Antimicrobial activity and toxicity of *Abarema cochliacarpos* against oral human microorganisms and human cells

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ABSTRACT

Abarema cochliacarpos popularly known as "Barbatimão" is a tree with medicinal properties, easily found in the Atlantic Forest and the Brazilian *Cerrado*, being little studied by scientists. This study aimed to evaluate the antimicrobial activity of 4 fractions (ethanol, hexane, ethyl acetate and methanol/water) of *A. cochliacarpos* against three mixed bacterial cultures of the oral cavity, and their cytotoxicity on human red blood cells. A narrative review was carried out using *A. cochliacarpos* and Barbatimão as descriptors to identify the main compounds with biological activities in the plant. Through a narrative review, it was seen that this species has saponin alkaloids, terpenes, flavonoids, steroids and tannins being responsible for the biological activities. Antimicrobial activity was determined by measuring the halo of inhibition of microbial growth, using ethanolic fraction at concentrations of 100, 50, and 25 mg/ml, and other fractions at concentrations of 25 and 10 mg/ml. The ethanol extract showed the best performance with halos of 10.60 mm at its lowest concentration, while ethyl acetate showed the worst performance, not forming halos in the 3 concentrations tested. The minimum inhibitory concentration (MIC) was 22 mg/ml, this concentration was able to inhibit microbial growth in all samples against the 3 inoculums. In this study, Barbatimão extracts did not present toxicity in hemolytic activity. In view of this research, it was possible to observe that Barbatimão showed antimicrobial activity and no toxicity, arousing the interest of new *in vivo* studies.

Keywords: Barbatimão, Anti-Infectious, Hemolysis, phytotherapy.

INTRODUCTION

Barbatimão (*Abarema cochliacarpos* (Gomes) Barneby & J.W.Grimes) is a medium-sized species that can reach up to 8 meters in height with compound leaves, slightly yellowish flowers and grayish white seeds (Tenório et al. 2016). *A. cochliacarpos* is a species rich in compounds of medicinal interest and has several biological activities proven by the literature such as: antimicrobial, anticancer, healing. These properties can be explained by the high content of phenolics, more specifically the condensed tannins. (Aguiar 2021; Farias 2021; Salazar 2021).

The oral mucosa is considered the most contaminated region in humans, more than 750 different species of gram-positive and gram-negative microorganisms can be found there (Vesna 2018). These microorganisms can cause several pathologies to this mucosa such as dental caries, gingivitis and periodontitis. To regulate the high level of contamination in the oral cavity, several hygiene methods are used at predetermined periods. Knowing that *A. cochliacarpos* has antimicrobial potential, it is possible to evaluate its therapeutic capacity for possible treatments against microorganisms in the oral cavity (Vesna 2018; Farias et al. 2021).

Dentistry has been looking for new formulas using herbal medicines and natural products, in order to manufacture oral hygiene drugs and materials to be used in dental treatments. Interest in this area has been growing due to the low cost and low level of toxicity of medicinal plants, in addition, it brings more options of substances to control the level of contamination of the oral cavity, mainly for the low-income population (Silva et al. 2020).

This study proposes to evaluate through a

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narrative review, the existing compounds in the bark of *A. cochliacarpos* already detailed in the literature, and also to evaluate its antimicrobial potential against microorganisms of the oral cavity and its toxicity in human erythrocytes *in vitro*.

METHODOLOGY

Narrative review

The narrative review was built from the organizational chart proposed by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (Prisma) methodology, without meeting all the criteria and oriented towards a non-clinical study (Page et al. 2021). The proposal adapted from the acronym PICOT created for reviews of clinical studies for PICO was used, where the Population refers to barbatimão; Intervention on findings in secondary compounds; the Comparator species of the same family and the Composite Outcome often found in the species of the same family.

Two evaluators individually used the descriptors crossed with the Boolean operator "OR" ("*Abarema cochliacarpos* OR Barbatimão"), scientific articles published in the last 5 years (from 01/2017 to 02/2022) were searched in CAPES journals (DOAJ Directory of Open Access Journals; ROAD: Directory of Open Access Scholarly Resources; Medline Complete; Latindex; SciELO Brazil; Academic Search Premier; Wiley-Blackwell Full Collection 2013; BioOne.1; BioMedCentral Open Access; Computers & Applied Sciences Complete; BioOne Open Access Titles), in English and Portuguese. Files that were not Scientific articles from experimental research, those that were repeated, those that were not related to any of the barbatimão species, those that were not experimental studies and those that did not have phytochemical analysis were excluded. During the analysis, a third author was designated to answer questions and review the entire methodological process (Figure 1).

Sample preparation and biological activities

Preparation of ethanolic extract, partitioning and standardization of extracts from the bark of *Abarema cochliacarpos*

To obtain the extract, 480 g of the stem barks of *A. cochliacarpos*, were provided by the Instituto de Agronomia de Pernambuco (IPA); geographic coordinates: 8°03'57" S, 34°55'29" W; Voucher Specimen Number: 192889; Herbarium: IPA – Dárdano de Andrade Lima; Identified by: F. Gallindo. The barks were dried in an oven at 40.0 ºC, then ground in a knife mill. To extract the constituents, 200 g of *A. cochliacarpos* trunk bark was ground, inserted in 2 l of 99% ethanol and kept in the solvent for 7 days. The solution obtained was filtered and concentrated in a rotary evaporator, then lyophilized, thus obtaining 33.75 g the crude ethanolic extract.

To partition the extract, solvents of different polarities were used, methanol/water, hexane and ethyl acetate in a 1:1 proportion, and the products obtained were rotary evaporator concentrated in under reduced pressure, obtaining 1.65 g of hexane extract, 1.34 g of ethyl-acetate extract, and 6.89 g of methanol/water extract.

Biological Activities

In order to use extracts of *A. cochliacarpos* and other plant samples with biological perspectives, it is necessary to carry out specific tests to map the secondary metabolites which are responsible for their biological properties, thus justifying its empirical use by the population. Tests such as those for hemolytic and antimicrobial activity are tests indicated in the literature, with the aim of quantifying the levels of toxicity in prokaryotes and eukaryotes (Salehi 2020).

Collection of samples of microorganisms from the oral cavity

Mixed cultures were obtained from the dental surface and dorsum of the tongue of three healthy volunteers, previously selected within the eligibility criteria approved by the Ethics Committee in Research with Human Beings (CAAE: 11397219.4.0000.5207). For this collection, sterilized swabs were used, offering no risk to volunteers. The collected material was inoculated into Brain Heart Infusion Broth (BHI) and incubated at 37.0 °C for 24 h (Chierrito et al. 2019).

Antimicrobial activity

The modified disc diffusion method according to NCCLS (National Committee for Clinical Laboratory Standards, 2003) in document M2-A8 (2003) was used. The microorganisms used were named by acronyms, as follow: CO1 (individual 1's oral cavity), CO2 (individual 2's oral cavity), and CO3 (individual's oral cavity 3). The extracts were solubilized in sterilized distilled water in the following proportions: ethanol: 100, 50, and 25 mg/ml; and its fractions 25 and 10 mg/ml.

The microorganisms were standardized in a spectrophotometer at 600 nm, which is equivalent to the number 5 standard of the Mcfarland scale, giving a final inoculum concentration of 1- $2x10^8$ CFU/ml in 1 ml of BHI broth. 20 ml of Mueller Hinton Agar were poured in Petri dishes, and the standardized microorganisms were spread evenly throughout the surface. Wells were perforated with a 6 mm diameter matrix and 30 µl of the extracts in their different

concentrations were inoculated into each well. The plates were incubated in a microbiology incubator at 37.0 ºC for 24 h. For the positive control, two solutions were used, 2% chlorhexidine and colgate plax, from the commercial producer Colgate. The reading of the inhibition halos was performed with the aid of a caliper and the statistical results were obtained through mathematical calculations of standard deviation.

Determination of the minimum inhibitory concentration (MIC)

To determine the MIC, it was used the broth microdilution method (NCCLS, 2003), using 96-well microplates. The microplates were prepared by dispensing 100 µl of each sample concentration, 90 µl of BHI broth and 10 µl of the standardized inoculum at 1-2x10^8 CFU/ml into each well. 2% chlorhexidine and colgate plax were used as positive controls. For the negative control, BHI broth with the standardized inoculum was used. The microplates were incubated at 35.0 °C for 24 h. After the incubation period, the reading was performed in a spectrophotometer for microplates. The qualitative result was induced by adding 30 µl of resazurin in an aqueous solution at a concentration of 0.02% to the wells and returned to the bacteriological oven at 37.0 °C for another 2 h. The tests were performed in triplicate.

Determination of the minimum bactericidal concentration (MBC)

To confirm bacterial death (CBM), 50 µl were removed from wells where no viable bacterial cells were identified during the MIC assay, and subculture was performed on Müeller Hinton Agar and incubated for 24 h at 37.0 °C. (Andrews 2001).

Hemolytic activity assay

The hemolytic activity assay was performed according to the methodology recommended by Oliveira et al. (2012) with adaptations. Human erythrocytes from the O blood system were used. After collection, erythrocytes were resuspended in 0.9% saline solution three times. A 1% solution of red blood cells was obtained by using 0.9% NaCl. The extract was solubilized in saline solution (NaCl 0.9%) at concentrations of 250, 500, 750, and 1000 mg/ml. 1.1 ml of the 1% red blood cell solution and 0.4 ml of extracts at different concentrations were added to tubes. For the positive and negative controls, 1% triton X and 1% red blood cell solution were used, respectively. The tubes were left to rest at room temperature for 1 h and then centrifuged at 1500 *x*g for 5 min. The supernatant obtained was read at 540 nm in a spectrophotometer. The entire experiment was performed in triplicate and the results expressed as a percentage of the degree of hemolysis compared to the absorbance of the positive control, applying the following formula:

$$\text{Hemolytic activity \%} = \frac{(As - Ab)}{(Ac - Ab)} \times 100$$

Where (Ab) was the absorbance of the negative control, (Ac) was the absorbance of the positive control and (As) was the absorbance of the 1% red blood cell solution in the presence of extracts at different concentrations. Each experiment was performed in triplicate and the results are expressed as the mean ± SD (standard deviation).

RESULTS AND DISCUSSION

Initially, a narrative review was built that followed part of the parameters found in the methodology proposed in PRISMA, without meeting all the criteria and oriented towards a non-clinical study (Page et al. 2021). Was carried out along the lines proposed by the PRISMA, in order to identify the secondary compounds existing in the "barbatimão" species. The descriptors for the search were "*Abarema cochliacarpos* and Barbatimão", the databases identified 558 initial articles that were treated as shown in Figure 1.

During the narrative literature review, it became clear that the plant popularly known as "barbatimão" is represented by two distinct genera of the Fabaceae Lindl. family (Moreira et al. 2018). Following the criteria adopted in this research, seven articles were selected to compose the literature review about the secondary compounds identified in research on "barbatimão" (Table 1).

Aguiar et al. (2021) in their phytochemical screening and HPLC analysis of the ethanolic extract of *S. adstringens* (Mart.) Coville suggested the presence of tannins. Quantitative phytochemical analyzes showed that the tannin content was 158.13 mg, similar to the results obtained by Gomes et al. (2021) who phytochemically analyzed *S. pulcherrimum* (Willd.) Hochr. finding that the total content of quantified condensed tannins in the ethanol extract was 128.87 mg.

Alcohols, terpenoids, phenolic derivatives, lipids, carotenoid-like compounds, alkaloids, flavonoids, polyketides, and glycerophospholipids were found in the ethyl acetate extract of *A. cochliacarpos* (Gomes) Barneby & J.W. Grimes after HPLC/HR-MS evaluation performed by Farias et al. (2021). This result differs from the findings of Salazar et al. (2021) who used the ethyl acetate extract of the species *S. rotundifolium* Mart. and showed phytochemically through HPLC the presence of secondary metabolites such as polyphenols, flavonoids, leucoanthocyanidins,

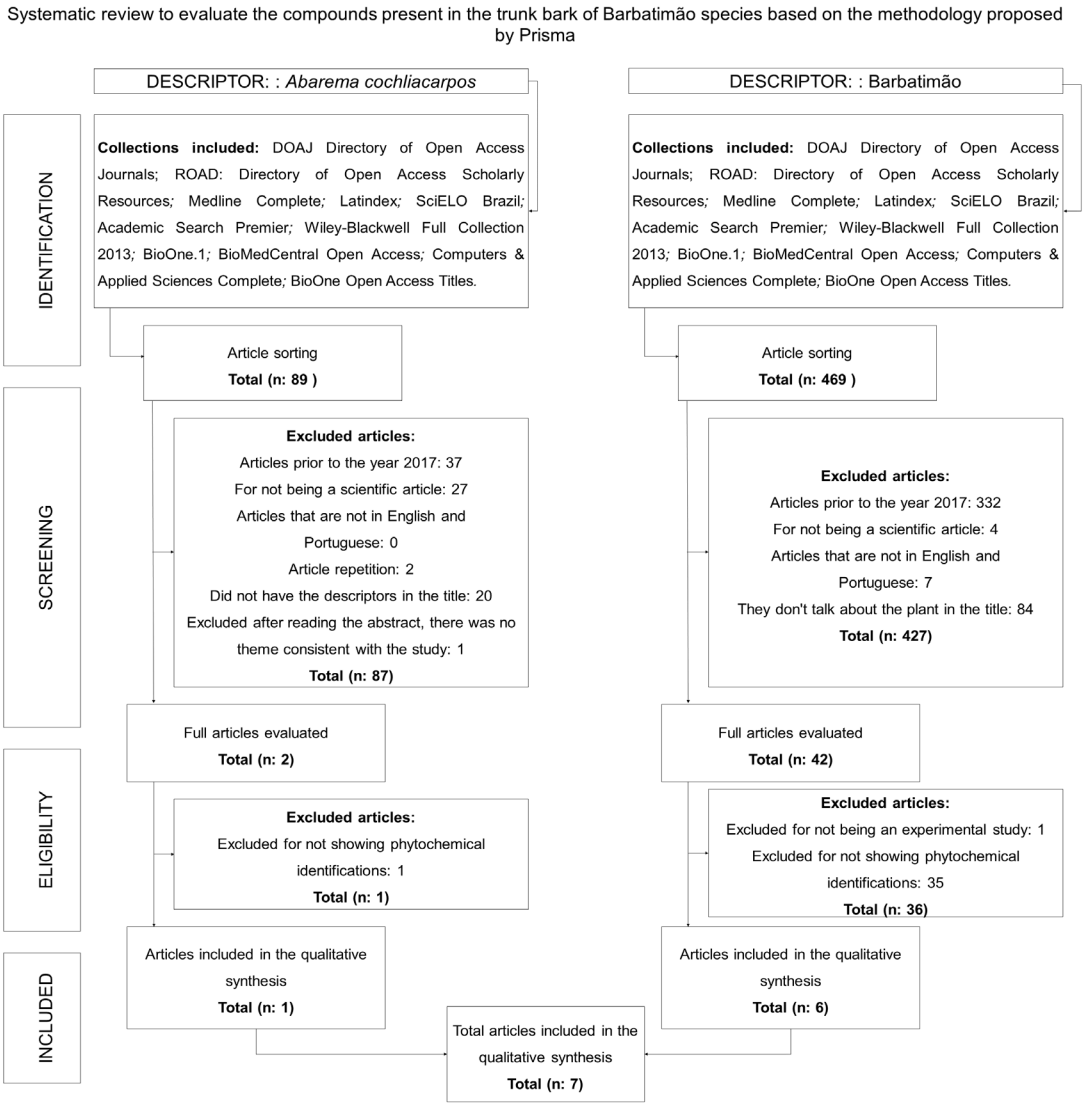

Figure 1. Flowchart of the selection of studies according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) using the descriptors *Abarema cochliacarpos* OR Barbatimão.

aurones, chalcones, catechins and hydrolysable and condensed tannins.

In the selected studies, it can be observed that gallic acid and different types of catechins are quite common in the species, both compounds have relevant antioxidant potential, as well as antimicrobial potential. Toledo et al. (2021), in their study, showed that flavonoids and tannins present in plants are hydroxylated phenolic substances with proven antimicrobial activities. Catechins (components of the condensable tannin class) are the main compounds due to their antimicrobial activity in addition to their antioxidant properties (Cavalcante et al. 2020).

The ethanolic extract of the trunk bark of *A. cochliacarpos* was able to inhibit the growth of the three mixed cultures of the oral cavity used in this study, in the three concentrations tested. The most expressive results were at the concentration of 100 mg/ml (Table 2), which is similar to the results obtained by Almeida et al. (2017), who evaluated the inhibition halos obtained through the ethanolic extract of *S. adstringens* against isolated strains of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) obtaining better results at higher concentrations (600 µl/ml).

In vitro antimicrobial activity assays of extracts (ethanolic, hexane, ethyl acetate and methanol/H_2O) from the trunk bark of *A. cochliacarpos* showed inhibition against CO1, CO2, and CO3 at all concentrations tested, except for the ethyl-acetate fraction, which did not show activity against CO3 (25 and 10 mg/ml) and CO2 (10 mg/ml). The hexane fraction showed antimicrobial activity at

Table 1. Synthesis of the articles included in the narrative review and the compounds found by the different species of "Barbatimão".

Authors/year	Species	Analysis technique	Compounds found
FARIAS, 2021	*Abarema cochliacarpos*	HPLC	(3*R*,7*R*)-1,3,7-Octanetriol; (8'*R*)-Neochrome; 1'-Acetoxyeugenol acetate; 2,6-Diamino-7-hydroxyazelaic acid; 22-Acetylperiverogenin B; 3β-(3-Methylbutanoyloxy)villanovane-13α,17-diol; 5α-Cholane; 7-(Methylthio)heptanenitrile; 7',8'-Dihydro-8'-hydroxycitraniaxanthin; All-*trans*-heptaprenyl diphosphate; Buxamine E; Caffeoylcycloartenol; DG(12:0/17:2(9Z,12Z)/0:0); DG (13:0/20:5(5Z,8Z,11Z,14Z,17Z)/0:0); Dulcoside A; Evocarpine; Germanicol cinnamate; Gingerglycolipid C; Lycoperoside D; Notoginsenoside R10; Octadecylphosphocholine; PA(16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)); PA(P-20:0/19:1(9Z)); Pandamine; Phenethyl rutinoside; PI(20:4(5Z,8Z,11Z,14Z)/0:0); Rebaudioside F; Solacapine; TG(18:4(6Z,9Z,12Z,15Z)/18:4(6Z,9Z,12Z,15Z)/20: 5(5Z,8Z,11Z,14Z,17Z)); Yiamoloside B.
SALAZAR, 2021	*Stryphnodendron rotundifolium* Mart.	HPLC	Unknown; 4'-O-methyl-epigallocatechin-(4→8)-Epigallocatechin; Gallic acid[3]; Epigallocatechin-(4→8) epigallocatechin-(4→8)-epigallocatechin; 4'-*O*-methyl-epigallocatechin-3-*O*-gallate-(4→6)-epigallocatechin-3-*O*-gallate; Epigallocatechin-(4β→6)-epigallocatechin;Epigallocatechin-(4→8)-epigallocatechin-(4→8)-epigallocatechin-3-*O*-gallate; Epigallocatechin; Epigallocatechin-(4→8)-epigallocatechin-3-*O*-gallate/ Epigallocatechin-3-*O*-gallate-(4→8)-epigallocatechin; Robinetinidol-4'-*O*-methyl-(4→8)-epigallocatechin; C-hexosyl-*O*-pentosyl-5,7- dihydroxychromone isomer; Procyanidin/Prodelphinidin type B.
AGUIAR, 2021	*Stryphnodendron adstringens*	HPLC	Gallic acid; Caffeic acid; Rutin.
JUNIOR, 2020	*Stryphnodendron adstringens*	HPLC	Sucrose; Prodelphinidin B5; Gallic acid; Protocatechuic acid; (epi)Gallocatechin; (epi)Gallocatechin methyl ether; (epi) Gallocatechin gallate; (epi)Gallocatechin-(epi)gallocatechin-*O*-(hydroxybenzoate); (epi)Gallocatechin-(epi)gallocatechin-*O*-gallate; Myricitrin; (epi)gallocatechin-*O*-vanillate; Quercetin-hexoside; (epi)Gallocatechin-*O*-(di-*O*-methylgallate); Myricetin; Methoxyapigenin (Chrysoeriol); Phloretin-glucoside; Kaempferol; Naringenin; Dimethoxy-trihydroxyflavone; Dihydroxycoumarin.
PELLENZ, 2018	*Stryphnodendron adstringens*	HPLC	Gallic Acid; Catechin; Epigallocatechin Gallate; Caffeic Acid; Quercetin; Kaempferol.
GOMES, 2021	SA: *Stryphnodendron adstringens;* SP: *Stryphnodendron polyphyllum;* SR: *Stryphnodendron rotundifolium.*	LC-HRMS.	Gallic acid: SA, SP, SR; catechin: SA, SR; Procyanidin-Prodelphinidin dimer (B type): SA.
OLIVEIRA, 2018	*Stryphnodendron rotundifolium*	HPLC	Gallic acid; Catechin; Caffeic acid; Rutin; Kaempferol.

concentrations of 25 and 10 mg/ml, in contrast to Tenório et al. (2016) who did not obtain good results with the hexane extract of the same species. The authors results showed no antimicrobial activity at concentrations 12.5 and 6.25 mg/ml while the other extracts showed inhibition at all concentrations tested.

In the analysis of variance through ANOVA, there were differences between the action of the extracts (p<0.05) concluding that there is a significant difference between the variables at a significance level of 5%.

The MIC of extracts from the trunk bark of *A. cochliacarpos* was determined at 25 mg/ml, this concentration was able to inhibit the development of microorganisms in all extracts when exposed to the three mixed cultures of the oral cavity. In 2021, Gomes et al. (2021) worked with the species *S. pulcherrimum* from "barbatimão" obtaining higher values for the MIC as follow: 100 mg/ml for gram

Table 2. Microbial inhibition halos (mm), minimum inhibitory concentration (MIC) and antimicrobial potential of extracts from the trunk of *Abarema cochliacarpos* against CO1, CO2, and CO3.

Extract	Halo Concentration (mg/ml)	CO1		CO2		CO3	
		Halo (Mean ± SD)	MIC (µg/ml)/ AP	Halo (Mean ± SD)	MIC (µg/ml)/ AP	Halos (Mean ± SD)	MIC (µg/ml)/ AP
Ethanol	100	16.778 ± 1.175	12.5 bacteriostatic	14.556 ± 0.768	25 bactericide	14.222 ± 1.938	12.5 bacteriostatic
	50	14.444 ± 1.130		12.333 ± 0.866		11.777 ± 1.460	
	25	13.688 ± 1.486		10.944 ± 0.583		10.600 ± 1.397	
Hexane	25	13.611 ± 1.932	25 bacteriostatic	9.666 ± 0.707	25 bacteriostatic	9.611 ± 0.601	25 bacteriostatic
	10	10.944 ± 1.333		7.666 ± 0.353		9.667 ± 0.560	
Ethyl Acetate	25	11.444 ± 1.014	25 bactericide	9.333 ± 0.661	25 bactericide	-	25 bactericide
	10	9.778 ± 0.939		-		-	
Methanol/H_2O	25	13.611 ± 1.616	12.5 bacteriostatic	10.722 ± 0.565	25 bacteriostatic	10.777 ± 1.325	12.5 bacteriostatic
	10	11.722 ± 1.064		9.333 ± 0.56		9.722 ± 1.523	
Controls	c+	23.000 ± 1.803	bactericide	19.000 ± 0.000	bactericide	25.000 ± 0.000	bactericide
	c++	11.417 ± 0.665	bactericide	10.000 ± 0.000	bactericide	10.500 ± 0.707	bactericide

Legend: Conc.= Concentration; MD= Mean reading on microplate spectrophotometer; SD= Standard Deviation; AP= Antimicrobial Potential; c+ = positive control with chlorhexidine; c++= positive control with Colgate Plax ®.

positive bacteria (*Staphylococcus aureus*) and 900 mg/ml for Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Since our study used a pool of microorganisms (mixed culture), the lower MIC found using *A. cochliacarpos* suggests better effectiveness than *S. pulcherrimum* used in the aforementioned study.

The ethanol extract when exposed to sample CO2 showed a bactericidal potential, and when tested on samples CO1 and CO3 it exhibited a bacteriostatic potential; the hexane and methanol/H_2O extracts presented bacteriostatic potential against the three mixed cultures of the oral cavity. The ethyl acetate extract showed bactericidal potential against CO1, CO2, and CO3. Santos et al. (2021) obtained bacteriostatic and fungistatic potential using the ethanolic extract of *S. barbatimam* Mart. when exposed to 15 mixed oral cavity cultures and 10 gram-positive and gram-negative ATCCs bacteria.

Baldivia et al. (2018) demonstrated that *S. adstringens* throughout the experimental period did not show hemolytic activity in human erythrocytes at any of the concentrations tested, a result that was similar to our study using the species *A. cochliacarpos*, where all the concentrations tested were not able to lyse the cells.

Based on the results obtained in the narrative review, *A. cochliacarpos* extracts were not identified as surfactant compounds, which may explain the absence of cellular hemolysis. According to Xavier (2021), the hemolytic action of the compounds is attributed to non-specific mechanisms such as surfactants, which produce their hemolytic effect by solubilizing the erythrocyte plasma membrane.

CONCLUSION

The extracts from the bark of *A. cochliacarpos* showed antimicrobial potential and low toxicity to human cells, which is justified by the high presence of secondary compounds already described in the literature that act in these biological activities. The low toxicity has the potential to stimulate and direct the development of new studies for the formulation of drugs for the treatment of several pathologies of microbial origin.

Table 3. Hemolytic activity using human erythrocytes exposed to extracts of *Abarema cochliacarpos* at different concentrations.

Extract	Conc. (µg/ml)	Mean ± SD	% hemolysis
Ethanol	1000	-0.152 ± 0.002	33.660
	750	-0.092 ± 0.001	43.504
	500	-0.078 ± 0.001	45.925
	250	-0.040 ± 0.023	53.208
Hexane	1000	-0.159 ± 0.009	32.659
	750	-0.137 ± 0.000	36.018
	500	-0.110 ± 0.003	40.324
	250	-0.085 ± 0.001	44.675
Ethyl-Acetate	1000	-0.157 ± 0.003	32.958
	750	-0.125 ± 0.003	37.922
	500	-0.098 ± 0.005	42.466
	250	-0.105 ± 0.004	41.162
Methanol/H_2O	1000	-0.174 ± 0.004	30.463
	750	-0.141 ± 0.002	35.446
	500	-0.108 ± 0.005	40.658
	250	-0.099 ± 0.004	42.238
Controls	-	-0.045±0.000	
	+	0.723±0.019	

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AUTHORS' CONTRIBUTIONS

The authors equally contributed to the manuscript.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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