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RESEARCH ARTICLE

EVALUATION OF ANTIBIOFILM ACTIVITY OF THE LATEX EXTRACTED FROM *Himatanthus drasticus* (Mart.) Plumel (JANAGUBA)

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Abstract

The secondary metabolites present in plants often have antibiofilm activity, this property attributed to plant compounds is of great importance, given the formation of biofilms in biomaterials, which further boosts the search for natural substances capable of minimizing this type of formation. *Himatanthus drasticus* (Mart.) Plumel is a tree species popularly known as janaguba, this is found in areas of caatinga, cerrado, rupestrian fields and rainforest. Community empirical knowledge attributes to this plant species therapeutic action against tumors, hemorrhoids, respiratory tract infections, digestive tract and urogenital tract. Considering the reduced number of scientific findings on the biological activities of *H. drasticus* latex, the objective of the present study was to verify the antibiofilm activity of its latex. The latex was extracted from *H. drasticus* specimens, where a small area of the vegetal bark (5x30cm) was removed, later the latex was dissolved in water and lyophilized. For the microbiological assays, the ATCC standards of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were cultured in BHI broth for 24 hours at 37 °C. The assay was performed with flat-bottomed 96 well plates, and the latex extracted from *H. drasticus* and bacterial suspensions were added to the BHI broth. Negative Control (NC) was used as broth BHI and Positive Control (PC), the PA01 strain of *P. aeruginosa* as this strain is indicated as a positive control for biofilm assays. The plates were then incubated at 37 °C for 24 hours. After this period, the absorbance was read in an

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ELISA reader with wavelength of 570 nm, then the samples were classified. The antibiofilm activity of the latex extracted from *H. drasticus* was classified as non-adherent on the species *P. aeruginosa* and *S. aureus*. Thus, the study revealed that *H. drasticus* latex has a potential antibiofilm action, however, additional studies should be carried out in order to evaluate other aspects associated with the biological activities performed by this plant species.

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Introduction:-

The formation of biofilms in biomaterials such as orthopedic prostheses, catheters, dental implants and probes, has been increasing over the years. These biomaterials are susceptible to microbial colonization and favor the development of infections, characterizing as an important source of transmission of resistant pathogens in hospitals and clinics. In order to minimize the formation of biofilms, several researches have been developed to identify substances capable of preventing the colonization and proliferation of these microorganisms (ARCIOLA et al., 2012; LIM et al., 2013).

Against this, microbicidal substances derived from medicinal plants have been tested for their applicability under the formation of bacterial biofilms. These, in turn, should be carefully analyzed, to be used safely in the development of new products. It is also worth noting that mechanisms of action that combine antibiofilm agents with conventional antibiotic therapy are primordial for the control of Healthcare Associated Infections – HAIs (CEGELSKI et al., 2008; BELOIN et al., 2014).

Himatanthus drasticus (Mart.) Plumel is a tree species, with dense foliage at the extremities of the branches, located only in Brazilian territory. It has as characteristics a milky latex that is commonly used in the treatment of cancer in some regions of the Northeast. According to Plumel (1991) and Soares et al. (2015), the popular names of this species are distinguished according to the regions, among the denominations are: tiborna, raivosa e jasmim-manga (in the Southeast), janaguba or pau-de-leite (in the Northeast) and sucuuba (in the North).

In Ceará, this species occurs with greater distribution in the Chapada do Araripe, its use predominates among the communities of the state, for anti-inflammatory purposes, in addition, several structures of the plant are used, in addition to latex, with therapeutic purpose, such as leaves and the shell (KAPLAN, 1967).

The biological activity is in full investigation, since its medicinal use is consolidated in several regions of Brazil for the treatment of several diseases, especially in the fight against cancer cells. Its extracts also act in the fight against infections of the respiratory tract, digestive tract, urogenital tract and hemorrhoids (KAPLAN, 1967), also revealing bactericidal, fungicidal, antiviral, analgesic and antiallergic activities (PATOCKA, 2003).

Therefore, the present study had as objective to evaluate the antibiofilm activity of latex extracted from *H. drasticus*.

Methodology:-

Latex was collected from specimens of *H. drasticus*, in the Araripe - Apodi National Forest, state of Ceará, Brazil. The botanical material was identified in Herbarium Dárdano de Andrade - Lima, IPA (Instituto Agronômico de Pernambuco, Recife, Brazil). The collection was done by cutting a small area (5x30cm) of bark from the trunk of the plant (LUCETTI, 2010). An exsicata of the plant was directed to the Herbarium of the IPA in Recife - PE, receiving the code 92.408.

The technique performed for the study was described by Stepanović et al. (2000), with some modifications. Isolates of *Pseudomonas aeruginosa* ATCC 278553 and *Staphylococcus aureus* ATCC 29213 were grown in BHI broth for 24 hours at 37 °C. Then, the bacterial suspensions were applied to polystyrene plates containing flat bottom 96 wells for microtiter, in triplicate, and BHI broth and latex extracted from *H. drasticus* at a concentration of 50 mg/mL were added. The BHI broth and Positive Control (PC), the PA01 strain of *P. aeruginosa*, were used as Negative Control (NC), since this strain is established as a positive control for biofilm assays.

The plates were then incubated at 37 °C for 24 hours, after which time the bacterial suspensions were removed and each well was washed three times with 250 µL of sterile physiological solution (0.9% NaCl). Subsequently, fixation was performed with 200 µL of methanol P.A. for 15 minutes, after which the methanol was removed, the plates were left at room temperature to dry and stained with 200 µL of crystal violet solution for five minutes. The plates were washed with running water and dried at room temperature, after which the absorbance reading was performed on an ELISA reader (BioRad, model 550), wavelength 570 nm and the samples were classified according to Stepanović et al. (2000).

The value of the Optical Densities for each isolate (DOi) was obtained from the mean of the three wells, this value being compared to the Optical Density of the negative control (DOc). The isolates were classified into four categories according to the mean of the Optical Densities (Dos) related to the DOc results. The categories were based on the following criteria: non-adherent: DOi ≤ DOc; weakly adherent (+): DOc <DOi ≤ 2xDOc; moderately adherent (++): 2x ODc <ODD ≤ 4xDOc or strongly adherent (+++): 4x ODc <DOi.

Results and Discussion:-

Considering the classification criteria established by Stepanović et al. (2000), the antibiofilm activity of latex extracted from *H. drasticus* was classified as non-adherent on *P. aeruginosa* and *S. aureus* species (Table 1).

Table 1:-Antibiofilm activity of latex extracted from *H. drasticus*.

Bacterial species	Latex antibiotic activity
P. aeruginosa	Non-stick
S. aureus	Non-stick

The data obtained are considered satisfactory, since *P. aeruginosa* is reported as a versatile Gram-negative bacterium and commonly associated with Healthcare-associated Infections (HAIs). This microorganism easily adheres to several types of biomaterials; in addition, due to virulence factors and high resistance to antibiotics, its proliferation is difficult to control (STREETER and KATOULI, 2016).

According to Tintino et al. (2013), *P. aeruginosa* is highly efficient in the formation of biofilms, a property used for protection of host defense and antibiotic action. As for the activity of *S. aureus*, this species resembles *P. aeruginosa* due to its capacity to form biofilms and its relation with HAIs. However, it behaves opportunistically and may cause, in more severe situations, bacteremia, pneumonia, osteomyelitis, meningitis and cardiac inflammation (endocarditis, myocarditis and pericarditis). This microorganism has potentially increased its resistance to antibiotic therapy, making its control increasingly complex (PEREIRA et al., 2004, TRABULSI and ALTERTHUM, 2009).

Trentin, Giordani and Macedo (2014) reported that the development of new strategies that prevent the formation of biofilm has received notoriety, especially in biomaterials, since the antibiofilm action can occur by blocking bacterial cell adhesion under a certain surface or through of the breakdown of bacterial cell communication (Quorum Sensing - QS).

Several studies have described the action performed by *P. aeruginosa* and *S. aureus* on the formation of biofilms. According to Grenho (2012), after evaluating the adhesion of *P. aeruginosa* and *S. aureus* on nanohydroxyapatite and zinc oxide (nanoHA-ZnO), it was verified that the bioamaterials used showed efficiency against the reduction of microbial growth and also in the constitution of biofilm.

Leite (2008) verified the colonization of bacteria and the formation of biofilms on both surfaces (polypropylene and polyurethane), strengthening the properties of *P. aeruginosa* and *S. aureus* under the formation of biofilm in facial dermosion wires. Bacteria of adhesion in the formation of biofilm in different types of materials, in addition, it was evidenced the importance of new studies that elucidate the viability of the antiaggregation of these bacteria against biomaterials in order to reduce the formation of biofilms.

A recent study by Moura (2016) revealed the antibacterial activity of latex extracted from *H. drasticus* against *P. aeruginosa*, *Acinetobacter baumannii* ATCC 19606, *Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 25922, *Salmonella* sp. ATCC 14028 and *Shigella* sp. ATCC 12022 and Gram-positive *S. aureus*, *Kocuria rhizophila*

ATCC 9341, Methicillin-resistant *Staphylococcus aureus* (MRSA) from wounds and *Streptococcus pyogenes* (clinical strain), corroborating with the present results in view, the function of latex inhibit bacterial growth.

In other findings, the properties of latex of *H. drasticus* were also presented, revealing functions: anti-inflammatory, antibacterial and antifungal due to the presence of triterpene, secondary metabolite (PATOCKA, 2003; SPARG et al., 2004; ZHANG et al., 2008; POPOVA et al., 2009; SAEIDNIA, et al., 2014; CARMO, 2015; MOURA, 2016).

The antibiofilm action developed by the latex *H. drasticus*, can be attributed to its chemical composition. According to Moura (2016) the species *H. drasticus* is constituted among other components by terpenes (monoterpernes, sesquiterpenes and triterpenes).

Borowski (2015), investigated the antibiofilm capacity of isolates of the *Capsicum baccatum* seed extract, found that these isolates inhibited the formation of biofilms in 60% and 80% for *P. aeruginosa* and *S. epidermidis*, respectively, due to possible functional activity by terpenes.

Evaristo et al. (2015) also evaluated the antimicrobial activity and antibiofilm activity of a triterpene isolated from leaves *Combretum leprosum* on *Streptococcus sobrinus* and *Streptococcus sanguinis* and suggested that the isolate is a possible biotechnological input with considerable potential for the treatment of infections associated with oral bacteria.

Therefore, it is paramount to search for innovative strategies capable of preventing or minimizing the formation of biofilms, including the study of new compounds, elucidation of possible mechanisms of action and development of materials with anti-infective surfaces.

Conclusions:-

The latex of *H. drasticus* has a potential antibiofilm action against Gram-negative bacteria (*P. aeruginosa* ATCC 278553) and Gram-positive (*S. aureus* ATCC 29213), and may be a viable alternative in the development of non-stick surfaces for clinical practice. However, additional studies should be performed with the purpose of analyzing the plant species from different perspectives, considering the mechanisms of action involved in biological activity.

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