAR correlation). Unlike SCEO, the MIC and MBC values found for the SCO were substantially correlated to chloramphenicol (ρ = 0.43 for MIC; ρ = 0.58 for MBC).

DISCUSSION

S. aureus is an extremely versatile, worldwide pathogen, which is able to cause from superficial to deep-seated skin infections that can lead to sepsis (Du Toit, 2014).

Table 4. Anti-*S. aureus* activity of oil and essential oil from seeds of *S. coronata*.

Strain	SCEO			SCO			Control		
	MIC[a]	MBC[a]	MBC/MIC	MIC[a]	MBC[a]	MBC/MIC	MIC[b]	MBC[b]	MBC/MIC
UFPEDA 02	0.002	0.002	1	0.16	0.16	1	0.04	0.04	1
MSSA 1	0.01	0.02	2	0.16	1.25	8	0.04	0.625	16
MSSA 2	0.01	0.04	4	0.16	2.5	16	0.04	0.625	16
MRSA 3	0.01	0.01	1	0.16	1.25	8	0.04	0.625	16
MRSA 4	0.005	0.02	4	1.25	2.5	2	0.312	10	32
MSSA 5	0.002	0.004	2	0.16	0.63	4	0.04	1.25	31
MSSA 6	0.01	0.01	1	0.63	2.5	4	0.04	0.625	16
MSSA 7	0.01	0.02	2	0.16	1.25	8	0.04	0.625	16
MSSA 8	0.01	0.02	2	0.16	0.16	1	0.04	0.625	16
MRSA 9	0.04	0.04	1	1.25	2.5	2	0.08	1.25	16
MSSA 10	0.04	0.04	1	1.25	2.5	2	0.08	1.25	16
MRSA 11	0.04	0.16	4	0.63	0.63	1	0.04	0.08	2
MSSA 12	0.08	0.31	4	2.5	2.5	1	0.08	0.625	8
MSSA 13	0.02	0.02	1	2.5	2.5	1	0.04	0.08	2
MSSA 14	0.01	0.02	2	1.25	2.5	2	0.625	10	16
MRSA 15	0.01	0.02	2	2.5	5	2	0.312	5	16
MRSA 16	0.01	0.02	2	2.5	5	2	0.312	10	32

[a]MIC and MBC values are expressed in µL/mL; [b]MIC and MBC values are expressed in µg/ml; MRSA: Methicillin-resistant *S. aureus* strain; MSSA: Methicillin-sensitive *S. aureus* strain. UFPEDA02: Standard *S. aureus* strain provided by the Culture Collection UFPEDA.

This bacterium has an exceptional capacity to acquire resistance to antibiotics (Gould et al., 2012). These combined features make *S. aureus* the most important pathogen in the Twenty-first Century and point to the urgent need for new anti-*S. aureus* agents. In the present study, we reports the antimicrobial action of *S. coronata* seed oils against MRSA and MSSA *S. aureus* strains and the chemical composition and fatty acid content of SCEO and SCO, respectively. While both oils showed antimicrobial activity, SCEO was more active (15.6 to 250 times greater) than SCO. Their MIC values were moderately correlated between themselves (ρ = 0.43). Nevertheless, while SCEO is a more effective bactericidal agent, SCO showed both bactericidal and bacteriostatic actions. The antimicrobial activity of compounds derived from *S. coronata* has been evaluated for aqueous and methanol extracts from different tissues (leaves, inflorescence, nut-shell, liquid and solid endosperm nuts). In that study, only the extracts obtained from inflorescence tissue showed antimicrobial activity by inhibiting *S. aureus* (including strains with antibiotic resistance) and *Bacillus cereus*. The authors did not report the chemical characterization of these active extracts (Hughes et al., 2013).

To the best of our knowledge, the composition of *S. coronata* essential oil and its biological activity have not been reported before. Only one study on the volatile fraction of *S. coronata* is known (Belviso et al., 2013). The study's authors evaluated the volatile fraction of raw and roasted seeds. A total of 59 volatile compounds were identified in *S. coronata* (34 in raw and 55 in roasted) belonging to 8 chemical classes. Among these, 30 compounds were found in both raw and roasted seeds. Carboxylic acids (such as octanoic and hexanoic acids) prevailed in raw *S. coronata* seeds, while after roasting, Strecker aldehydes (δ-lactones and alkyl pyrazines) were the most abundant. These data corroborate with our results, which showed that the essential oil of *S. coronata* seeds is also predominantly composed of fatty acids such as octanoic, dodecanoic and hexanoic acids.

Although less active than SCEO, the seed oil of *S. coronata* also showed a significant anti-*S. aureus* activity. SCO is predominantly composed of saturated fatty acids, with lauric acid (dodecanoic acid) the main constituent. The levels of saturated fatty acids were approximately three times higher than unsaturated fatty acids. Saturated fatty acids with medium chain length, such as lauric acid, have been found to be major components of other oils from Arecaceae plants, such as *Syagrus oleraceae, Syagrus romanzoffiana* and *Acrocomia aculeate* (Coimbra and Jorge, 2011). This study demonstrated that *S. coronata* seed oil is a rich source of medium-chain fatty acids, which could be suitable for biomedical applications (cosmetic and pharmaceutical industries), as showed by Leal et al. (2013). Our data are in agreement with the work of Bauer et al. (2013), which showed that saturated fats with high levels of medium-chain fatty acids (such as lauric and myristic fatty acids) are

prevalent in kernel and fruit oil of *S. coronata* collected in Bahia, Brazil. These authors commented that this composition is very similar to coconut oil. The presence of saturated chains made a biodiesel derived from *S. coronata* less viscous and more stable to oxidation and these physico-chemical properties showed that it has good potential for use in engines (Iha et al., 2014).

Various studies have reported the antimicrobial action of saturated and unsaturated fatty acids, in the form of oil (mixture) or individual compounds (Dilika et al., 2000; Yff et al., 2002; Kitahara et al., 2006; Narasimhan et al., 2006). For instance, lauric acid and related compounds have shown inhibitory action against a range of bacteria, such as *S. aureus* (Kitahara et al., 2006). Likewise, the second-most important compound of SCO, oleic acid (an unsaturated fatty acid), has shown anti-*S. aureus* activity, as has olinoleic acid (9,12-octadecadienoic acid), the only polyunsaturated fatty acid detected (Dilika et al., 2000). Other fatty acids present in SCO have antimicrobial action, such as myristic (tetradecanoic acid) (Narasimhan et al., 2006) and palmitic acid (hexadecanoic acid) (Yff et al., 2002).

In this study, the strong anti-staphylococcal properties of *S. coronata* seed oils were demonstrated. These are promising results that encourage further research on the toxicological and pharmacological aspects of this species, as well the determination of the action mechanisms involved. Such research would clarify these substances' suitability in any potential application as antimicrobial agents for therapy, food practices and/or cosmetic industry.

Conflict of Interests

The authors have not declared any conflict of interests.

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Ethnobotanical and ethnoveterinary study of medicinal plants used in the municipality of Bom Princípio do Piauí, Piauí, Brazil

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Knowledge of medicinal plants has accumulated over centuries and often represents the only therapeutic resource of small municipalities in the interior of Brazil. The objective of this study was to evaluate the knowledge and use of medicinal plants by the population of the municipality of Bom Princípio do Piauí, Piauí state, for the treatment of diseases in humans and domestic animals. Interviews were conducted with 38 residents using standardized questionnaire forms, with the "snowball" technique. Fifty nine families, 98 genera and 112 species were recorded. Of these, 22.3% were indicated for the treatment of diseases in animals and 9.8% were said to cause adverse effects. The families with most species were Fabaceae (14), Euphorbiaceae (11) and Lamiaceae (6). The species with greatest use value (UV) were *Myracrodruon urundeuva* Allemão (0.65), *Dysphania ambrosioides* (L.) Mosyakin and Clemants (0.63) and *Amburana cearensis* (Allemão) A.C.Sm. (0.42). The leaves were the parts most frequently used (26.8%), followed by bark (21.0%). Of the 15 used categories listed in this study, those with the highest number of recorded species were related to illnesses associated with the digestive tract (102), diseases of the genitourinary system (72) and diseases of the respiratory system (60). This study revealed the importance of knowledge and use of medicinal plants in caring for the health of people and domestic animals in the municipality. In addition, the study provided information on plants of the local flora with pharmacological potential.

Key words: Caatinga, medicinal plant, herbal medicine, popular knowledge.

INTRODUCTION

Around 50,000 plant species have recorded medicinal uses and the World Health Organization estimates that

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about 80% of the world´s population still rely on such plants for their primary source of medicines (Wood et al., 2010). Brazil is one of the countries with the greatest diversity of plants in the world, with an estimated 20% of all species on the planet (Carvalho et al., 2007), and more than 46,000 accepted species currently recorded (Lista de Espécies da Flora do Brasil, 2015).

Research in Brazil shows that 91.9% of the population make use of some or other medicinal plants, and that 46% cultivate these plants in home gardens (Ethur et al, 2011). Traditional knowledge about the use of these plants is vast and it is in many cases the only practical recourse available to the rural population for treating ailments (Pasa et al., 2005).

Much knowledge of native plants held by rural communities has been lost due to rapid environmental degradation and reduction of plant diversity in areas where the original vegetation has been replaced by croplands and pastures (Castelleti et al., 2003; Shen et al., 2010). Environmental degradation can lead to desertification, especially in areas within the semi-arid caatinga biome (MMA, 1998). As traditional links with the land are weakened by modernization and more intensive contact with urban centers, the transmission of folk knowledge of medicinal plants may be reduced and ultimately lost (Pilla et al., 2006). Salvaging popular knowledge of medicinal plants is important not only as part of cultural preservation but also because it can provide reliable information for modern bio-prospection and environmental management models that promote the conservation of natural resources (Albuquerque and Andrade, 2002).

Ethnoveterinary medicine embodies traditional knowledge and veterinary practices concerning the health care of domestic animals in rural areas (Mathias-Mundy et a., 1989) and represents an affordable and inexpensive option for farmers (Mathias, 2004). Despite the crucial role of ethnoveterinary medicine in most developing countries, very little of this knowledge has been documented (Yinenger et al., 2007). In Brazil, although such knowledge is widespread (Confessor et al., 2009), we know of no published ethnoveterinary studies for the state of Piauí.

Given the above, the objective of the present study was to carry out a survey of plant species used in traditional medicine for the treatment of humans and domestic animals and also to record their adverse effects as recognized by residents of the municipality of Bom Princípio do Piauí.

MATERIALS AND METHODS

The municipality of Bom Princípio do Piauí has an area of 521,572 km^2 with its principal town situated at 03°11'27"S and 41°38'42"W in the *Litoral Piauiense* micro-region. To the north it borders with the municipalities of Luís Correia and Parnaíba, to the south with Buriti dos Lopes and Cocal, to the east with Cocal and Luís Correia, and to the west with Buriti dos Lopes and Parnaíba. Minimum temperatures are 22°C and maximum 36°C, average annual rainfall is 800-1600 mm, and the area has a warm tropical climate, a five to six month rainy season and a dry season for the rest of the year (Aguiar et al., 2004; IBGE, 2014). The vegetation of the study area includes hypoxerophytic caatinga, transitional vegetation types, hyperxerophytic caatinga and areas of intermixed cerrado and carrasco vegetation (Aguiar et al., 2004).

The population of the municipality is estimated at 5,304 inhabitants, of which 68.8% live in the countryside, having a nominal average monthly per capita income of R$ 201 (IBGE, 2014). There is only one health center, a single Joint Health Unit and a single pharmacy. The incidence of poverty is 49.06% (IBGE, 2014). The nearest hospital is located 51 km away in the town of Parnaíba, Piauí.

The study was conducted between November 2012 and March 2013. Standardized questionnaire forms were used. These contained structured and semi-structured questions to measure independent variables of the interviewee (gender, age, education, income level) and issues related to knowledge of the use of medicinal plants, such as which plants are known to the interviewee as medicinals, reason for their use, method of use, how the plant is acquired, plant part(s) used and undesirable effects observed. The information obtained in the interviews was recorded or transcribed only after the purpose and methods of the study had been explained and the respondents had given their prior permission and signed a document registering their Free and Informed Consent (Termo de Consentimento Livre e Esclarecido).

The "snowball" technique was used to identify interviewees and followed this sequence: a local mediator indicates the key community informants who have the greatest knowledge about medicinal plants, and these, in turn, will indicate new informants, until the cycle is closed and no further informants are indicated (Bailey, 1994).

Medicinal plant species in the municipality were collected in flower or fruit by means of excursions guided by local informants (Albuquerque et al., 2010). The collected plant specimens were labelled and preserved as herbarium vouchers following the methodology described by Mori et al. (1989). Species identification was carried out by consultation of the specialized taxonomic literature, using identification keys and comparison with herbarium specimens already identified in the Herbaria of the Universidade Federal do Piauí (HDELTA), where the entire collection of this study was later incorporated. Unidentified material was sent to taxonomic experts. The family classification follows APG III (2009). The names and authors of botanical taxa are in accordance with the standard works by Brummitt et al. (1992) and The International Plants Names Index (2015). Data on the origin of the species (native to Brazil or exotic) followed Lista de Espécies da Flora do Brasil (2015).

To quantify the survey results, we employed the Use Value (UV) for each species cited by informants, a methodology proposed by Phillips et al. (1993) and modified by Rossato et al. (1999). Use Value is given by the formula $UV = \Sigma U/n$, where UV = Use Value; U = number of citations (or uses) of the ethnospecies per informant and n = total number of informants.

A measure of agreement among informants in the use of the species was calculated as the Informant Consensus Factor (FCI), according to the formula: $FCI = (nur - nt)/ (nur - 1)$, adapted from Trotter et al. (1986), where nur = number of use citations made by informants to a category of ailments; nt = number of species listed in each ailment category. The maximum value of FCI is 1 when there is complete consensus among informants within the ailment category.

The therapeutic indications were grouped into 15 ailment categories based on Almeida and Albuquerque (2002) and Cartaxo

et al. (2010) with modifications; namely, ailments of the respiratory system, nervous system, digestive system, genitourinary system, musculoskeletal and connective tissue system, injuries caused by poisoning and its consequences, inflammation and pain in general, infectious and parasitic diseases, neoplasms, ailments of the circulatory system, the skin and subcutaneous tissue, endocrine glands, nutrition and metabolism, disorders of the sensory system (eyes), the sensory system (ears) and "cultural illness". This last category was used for plants indicated for other non-medical purposes, such as 'evil eye' or "envious eye" (Amorozo, 2002).

RESULTS AND DISCUSSION

Medicinal plants are used by the population of the municipality of Bom Princípio for the treatment of ailments that affect both people and domestic animals. Respondents varied in age from 35 to 86 years, with a mean of 60 years, showing that older people tend to have more knowledge of medicinal plants. This has been observed in another study (Silva et al., 2015), where people over 50 years had most knowledge of medicinal plants and younger people showed very little interest in the subject. More men (55.26%) than women were represented among the respondents, also observed by Oliveira et al. (2010). This could be because men's daily work generally requires closer and more regular contact with local native plants and vegetation, promoting greater acquisition of knowledge about the medicinal properties of plants (Ming, 2006).

Among the respondents, 42.1% had not completed elementary school education, 39.5% were illiterate, 15.8% could read and write and 2.6% had completed elementary school. Most respondents (72.0%) were from the rural zone and 55.3% had a family income of a single minimum wage. These figures may reflect the limited availability of local employment coupled with low wages and the main economic activities in the municipality being subsistence agriculture and small businesses. A prevailing family income of a single minimum wage was also recorded by Oliveira (2010) in rural communities of Oeiras in the semi-arid region of Piauí state, and by Silva et al. (2015), in communities of the municipality of Luís Correia, in Piauí's littoral region.

Overall, this study recorded 112 species of plants used in traditional medicine, distributed in 98 genera and 59 families (Table 1). The families with most species were Fabaceae (14), Euphorbiaceae (11) and Lamiaceae (6). Similar results were obtained in studies conducted in the municipality of Abreu e Lima, in Pernambuco state (Rodrigues et al., 2014).

The large number of medicinal plant species recorded in this study shows that they are widely used in the area, as also observed in studies such as that of Ribeiro et al. (2014) conducted in a caatinga area the municipality of Assaré, Ceará state. The use of medicinal plants to cure various ills has a long tradition in some regions of Brazil and demonstrates people's wealth of knowledge. A chronic lack of industrialized medical drugs in the public health services (Matos, 1998) and their high cost (Souza et al., 2012), tend to make the population more reliant on medicinal plants.

As regards the origin of the medicinal species, 66.1% are native to Brazil, which demonstrates the importance of the region's useful plants to local people. A different result was reported by the study of Ribeiro et al. (2014), where the use of exotic species predominated over natives.

The species with the highest UV were *Myracrodruon urundeuva* Allemão (0.65), *Dysphania ambrosioides* (L.) Mosyakin and Clemants (0.63) and *Amburana cearensis* (Allemão) A.C.Sm. (0.42) (Table 1). These use values are a measure of the importance of these taxa to the region's population and also underlines the importance of biological conservation of the local flora. Lack of guidance for the sustainable harvesting of native species for therapeutic use could lead to the disappearance or drastic reduction of natural populations, as with *M. urundeuva* and *A. cearensis*. These two species have been included in the Brazilian Ministry of Environment's official list of endangered species, according to Normative Instruction Nº 6, of 23 September 2008 (Brasil, 2008). The threat of extinction of these two species is enhanced by their popularity as medicinal plants which leads to unsustainable harvesting of their bark and consequent death of individual trees. Sustainable management would allow these plants a long life as the source of medicinal raw material for local people and such practices need to be implemented as soon as possible (Shiki, 1997).

In this study, the use of *M. urundeuva* was recorded as an anti-inflammatory for the human intestine and uterus and for the treatment of reproductive problems in cows (Table 1). This taxon is also used for inflammation of the reproductive tract in traditional communities in the municipality of Soledade, state of Paraíba (Lucena et al., 2011). The pharmacological potential of this species has been indicated by its antimicrobial and antiulcerogenic effects and as a protecting agent for gastric mucosa (Alves et al., 2009; Carlini et al., 2010).

It is noteworthy that 22.3% of the species recorded in this study were cited for veterinary use by the majority of respondents (73.7%). Such species are used primarily for the treatment of parasitic diseases (10) and for retention of the placenta in cows (7) (Table 1). Ethnoveterinary medicine is commonly carried out in the municipality of Bom Princípio do Piauí, where cattle, horses and dog species are treated.

D. ambrosioides is one of the most commonly used plants in folk medicine in almost all of Brazil, especially in the Northeast (Matos, 2007). Some effects indicated by respondents, such as its ability to combat flu, treat worms and consolidate fractures (Table 1) are also described by

Table 1. Plant species used in traditional medicine by the people of the municipality of Bom Princípio do Piauí, Piauí, Brazil.

Family/Species	CN	PU	FU	TI	VS	UV	St
Acanthaceae							
Justicia pectoralis Jacq.	Anador	Le	Infusion	Headache	Castro, KNC 114	0.03	N
Amaranthaceae							
Alternantera dentata (Moench) Stuchlík ex R.E.Fr.	Cibalena	Le	Infusion	Headache	Castro, KNC 121	0.03	N
Alternanthera brasiliana (L.) Kuntze	Penicilina	Le	Decoction	Stomach ache, anti-inflammatory	Castro, KNC 115	0.03	N
Dysphania ambrosioides (L.) Mosyakin & Clemants	Mastruz	Le, Sm	Maceration, juice, cataplasm, bath	Nerves, fever, ovarian cyst, worms, tuberculosis, pneumonia, healing, flu and animal ticks	Castro, KNC 13	0.63	Ex
Gomphrena elegans Mart.	Pustemeira	Sb	Decoction	Prostatitis, uterine inflammation	Castro, KNC 78	0.03	N
Anacardiaceae							
Anacardium occidentale L.	Cajuí-da-mata	Sb	Cataplasm, Decoction	Inflamed tooth, healing, inflamed throat, anti-inflammatory	Castro, KNC 72	0.16	N
Myracrodruon urundeuva Allemão	Aroeira	Sb	Maceration, Decoction	Back pain, healing, anti-inflammatory for bowel and uterus, fungal dermatitis, cow placenta retention	Castro, KNC 19	0.65	N
Spondias purpurea L.	Seriguela	Le	Decoction	Diarrhoea	Castro, KNC 26	0.03	Ex
Annonaceae							
Annona muricata L.	Graviola	Le	Decoction	High cholesterol	Castro, KNC 48	0.03	Ex
Asparagaceae							
Asparagus pyramidalis Kar, D.K.	Milindo	Wp	Decoction	Accelerated heart	Castro, KNC 44	0.05	Ex
Apocynaceae							
Aspidosperma pyrifolium Mart.	Pereira	Sb	Maceration, bath	Animal fleas and ticks	Castro, KNC 06	0.03	N
Himatanthus drasticus (Mart.) Plumel	Janaguba	La	Ingestion, *in natura*	Fracture, anti-inflammatory, for everything	Castro, KNC 15	0.16	N
Campanulaceae							
Hippobroma longiflora (L.) G.Don	Arrebenta-boi	Vi, Ro	Infusion, Decoction	Anti-inflammatory, kidney stone, back pain	Castro, KNC 14	0.05	N
Araceae							
Dieffenbachia seguine (Jacq.) Schott	Comigo-ninguém-pode	Wp	Planted	Evil eye	Castro, KNC 107	0.05	N
Arecaceae							
Cocos nucifera L.	Côco-da-praia	Fr	Decoction	Diarrhoea	Castro, KNC 126	0.03	Ex
Asteraceae							
Acanthospermum hispidum DC.	Maroto	Le	Decoction	Inflamed tooth	Castro, KNC 114	0.03	N
Bignoniaceae							
Fridericia dichotoma (Jacq.) L.G.Lohmann	Açoita-cavalo	Sb, Ro, Le	Maceration, decoction	Anemia, thinning the blood, diarrhoea	Castro, KNC 07	0.13	N
Handroanthus impetiginosus (Mart. ex DC.) Mattos	Ipê-roxo	Sb	Maceration	Prostatitis, anti-inflammatory, anemia	Castro, KNC 09	0.11	N

Table 1. Cont'd.

Handroanthus serratifolius (Vahl) S.Grose	Ipê-amarelo	Sb	Maceration, infusion	Prostatitis, anti-inflammatory	Castro, KNC 85	0.05	N
Bixaceae							
Bixa orellana L.	Urucum	Se	Maceration Decoction	Intoxication, flu, asthma	Castro, KNC 41	0.05	N
Boraginaceae							
Heliotropium indicum L.	Crista-de-galo	Le	Cataplasm	Canine and bovine scabies	Castro, KNC 47	0.03	N
Burseraceae							
Commiphora leptophloeos (Mart.) J.B.Gillett	Imburana-de-espinho	Sb	Maceration,inhalation decoction	Bladder inflammation, stomach ache, sinusitis	Castro, KNC 58	0.08	N
Cactaceae							
Cereus jamacaru DC.	Mandacaru	Wp, Fr	Planted, maceration	Evil eye, colic	Castro, KNC 84	0.05	N
Capparaceae							
Crateva tapia L.	Jenipapinho	Sb	Cataplasm	Fractures, dislocation	Castro, KNC 124	0.11	N
Caricaceae							
Carica papaya L.	Mamão	Fl, Se	Decoction with salt, *in natura*	Gastritis, worms	Castro, KNC 122	0.05	Ex
Cecropiaceae							
Cecropia cf. saxatilis Snethl.	Torém	Le	Maceration	Kidney inflammation	Castro, KNC 61	0.03	N
Chrysobalanaceae							
Licania rigida Benth.	Oiticica	Sb	Maceration	Cow placenta retention	Castro, KNC 77	0.05	N
Cleoamaceae							
Tarenaya spinosa (Jacq.) Raf.	Muçambê	Ro, Sb	Maceration, decoction	Tuberculosis, flu	Castro, KNC 02	0.13	N
Clusiaceae							
Platonia insignis Mart.	Bacuri	Se	Oil	Inflamed tooth	Castro, KNC 24	0.03	N
Combretaceae							
Combretum leprosum Mart.	Mufumbo	Sb, Ro, Fl	Cataplasm, infusion, bath, decoction	Bleeding, anti-inflammatory, liver, diarrhoea, colic, itching and hair loss in horse	Castro, KNC 68	0.26	N
Convolvulaceae							
Ipomoea batatas (L.) Lam.	Batata-doce	Le	Decoction, gargle	Inflamed tooth	Castro, KNC 90	0.03	Ex
Operculina alata (Ham) Urb	Batata-de-tiú	Ro	Decoction, infusion	Intoxication, snakebite and bovine worms	Castro, KNC 63	0.11	N
Operculina hamiltonii (G.Don) D.F.Austin & Staples	Batata-de- purga	Ro	Decoction, maceration	Diarrhoea, worms, horse appetite stimulant, thinning the blood, flu, soothing	Castro, KNC 64	0.13	N

Table 1. Cont'd.

Crassulaceae							
Kalanchoe pinnata (Lam.) Pers.	Coirama	Le	Juice, cataplasm	Gastritis, expel foreign body, pneumonia, bleeding	Castro, KNC 43	0.24	Ex
Cucurbitaceae							
Luffa operculata (L.) Cogn.	Paulista	Fr	Maceration, *in natura*, decoction	Anemia, worms, indigestion, sinusitis, cough animal, appetite stimulant, cow placenta retention	Castro, KNC 60	0.18	Ex
Momordica charantia L.	Melão-de-são-caetano	Fr	Maceration, bath	Fleas, ticks and animal mange	Castro, KNC 35	0.08	Ex
Cyperacaceae							
Schoenoplectus californicus (C. A. Mey.) Soják	Junco	Ro	*In natura*	Snakebite	Castro, KNC 89	0.03	N
Dilleniaceae							
Curatella americana L.	Sambaíba	Sb	Maceration	Thinning the blood, get pregnant	Castro, KNC 10	0.03	N
Euphorbiaceae							
Cnidoscolus urens (L.) Arthur	Cansanção	La	*In natura*	Inflamed tooth	Castro, KNC 81	0.03	N
Croton heliotropiifolius Kunth	Velame	Le, Wp	Juice, Decoction	Toothache, painkiller, gastritis, sexually transmitted disease	Castro, KNC 52	0.25	N
Croton sonderianus Mull.Arg.	Mameleiro preto	Sb	Decoction, maceration	Diarrhoea, indigestion, liver, stomach, nausea, sinusitis	Castro, KNC 10	0.24	N
Croton urucurana Baill.	Urucurana	La	Cataplasm	Itch	Castro, KNC 102	0.03	N
Euphorbia tirucalli L.	Cachorro-pelado	La	Cataplasm	Erisipela	Castro, KNC 91	0.03	Ex
Jatropha gossypiifolia L.	Pinhão-roxo	Le	Cataplasm	Evil eye, headache	Castro, KNC 70	0.16	N
Jatropha mollissima (Pohl) Baill.	Pinhão manso	La, Se	*In natura*	Healing, worms in dog	Castro, KNC 50	0.05	N
Jatropha sp.	Pinhão branco	La	*In natura*	Convulsion	Castro, KNC 44	0.03	N
Manihot sp.	Manipeba	Ro	Cataplasm	Healing, burns	Castro, KNC 101	0.03	N
Phyllanthus orbiculatus Rich.	Quebra-pedra	Ro, Wp	Decoction	Kidney stones, gallbladder stones	Castro, KNC 33	0.24	Ex
Ricinus communis L.	Mamona	Se, Le	Oil, cataplasm	Laxative for pets and children, worms, headache	Castro, KNC 108	0.11	Ex
Fabaceae							
Amburana cearensis (Allemão) A.C.Sm.	Imburana-de-cheiro	Sb, Se	Maceration, decoction, bath, *in natura*	Fungal dermatitis, snakebite, cow placenta retention, conjunctivitis, flu, sinusitis, headache, vaginal discharge.	Castro, KNC 82	0.42	N
Anadenanthera peregrina (L.) Speg.	Angico-branco	Sb	Maceration	Malaria, anemia, healing, analgesic	Castro, KNC 25	0.16	N
Bauhinia sp.	Mororó	Le, Sb	Decoction	Diabetes	Castro, KNC 37	0.03	N
Bowdichia nitida Spruce ex Benth.	Sucupira	Se	Grind and add honey	Sore throat, indigestion in cow	Castro, KNC 20	0.05	N
Hymenaea courbaril L.	Jatobá ou jataí	Sb, Re	Infusion, bath, inhalation	Prostate cancer, gastritis, anti-inflammatory, back pain, vaginal discharge, nosebleed.	Castro, KNC 16	0.21	N
Libidibia ferrea (Mart. ex Tul.) L.P.Queiroz	Jucá	Sb, Fr, Se	Maceration	Anti-inflammatory, kidneys, bruises, back pain, healing, analgesic	Castro, KNC 125	0.24	N
Mimosa acutistipula (Mart.) Benth.	Jurema-preta	Sb	Maceration	Diarrhoea	Castro, KNC 13	0.03	N
Mimosa caesalpiniifolia Benth.	Sabiá	Sb	Decoction, cataplasm	Diarrhoea, healing	Castro, KNC 17	0.05	N
Poincianella bracteosa (Tul.) L.P.Queiroz	Catingueira-preta	Sb	Decoction	Diarrhoea	Castro, KNC 11	0.03	N
Senna alata (L.) Roxb.	Mata-pastão	Fl, Ro	Decoction	Flu, worms	Castro, KNC 100	0.05	N

Table 1. Cont'd.

Stryphnodendron adstringens (Mart.) Coville	Barbatimão	Sb	Decoction, maceration	Healing, bone pain	Castro, KNC 21	0.05	N
Tamarindus indica L.	Tamarindo	Le	Decoction, cataplasm	Hemorrhoid, varicose veins	Castro, KNC 22	0.05	Ex
Vachellia farnesiana (L.) Wight & Arn.	Coronha	Fr, Se	Decoction, maceration	Fever, anemia, dores, kidney stone, liver, colics, infection, the flu	Castro, KNC 30	0.24	N
Vigna unguiculata (L.) Walp.	Feijão	Se	Maceration	Infection	Castro, KNC 95	0.03	Ex
Iridaceae							
Eleutherine bulbosa (Mill.) Urb.	Palmeirinha	Ro	Decoction, infusion	Diarrhoea	Castro, KNC 29	0.16	Ex
Lamiaceae							
Hyptis suaveolens (L.) Poit.	Bamburral	Le	Maceration, decoction	Colics on horseback, heart	Castro, KNC 75	0.05	N
Mentha arvensis L.	Vick	Le	Infusion, chew	Flu, expectorant, headache	Castro, KNC 45	0.05	Ex
Mentha sp.	Hortelã	Le	Decoction, juice, infusion	Period pains, stomach ache, high cholesterol, constipation, cough, flu, hiccups, bronchitis, fever	Castro, KNC 28	0.21	Ex
Ocimum basilicum L.	Alfavaca	Le	Decoction, juice	Indigestion, flu, earache	Castro, KNC 65	0.11	Ex
Plectranthus barbatus Andrews	Boldo [1]	Le	Infusion, decoction	Laxative, stomach pain, indigestion, nausea, liver, thinning blood	Castro, KNC 46	0.37	Ex
Rosmarinus officinalis L.	Alecrim	Le	Decoction, maceration	Animal ticks, sinusitis	Castro, KNC 87	0.05	N
Lauraceae							
Persea americana Mill.	Abacate	Se	Moer, cataplasm	Healing	Castro, KNC 112	0.03	Ex
Lecythidaceae							
Lecythis pisonis Cambess.	Sapucaia	Fl	*In natura*	Animal ticks	Castro, KNC 21	0.03	N
Lythraceae							
Cuphea carthagenensis (Jacq.)J.F.Macbr.	Sete-sangria	Le	Maceration	Thinning the blood	Castro, KNC 106	0.03	N
Malvaceae							
Gossypium hirsutum L.	Algodão	Le, Fr	Infusion, decoction, gargle	Asthma, cough, the flu, tiredness, inflamed tooth, retained placenta cow	Castro, KNC 22	0.11	N
Hibiscus sabdariffa L.	Quiabo	Se	Toast	Asthma	Castro, KNC 88	0.05	Ex
Meliaceae							
Azadirachta indica A. Juss.	Nim	Le, Fr	Maceration, bath	Animal ticks	Castro, KNC 26	0.03	Ex
Cedrela odorata L.	Cedro	Sb	Maceration	Joint pain, abortive	Castro, KNC 48	0.05	N
Moraceae							
Ficus insipida Willd.	Gameleira	La	Cataplasm	Fracture, wart, healing for man and animal	Castro, KNC 103	0.08	N
Rubus brasiliensis Mart.	Amora	La, Sb	Maceration, cataplasm	Analgesic, thin the blood, itch in skin	Castro, KNC 53	0.08	Ex
Musaceae							
Musa paradisiaca L.	Bananeira	Sm	Juice	Indigestion on animal, lung, healing	Castro, KNC 99	0.05	Ex

Table 1. Cont'd.

Myrtaceae							
Eucalyptus globulus Labill.	Eucalipto de cheiro	Le	Decoction	Fever, body ache	Castro, KNC 49	0.08	Ex
Psidium guajava L.	Goiabeira	Le	Decoction	Diarrhoea	Castro, KNC 27	0.32	Ex
Eugenia sp.	Ubaia	Le, Ro	Decoction	Diarrhoea, colic	Castro, KNC 69	0.03	N
Eugenia uniflora L.	Pitangueira	Le	Decoction	Diarrhoea	Castro, KNC 76	0.03	N
Nyctaginaceae							
Guapira tomentosa (Casar.) Lundell	João mole	Sb	Decoction, maceration	Retained placenta cow, fungus	Castro, KNC 18	0.21	N
Olacaceae							
Ximenia americana L.	Ameixa	Sb	Maceration, cataplasm, gargle	Gastritis, diarrhoea, liver, cancer, anemia, antibiotic, tooth inflammation, menstrual pain, analgesic, anti-inflammatory, healing, retained cow placenta	Castro, KNC 74	0.37	N
Oxalidaceae							
Averrhoa carambola L.	Carambola	Fr	Juice	Kidney pain	Castro, KNC 113	0.03	Ex
Passifloraceae							
Passiflora edulis Sims	Maracujá	Le	Decoction	Insomnia	Castro, KNC 120	0.03	N
Passiflora sp.1	Maracujá da mata	Le	Decoction	Soothing	Castro, KNC 116	0.03	N
Passiflora sp.2	Maracujazinho	Fr	Juice	Prostatitis, urinary infection	Castro, KNC 80	0.03	N
Pedaliaceae							
Sesamum indicum L.	Gergelim	Se, Ol	Maceration, cataplasm	Fever, the flu, malaria, headache	Castro, KNC 94	0.13	N
Phytolaccaceae							
Petiveria alliacea L.	Tipi	Ro, Wp	Decoction	Rheumatism, body aches	Castro, KNC 05	0.08	N
Piperaceae							
Piper aduncum L.	Pimenta de macaco	Se	Grind, infusion	Indigestion, gases	Castro, KNC 38	0.03	N
Plantaginaceae							
Scoparia dulcis L.	Vassourinha	Le	Decoction, juice	Evil eye, kidney ailments, calf worms	Castro, KNC 42	0.11	N
Poaceae							
Cymbopogon citratus (DC.) Stapf	Capim-santo	Le	Decoction	High pressure, soothing	Castro, KNC 45	0.08	Ex
Saccharum officinarum L.	Cana-de-açúcar	Sm	Decoction, juice	High pressure, weight loss, increase milk production	Castro, KNC 127	0.08	Ex
Polygonaceae							
Punica granatum L.	Romã	Fr	Juice, chew	Conjunctivitis, indigestion, sore throat, hoarseness	Castro, KNC 40	0.26	Ex

Table 1. Cont'd.

Punicaceae							
Triplaris gardneriana Wedd.	Pajeú	Sb	Decoction, cataplasm	Inflamed tooth and mouth, healing	Castro, KNC 03	0.05	N
Rhamnaceae							
Ziziphus joazeiro Mart.	Juá	Sb	Maceration, mounthwash	Gingivitis	Castro, KNC 56	0.03	N
Rubiaceae							
Carapichea ipecacuanha (Brot.) L.Andersson	Papeconha	Wp	Decoction	Worms	Castro, KNC 79	0.03	N
Coutarea hexandra (Jacq.) K.Schum.	Quina-quina	Sb	Maceration	Prostate inflammation, abortion, malaria, diabetes	Castro, KNC 55	0.11	N
Morinda citrifolia L.	Noni	Fr	*In natura*, maceration	Uterine inflammation, cancer, prostatitis	Castro, KNC 27	0.05	Ex
Rutaceae							
Citrus aurantium L.	Laranja	Le, Fb	Infusion, decoction	High pressure, soothing, indigestion, diarrhoea, stomach ache	Castro, KNC 71	0.18	Ex
Citrus limon (L.) Burm. f.	Limão	Fr	Juice, decoction	Sore throat, cholesterol, high blood pressure	Castro, KNC 36	0.11	Ex
Citrus sp.	Mexirica	Fb	Maceration, inhalation	Sinusitis	Castro, KNC 46	0.03	Ex
Ruta graveolens L.	Arruda	Le	Decoction	Evil eye, thinning blood, uterine inflammation, indigestion, muscle pain	Castro, KNC 109	0.11	Ex
Solanaceae							
Capsicum frutescens L.	Pimenta malagueta	Ro	Grind, infusion	Cardiac arrhythmia	Castro, KNC 39	0.03	Ex
Turneraceae							
Turnera ulmifolia L.	Shanana	Ro, La	Decoction, *in natura*	Uterine inflammation, pain, anti-inflammatory	Castro, KNC 04	0.11	N
Urticaceae							
Urtica dioica L.	Urtiga mansa	Ro	Decoction	Gallstones	Castro, KNC 59	0.03	N
Verbenaceae							
Lippia alba (Mill) N.E. Br. ex Britton & P. Wilson	Erva cidreira	Le	Infusion, decoction	High pressure, soothing, indigestion, diarrhoea	Castro, KNC 32	0.21	N
Xanthorrhoeaceae							
Aloe vera (L.) Burm. f.	Babosa	Le	In natura, cataplasm	Prostate cancer, burns, itch, hair loss in man and horse, healing	Castro, KNC 30	0.18	Ex
Zingiberaceae							
Zingiber officinale Roscoe	Gengibre	Ro	Juice, gargle	Hoarse Hoarseness	Castro, KNC 123	0.05	N

Legend: CN: common name; PU: portion used; FU: form of use; TI: therapeutic indication; VS: Voucher sample number in the herbarium of HDelta- UFPI, Brasil; UV: use value; St: status; Sm: stem; Fb: fruit bark; Vi: Vine; Sb: stem bark; Fl: flower; Le: Leaf; Fr: Fruit; La: latex; Ol: Oil; Wp: Whole plant; Ro: Root; Re: Resin; Se: seed; N: Native; Ex: exotic.

Morais et al. (2005) and Matos (2007). Action on fractures has been demonstrated in a test with rabbits (Pinheiro Neto et al., 2005), while wound healing activity was observed in experiments with rats (Sérvio et al., 2011).

The efficacy of extracts of *A. cearensis* as a bronchodilator, analgesic and antinflammatory was observed in preclinical studies by Leal et al. (2000) and its antibacterial action against

Table 2. Informant Consensus Factor (FCI) based on the use of medicinal species by the informants of the municipality of Bom Princípio do Piauí, Piauí, Brazil.

Categories of medicinal use	No. of citations given by informants	No. of species	FCI
Diseases involving the digestive system	102	48	0.5
Diseases of the genito-urinary system	72	29	0.6
Respiratory conditions	60	27	0.6
Diseases of the circulatory system	36	26	0.3
Diseases associated with inflammation, pain and fever	47	25	0.5
Infectious and parasitic diseases	32	24	0.3
Diseases of the skin and nails	39	22	0.4
Diseases of the musculoskeletal system	41	19	0.6
Mental and behavioral disorders	21	15	0.3
Endocrine, nutritional and metabolic disorders	19	14	0.3
Culture diseases	11	5	0.6
Injuries caused by poisoning and its consequences	7	4	0.5
Neoplasms	4	4	0.0
Disorders of the sensory system (eyes)	3	2	0.5
Disorders of the sensory system (ears)	2	1	1.0

Staphylococcus aureus and *Escherichia coli* was shown by Figueiredo et al. (2013).

The leaf was the plant part most frequently reported for medicinal use, representing 26.8% of the citations, followed by the bark (21.0%). The leaves of many species are not available throughout the year because they fall during the dry season and in these cases the collectors dry and store them for later use (Silva et al., 2015).

The category of therapeutic agents for problems of the digestive system had the highest number of citations (102) and the highest number of species (48), *P. barbatus* being the most frequently cited. According to Matu and Staden (2003), extracts of *P. barbatus* have antibacterial and antiinflammatory activities. However, this plant should not be used by pregnant women, infants, children, hypertensive patients and those with biliary obstructions, because this species has toxic and abortifacient properties (ANVISA, 2011).

The greatest value of the Informant Consensus Factor (FCI) was related to disorders of the sensory system (ears) (FCI = 1), followed by ailments of the genitourinary system and musculoskeletal, respiratory and cultural problems (FCI = 0.6 each) (Table 2). There is greater consensus when a species is indicated by several informants for signs and symptoms of a disease category (Trotter et al., 1986).

The Agência Nacional de Vigilância Sanitária (ANVISA) maintains a list of 66 traditional medicinal plants which have scientifically proven medicinal effects and indicates their correct usage. Fifteen species cited by informants are included in this list and despite their adverse effects according to ANVISA (2013), none of the respondents cited these effects, suggesting they were unaware of the health risks linked to their usage. On the other hand, 18.4% of respondents indicated eleven species as the cause of adverse effects (Table 3), demonstrating that there is partial knowledge within the population of the municipality of Bom Princípio do Piauí of the health risks associated with the use of some medicinal plant species. The World Health Organization recognizes the importance of the therapeutic potential of plants, but it cautions against their use because of the dangers posed by inadequate preparation of traditional medicines and the frequent lack of knowledge of their possible adverse side effects (Calixto, 2000).

Various traditional medicinal species cited in this study possess toxicity, even when this was not recognized by informants. The seeds of *Ricinus communis* L., for example, may cause irritation of the gastrointestinal mucosa and in severe cases can lead to convulsions, coma and death (Plantas Tóxicas no Brasil, 2009), *Acanthospermum hispidum* DC. has abortifacient and teratogenic action (Lemonica et al., 1994), while *Aloe vera* (L.) Burm. f. exhibits properties toxic to the kidneys (Wagner et al., 2006) and liver (Yang et al., 2010). Moreover, the toxicity of some plants used in traditional medicine is sometimes described only after the occurrence of many cases of intoxication (Silveira et al., 2008) and the toxic potential of many other species has not yet been identified.

Medicinal plants are recognized as such by the effects they produce and many of the therapeutic indications provided by local informants are in agreement with results reported in the scientific literature, even though the local population is unaware of the active plant

Table 3. Adverse effects from the use of medicinal plants cited by the informants of Bom Princípio of Piauí, Piauí, Brazil.

Species	Adverse effects
Asparagus pyramidalis Kar, D.K.	Pressure fall
Bowdichia nitida Spruce ex Benth.	Red spots on the skin, intoxication
Cereus jamacaru DC.	Nausea
Cnidoscolus urens (L.) Arthur	Breaking the tooth
Coutarea hexandra (Jacq.) K.Schum.	Vomit
Dysphania ambrosioides (L.) Mosyakin & Clemants	Pressure drop, abortive, weakens bones
Himatanthus drasticus (Mart.) Plumel	Stiffens the joints
Luffa operculata (L.) Cogn.	Nosebleed
Myracrodruon urundeuva Allemão	Bleeding
Operculina hamiltonii (G.Don) D.F.Austin & Staples	Diarrhoea, vomiting and malaise
Senna alata (L.) Roxb.	Tremors

constituents (Bertini et al., 2005). However, plants contain complex mixtures of chemicals that can elicit biological and pharmacological actions (Sousa et al., 2008) and while some may have beneficial effects, others may be injurious to humans or animals.

Conclusion

This study verified the existence of an important practical dependence of the people of the municipality of Bom Princípio do Piauí on local medicinal plant species used for both humans and domestic animals. The knowledge of medicinal plants harboured by this community was shown to be important for local health care and to provide a strong additional argument for the conservation of the local flora.

Conflict of Interests

The authors have not declared any conflict of interests.

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Antimicrobial activity of *Piper aduncum* leaf extracts against the dental plaque bacteria *Streptococcus mutans* and *Streptococcus sanguinis*

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Piper aduncum **has been widely used for medicinal purposes, and it has also been known to possess antimicrobial activity. Dental plaque is a complex ecosystem that harbors benign and pathogenic bacteria. It is desirable that compounds targeted to treat dental plaque-related diseases should be selective in their action, preserving the benign bacteria and inactivating the pathogenic ones. Thus, the study evaluated the antibacterial activity of *P. aduncum* leaf extracts against cariogenic (*Streptococcus mutans*) and health-associated (*Streptococcus sanguinis*) bacterium. For this, ethanol extracts were obtained by decoction, maceration, Soxhlet or turbo-extraction. The minimum inhibitory concentration (MIC) of the extracts was determined using the broth microdilution method. The influence of extracts on virulence traits of *S. mutans* was evaluated by the adherence assay to glass surface and by the glycolytic pH drop assay. *S. mutans* was more susceptible to crude extracts of *P. aduncum* than *S. sanguinis* and the highest activity was obtained with the maceration extract (MAC). Thus, MAC was further fractionated by gel permeation chromatography and the most active fraction against *S. mutans* (MIC of 0.08 mg/mL) had a MIC of 0.62 mg/mL for *S. sanguinis*. In addition, this fraction inhibited sucrose-dependent adherence of *S. mutans* and also reduced the level of acid production by this bacterium. The preferential activity of *P. aduncum* extracts towards *S. mutans* compared with *S. sanguinis*, in addition to their ability to inhibit sucrose-dependent adherence and reduce the level of acid production by *S. mutans*, suggest that this plant may have a potential to prevent dental caries.**

Key words: Plant extracts, *Piper aduncum*, antibacterial activity, *Streptococcus mutans*, caries prevention.

INTRODUCTION

Dental plaque or dental biofilm is a dynamic and complex community of microorganisms found on a tooth surface, embedded in a matrix of polymers of host and bacterial origin. This structure forms via an ordered sequence of

events, resulting in a structurally- and functionally-organized, species-rich microbial community (Marsh, 2004). Under health conditions, dental plaque plays an essential role in natural host defense mechanisms, protecting the host from invasions by exogenous pathogens; however, when the homeostasis is disrupted, changes in the species composition of dental biofilms can lead to diseases such as caries (Filoche et al., 2010).

During caries development, remarkable changes occur in the microbiota on the tooth surface towards the predominance of acidogenic and aciduric bacterial species such as *Streptococcus mutans* (Takahashi and Nyvad, 2011; He et al., 2015). It is widely accepted that *S. mutans* is one of the main caries-related bacteria, since it is responsible for the beginning of the caries process on enamel and root surfaces (Tanzer et al., 2001).

Streptococcus sanguinis is among the most abundant species within the first few hours of biofilm formation of newly cleaned tooth surfaces (Li et al., 2004). This pioneer colonizer is thought to play a beneficial role in the oral cavity (Caufield et al., 2000). It is of interest that both *S. mutans* and *S. sanguinis* have been known for their antagonism at the ecological level (Giacaman et al., 2015). Epidemiological studies showed that early colonization by *S. sanguinis* in infants results in delayed colonization of *S. mutans.* Conversely, high levels of *S. mutans* in the oral cavity correlate with low levels of *S. sanguinis* (Caufield et al., 2000). A possible molecular mechanism underlying these fascinating interspecies interactions relies, at least in part, on antimicrobial compounds such as bacteriocins and hydrogen peroxide, which are produced by *S. mutans* and *S. sanguinis*, respectively, ultimately to create an environment that favors the colonization of one group of organisms over the other (Kreth et al., 2005; Giacaman et al., 2015).

Based on the notion that dental plaque is a complex ecosystem constituted by both benign and pathogenic bacteria populations, it is desirable that compounds targeted to treat dental plaque-related diseases be selective in their action, preserving the benign bacteria and inactivating the pathogenic ones in order to lead to the re-establishment of a health-compatible species composition (Filoshe et al., 2010). Many studies have shown that plant products can be promising agents for the prevention of dental caries, especially due to their antimicrobial properties against *S. mutans* (Limsong et al., 2004; Yatsuda et al., 2005; Percival et al., 2006; Rukayadi and Hwang, 2006; Tomczyk et al., 2011).

Piper aduncum L. (*Piperaceae*) is a tropical shrub widespread in South and Central America, growing naturally in the Amazon and in the Atlantic Forests of Brazil. This plant has been widely used for medicinal purposes and its antibacterial properties have also been described, including against *Streptococcus* species (Lentz et al., 1998; Kloucek et al., 2005). Thus, the main aim of this study was to evaluate the *in vitro* antimicrobial activity of extracts and fractions of *P. aduncum* on the growth, sucrose-dependent cell adherence and acidogenicity of *S. mutans*. Furthermore, to assess the possible effects of these plant products towards maintaining or restoring the health-compatible state of dental plaque, their inhibitory activity on growth of *S. sanguinis* was also examined.

MATERIALS AND METHODS

Plant material

Leaves of *P. aduncum* (adult plants) were collected between June and July, 2009 in the region of Governador Valadares city, state of Minas Gerais, Brazil. The plant was identified by Dr. Beatriz Gonçalves Brasileiro, formerly from the Faculdade de Ciências Biológicas e da Saúde, Universidade Vale do Rio Doce, where voucher specimen was deposited under the number 423.

Preparation of crude extracts

The powder of the air-dried leaves (15 g) was extracted with 80% ethanol (150 mL) by different extraction techniques:

(i) Maceration for a week at room temperature;
(ii) Soxhlet apparatus for 4 h at 78-80°C;
(iii) Decoction for 6 h at 78-80°C.
(iv) turbo-extraction (2,000 rpm) for 20 min at room temperature

After filtration, the resulting solution was concentrated to dryness under reduced pressure using a rotary evaporator at a temperature lower than 40°C.

Preparation of fractions

The most active antimicrobial extract (5 g) was dissolved in 15 mL of ethanol and further fractionated by gel permeation chromatography (GPC). GPC system was constituted by a glass column of 50 mm diameter and 250 mm length coupled in series to the two other similar columns of 50 mm diameter and 480 mm length, filled with Sephadex^{TM} LH-20 gel (GE Healthcare, USA). The system was pumped by means of a P-500 (Pharmacia, USA) pump. Distilled ethanol was used as mobile phase pumped at 2 mL/min. Two hundred fractions of 20 ml each were collected by SF 2120 collector (Advantec, JP) and grouped according to the profile obtained by thin layer chromatography (TLC), making a total of 17 fractions. TLC was developed using silica 20x20 cm^2 HF_{254} plates (Merck). Ethyl acetate, hexane, dichloromethane and methanol were used as solvents.

The chromatograms were revealed by means of vanillin-sulfuric acid or NP/PEG (diphenylborinic acid ethanolamine ester - polyethylene glycol).

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Table 1. *In vitro* antimicrobial activity of extracts and fractions from *P. aduncum* leaves against *S. mutans* and *S. sanguinis*.

Micro-organisms	Minimum inhibitory concentration (MIC) in mg/ml										
	Crude extracts				Fractions (MAC extract)						
	DEC	MAC	SOX	TUR	G	I	J	L	M	N	Q
S. mutans	0.62	0.16	0.31	0.31	>10.0	1.25	0.08	0.31	5.0	>10.0	>10.0
S. sanguinis	2.5	0.31	0.62	1.25	>10.0	1.25	0.62	0.31	1.25	>10.0	>10.0

DEC: Decoction; MAC: Maceration; SOX: Soxhlet apparatus; TUR: Turbo-extraction.

Microorganisms

The microorganisms used in this study were *S. mutans* IM/UFRJ and *S. sanguinis* ATCC 10557. The culture was grown in Brain Heart Infusion (BHI) broth at 37°C for 24 h, under anaerobic conditions (anaerobic chamber model 1025; Forma Scientific Company, Marietta, OH, USA, containing an atmosphere of 85% N_2, 10% H_2 and 5% CO_2). The stock organism was stored in BHI broth containing 50% glycerol at -80°C.

Determination of minimum inhibitory concentration (MIC)

The MIC values were determined based on the broth microdilution method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2006), with modifications. Briefly, the assays were performed in polystyrene microtiter plates with 96 flat-bottomed wells. Two-fold dilution series of extracts and fractions (concentrations ranging from 10 to 0.08 mg/mL) were tested. Diluted suspensions (100 µL) of each bacterial strain were added to 100 µl of various concentrations of vegetal products diluted with the BHI broth to reach a final bacterial count of approximately 5×10^5 CFU/mL. Growth and sterility controls were included for each assay and tests were performed in triplicate in at least three independent experiments. The vehicle (DMSO) served as negative control and was used at the final concentration ≤ 4%. Chloramphenicol MIC for *S. aureus* ATCC 29213 was included for quality control, and its value (8 µg/mL) was within established ranges as published by the CLSI guidelines. The microdilution plates were incubated at 37°C for 24 h in an anaerobic atmosphere (85% N_2, 10% H_2 and 5% CO_2). The MIC was defined as the lowest concentration of extract that completely inhibited visible growth of microorganisms in the microdilution wells.

Inhibition of bacterial adherence to glass surface

To assess the bacterial adherence of *S. mutans* to a smooth glass surface, the bacteria (approximately 10^6 CFU/mL) were grown at 37°C for 6 h at an angle of 30° in a glass test tube with 1ml of BHI containing 1% (weight by volume (wt/vol)) sucrose plus two-fold dilution series of extracts and fractions (concentrations ranging from 0.62 to 0.04 mg/ml), as described earlier (Limsong et al., 2004), with modifications. After incubation, the tubes were washed three times with 5 ml of saline solution (NaCl 0.85%) and attached cells were stained with 1% crystal violet. The concentration for total bacterial adherence inhibition (TBAI) was defined as the lowest concentration that allowed no visible cell adherence on the glass surface. All determinations were performed in triplicate.

Effect of extracts and fractions on acid production

The assay was performed by standard pH drop with dense cell suspensions, according to Belli et al. (1995), with modifications. *S. mutans* cells from suspension cultures were harvested, washed three times in salt solution (50 mM KCl and 1 mM $MgCl_2$) and resuspended in 5 ml of the same salt solution, which was titrated to pH 7.2 with 0.1 M KOH. Glucose (100 mM final concentration), with and without different concentrations of extracts and fractions was added and the pH fall was assessed with glass electrode over a period of 1 h. Three independent assays were performed, and a statistical analysis was carried out using the Student's *t*-test. Differences between control (no treatment) and treatment with plant products were considered statistically significant if $p < 0.05$.

Time-kill assays

Time-kill curves were obtained according to Santos et al. (2007), with adaptations. Starting inocula of approximately 10^6 CFU/ml of each bacterial strain was grown anaerobically at 37°C in BHI broth until the middle of the exponential growth phase (approx. 3 h for *S. sanguinis* and 6 h for *S. mutans*). Then, the crude ethanol extract (final concentration of 0.31 or 0.16 mg/ml for *S. sanguinis* and *S. mutans*, respectively) or its fractions (final concentration of 0.62 mg/ml for J or 0.31 mg/ml for L) were added to each test vial. No vegetal product was added to the control vial. Cultures were then incubated at 37°C under anaerobic conditions. Samples were removed to determine viable counts every 30 min over a 2.5 h period. Ten-fold serial dilutions were prepared in sterile saline and 0.1 ml of each dilution was plated onto BHI agar. The plates were incubated at 37°C for 24 h, at which time the number of colonies was determined.

RESULTS

All four crude extracts inhibited the growth of both bacteria tested with MIC values ranging from 0.16 to 2.5 mg/mL (Table 1). The MICs for *S. sanguinis* were consistently higher (2 to 4-fold) than those found to *S. mutans,* and the crude extract yielded by maceration technique (MAC) was the most effective. Thus, MAC was fractionated by gel permeation chromatography, yielding 17 fractions, designated as A to Q (data not shown). Only seven of them (G, I, J, L, M, N, Q) were available in sufficient amounts to test their inhibitory effect on bacterial growth (Table 1). The G, N and Q fractions displayed no inhibitory activity, whereas the J and L fractions exhibited the highest activities against *S. mutans* and *S. sanguinis*, respectively. It is noteworthy that the J fraction was approximately eight times more active against *S. mutans* than on *S. sanguinis*, showing also a lower MIC than that of the MAC.

MAC and its J and L fractions had the strongest

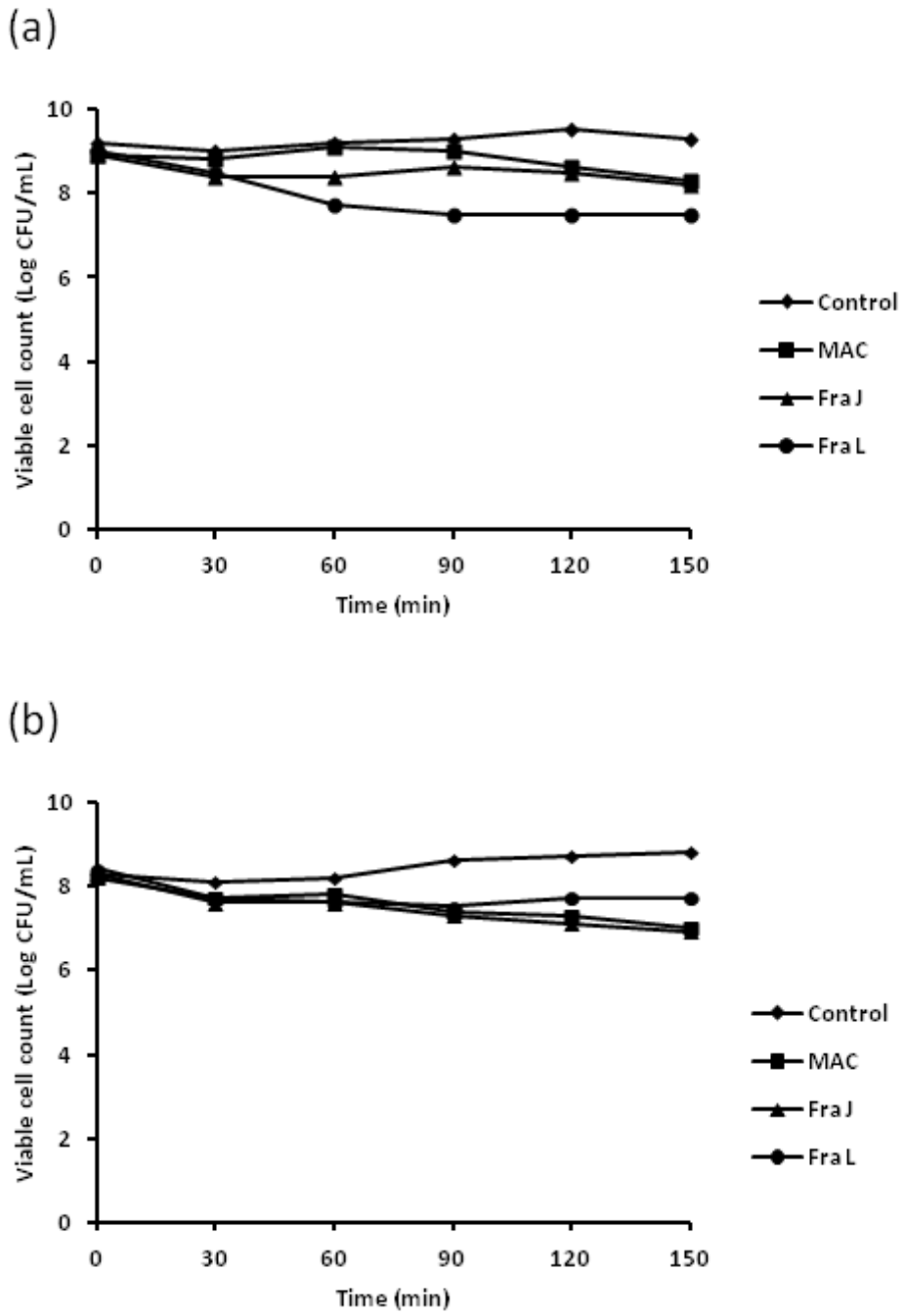

Figure 1. Time-kill curves of maceration extract of *P. aduncum* (MAC) and its both fractions (Fra J and Fra L) against *S. sanguinis* (a) and *S. mutans* (b). A growth control is also shown (Control). The tested concentrations were as follows: (a) MAC 0.31 mg/mL, Fra J 0.62 mg/mL, Fra L 0.31 mg/mL; (b) MAC 0.16 mg/mL, Fra J 0.08 mg/mL, Fra L 0.31 mg/mL. The figure shows representative data from two independent experiments.

antibacterial activity against planktonic cells, and were thus selected for the time kill test. The results revealed that these agents at MIC concentrations exhibited a bacteriostatic, but not a bactericidal effect against both *S. sanguinis* and *S. mutans* in exponential growth, since the decrease in the bacterial count for every species, relative to the starting inoculum, was <3 log (Figure 1).

To determine the effect of plant products on bacterial acid production, the pH of dense suspension of *S. mutans* was recorded during 60 min after glucose pulse. The pH of the suspension not exposed to the plant products (control) decreased rapidly from pH 7.20 to pH

Table 2. Inhibitory effects of maceration extract of *Piper aduncum* (MAC) and its both J and L fractions (Fra) on acid production by *Streptococcus mutans* at different time periods.

Treatment (mg/ml)	pH (mean + S.D.)				
	5 min	15 min	30 min	45 min	60 min
None - control	4.22±0.16	3.95±0.09	3.79±0.12	3.65±0.13	3.57±0.15
MAC (0.16)	4.51±0.01*	4.52±0.11***	4.27±0.07***	4.09±0.01***	3.98±0.05**
Fra J (0.08)	4.46±0.08*	4.24±0.16*	4.15±0.10**	3.99±0.11**	3.85±0.14*
Fra L (0.31)	4.39±0.06	4.16±0.01**	3.94±0.01*	3.81±0.02	3.71±0.02

Statistically significant difference between control (no treatment) and treatment with plant products (*p<0.05; **p<0.01; ***p<0.001) assessed by the Student's *t*-test.

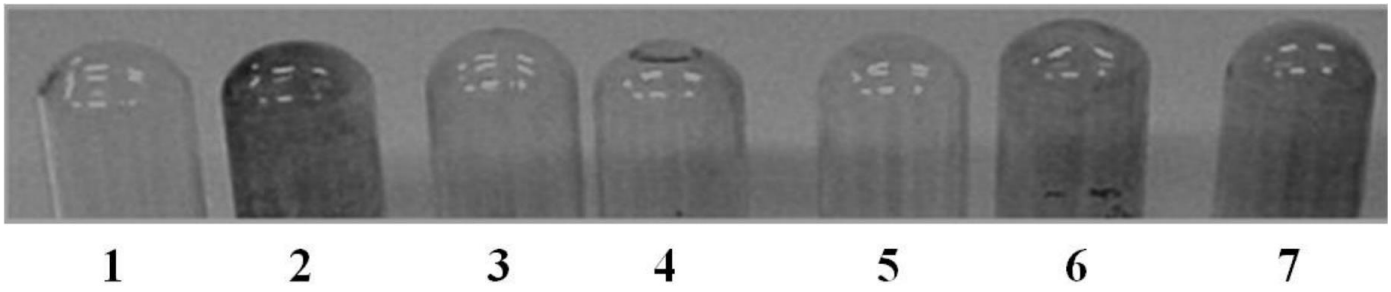

Figure 2. Inhibitory effect of maceration extract of *P. aduncum* (MAC) on sucrose-dependent adherence to glass surface of *S. mutans*. *S. mutans* were grown at 37°C for 6 h at an angle of 30° in a glass test tube with 1ml of BHI broth containing 1% [weight by volume (wt/vol)] sucrose plus two-fold dilution series of MAC. After incubation attached cells were stained with 1% crystal violet. (1) Negative control: bacteria alone; (2) Positive control: bacteria plus sucrose; (3-7) Bacteria plus sucrose with MAC at (3) 0.62 mg/mL, (4) 0.31 mg/mL, (5) 0.16 mg/mL, (6) 0.08 mg/mL e (7) 0.04 mg/mL.

4.22 and 3.57 after 5 min and 60 min, respectively (Table 2). However, the presence of both MAC and J fraction, at MIC concentrations, significantly reduced the rate of acid production by *S. mutans*, when compared to the control, at all the time intervals tested (5 to 60 min). MAC was actually somewhat more potent than its J fraction. The L fraction showed the weakest inhibition of acid production, significantly reducing the pH drop only at 15 and 30 min after glucose pulse.

In order to study the effect of the plant products on sucrose-dependent adherence of *S. mutans*, the study determined their concentration for total bacterial adherence inhibition (TBAI) to a glass surface. MAC (Figure 2) and its L fraction (data not shown) had a similar inhibitory activity (TBAI = 0.16 mg/ml). However, the J fraction was the most effective agent, showing inhibitory effect at a concentration of 0.08 mg/mL (data not shown).

DISCUSSION

Dental caries remains the most prevalent dental disease in many countries therefore being one of the greatest challenges in oral health care (Bagramian et al., 2009). Although the oral microbiota is quite diverse and complex, *S. mutans* has been recognized as an important etiological agent in human dental caries (Loesche, 1986; He et al., 2015).

It is widely accepted that this disease appears as a result of the breakdown of the microbial homeostasis due to a more frequent exposure of plaque to low pH following an increased frequency of sugar intake. This acidic condition provides a selective pressure that allows overgrowth of acidogenic and acid-tolerant species, such as *S. mutans*, whereas at the same time suppressing acid-sensitive bacteria such as *S. sanguinis* (Marsh, 1994).

Consequently, caries control can involve direct use of antibacterial agents to suppress bacterial overgrowth. Nevertheless, the major drawback is that antibacterial products currently in use are not selective in their action, affecting both pathogenic and beneficial bacteria (Marsh, 2010). Thus, the searches continue to find an ideal chemical agent that could control the levels of pathogenic bacteria while preserving the beneficial properties of the resident oral microbiota.

In the present study, *S. mutans* was more sensitive to *P. aduncum* extracts than *S. sanguinis.* Among all of the tested extracts, the J fraction yielded by gel permeation chromatography from MAC was the most effective, inhibiting the growth of *S. mutans* at MIC value

approximately eight times lower than that of *S. sanguinis* (0.08 and 0.62 mg/ml, respectively). In an oral environment in which *S. mutans* is dominant, this differential action, in addition to well-known molecular mechanisms of interspecies competition between *S. sanguinis* and *S. mutans* (Kreth et al., 2005), would result in microbial shifts, and *S. sanguinis* would take the place of *S. mutans* in the ecosystem, restoring the health-compatible state of dental plaque.

According to Ríos and Recio (2005), a significant problem in many studies is to claim positive activity for excessively high concentrations. They consider that plant extracts that are active at concentrations lower than 0.1 mg/ml have a good potency level. Thus, based on this criterion, the study showed that J fraction has promising activity against *S. mutans*. It is possible to speculate that there are specific compounds in this fraction that can inhibit the growth of *S. mutans* at low concentrations. In addition, the study fractionation process had good result towards enhancing the antibacterial activity against *S. mutans*, since the J fraction showed higher activity than its corresponding crude extract (MAC) only on this bacterium.

Results of a previous study have shown that there were differences between the chromatographic profiles of the *P. aduncum* extracts yielded by different extraction methods. The MAC had the highest content of sesquiterpenes, which accounted for more than 97% of the total identified compounds (Santos et al., 2013). Sesquiterpenes have been extensively described in the literature for their antibacterial properties (Paduch et al., 2007; Saleem et al., 2010), including against *S. mutans* (Kubo et al., 1992). These latter authors reported that nerolidol, which was one of the most abundant sesquiterpenes present in MAC (Santos et al., 2013), has potent activity against *S. mutans*, with MIC of 0.025 mg/ml (Kubo et al., 1992). Thus, the good performance of the MAC and its J fraction can be related to their high content of sesquiterpenes. The mechanism of action of terpenes is not fully under-stood, but it is thought to involve membrane disruption associated with their lipophilic character (Cowan, 1999; Paduch et al., 2007). However, the reason for which MAC and its J fraction inhibited *S. mutans* and *S. sanguinis* to varying extent remains to be clarified.

It is important to note that the present results are different from those of Lentz et al. (1998). They reported that ethanol extract from *P. aduncum* collected in Honduras had no activity against *S. mutans* but revealed measurable antibiosis on *S. sanguinis*. Since in this study the agar well test was employed, MIC values were not determined. These divergent results can be explained by many factors, including differences in the geographical locations of the plants (Honduras × Brazil), extraction methods (percolation × maceration) and antibacterial assays (agar-diffusion method x dilution method). Accor-ding to Cos et al. (2006), several methods to detect the extract activity are available; however, since they are not equally sensitive or not based upon the same principle, results may be profoundly influenced by the chosen method. The diffusion method is not appropriate for testing non-polar samples or samples that do not easily diffuse into agar. Contrarily, in general, dilution methods are appropriate for assaying polar and non-polar samples. Kubo et al. (1992) showed that some active sesquiterpenes against *S. mutans* by broth dilution method did not have any activity by an agar-diffusion method.

Besides inhibiting bacterial growth, J fraction at a concentration of 0.08 mg/ml had also biological activity against two of the main virulence traits of *S. mutans*: sucrose-dependent adherence and acidogenicity. Since the concentration that inhibited bacterial growth was the same as that affected the virulence factors, it can be suggested that the anti-virulence effect of this fraction may be due to its antibacterial activity rather than due to a direct effect on specific virulence traits. However, at least for acidogenicity, the effect of J fraction occurred at early times (5 to 60 min) in which it has not bactericidal activity, according to the time kill assays.

The time kill assays were carried out for the maximum length of time of 2.5 h because, based on the present results, *P. aduncum* extracts could be thought to be used in oral care products. Thus, whether in this exposure time a bactericidal effect was not yet achieved *in vitro*, where there is fixed concentration of agent, probably under the *in vivo* conditions these extracts also will fail to show bactericidal activity because of their lack of persistence in the mouth. Chemical antimicrobial compounds used as a mouthwash tend to be rapidly dislodged, diluted or removed (Addy, 1994; Marsh, 2010).

In order to analyze the inhibitory effect of the extracts on adherence of *S. mutans*, we performed the adherence assay to glass surface. According to Limsong et al. (2004), in this assay, the adherence is mediated by glucan as well as the *in vivo* situation. This is an interesting issue because the establishment of *S. mutans* on the tooth surface is rendered irreversible only after the synthesis of sticky water-insoluble glucan from sucrose by enzymatic action of glucosyltransferase and the subsequent cell-to-cell aggregation (Nostro et al., 2004). Thus, the effect of the extracts from *P. aduncum* can be of value to prevent both adherence and accumulation of *S. mutans* on the tooth surface.

Conclusion

The findings that *P. aduncum* extracts exhibit a preferential antimicrobial activity towards *S. mutans* compared with *S. sanguinis*, in addition to their ability to inhibit sucrose-dependent adherence and reduce the level of acid production from *S. mutans*, suggest that this plant may have a potential for further exploitation in

dentistry to prevent dental caries. Future studies should be conducted in order to find the compounds of *P. aduncum* responsible for its anti-*S. mutans* properties.

Conflict of Interests

The authors have not declared any conflict of interests.

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