



CHEMICAL PROFILE, ANTIBACTERIAL AND TOXICOLOGICAL STUDY OF *Asparagus setaceus* (KUNTH)

José Walber Gonçalves Castro¹
Cicero Lucas Marques Macedo²
José Weverton Almeida-Bezerra³
Adrielle Rodrigues Costa⁴
Luciene Ferreira de Lima⁵
Luiz Filipi Teles Feitosa⁶
Elaine Cristina Conceição de Oliveira⁷
Gabriel Messias da Silva Nascimento⁸
Nathallia Correia da Silva⁹
Francisca de Fátima Silva de Sousa¹⁰
Eveline Naiara Nuvens Oliveira¹¹
Fabiola Fernandes Galvão Rodrigues¹²

ABSTRACT

Objective: The objective of this work was to obtain the chemical profile and verify the biological activities of ethanolic extract from the leaves of *Asparagus setaceus*.

Method: The extract was obtained with cold ethanol and the classes of secondary metabolites were identified with specific reagents. The antibacterial assays were performed using the microdilution method against the standard bacteria, Gram +: *Staphylococcus aureus*, *Streptococcus mutans* and *Enterococcus faecalis* and Gram -: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and two multidrug-resistant strains: *Escherichia coli* 27 and *Staphylococcus aureus* 358 and the resistance modulation assay was performed using aminoglycoside and beta- lactam antibiotics. Toxicity was tested against the microcrustacean *Artemia salina* (Leach) ranging in concentrations from 10 to 500 µg/mL

¹ Universidade Regional do Cariri, Crato, Ceará, Brazil. E-mail: josewalber@leaosampaio.edu.br

Orcid: <https://orcid.org/0000-0002-5791-5880>

² Centro Universitário Dr. Leão Sampaio, Juazeiro do Norte, Ceará, Brazil. E-mail: cicerolucasm@gmail.com

Orcid: <https://orcid.org/0009-0002-3649-8536>

³ Universidade Regional do Cariri, Crato, Ceará, Brazil. E-mail: weverton.almeida@urca.br

Orcid: <https://orcid.org/0000-0002-0966-9750>

⁴ Universidade Federal do Cariri, Crato, Ceará, Brazil. E-mail: adrielle.arcg@gmail.com

Orcid: <https://orcid.org/0000-0003-1518-0115>

⁵ Universidade Regional do Cariri, Crato, Ceará, Brazil. E-mail: luciene.ferreira@urca.br

Orcid: <https://orcid.org/0000-0003-3317-3269>

⁶ Centro Universitário Estácio do Ceará, Fortaleza, Ceará, Brazil. E-mail: filipiteles13@gmail.com

Orcid: <https://orcid.org/0009-0005-1327-9674>

⁷ Universidade Regional do Cariri, Crato, Ceará, Brazil. E-mail: elainecryca@gmail.com

Orcid: <https://orcid.org/0000-0002-2593-4390>

⁸ Universidade Regional do Cariri, Crato, Ceará, Brazil. E-mail: gabrielmessias0397@gmail.com

Orcid: <https://orcid.org/0000-0002-6487-2890>

⁹ Universidade Regional do Cariri, Crato, Ceará, Brazil. E-mail: nathallia.silva@urca.br

Orcid: <https://orcid.org/0000-0003-3562-7973>

¹⁰ Universidade Regional do Cariri, Crato, Ceará, Brazil. E-mail: fatimabiohnd@bol.com.br

Orcid: <https://orcid.org/0009-0005-8021-2664>

¹¹ ¹¹ Universidade Regional do Cariri, Crato, Ceará, Brazil. E-mail: evelineprofenfa@gmail.com

Orcid: <https://orcid.org/0009-0003-3926-7132>

¹² Universidade Regional do Cariri, Crato, Ceará, Brazil. E-mail: fabiolafer@gmail.com

Orcid: <https://orcid.org/0000-0003-3901-6758>



Results and Discussion: The ethanolic extract of *Asparagus setaceus* (EEAS) indicated the presence of phenols, condensed tannins, anthocyanins, anthocyanidins, flavones, flavonols, xanthenes, chalcones, aurones, flavononols, catechins, leucoanthocyanidins, flavones and saponins. The EEAS presented CL₅₀ of 90.22 µg/ mL. The microbial assay showed inhibition for standard strains of Gram + and Gram bacteria - but in the multidrug-resistant strains it showed clinically non-signific results. For the modulation of antibiotic action against bacterial strains, EEAS presented both synergistic and antagonistic action, with the synergistic action being more expressive on benzylpenicillin, amikacin and gentamicin in front of *Escherichia coli* 27. The aminoglycoside antibiotics showed synergistic activity on all bacteria tested.

Research Implications: Given the results, it was found that the ethanolic extract of the leaves of *Asparagus setaceus* (Kunth) disp of chemical and biological properties that can contribute to the elaboration of therapeutic resources against inflammatory and bacterial infectious processes.

Keywords: Antibacterial Assay, Secondary Metabolites, Modulation, *Asparagus setaceus*.

PERFIL QUÍMICO, ESTUDO ANTIBACTERIANO E TOXICOLÓGICO DE *Asparagus setaceus* (KUNTH)

RESUMO

Objetivo: O objetivo deste trabalho foi obter o perfil químico e verificar as atividades biológicas do extrato etanólico das folhas de *Asparagus setaceus*.

Método: O extrato foi obtido com etanol frio e as classes de metabólitos secundários foram identificadas com reagentes específicos. Os ensaios antibacterianos foram realizados pelo método de microdiluição contra as bactérias padrão, Gram +: *Staphylococcus aureus*, *Streptococcus mutans* e *Enterococcus faecalis* e Gram -: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* e duas cepas multirresistentes: *Escherichia coli* 27 e *Staphylococcus aureus* 358 e o ensaio de modulação da resistência foi realizado com antibióticos aminoglicosídeos e beta-lactâmicos. A toxicidade foi testada contra o microcrustáceo *Artemia salina* (Leach) variando em concentrações de 10 a 500 µg/mL. O extrato etanólico de *Asparagus setaceus* (EEAS) indicou a presença de fenóis, taninos condensados, antocianinas, antocianidinas, flavonas, flavonóis, xantonas, chalconas, auronas, flavononóis, catequinas, leucoantocianidinas, flavonas e saponinas. A SEAE apresentou CL₅₀ de 90,22 µg/mL.

Resultados e Discussão: O ensaio microbiano mostrou inibição para cepas padrão de bactérias Gram + e Gram – mas nas cepas multirresistentes mostrou resultados clinicamente não significativos. Para a modulação da ação antibiótica contra cepas bacterianas, o SEAE apresentou ação tanto sinérgica quanto antagônica, sendo a ação sinérgica mais expressiva sobre benzilpenicilina, amicacina e gentamicina frente a *Escherichia coli* 27. Os antibióticos aminoglicosídeos apresentaram atividade sinérgica sobre todas as bactérias testadas.

Implicações da Pesquisa: Diante dos resultados constatou-se que o extrato etanólico das folhas de *Asparagus setaceus* (Kunth) dispõe de propriedades químicas e biológicas que podem contribuir para a elaboração de recursos terapêuticos contra processos inflamatórios e infecciosos bacterianos.

Palavras-chave: Ensaio Antibacteriano, Metabólitos Secundários, Modulação, *Asparagus setaceus*.

PERFIL QUÍMICO, ESTUDIO ANTIBACTERIANO Y TOXICOLÓGICO DE *Asparagus setaceus* (KUNTH)

RESUMEN

Objetivo: El objetivo de este trabajo fue obtener el perfil químico y verificar las actividades biológicas del extracto etanólico de hojas de *Asparagus setaceus*.

Método: El extracto se obtuvo con etanol frío y las clases de metabolitos secundarios se identificaron con reactivos específicos. Los ensayos antibacterianos se realizaron mediante el método de microdilución frente a bacterias estándar, Gram +: *Staphylococcus aureus*, *Streptococcus mutans* y *Enterococcus faecalis* y Gram -: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* y dos cepas multirresistentes: *Escherichia coli* 27 y



Staphylococcus aureus 358 y la El ensayo de modulación de la resistencia se realizó con antibióticos aminoglucósidos y betalactámicos. La toxicidad se probó contra el microcrustáceo *Artemia salina* (Leach) en concentraciones de 10 a 500 µg/ml. El extracto etanólico de *Asparagus setaceus* (EEAS) indicó la presencia de fenoles, taninos condensados, antocianinas, antocianidinas, flavonas, flavonoles, xantonas, chalconas, auronas, flavonoles, catequinas, leucoantocianidinas, flavonas y saponinas. SEAE presentó una CL₅₀ de 90,22 µg/mL.

Resultados y Discusión: El ensayo microbiano mostró inhibición para cepas estándar de bacterias Gram + y Gram –, pero en cepas multirresistentes mostró resultados clínicamente no significativos. Para la modulación de la acción antibiótica contra cepas bacterianas, SEAE mostró acción tanto sinérgica como antagonista, siendo la acción sinérgica más significativa la bencilpenicilina, amikacina y gentamicina contra *Escherichia coli* 27. Los antibióticos aminoglucósidos mostraron actividad sinérgica en todas las bacterias analizadas.

Implicaciones de la investigación: Ante los resultados, se encontró que el extracto etanólico de las hojas de *Asparagus setaceus* (Kunth) tiene propiedades químicas y biológicas que pueden contribuir al desarrollo de recursos terapéuticos contra procesos bacterianos inflamatorios e infecciosos.

Palabras clave: Ensayo Antibacteriano, Metabolitos Secundarios, Modulación, *Asparagus setaceus*.

RGSA adota a Licença de Atribuição CC BY do Creative Commons (<https://creativecommons.org/licenses/by/4.0/>).



1 INTRODUCTION

Since antiquity populations have made use of plants in the interest of treating or curing diseases. Therefore, the use of these vegetables in primary health care besides being old is related to the evolution of man, who found in nature the only resource of survival. Myths, superstitions or own scientific evidence strengthen the use of medicinal plants as a font and therapy (Ibiapina et al. 2014; Valli & Bolzani, 2019).

Brazil has become an important source of natural products due to its great plant diversity, especially for medicinal plants that have been included in the treatment and prevention of various diseases. In their metabolism there are various chemical components with pharmacological properties that perform the function of defense, protection and adaptation to the environment, these components are researched and studied through phytotherapy (Cardoso et al. 2019; Stangarlini et al. 2011).

The study of medicinal plants with healing or remediating properties that are part of the practice of folk medicine. This practice is associated with the set of knowledge disseminated by several users and practitioners. The use of these medicinal plants by the population is quite common and is often the only artifice of primary health care (Alves & Povh, 2013; Bruning et al. 2012; Cross, 2017).

The use of herbal medicines has been constantly contributing to the improvement of new therapeutic techniques due to their secondary metabolites. These are known to contribute



directly or indirectly to the human organism and may inhibit or antagonize important molecular and cellular targets in microorganisms (Araujo et al., 2012; Lacerda et al., 2016).

Treatment using herbal medicines to the detriment of synthetic drugs is mainly due to the chemical composition of these plant products, as described above. The numerous secondary metabolic sites on the function of inhibiting or delaying the biochemical processes of microorganisms contributing directly to the human organism. The antimicrobial properties that make the extracts and essential oils of plants have become the main guidelines of study because they have compounds that can be the basis of less harmful compounds and with more specific antimicrobial properties (Carvalho et al., 2017).

Currently, the population has questioned the indiscriminate use of synthetic drugs, looking for alternatives in herbal medicines, due to the lower amount of side effects acquired using these natural and healthy methods, low cost compared to industrialized drugs and the increasing antimicrobial proof of therapeutic action. (Silva et al., 2022; Bruning et al., 2012; Leg & Ferreira, 2014).

The number of microorganisms resistant to conventional antibiotics is increasing. The resistance of a microorganism to an antibiotic can be reduced or eliminated by the combination of two or more antibiotics, through the synergistic effect. Often, this synergistic effect occurs through modulation with herbal medicines, making the natural compound a low-cost alternative with scientific proof. (Coast et al., 2017).

The use of natural products in the process of limiting antimicrobial resistance is a very evident characteristic since herbal medicines can act synergistically with drugs thus enhancing the therapeutic effect of these drugs. In addition, vegetable compounds that present scientific evidence of their therapeutic action may have a low cost when compared to industrialized drugs (Tintino et al., 2013).

These characteristics are observed in plants of the family Asparagaceae as is the case of *Asparagus setaceus* (Kunth), popularly known as “melindro”. The species of this family have a chemical composition that consists mostly of phenolic compounds, terpenes, and ascorbic acid responsible for antioxidant action. The antimicrobial action is due to the presence of high solubility lipophilic compounds, which have degenerative actions on the membranes of microorganisms (El-Seedi et al., 2012). Therefore, one should consider this resource of natural popular origin in daily practice, as a strong attribute to mediate the health-disease process. Therefore, this study aimed to obtain the chemical profile, antibacterial and toxicological study of ethanolic extract from the leaves of *Asparagus setaceus* (Kunth) in the morning (8 hours).



2 MATERIAL AND METHODS

2.1 PLACE OF STUDY

The experiments were carried out in the Microbiology and Bromatology laboratories of the University Center Doctor Leão Sampaio - UNILEÃO and inside the Research Laboratory of Natural Products - LPPN of the Regional University of Cariri-URCA.

2.2 PLANT MATERIAL

The species was acquired commercially by the researcher at a free fair in the city of Juazeiro do Norte - CE and later cultivated in a private ornamental garden. The leaves of *A. setaceus* (Kunth) were collected from this garden in the morning (8 hours) in July 2021, Crato-CE with latitude coordinates: 7° 13' 44,049" S, longitude: 39° 23' 38,438" W, and altitude: 400.

2.3 PEXTRACT REPAIR

The leaves (122, 8 g) of *A. setaceus* (Kunth) were dried at room temperature and submitted to extraction for 72 hours and ethanol was chosen as the extractor solvent, according to Simões et al. (2010). After this period the mixture was submitted to filtration for removal of impurities, and the distillation of the solvent occurred in a rotary evaporator under pressure reduced at a controlled temperature between 30-40 °C, with a yield of 1.66% (v/v).

2.4 CHEMICAL PROSPECTING

Chemical prospecting was carried out according to the methodology of Matos (2009) and Simões et al. (2010) to identify the classes of metabolites present. This test is based on visual observation, color intensification, or precipitate formation after adding specific reagents to the sample solutions.

2.5 TOXICITY ASSESSMENT

Toxicity was tested against *artemia saline microcrustacean* (Leach). The test was performed in triplicate, with concentrations of 10, 25, 50, 100, 250, and 500 µg/mL,



accompanied by positive control prepared with potassium dichromate ($K_2Cr_2O_7$), and negative control with marine water. After 24 h, surviving larvae were read. The calculation of the median lethal concentration was performed by linear regression, being considered significant when $CL_{50} < 1000 \mu g/mL$ (Meyer et al., 1982; Hirota et al., 2012)

2.6 ANTIBACTERIAL EVALUATION AND MINIMUM INHIBITORY CONCENTRATION (MIC)

Antibacterial activity was evaluated by the microdilution method based on CLSI (2012). Standard bacteria were used, three Gram (+): *Bacillus cereus* ATCC 33018, *Streptococcus mutans* ATCC 00446 and *Staphylococcus aureus* ATCC 6538, and three Gram (-): *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442 and *Klebsiella pneumoniae* ATCC 10031 and two multi-resistant strains: *Escherichia coli* 27 e *Staphylococcus aureus* 358. All strains were granted by the Natural Products Research Laboratory (LPPN) of the Regional University of Cariri-URCA.

Initially, a mother solution containing 10 mg of ethanolic extract dissolved in 1 mL of DMSO and 9 mL of distilled water was prepared, obtaining this solution at a concentration of 1024 $\mu g/mL$.

Bacterial strains were activated in *Brain Heart Infusion Broth* (BHI 3.8 %) medium and kept in the greenhouse for 24 h. After the first cultivation, the inoculum was standardized from the concentration of approximately 1×10^8 CFU/mL (turbidity of 0.5 on the McFarland scale). Then, this suspension was diluted in BHI broth at 10 % and volumes of 100 μL were added and homogenized in the wells of a microdilution plate plus the plant product with the initial concentration of 1024 $\mu g/mL$. Serial dilutions were performed and concentrations ranged from 512 to 0.5 $\mu g/mL$. The plates were incubated at 37°C for 24 h. The experiments were carried out in triplicate (CLSI, 2012).

Antibacterial activity was detected using the colorimetric method using 25 μL of sodic resazurin (0.01%) after the incubation period. The last well of the microdilution plate contained only the bacterial inoculums, so it was regarding the positive control. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the extract capable of inhibiting bacterial growth (CLSI, 2012).



2.7 EVALUATION OF MODULATING ACTIVITY

The modulation test was performed in the presence and absence of the natural compound through microdilution in triplicate. To evaluate the modulating activity, MIC was used, from the ethanolic extract against antibiotics of the class aminoglycosides (gentamicin and amikacin), and beta-lactams (benzylpenicillin and cefalotin).

The amount of ethanolic extract was calculated by the sub-inhibition concentration ($Im/8$ C). Bacterial inoculums in BHI at 10 % associated with the extract were distributed in the microplate followed by the microdilution of 100 μ L of antibiotic solutions (1024 μ g/ mL). There were then dilutions obtaining the concentrations of the antibiotic ranging from 512 to 0.5 μ g/ mL (Coutinho et al., 2008).

The test was monitored with an inhibition control containing only antibiotics and microorganisms. The plates managed with microdilution were incubated at 37 °C for 24 hours and the reading was performed with sodic resazurin assistance as described earlier (Coutinho et al., 2008).

2.8 STATISTICAL ANALYSIS

The results of the evaluation of toxicological activity were tabulated using the linear regression model and Tukey test by multiple comparisons. The microbiological tests were analyzed by the two-way ANOVA followed by the Bonferroni test using GraphPad Prism 6.0 software. The results in $p < 0.05$ were considered statistically significant.

3 RESULTS E DISCUSSION

3.1 PHYTOCHEMICAL PROSPECTING

The chemical prospection of the ethanoic extract of *A. setaceus* (EEAS) indicated the presence of phenols (condensed tannins) and flavonoids (anthocyanins, anthocyanidins, flavones, flavonols; xanthonenes, chalcones, aurones and flavonols). The flavonoids that presented lower color intensity and precipitate formation were: catechins; leucoanthocyanidins and flavones (Table 1).

**Table 1**

Result of phytochemical prospection of Ethanolic Extract from *Asparagus setaceus* leaves (EEAS).

CLASS OF SECONDARY METABOLITES																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
EEAS	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

1: Phenols; 2: Hydrolysable tannins; 3: Condensed tannins; 4: Anthocyanins; 5: Anthocyanidins; 6: Flavones; 7: Flavonols; 8: Xanthones; 9: Chalcones; 10: Aurones; 11: Flavononols; 13: Catechins; 14: Leukoanthocyanidins; 15: Flavones; 16: Saponins. (+) present; (-) absent.
Source: authors (2023)

The ethanolic extract of *A. setaceus* presented a diversity of secondary metabolites such as phenolic compounds, for example, which are responsible for protecting vegetables from the attack of bacteria by binding to certain antimicrobial proteins leading to a reduction of their solubility. Studies by Nindo et al. (2003) reported that asparagus is a species with several chemical compounds mainly phenolic and flavonoid compounds (such as rutin) making it one of the most consumed foods for acting in the regulation of gastric disorders.

Other phenols such as condensed tannins are responsible for characterizing foods with a bitter taste and strong odor, and sensory characteristics were also observed in the species of the present study. Lima et al. (2018), the importance of tannins present in ornamental plant leaves when it demonstrates that they play an important role in protecting against microorganisms (including fungi), besides interfering in the symbiotic process and having great relevance in pharmacological activities.

Flavonoids are plant compounds that have high antioxidant power and can interact with free radicals reducing cell aging, it also has analgesic, vasodilator, and anti-inflammatory functions. Studies conducted by Cabral et al. (2014) characterize the family of Asparagaceae with high content of flavonoids and anthocyanins, which gives this family cardiogenic activity, so this study corroborates the findings of these metabolites in the species.

The species of this genus are widely used as antiseptics because they also contain saponins, which have properties of detergents and surfactants. El-Seedi et al. (2012) and Zhong et al. (2015), attribute the genus to the presence of triterpenic saponins that are lipophilic compounds with high solubility function, having action on cell membranes, disorganizing them. In addition to being able to add to proteins, carbohydrates, vitamin complexes, and amino acids



attributing to the species actions: anti-inflammatory, diuretic, vasodilator, analgesic, and control of cell permeability.

3.2 TOXICITY ASSESSMENT

Plants popularly known as therapeutic often has toxic characteristics that are only discovered at the time of the search for pharmacological properties or also by excessive popular use and without prior knowledge. According to Campos et al. (2016), the toxicity of a plant species may be related to certain secondary metabolic diseases and may be influenced by the mode of cultivation, plant use, and environmental factors.

The extract of the species of *A. setaceus* presented an LC_{50} of 90.22 $\mu\text{g}/\text{mL}$. In the palliative studies of Molina-Fajardo et al. 2011, with *Sansevieria trifasciata* (Prain) plant also belonging to the family Asparagaceae, the total ethanolic extract presented an average lethal concentration of 146.41 $\mu\text{g}/\text{mL}$ from *Artemia salina*, the same did not demonstrate cytotoxic activity against Vero cells or Myeloma cells of antitumor mice.

According to Santos et al. (2015), a percentage of 60% of poisonings in Brazil occur by plants and about 50% of these occur by ornamental species. The survey of the study on the main plants and their families with recognized toxicity found the family Asparagaceae having as representative *Dracaena* sp and *Sansevieria trifasciata*. Some signs of intoxication reported appeared after direct ingestion or exudate, thus corroborating the present study because it is an ornamental plant with viscous exudate belonging to the same family.

3.3 ANTIBACTERIAL ASSAYS AND MINIMUM INHIBITORY CONCENTRATION (MIC)

The results against gram⁺ and gram bacteria - showed that the extract has an inhibiting effect. For gram bacteria - standard strains obtained a MIC of 512 $\mu\text{g}/\text{mL}$, while gram-positive bacteria standard lineage presented a MIC that varied between 512 and 256 $\mu\text{g}/\text{mL}$ depending on the bacterial species evaluated. On the other hand, the multidrug-resistant bacteria presented a $\geq 1024 \mu\text{g}/\text{mL}$ (Table 2).

**Table 2**

The minimal inhibitory concentration of EEAS against Gram-negative and Gram- positive bacterial strains.

EEAS	
BACTÉRIAS	MIC (µg/mL)
<i>Pseudomonas aeruginosa</i> ATCC 15442	512
<i>Escherichia coli</i> ATCC 25922	512
<i>Klebsiella pneumoniae</i> ATCC 10031	512
<i>Bacillus cereus</i> ATCC 33018	256
<i>Streptococcus mutans</i> ATCC 00446	256
<i>Staphylococcus aureus</i> ATCC 6538	512
<i>Escherichia coli</i> 27 multirresistente	≥ 1024
<i>Staphylococcus aureus</i> 358 multirresistente	≥ 1024

Source: authors (2023)

The plant product showed greater effectiveness compared to Gram-positive bacteria, which is according to Lima et al. (2016) and Tian et al. (2009), which confirm that these bacteria are more susceptible to natural extracts. Gram-negative bacteria have in morphology the cell wall covered by the lipopolysaccharides layer, which thus limits the input of some compounds, so the action of the product is reduced.

As for multi-resistant bacteria such as *Escherichia coli* 27 and *Staphylococcus aureus* 358, an inhibitory concentration greater than 1024 µg/mL was shown (Table 2). According to Moura et al. (2017) and Houghton et al. (2007), in this concentration the extract does not show a reliable clinical level, that is, it does not interfere materially with biological activity, and it is necessary for large amounts of the extract to reach plasma levels.

João (2015) corroborates the result of this research when evaluating the antimicrobial activity of two species of the genus *Asparagus*, not finding reliable clinical activity against *Staphylococcus aureus*, and the evaluated product had an effect when using a concentration above 2000 µg/mL (higher concentration). In addition, it justified this result because the bacterial lineage is one of the main microorganisms causing hospital infection and produces numerous resistance factors (flow pump, enzymatic inactivation, and alteration of the binding site).

The study by Batista et al. (2018) showed the incidence of *Bacillus cereus* that may be associated with food poisoning by the production of enterotoxin and hemolysins, being an alternative to control the use of natural products that inhibit the exponential growth of the



bacterium. The present research is following this statement since the ethanolic extract was able to inhibit the growth of the microorganism at a concentration of 256 µg/ mL.

The same MIC of 256 µg/mL was also observed when the EEAS was used *front Streptococcus mutans* (Table 2). In the Ferreira (2017) assays, where three species of *the genus Dracaena were used*, it was possible to verify that some plants that make up the family have resin with the ability to interact with membranes of microorganisms obtaining antimicrobial and cytotoxic action, especially gram-positive bacteria.

It is possible to observe that there are studies related to the antibacterial activity of species of the family Asparagaceae that are in different parts in this study. The tests of Bozorgi et al. (2017), using *drimia maritima* Stearn (Asparagaceae) exhibited high antibacterial activity against strains of *Staphylococcus aureus* multi-resistant, *Bacillus megaterium*, and *Neisseria gonorrhoeae*. As seen in the present study, the least significant minimum inhibition concentration was compared to multiresistant bacteria.

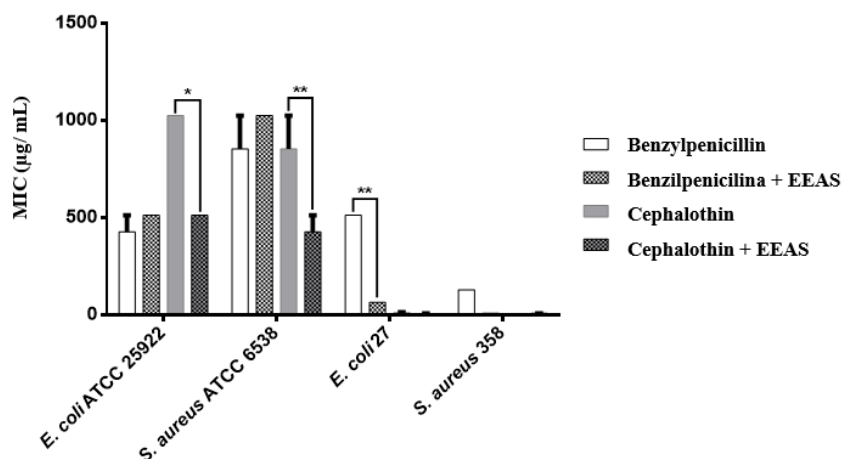
3.4 MODULATOR ACTIVITY

The search for natural products with modulating properties has intensified, due to natural compounds being able to interact with drugs altering the permeability of membranes and potentiating the influx of the drug. Using these products with considerable synergistic effects, the field of action of the drug is increased while decreasing microbial resistance (Seukep et al., 2019).

For the modulation of antibiotic action against bacterial strains, the EEAS presented both synergistic and antagonistic action as observed in Figures 1 and 2. The extract exerted more expressive synergistic action on benzylpenicillin, amikacin, and gentamicin front *Escherichia coli* 27 with the decrease of MIC from 512 µg/ mL to 64 µg/ mL.

**Figure 1**

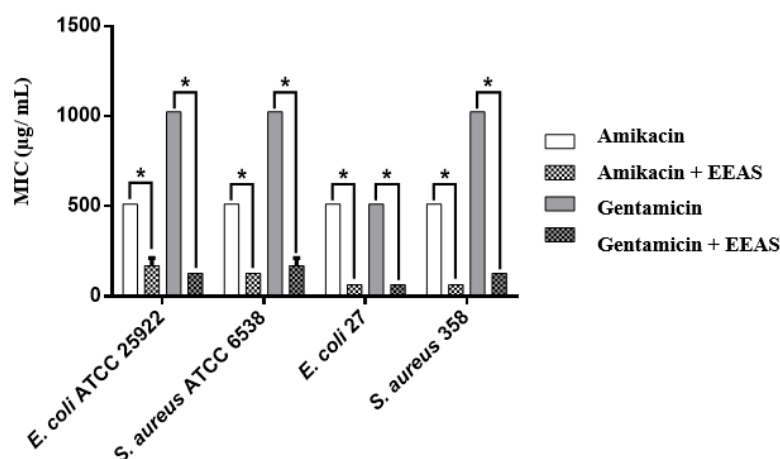
Result of the modulator potential of EEAS in the antibiotic activity of lactams beta against *e.coli* ATCC 25922 strains; *S.aureus* ATCC 6538; *E. coli* 27 (multi-resistant) and *S. aureus* 358 (multiresistant). * $p < 0.0001$; ** $p < 0.0005$.



The antagonism of the cephalexin front *Staphylococcus aureus* 358 observed in Figure 1 is mainly due to the multiple resistance of the strain, responsible for limiting therapeutic options and prolonging the treatment of some infections. According to Lima et al. (2015), most strains of *S. aureus* can acquire plasmids that encode penicillins and inactivate the antibiotic by hydrolysis of the bethalactamic ring even though they are in association with a modulating agent.

Figure 2

Result of the EEAS modulator potential in the antibiotic activity of aminoglycosides against the strains of *E.coli* ATCC 25922; *S.aureus* ATCC 6538; *E. coli* 27 (multiresistant) and *S. aureus* 358 (multiresistant). * $p < 0.0001$; ** $p < 0.0005$.





In front of standard *Escherichia coli*, synergism was observed on cephalothin decreasing the MIC from 1024 µg/mL to 512 µg/mL, gentamicin with the decrease of MIC from 1024 µg/mL to 512 µg/mL, and amikacin with a reduction from 512 µg/mL to 128 µg/mL, as to benzylpenicillin the extract did not alter the MIC remaining with 512 µg/mL.

Against standard *Staphylococcus aureus*, the EEAS reduced the MIC: from amikacin from 512 µg/mL to 128 µg/mL, cephalothin from 1024 µg/mL to 512 µg/mL, and gentamicin from 1024 µg/mL to 128 µg/mL and the most statistically significant reduction. As for benzylpenicillin, MIC remained at 1024 µg/mL.

In *Staphylococcus aureus*, however, the multidrug-resistant strain was antibiotically potentiated on amikacin reducing the MIC from 512 µg/mL to 64 µg/mL, as well as on gentamicin with a reduction from 1024 µg/mL to 128 µg/mL. It observed an unexpressive antagonistic effect against cephalothin, increasing the MIC from 4 µg/mL to 8 µg/mL.

Figure 2, all antibiotics of the aminoglycoside class obtained synergistic activity on all bacteria tested belonging to standard or multidrug-resistant strains. This characteristic cannot be observed in beta-lactam antibiotics since they were unable to synergistically inhibit all bacteria, only cephalothin the two bacteria belonging to the standard strain and benzylpenicillin inhibited *Escherichia* multidrug-resistant coli 27 and *Staphylococcus Aureus* Multidrug resistant 358.

Beta-lactams are a class of antibiotics commonly used due to their tolerance by the body, but the inappropriate use of this class generates resistance mechanisms, one of the most used by bacteria is the production of inactivation enzymes called beta-lactamases. According to Ribeiro 2018, an alternative to overcome this barrier of resistance is the combination of these drugs with natural products.

Characterizing the aminoglycosides, Brazil (2010), points out that they are antibiotics with strong penetrating power and may have more immediate action because they interfere in protein synthesis, especially amikacin and gentamicin presenting greater activity against multidrug-resistant strains of the Enterobacteriaceae family.

Aminoglycosides are antibiotics that present very important cellular toxicity and can cause nephrotoxicity and even block muscles. According to Nonato (2018), these toxic effects can be solved with the combination of antibiotic classes with natural products, this will lead to a decrease in the effective therapeutic dose from the significant reduction of the minimum inhibitory concentration, as verified in modulation.

According to Pindel (2017), the Family Asparagaceae has the ability to morphogenetic alteration of some microorganisms whether standard or multiresistant, this genetic modification



is related to numerous factors such as climate, seasonality or even the microorganism tested, these conditions may be responsible for reducing or antagonizing the potential of some species, which reinforces the antagonistic result observed by benzylpenicillin in Figure 1.

Synergism occurs when an antibiotic has its effect increased in conjunction with another substance, this situation can be justified by increased bacterial permeability which facilitates the entry of the antibiotic. The aminoglycosides showed a synergistic effect when tested against the strains of *Escherichia coli* (Figure 2), corroborating with these data Souza et al. (2016), found that the extracts produced in these same conditions presented a synergistic effect when tested against amikacin and gentamicin.

4 CONCLUSIONS

The results obtained in this work demonstrated that the ethanolic extract of *the leaves of Asparagus setaceus* (Kunth) presented CL₅₀ 90.22 µg/ mL, which can be explained by the variety of metabolic as well as by the form of cultivation and experimental conditions for the use of the leaves of the plant and thus, further studies are necessary to verify the cytotoxicity of the species.

In addition, these chemical compounds are responsible for *in vitro* microbiological properties of the extract. Therefore, this is a promising alternative in antibiotic combined antibacterial therapy, indicating that the plant has the potential to be used as an alternative in the treatment against resistant bacteria. The data highlight the need for studies that quantify the compounds of the species to better report and interpret its biological activities.

REFERENCES

- Alves, G. S. P. et al. (2013). Ethnobotanical study of medicinal plants in the community of Santa Rita, Ituiutaba-Minas Gerais, Brazil. *Biotemas*, 26(3), 231-242, 2013.
- Araújo, K. R. M. et al. (2012). Medicinal plants in the treatment of respiratory diseases in childhood: a view from popular knowledge. *Rev Rene*, 13(3).
- Arimura, G. et al. (2004). Herbivore-induced defense response in a model legume. Two-spotted spider mites induce emission of (E)-β-ocimene and transcript accumulation of (E)-β-ocimene synthase in *Lotus japonicus*. *Plant physiology*, 135(4), 1976-1983.
- Batista, R. D. et al. (2018). *Bacillus cereus* contamination and the risks generated by food poisoning. *Rev Desaf.*, 5, 30-40.
- Bozorgi, M. et al. (2017). Traditional medical uses of *Drimys* species in terms of



- phytochemistry, pharmacology and toxicology. *Journal of Traditional Chinese Medicine*, 37(1), 124-139.
- BRAZIL. (2010). Ministry of Health. Secretary of Science, Technology and Strategic Materials. Department of Pharmaceutical Assistance and Strategic Materials. *National List of Essential Medicines: Rename*. Ministry of Health.
- Bruning, M. C. R.; Mosegui, G. B. G.; Vianna, C. M. D. (2012). The use of phytotherapy and medicinal plants in primary healthcare units in the cities of Cascavel and Foz do Iguaçu-Paraná: the viewpoint of health professionals. *Ciencia & saude coletiva*, 17(10), 2675.
- Cabral, C; Pita, J. R.; Salgueiro, L. (2014). *Medicinal plants: between the past and the present*. University Press.
- Campos, S. C. et al. (2016). Toxicity of plant species. *Revista Brasileira de Plantas Mediciniais*, 18, 373-382.
- Cardoso, J. C.; Oliveira, M. E. B.; Cardoso, F. C. (2019). Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. *Horticultura Brasileira*, 37(2), 124-132.
- Carvalho, J. A. M. et al. (2017). Chemical composition and evaluation of the antimicrobial activity of pink peppercorn oil (*Schinus terebinthifolius*). *Anais da V Semana de Engenharia Química UFES*, 1, 1-5.
- CLSI. (2012). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard*. CLSI document Clinical and Laboratory Standards Institute.
- Costa, A. P. M. et al. (2017). Antimicrobial resistance and the implementation of RDC 20/2011. *Úni cad acad*, 3, 1-6.
- Coutinho, H. D. M. et al. (2008). Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. and chlorpromazine. *Chemotherapy*, 54, 1250-1318.
- Cross, D. B. (2017). *Antioxidant activity and cytotoxicity of plants of the Brazilian rupestrian field: Gomphrena arborescens L.F., Gomphrena virgata Mart., Miconia ferruginata DC. and Vochysia elliptica Mart.* (Dissertação de Mestrado). Universidade Federal dos Vales do Jequitinhonha e Mucuri.
- Dodt, R. C. M. et al. (2015). Experimental study of an educational intervention to promote maternal self-efficacy in breastfeeding. *Revista Latino-americana de Enfermagem*, 23, 725-732.
- El-Seedi, H. R. et al. (2012). Anti-schistosomiasis triterpene glycoside from the Egyptian medicinal plant *Asparagus stipularis*. *Revista Brasileira de Farmacognosia*, 22, 314-318.
- Ferreira, S. L. (2017). *Phytochemistry and pharmacological evaluation of Dracaena sp.* (Dissertação de mestrado). Instituto Superior de Ciências da Saúde Egas Moniz.



- Hirota, B. C. K. et al. (2012). Toxicity assessment in vitro: applicability of the lethality test against *Artemia salina*. *Visão Acadêmica*, 13(2), 42-48.
- Houghton, P. J. et al. (2017). Uses and abuses of in vitro tests in ethnopharmacology: visualizing an elephant. *Journal of Ethnopharmacology*, 110(3), 391-400.
- Ibiapina, W. V. et al. (2014). Inserção da fitoterapia na atenção primária aos usuários do SUS. *Revista de Ciências da Saúde Nova Esperança*, 12(1), 60-70.
- João, A. A. (2015). *Evaluation of the antimicrobial activity of Baker asparagus plumosus and Asparagus africanus roots*. (Trabalho de Conclusão de Curso). Eduardo Mondlane University.
- Lacerda, G. M. et al. (2016). Modulating activity on antibiotics by the aqueous extract of the leaves of *Bauhinia unguolata* L. *Revista cubana de plantas medicinales*, 21, 309-317.
- Lima, J. L. S. et al. (2016). Identification and susceptibility of a micro-organism from a public hospital unit. *Anais da X Mostra Científica da Farmácia – UNICATÓLICA*, 1, 1-6.
- Lima, M. F. P. et al. (2015). *Staphylococcus aureus* e as infecções hospitalares–Revisão de Literatura. *Uningá Review*, 21(1).
- Lima, R. et al. (2018). Phytochemical prospection of *piper tuberculatum* jacq plant extract. (Piperaceae) and its antimicrobial potential. *Rev Electtr da FAINOR*, 11, 316-334.
- Matos, F. J. A. (2009). *Introdução a fitoquímica experimental*. UFC.
- Meyer, B. N. et al. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica*, 45(05), 31-34.
- Molina-Fajardo, L.; Gomez-Barrera, M.; Castano-Osorio, J. C. (2011). Contribution to phytochemical study of the species "sansevieria trifasciata prain" and their behavior in biological assays. *Revista de investigaciones-universidad del quindio*, 22(1), 121-128.
- Moura, W.C.S. et al. (2017). Antibacterial profile of new replaced N-acylhydrazonic derivatives. *Biofarm Magazine*, 13(4), 59-66.
- Nindo, C. I. et al. (2003). Evaluation of drying technologies for retention of physical quality and antioxidants in asparagus (*Asparagus officinalis*, L.). *LWT-Food Science and Technology*, 36(5), 507-516.
- Nonato, C. F. A. (2018). *Chemical characterization and biological evaluations of the pulp of mauritia flexuosa L. f. (Arecaceae) of Cariri from Ceará*. (Dissertação de Mestrado). Federal Rural University of Pernambuco.
- Perna, T. A.; Ferreira, A. P. N. L. (2014). Revisão bibliométrica sobre o cultivo de plantas medicinais em quintais urbanos em diferentes regiões do Brasil (2009-2012). *Journal of Health Sciences*, 16(1).
- Pindel, A. (2017). Regeneration capacity of *Asparagus setaceus* (Kunth) Jessop 'pyramidalis' in



- in vitro cultures. *Acta Scientiarum Polonorum Hortorum Cultus*, 16(1), 85-93.
- Ribeiro, D. A. (2018). *Variability of chemical composition and biological activities of *Secondatia floribunda* A.DC. as a function of seasonality and in different phenolic phases*. (Dissertação de Mestrado). Federal Rural University of Pernambuco.
- Santos, F. P.; Fukushima, A. R.; Fávero, O. A. (2015). Verificação da ocorrência de plantas com potencial de toxicidade nos jardins do campus Mooca da Universidade São Judas Tadeu (São Paulo/SP). *Periódico Eletrônico Fórum Ambiental da Alta Paulista*, 11(8).
- Seukep, A. J. et al. (2020). Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *Journal of pharmaceutical analysis*, 10(4), 277-290.
- Silva, J. M. D. et al. (2020). Use of herbal medicines in dentistry: integrative review. *Research Soci and Devel*, 9, 1-17.
- Simões, C. M. O. et al. (2010). *Pharmacognosy from plant to drug*. Editor of UFSC.
- Sousa, A. T. L. et al. (2016). Evaluation of the antibacterial activity of methanolic and hexanic extracts of puff pastry stem, *Melissa officinalis* L. *Revista Ciencias de la Salud*, 14(02), 201-210.
- Stangarlin, J. R. et al. (2011). The plant defense against pathogens. *Scient Agrar Paran*, 10, 18-46.
- Tian, F. et al. (2009). Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*: The polarity affects the bioactivities. *Food chemistry*, 113(1), 173-179.
- Tintino, S. R. et al. (2013). Modulatory activity of ethanol and hexane extracts of *Costus cf. arabicus* roots on antimicrobial drugs. *Revista Brasileira de Biociências*, 11, 157-162.
- Valli, M.; Bolzani, V. S. (2019). Natural products: perspectives and challenges for use of Brazilian plant species in the bioeconomy. *Anais da Academia Brasileira de Ciências*, 91, e20190208.
- Zhong, C. et al. (2015). Antihepatic fibrosis effect of active components isolated from green asparagus (*Asparagus officinalis* L.) involves the inactivation of hepatic stellate cells. *Journal of agricultural and food chemistry*, 63(26), 6027-6034.