BIOGUIDED ASSAY FOR INVESTIGATING THE CYTOTOXIC POTENTIAL OF THE MARINE SPONGE *Ectyoplasia ferox*

Silva, S. M. P.[1*]; Brito, M. de F.[1]; Jimenez, P. C. [2]; Costa-Lotufo, L. V.[3]; Muricy, G.[4]; Abreu, L. S.[5]; Valverde, A. L.[1]

[1]*UFF/LaProMar, Niterói, RJ, Brasil;*
[2]*UNIFESP/BioproSP, Santos, SP, Brasil;*
[3]*USP/LAFARMAR, Butantã, SP, Brasil;*
[4]*UFRJ/MNRJ, Museu Nacional, RJ, Brasil.*
[5]*UFF/LQPN, Niterói, RJ, Brasil.*
**stephanie_montechiari@id.uff.br*

Introduction

Currently, cancer is the leading cause of death from non-communicable diseases worldwide. According to estimates from the National Cancer Institute (INCA), in the year 2020, there were 20,540 cases (9.1%) of colon and rectal cancer in men and 20,470 cases (9.2%) in women.[1] There is an ongoing effort to discover new cancer treatments, and the study of natural products has become a thriving field of research, particularly in countries like Brazil, which boasts rich biodiversity both on land and in its 7,367-kilometer-long coastline.[2] Therefore, the purpose of this study is to investigate the cytotoxic potential of the sponge *Ectyoplasia ferox* collected in the archipelago of Fernando de Noronha, Pernambuco, as well as to isolate and characterize the substances responsible for cytotoxic activity through bioguided fractionation. The project is registered in the National System for Management of Genetic Heritage (SISGEN) under the code AB724BB.

Material and Methods

First, the sample was extracted through static maceration, in which one extraction was performed with ethanol, followed by two extractions with a mixture of methanol and ethyl acetate (1:1). Next, with this extract a partition by polarity gradient was performed using the solvents hexane, ethyl acetate, butanol, and water. To purify these samples, several chromatographic techniques were employed, including thin-layer chromatography (TLC) and vacuum liquid chromatography. Pharmacognostic test were additionally carried out to categorize secondary metabolite classes in some fractions, while cytotoxicity assessments were conducted on all fractions acquired as part of the bioguided study.

Results and Discussion

Out of the six samples of *E. ferox* collected in the Fernando de Noronha archipelago, the sample obtained from the Cagarras Rasa region was identified and assigned the accession number (21314), and it has been deposited in the collection of the National Museum of Rio de Janeiro. The crude extract (FN98 017) and the fractions from the partition (F.hex P, F.act P, F.act + but P (Fact'), F.but P, and F.aq P) were evaluated for cytotoxic activity against the human colorectal cancer cell line (HCT-116) using the MTT method.[3] These samples were assessed at a concentration of 50 µg/mL and demonstrated rates of cell proliferation inhibition (Table 1).

Table 1: Cytotoxicity of partition samples.

Samples	% Inhibition
FN98-017	62.21
FN98-017 Hex P	28.73

FN98-017 Act P	19.18
FN98-017 Act + But P	27.42
FN98-017 But P	103.15
FN98-017 Aq P	38.40

The FN98-017 But P fraction was investigated due to its high inhibition rate (103.15). According to the literature, it was observed that out of the 17 substances already isolated from this species, 8 belong to the class of saponins. According to Campagnouolo *et al.*[4], the butanolic fraction of the partition is rich in saponins. To confirm this information, a qualitative foam test was conducted, where the formation of stable foam indicated the presence of saponins. Therefore, the FBut P was fractionated by Vacuum Liquid Chromatography (VLC), in a reverse-phase, following the adapted method of Lins *et al.*[5] The chromatography was carried out using the mobile phase H_2O:MeOH 80:20 to 100% MeOH. At the end of the process, 13 fractions were obtained, which were evaluated for cytotoxicity. The fractions that were eluted with a higher proportion of MeOH showed better values of cell proliferation inhibition in the cytotoxicity test on HCT-116 cells. To obtain more precise information about the presence of saponins, fraction 17-B-CLV-9 was chosen because it had one of the highest yields (17.45%) and a good rate of cell proliferation inhibition (95.72%). The fraction was analyzed by ^{1}H NMR spectroscopy. In the spectrum, characteristic singlets of methyl groups from terpenoids (δ_H between 0.6 and 1.5) were observed. The glycosidic fraction of the saponin containing three sugar residues was identified by the presence of three characteristic doublets of anomeric hydrogens. Thus, this analysis aided in the identification of the saponin, but to complete the structural elucidation, it is necessary to perform ^{13}C NMR analysis and two-dimensional analyses such as COSY, NOESY, HMBC, and HSQC.

Conclusion

Through the chemical profiling of the *E. ferox* specie, it was observed that there were no reports in the literature regarding the cytotoxic evaluation of this species on the HCT-116 cell line, making this study pioneering. It is also the first time that the class of saponins has been investigated in E. ferox collected in Brazilian territory. The bioguided approach allowed for a more targeted investigation, highlighting promising fractions and substances. Chromatographic techniques played a crucial role in the purification and identification of the substances, and the foam formation test indicated the presence of saponins, which was later confirmed by ^{1}H NMR spectroscopy analysis.

Acknowledgments

The authors would like to express appreciation for the support of the sponsors FAPERJ (APQ1 2019 E-26/210.249/2021 and CNE E-26/200.515/2023). PPG-CAPS students should include FAPERJ (E-26/210.068/2021 and E-26/210.915/2021) and CAPES (Finance Code 001).

Bibliographic References

[1] INCA. Instituto Nacional de Câncer. Estatísticas de Câncer. Disponível em: < https://www.inca.gov.br/numeros-decancer > Accessed on September 19, 2023.

[2] Marinha do Brasil. Marinha, Sudene e a Amazônia Azul, 2019. Disponível em: < https://www.marinha.mil.br/cm/marinha-sudenee-amazonia-azul > Accessed on September 20, 2023.

[3] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays', J. Immunol. Methods, 1983, 65, (55).

[4] Campagnuolo, C. O. *et al.* Feroxosides AB, two norlanostane tetraglycosides from the Caribbean sponge *Ectyoplasia ferox*, Tetrahedron, 2001, 57, (18), pp. 4049-4055.

[5] Lins, F.S.V. *et al.* New Pregnane Glycosides from *Mandevilla dardanoi* and their Anti-Inflammatory Activity, Molecules, 2022, 27, (18), pp. 5992.