

Interaction between bark extract of *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul with antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA)
Interação entre o extrato de casca de *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul com antibióticos contra *Staphylococcus aureus* resistente à meticilina (MRSA)
Interacción entre extracto de corteza de *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul con antibióticos contra *Staphylococcus aureus* resistente a la meticilina (MRSA)

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Abstract

Anadenanthera colubrina var. *cebil* (Griseb.) Altschul, a plant often found in areas of the Caatinga in northeastern Brazil, is widely used in unconventional medicine for the treatment of infections and inflammations. Thus, the aim of the present study was to evaluate the antibacterial activity of *A. colubrina* bark extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates, to investigate if intact and regenerated bark extracts have the same effect against MRSA and to determine the interaction between these extracts and antibiotics. The antibacterial activity was performed by the determination of the minimum inhibitory concentration (MIC) according to the Clinical and Laboratory Standards Institute (CLSI) and the interaction assay was performed by the checkerboard method. *A. colubrina* extracts showed bacteriostatic activity (MIC = 8-32 mg/L) against MRSA clinical strains and no difference was found in antibacterial activity between intact and regenerated barks, suggesting that even after regeneration, the barks of this species have the same antibacterial activity. Moreover, the *in vitro* interaction of *A. colubrina* extracts with ciprofloxacin or erythromycin was additive (FICI = 0.52). Thus, the bark extracts of *Anadenanthera colubrina* exhibit antibacterial activity and can be used alone or in combination with antibiotics against MRSA clinical isolates.

Keywords: Fabaceae; *Staphylococcus aureus*; Angico; Caatinga; MRSA.

Resumo

Anadenanthera colubrina var. *cebil* (Griseb.) A Altschul, planta frequentemente encontrada em áreas da Caatinga, no Nordeste do Brasil, é amplamente utilizada em medicamentos não convencionais para o tratamento de infecções e inflamações. Assim, o objetivo do presente estudo foi avaliar a atividade antibacteriana dos extratos de casca de *A. colubrina* contra isolados clínicos *Staphylococcus aureus* resistentes à meticilina (MRSA), investigar se extratos de casca intactos e regenerados têm o mesmo efeito contra o MRSA e determinar a interação entre esses extratos e

antibióticos. A atividade antibacteriana foi realizada pela determinação da concentração inibitória mínima (MIC) de acordo com o Instituto de Normas Clínicas e Laboratoriais (CLSI) e o ensaio de interação foi realizado pelo método de tabuleiro de xadrez. Os extratos de *A. colubrina* mostraram atividade bacteriostática (MIC = 8-32 mg/L) contra cepas clínicas MRSA e nenhuma diferença foi encontrada na atividade antibacteriana entre cascas intactas e regeneradas, sugerindo que mesmo após a regeneração, as cascas desta espécie têm a mesma atividade antibacteriana. Além disso, a interação *in vitro* de extratos de *A. colubrina* com ciprofloxacina ou eritromicina foi aditiva (FICI = 0,52). Assim, os extratos de casca de *Anadenanthera colubrina* apresentam atividade antibacteriana e podem ser usados sozinhos ou em combinação com antibióticos contra isolados clínicos MRSA.

Palavras-chave: Fabaceae; *Staphylococcus aureus*; Angico; Caatinga; MRSA.

Resumen

Anadenanthera colubrina var. *cebil* (Griseb.) Altschul, una planta que a menudo se encuentra en áreas de la Caatinga en el noreste de Brasil, es ampliamente utilizada en medicina no convencional para el tratamiento de infecciones e inflamaciones. Así, el objetivo del presente estudio era evaluar la actividad antibacteriana de los extractos de corteza de *A. colubrina* contra aislados clínicos *Staphylococcus aureus* (MRSA) resistentes a la meticilina, investigar si los extractos de corteza intactos y regenerados tienen el mismo efecto contra el SARM y determinar la interacción entre estos extractos y antibióticos. La actividad antibacteriana se realizó mediante la determinación de la concentración inhibitoria mínima (MIC) según el Instituto de Normas Clínicas y de Laboratorio (CLSI) y el ensayo de interacción fue realizado por el método de tablero de ajedrez. Los extractos de *A. colubrina* mostraron actividad bacteriostática (MIC = 8-32 mg/L) contra cepas clínicas MRSA y no se encontró ninguna diferencia en la actividad antibacteriana entre cortezas intactas y regeneradas, lo que sugiere que incluso después de la regeneración, las cortezas de esta especie tienen la misma actividad antibacteriana. Además, la interacción *in vitro* de extractos de *A. colubrina* con ciprofloxacina o eritromicina fue aditiva (FICI = 0,52). Por lo tanto, los extractos de corteza de *Anadenanthera colubrina* exhiben actividad antibacteriana y se pueden utilizar solos o en combinación con antibióticos contra aislados clínicos MRSA.

Palabras clave: Fabaceae; *Staphylococcus aureus*; Angico; Caatinga; MRSA.

1. Introduction

Staphylococcus aureus is a microorganism of great interest in studies assessing antibacterial activity given this bacterium is one of the main etiological agents of hospital-acquired infections. Also, the ability of *S. aureus* to develop resistance to antibiotics is a major concern in the community (Faria et al., 2005; Mairi, Touati, & Lavigne, 2020) and has prompted researchers in the search for novel therapeutic options such as natural products.

Anadenanthera colubrina var. *cebil* (Griseb.) Altschul, (synonymous: *Mimosa colubrina* Vell.; *Piptadenia colubrina* (Vell.) Benth.; *Piptadenia macrocarpa* Benth.) popularly known as “angico” and “angico de caroço” is a plant that is part of the Fabaceae family. This species is found in areas of the Caatinga, in Northeast region of Brazil. Its bark is very sought after for use in leather tannery. In addition, it is one of the botanical species most cited by the local population due to its medicinal properties (Agra, Baracho, Nurit, Basílio, & Coelho, 2007; A. R. N. Lima et al., 2020; Weber et al., 2011).

The literature reports the medicinal use of *A. colubrina* bark extracts for inflammation of the throat, rheumatic pain, skin inflammations, external ulcers, bronchitis, asthma, among other therapeutic applications (A. R. N. Lima et al., 2020; Pessoa et al., 2015; Santos et al., 2013; Weber et al., 2011). Additionally, the *A. colubrina* bark is used as folk medicine for infections and inflammation and there are scientific evidence showing its potential for fighting microorganisms (A. R. N. Lima et al., 2020; R. D. F. Lima et al., 2014; S. W. C. Silva et al., 2019; Weber et al., 2011). Typically, *A. colubrina* bark extracts are prepared from their cooking or macerated with “cachaça” (T. A. de S. Araújo, Alencar, Amorim, & Albuquerque, 2008; R. D. F. Lima et al., 2014; Vieira Pereira, Santana, Góis, & Sant’ana, 2015).

Phenolic compounds are the vast majority of substances extracted from the bark of *A. colubrina* (A. R. N. Lima et al., 2020). These compounds are responsible for conferring their antimicrobial and antibiofilm properties (T. A. de S. Araújo et al., 2008; Katalinic et al., 2013; Kluczyński et al., 2010; R. D. F. Lima et al., 2014; Palmeira et al., 2010). However, there is a lack of studies that are able to evaluate the antibacterial activity of these extracts against antibiotic-resistant microorganisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA).

Furthermore, there are studies that explore the potential of intact and regenerated *A. colubrina* bark for the bioprospection of medicines, since natural compounds have been proposed as promising strategies for the treatment of infections (Hiramatsu, 2001; Holetz et al., 2002; Jandú, Silva, Silva, & Correia, 2015; Marques et al., 2018; S. W. C. Silva et al., 2019), especially because it contains three large phenolic compounds (epigallocatechin gallate, tohiperina and gallate) (T. A. de S. Araújo et al., 2015). Additionally, the association of substances with antimicrobial properties represents another important tool for infection treatment caused by resistant bacteria, such as MRSA, since combination therapy can help prevent the selection of resistant isolates and broaden the spectrum of action of antimicrobial agents (Cavalcanti et al., 2018; Jackson, Agboke, & Nwoke, 2009; J. L. da Silva, Mesquita, & Ximenes, 2009).

Thus, the aim of the present study was to ascertain the antibacterial activity of *A. colubrina* bark extracts against MRSA clinical isolates, to verify if intact and regenerated bark extracts have the same effect against MRSA and to determine the interaction between these extracts and antibiotics.

2. Methodology

2.1 Plant material and extract preparation

The plant material was collected from the rural area of the city of Altinho, Northeast Brazil (08°35'13.5" S and 36°05'34.6" W). This location is characterized by Caatinga scrub land, highly irregular rainfall, a hot, semi-arid climate (Bsh) and an average temperature exceeding 26 °C (R. M. S. De Araújo et al., 2012). A voucher specimen (48633) was deposited in Herbário Geraldo Mariz, Universidade Federal de Pernambuco (UFPE) and the plant was identified by Dra. Viviany Teixeira do Nascimento, Universidade do Estado da Bahia (UNEB).

Intact and regenerated bark was collected from the same tree specimens, giving a total of 12 samples in order to rule out genetic interference. The bark was also taken from a similar region of the trunk so as to reduce environmental effects such as sunlight exposure and mechanical impact.

The material was dried at an ambient temperature of 25 ± 2 °C and ground in a Wiley cutting mill to obtain a granulometry of 20 Mesh. The powder was submitted to extraction using 80% methanol at the proportion of 1:20 (w/v) for 72 hours and then filtered. The liquid extracts were evaporated in a rotary evaporator at 40 ± 2 °C under reduced pressure to obtain a dry solid extract.

2.2 Antimicrobial activity

2.2.1 Bacterial clinical strains

The antibacterial activity of the *Anadenanthera colubrina* extracts was assessed against 10 methicillin-resistant *Staphylococcus aureus* (MRSA) clinical strains obtained from Clinical Hospital of Federal University of Pernambuco and preserved at the Laboratory of the Microbiology and Immunology (LMB) of the Academic Center of Vitória of the Federal University of Pernambuco (CAV/UFPE). These strains were previously identified as MRSA by the disc diffusion method with cefoxitin and oxacillin as drug reference, as well as by screening using MHA supplemented with 4% NaCl and 6 µg/mL of oxacillin according to *Clinical and Laboratory Standards Institute* guidelines (2020) (CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI), 2020) (LMB 01, LMB 02, LMB 03, LMB 04, LMB 05, LMB 06, LMB 07, LMB 08, LMB 09 and LMB 10). Methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA) and methicillin-sensitive *Staphylococcus aureus* ATCC 29213 (MSSA) were used as controls.

Antibacterial activity can be classified by MIC as: inactive (MIC > 1000 mg/L); weak activity (500 < MIC < 1000 mg/L); moderate activity (100 < MIC < 500 mg/L) and good activity (MIC < 100 mg/L) (Akio Tanaka et al., 2005).

2.2.2 Antibacterial activity

The antibacterial activity of intact and regenerated bark extracts of *A. colubrina* and the antibiotics, ciprofloxacin (CIP) and erythromycin (ERY), was performed by the broth microdilution method according to Clinical and Laboratory Standards Institute guidelines (CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI), 2020). Initially, 96-well microtitre plates were filled with Müeller-Hinton broth (MHB) and then each extract or antibiotic were added to obtain different extract and CIP concentrations ranging from 0.5 to 250 mg/L and ERY concentrations ranging from 2 to 1024 mg/L. Subsequently, a bacterial suspension, suitably diluted to achieve a final concentration of 10^5 CFU/mL in each well, was added. The microplates were then incubated at $35 \pm 2^\circ\text{C}$ for 24 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the drug that causes complete inhibition by spectrophotometry (Ultrospec® 3000 pro – Amersham pharmaceutical biotech) at 630 nm. Minimum bactericidal concentration (MBC) was determined from the well where MIC results showed no bacterial growth, which was then seeded in Müeller-Hinton agar and incubated at $35 \pm 2^\circ\text{C}$ for 24 h. MBC was defined as the lowest concentration of the drug that resulted in $> 99.9\%$ decrease in the initial bacterial inoculum. All the experiments were performed in triplicate.

2.2.3 In vitro interaction assay

The *in vitro* interaction between *A. colubrina* extracts and antibiotics was performed by the checkerboard method (Sopirala et al., 2010). The clinical isolates used in this study were the five most resistant to ERY and CIP. Initially, the 96-well microplates were seeded by dispersing MHB into each well. Next, it was dispensed in the X-axis of the 96-well microdilution plates the serially diluted antibiotics (ERY or CIP) and in the Y-axis the testing extracts to obtain a final concentration equal to the MIC or dilutions lower than the MIC of the respective drugs. Finally, each plate received the adjusted bacterial suspension (10^5 CFU/mL) and they were then incubated at $35 \pm 2^\circ\text{C}$ for 24 h.

The fractional inhibitory concentration index (FICI) was used to evaluate the interaction effect. $\text{FICI} = (\text{MIC A} + \text{B} / \text{MIC A}) + (\text{MIC B} + \text{A} / \text{MIC B})$, where: MIC A + B represents the MIC of drug A when combined with drug B. MIC B + A represents the MIC of drug B when combined with drug A. MIC A and MIC B represent the MICs of drugs A and B when tested alone, respectively. The interaction is considered synergic for $\text{FICI} \leq 0.5$; additive ($0.5 < \text{FICI} \leq 1$), indifferent ($1 < \text{FICI} \leq 2$) and antagonistic ($\text{FICI} > 2$) (Cavalcanti et al., 2018).

3. Results and Discussion

3.1 Antibacterial activity

All the *A. colubrina* extracts exhibited a bacteriostatic effect (MIC = 8 - 32 mg/L), but none showed bactericidal effect (MBC > 250 mg/L) against MRSA clinical strains (Table 1). The MIC values of *A. colubrina* extracts against MSSA ATCC 29213 and MRSA ATCC 33591 were 8 and 32 mg/L, respectively. Based on the classification of antibacterial activity, the *A. colubrina* extracts exhibited good activity against MRSA. In addition, no difference in MIC values was found between extracts derived from intact or regenerated barks, suggesting that even after regeneration, the barks of this species have the same antibacterial activity (Table 1). Study of intact and regenerated bark about antibacterial activity can be valuable for the local population and the pharmaceutical industry by demonstrating whether regenerated bark has the same pharmacological effects, given that its metabolic composition may change following regrowth. If similar action of the bark is observed, this will confirm the species as a renewable resource for use in the manufacture of antibacterial medications.

In studies with extracts of traditional medicinal plants from the Brazilian flora against MRSA strains, it was found that ethanol extracts of *Punica granatum* (Romãzeira) and *Handroanthus impetiginosus* (Ipê-roxo) exhibit MICs ranging from 125 to 250 mg/L (Machado et al., 2003). Sousa (2019) studied the effect of *A. colubrina* gum and its derivatives against bacteria of

the genus *Staphylococcus*. The best results were found for the derivate GAQ-B that exhibit bactericidal effect against *S. epidermidis* ATCC 12228, *S. aureus* ATCC 29213 and *S. aureus* MRSA 43300 (MIC = 62.5 mg/L, 250 mg/L and 250 mg/L, respectively). Thus, *A. colubrina* is a promising extract against MRSA strains due to the low dose presented (SOUSA, 2019).

The literature reports the antimicrobial activity of *Anadenanthera colubrina* extracts against other microorganisms. The MIC of *A. colubrina* against *Streptococcus mutans*, *Streptococcus oralis* and *Streptococcus parasanguis*, was 50, 25 and 12.5 mg/L, respectively (Rocha et al., 2013). These values proved to be similar to those found for MRSA, showing very promising antimicrobial potential of *A. colubrina* against a variety of microorganisms.

Regarding the susceptibility of these isolates to erythromycin and ciprofloxacin, MIC values ranged from 64 to 1024 mg/L for ERY and 4 to 32 mg/L for CIP. These same values were also found in the others studies (Macêdo et al., 2013; Segatore et al., 2012).

It is important mention that, despite the proven antibacterial activity of *A. colubrine*, its effect against the MRSA was only bacteriostatic limiting its use in the treatment of infections caused by this microorganism. Thus, the interaction studies with antibacterial agents are an option to propose the use of this extract in therapy.

Table 1. Antibacterial activity of *Anadenanthera colubrina* intact or regenerated bark extracts against MRSA clinical strains.

MRSA clinical strains	<i>Anadenanthera colubrina</i> intact and regenerated bark extracts													
	MIC/MBC (mg/L)													
	ERY	CIP	A01	AR01	A03	AR03	A04	AR04	A06	AR06	A09	AR09	A11	AR11
LMB 01	1024/>1024	32/>256	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250
LMB 02	1024/>1024	32/>256	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250
LMB 03	256/>1024	4/8	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250
LMB 04	1024/>1024	32/>256	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250
LMB 05	256/>1024	4/64	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250
LMB 06	128/>1024	4/8	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250
LMB 07	1024/>1024	32/>256	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250	8 />250
LMB 08	1024/>1024	32/>256	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250	16 />250
LMB 09	64/>1024	4/8	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250
LMB 10	128/>1024	4/8	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250	32 />250

MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration; MRSA: methicillin-resistant *Staphylococcus aureus*; LMB: Laboratory of Microbiology and Immunology; ERY: erythromycin; CIP: ciprofloxacin; A: *Anadenanthera colubrina* intact extracts; AR: *Anadenanthera colubrina* regenerated extracts (Nascimento et al., 2021).

3.2 *In vitro* interaction assay

The combination of the intact and regenerated *A. colubrina* bark extracts with ERY or CIP resulted in an additive effect for most of the isolates (FICI = 0.52), except for the combination of A03 and AR03 extracts with ERY and CIP, which produced a synergic effect against most of the MRSA isolates (FICI = 0.27) (Table 2), allowing that previously ineffective antibiotics can act against these bacteria.

Antibacterial therapy based on a combination of drugs has shown positive results in preventing the emergence of bacterial resistance and producing beneficial effects in the treatment of infections caused by bacteria. The interaction between antibiotics and bioactive plant extracts is a relatively recent concept and one which can yield beneficial effects such as synergistic or additive interaction (Albano et al., 2016; Gibbons, 2004; Manekeng et al., 2018). This strategy, utilization of plants and drugs in a technique that combines mono- or multi-extracts, is known as “herbal shotgun” or “synergistic multi-effect targeting”. This method is capable of interacting with not only a single target but various targets, in which the different therapeutic components act together in a synergistic way (Matias et al., 2015). Comparatively, natural products can vary and have an antibacterial activity or resistance-modifying activity, when considering the existence of variabilities in polarity and secondary metabolites, which are related to affinities for biological action (Matias et al., 2015). The mechanisms by which the natural compounds can interfere with the growth of microorganisms are diverse and can be related to the chemical nature of some components. In consequence, phytochemicals can demonstrate a greater interaction with the lipid bilayer of the cell membrane, acting on the respiratory chain and energy production, or even make the cell more permeable to antibiotics, leading to the interruption of vital cellular activity (Menezes et al., 2015). Thus, the combination of antibiotics with *A. colubrina* bark extract can be an alternative to minimize the side effects of these antibiotics, since the association leads to a synergistic effect, significantly reducing the MIC of these drugs, decreasing the dose needed for therapeutic usage (Fernando G. Figueredo et al., 2013).

Braga et al. (2005) assessed the interaction between *Punica granatum* and the antibiotics chloramphenicol, gentamicin, ampicillin, tetracycline and oxacillin against MSSA and MRSA clinical isolates. The combinations showed synergistic and indifferent effects (Braga et al., 2005). Silva et al. (2019) studied the antibacterial activity of the dichloromethane fraction (DCMF) from the stem bark of *Mimosa caesalpiniiifolia* and its effect on the activity of conventional antibiotics against *Staphylococcus aureus* strains overexpressing specific efflux pump genes. They concluded that such compounds could be used as adjuvants of norfloxacin, ciprofloxacin or tetracycline for treatment of infections caused by *S. aureus* strains overexpressing efflux pumps (S. W. C. Silva et al., 2019).

A possible action mechanism for natural compounds in antibiotic-resistant microorganisms is the interaction with efflux pumps, which are energy-dependent proteins that promote the elimination of antimicrobial agents into the extracellular environment faster than plasma membrane diffusion to aid bacterial resistance. In *S. aureus*, NorA, NorB, NorC and Tet38 are chromosome-encoded efflux pumps of which the overexpression can confer resistance to multiple drugs (MDR), quinolones and other compounds (Nor pumps) or tetracyclines (Tet38). Natural products such as silybin, terpinene, tannic acid, or polyphenols such as gallic and caffeic acids have been shown to inhibit NorA efflux in *S. aureus* and, thereby, restore sensitivity to antibiotics in MRSA (Fernando Gomes Figueredo et al., 2020).

Thus, in our study, the combination of *A. colubrina* extracts with the antibiotics ERY and CIP substantially reduced the MIC of these drugs, increased their activity and also reduced the toxic effects of the antibiotics given that lower doses can be used.

Table 2. *In vitro* interaction between *Anadenanthera colubrina* intact or regenerated extracts and antibiotics against MRSA clinical strains.

Antimicrobial agents/ Extract	MRSA clinical strains	MIC (mg/L)		FICI	Antimicrobial agents/ Extract	MRSA clinical strains	MIC (mg/L)		FICI
		Combination Antibiotic/Extract	Mean	Interaction			Combination Antibiotic/Extract	Mean	Interaction
CIP/A03	LMB 01	8/0.125	0.266	Synergistic	ERY/A03	LMB 01	256/0.125	0.266	Synergistic
	LMB 02	8/0.125	0.266	Synergistic		LMB 02	512/0.125	0.516	Additive
	LMB 04	8/0.125	0.266	Synergistic		LMB 04	256/0.125	0.266	Synergistic
	LMB 07	8/0.125	0.266	Synergistic		LMB 07	256/0.125	0.266	Synergistic
	LMB 08	8/0.125	0.266	Synergistic		LMB 08	256/0.125	0.266	Synergistic
CIP/AR03	LMB 01	16/0.125	0.516	Additive	ERY/AR03	LMB 01	512/0.125	0.516	Additive
	LMB 02	16/0.125	0.516	Additive		LMB 02	512/0.125	0.516	Additive
	LMB 04	16/0.125	0.516	Additive		LMB 04	512/0.125	0.516	Additive
	LMB 07	16/0.125	0.516	Additive		LMB 07	512/0.125	0.516	Additive
	LMB 08	16/0.125	0.516	Additive		LMB 08	512/0.125	0.516	Additive
CIP/A04	LMB 01	4/0.125	0.144	Synergistic	ERY/A04	LMB 01	256/0.125	0.266	Synergistic
	LMB 02	16/0.125	0.516	Additive		LMB 02	512/0.125	0.516	Additive
	LMB 04	16/0.125	0.516	Additive		LMB 04	512/0.125	0.516	Additive
	LMB 07	16/0.125	0.516	Additive		LMB 07	512/0.125	0.516	Additive
	LMB 08	16/0.125	0.516	Additive		LMB 08	512/0.125	0.516	Additive
CIP/AR04	LMB 01	16/0.125	0.516	Additive	ERY/AR04	LMB 01	512/0.125	0.516	Additive
	LMB 02	16/0.125	0.516	Additive		LMB 02	512/0.125	0.516	Additive
	LMB 04	16/0.125	0.516	Additive		LMB 04	512/0.125	0.516	Additive
	LMB 07	16/0.125	0.516	Additive		LMB 07	512/0.125	0.516	Additive
	LMB 08	16/0.125	0.516	Additive		LMB 08	512/0.125	0.516	Additive

MIC: Minimal Inhibitory Concentration; FICI: fractional inhibitory concentration index; MRSA: methicillin-resistant *Staphylococcus aureus*; LMB: Laboratory of Microbiology and Immunology; ERY: erythromycin; CIP: ciprofloxacin; A: *Anadenanthera colubrina* intact extracts; AR: *Anadenanthera colubrina* regenerated extracts (Nascimento et al., 2021).

4. Conclusion

A. colubrina bark extracts exhibited bacteriostatic activity and a highly effective therapeutic option against MRSA. The intact and regenerated bark showed the same affect against MRSA reiterating that the process of bark regeneration of this plant had no negative impact on the antibacterial activity of the extract against MRSA. *A. colubrina* bark extracts potentiated the antibacterial activity of antibiotics against MRSA clinical isolates. Thus, the results suggest that *A. colubrina* intact and regenerated bark extracts offer potential as an antibacterial agent and may, in the future, be used alone or in combination with antibiotics for the treatment of infections caused by resistant microorganisms, especially MRSA.

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