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In Silico Analysis Applied to the Study of Cytotoxicity in Natural Products †

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Abstract: With the emergence of technological advances, computational analysis in research has become promising by enabling the emergence of scientific productions without the need for an experimental laboratory and began to be used in various sectors, including cytotoxicity. From this perspective, the main objective of this study is to understand the importance of computational analysis for the study of cytotoxicity in natural products, in addition to understanding scientific advances on this topic. Thus, this is a narrative-type bibliographic review, carried out between March and September 2022, through the digital databases Pubmed, SciELO and the Virtual Health Library with the adoption of the search formula configured with the available descriptors: "in silico analysis", "cytotoxic" and "natural products". This work reaffirmed the importance of computer simulations on cytotoxicity in natural products, allowing the verification that these analyzes are a source of knowledge about the structures of natural products, highlighting a great emphasis on the use of the analysis of cytotoxic agents, the anticancer action and treatment of other pathologies. With regard to effectiveness, it is clear that the software reflects results that are similar to studies carried out in vivo. It is noteworthy that the in silico method has an accentuated scientific importance as it allows for a greater adaptability and a lower cost of time and space for research. Thus, such methodology becomes essential in the process of cytotoxicity analysis, obtaining great potential in pharmacological research.

Keywords: in silico; natural products; cytotoxicity; computer simulation; biological products

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1. Introduction

The in silico study is a revolutionary method of analysis by enabling research without the need for an experimental laboratory, in this context, in silico methodologies have become essential in the process of analyzing natural products and discovering new drugs. This is because computational analysis impacts the entire drug development trajectory with a reduction in cost and time [1].

The origin of the term in silico is unclear, but nowadays, it is known that it refers to computational models responsible for investigating pharmacological and biological hypotheses using databases, analysis tools, data mining, learning machine and network analysis [2]. In silico methods are mainly used along with in vitro data generation, both to create models and to test them [3]. In this scenario, computational analysis began to be used in several research sectors. In the field of toxicity, this study provides an overview of the importance of in silico methodology in cytotoxicity in natural products and checks

whether it can be a source of knowledge about the structures of natural products and their pharmacological implications.

Several animal welfare organizations and broad social sectors speak out against the use of REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) experiments, as they cannot be produced or imported into the industry without approval from ECHA (European Chemicals Agency). The REACH regulation brought about a revolution in the regulation of chemical compounds, as the industry began to take control of the potential risk of the products it generates and their potential impact on human health [4].

The European Commission launched 1991 the European Center for the Validation of Alternative Methods (ECVAM), with the focus of finding alternative methods to reduce experiments on living beings, among them, are the in silico and the in vitro. However, the in vitro method, due to its procedures and multiple substances, can take many years to obtain reliable results, on the other hand, in silico constitutes a tool to accelerate the rate of discoveries without the use of expensive equipment and clinical trials [4].

Thus, the in silico study represents a promising alternative to chemical analysis of natural products, because it is simple and effective. In addition, computational analysis represents great importance in the evaluation of organic compounds employed in biological and pharmaceutical activities [5]. Therefore, this study aims to understand the importance of in silico analysis for understanding the cytotoxicity of substances taken from nature. For this, it is intended to acquire knowledge related to new ideas about the computational analysis of natural products and identify scientific advances from the in silico study about them.

2. Methods

It is a review of narrative-type literature, with a qualitative, exploratory, descriptive, and theoretical approach, which uses indirect documentation from secondary sources. The bibliographic research is carried out from the available record, resulting from previous research in articles.

In this way, the guiding question was "What is the importance of in silico analysis in the study of cytotoxicity in natural products?". From this perspective, the theme axis was defined as cytotoxicity focused on the in silico analysis of the study of the cytotoxicity of natural products. The content was obtained using the general formula, avoiding content bias.

The search was carried out, in March 2022, in the Pubmed and Virtual Health Library databases. For this, the following terms were used in combination: "in silico analysis", "cytotoxic" and "natural products" with the Boolean operators "AND" and "OR" to form the search formula. The time interval delimited was the last ten years, that is, from 2012 to 2022. Thus, the works were selected from reading the abstract and/or title, which should explicitly relate to the terms computational analysis and natural products.

In this sense, works published in the form of scientific articles received priority and the selected languages were Portuguese, English, and Spanish. The exclusion of articles followed the proposition that those that did not present relevant information about the central theme were excluded, and those that satisfactorily portrayed the central theme were included. Then, it was made the reading of the listed works, emphasizing the contributions of in silico analysis in the scientific development of natural products in the context of biological and/or pharmacological applications. Such reading occurred individually for each article.

After that, 189 articles resulted in Pubmed, which with the time frame of 10 years resulted in 180 articles. Subsequently, the studies were selected according to the reading of the title and abstract, totaling 12 articles; in the Virtual Health Library search engine, 37 articles were found, from the reading of titles and abstracts resulted in 2 articles. In the end, the total number of works analyzed in the research was 14 articles. The sequence elucidated above is contained in Figure 1.

As this research was based on data made publicly available in electronic media, consideration by the Research Ethics Committee was waived.

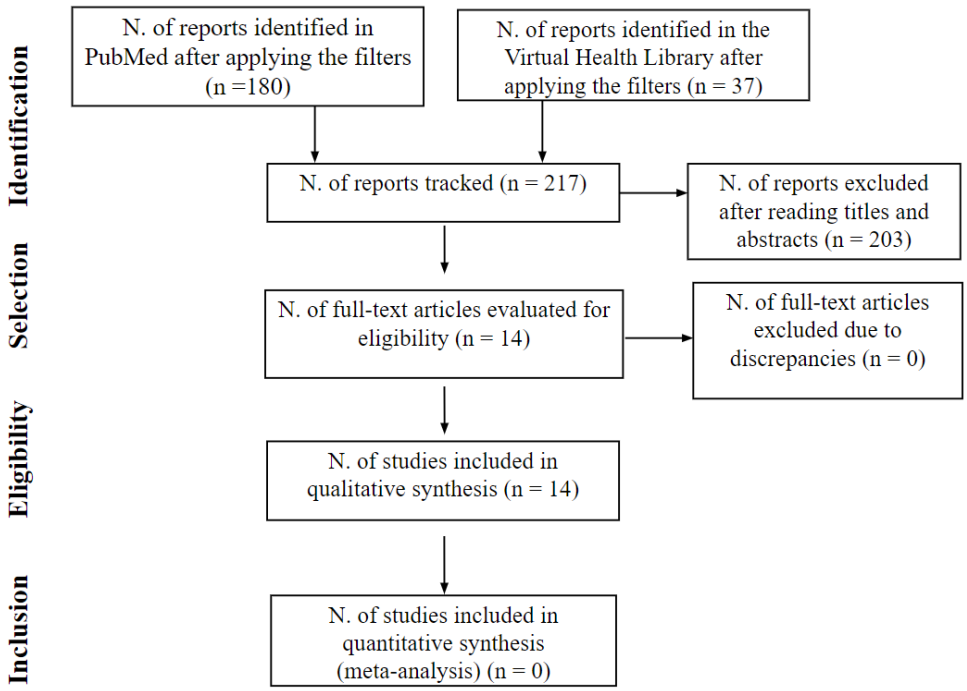

Figure 1. Flowchart of the methodology used.

3. Results and Discussion

3.1. Anticancer Action

With in silico parameters, it is possible to determine the anticancer potential of natural products. In a research carried out with a crude extract of *Coscinoderma* sp., it was found, through computational analysis, that this extract acts on two types of cancer cell lines in an antiproliferative manner: Pin-1 and SHP2 cell lines, inside liposomal vesicles; and HepG2 (liver), MCF-7 (mammary) and Caco-2 (colorectal) cell lines. Therefore, such a compound has great anticancer potential. In this analysis, predictions based on PASS (Prediction of Activity Spectra for Plants) were used to verify the anticancer effect. Therefore, this research highlights the importance of computational analysis to outline new ways to fight cancer [6].

In addition, it is known that analyzing the antitumor action of natural products with the in silico method constitutes a new tool for the production of drugs with the aim of combating tumors. From this perspective, another natural substance analyzed was solasonine. To verify the anticancer action of the compound, tests were carried out with semi-maximal inhibitory concentrations (IC50) at time intervals (every 6, 12, 24, and 48 h). The result showed that solasonine has high cytotoxic activity in HepG2 and Hep3b cell lines, however, the inhibitory potential in the 48 h interval of this natural product is around three times greater in HepG2 cell lines than in Hep3b. Thus, these two studies demonstrated the importance of algorithms in the analysis of possible drugs from natural sources [7].

In the matter of cancer metastases, computational analysis has shown to be promising in providing a broad view of the reactional behavior of natural products against the proliferation of tumor cells. In this scenario, in silico assays performed with *Heterotheca inuloides* cadnans in human uterine sarcoma cells demonstrated a high sensitizing activity to chemotherapy, which constitutes a potential to modulate drug resistance [8].

Furthermore, nowadays, there is an expansion of research with wild products. This was the case of *Heterotheca inuloides* derivatives, which sensitize resistant uterine sarcoma

cell lines (MES-SA/MX2), and potentiate the cytotoxicity of doxorubicin and mitoxantrone in these cells. One way of administering these drugs against multidrug resistance results from increasing the intracellular concentration, inhibiting the expulsion of their toxicity, caused by the overexpression of ABC membrane transport proteins. It was also tested on other resistance proteins, within which the multidrug resistance (MDR) protein was modulated by cadians [8].

From another perspective, research was carried out with the plant *Tabernaemontana catharinensis* to analyze it is in silico toxicity and antitumor activity. In that study, the indole alkaloid compounds of the plant were revealed to exhibit selective cytotoxicity towards A375 tumor cells [9]. About cancer cells, the computational study with green propolis derivatives highlighted this compound as a potential new cytotoxic inhibitor against breast tumor cells [10].

In this reasoning, the prediction of the phytocomponents of the Moringa oleifera fruit (MOF), exhibited a high binding interaction with the executioner protein caspase-3, because of these results, MOF was considered a potential anticancer therapeutic in the liver cell line (HepG2). However, the reduction in HepG2 viability depends on the amount of MOF extract, for example, doses of 50 and 75 µg/mL of MOF extract reduced cell viability to 77.12 and 46.31%, respectively, compared to the control [11].

3.2. Disease Treatment

Due to the lack of adequate diagnostic and therapeutic strategies, the prevention and treatment of some diseases remain a global challenge, on the other hand, conventional therapies are not free from complications and side effects. In this context, the in silico study appears as an alternative for the investigation of pathologies that are difficult to treat. On this subject, there is the example of the tuberous sclerosis complex genetic disease (TSC) which is caused by mutations in the TSC1 and TSC2 genes encoding the hamartin and tuberin proteins, respectively. Hamartin and tuberin form a cytosolic complex, which regulates the mammalian target of rapamycin (mTOR) in the control of cell proliferation and growth [12].

In this perspective, in silico research carried out to evaluate the cytotoxicity of asiaticoside and asiatic acid from a Malaysian plant revealed that such products can serve as inhibitors of the mTOR protein, and consequently, potential inhibitors of the genes that cause the disease TSC tuberous sclerosis complex [13]. This is one of the examples of how computational analysis can bring benefits to the health area by enabling discoveries of cellular interactions that fight diseases.

However, it is clear that in silico research can be used in conjunction with in vitro research, as one complements the other. This was the case of the analysis of the anti-hepatitis B activity of quercetin and kaempferol derivatives from the plant Euphorbia schimperi through interference with viral polymerase and capsid proteins. In this research, first an in vitro study was performed, and then a computational analysis to prove what had been observed. It was found, both in the algorithm and in the experiment, that such products have a high antiviral potential through interference with the HBV-Pol and HBV-Core proteins of the hepatitis virus [14].

In the drug development sector, computational analysis is combined with cytotoxicity bioassays to verify the relevance of plant extracts in the treatment of diseases. In this perspective, research was carried out with the PASS program that presents two parameters, probable activity (Pa) and probable inactivity (Pi), used to evaluate the biological activity of five natural products (nyasicoside, glucomannan, grandifloric acid, serine, and alanine). As a result, glucomannan showed the highest Pa for almost all biological activity in the range of 0.331–0.804 [15].

In the anticarcinogenic parameter of the five products, serine had the highest Pa value. As for the cytotoxic assay, a lethality test with brine shrimp (LC50, 24 h) was performed. In it, brine shrimp lethality LC50 < 10 ÿg/mL (LD50 between 100 and 1000 mg/kg) is considered the cut-off value for cytotoxicity. According to the measured LC50 values of

the extracts, none were found to be severely lethal or toxic to be processed as a pharmaceutical. Therefore, in silico analysis showed that these natural products can be used as potential drugs. In this way, the contributions of computational analysis to the field of health are perceptible, especially in the treatment of diseases and the production of medicines, given that there have been significant scientific advances demonstrated in the aforementioned studies that have contributed to the search for improving the health of individuals [15].

3.3. Cytotoxic Agents

Reducing the multidrug resistance of cancer cells should significantly improve the response of cancer cells to cytotoxic agents. In this perspective, these compounds may be the main ones for the development of new cytotoxic inhibitors against tumor cells, especially breast and prostate cancer. This is the case of the research carried out with the sponge *Amphimedon sp.*, which presented, through an in silico study, a potential source of cytotoxic agents against three cell lines HEPG2 (liver), MCF7 (breast) and CACO2 (colon) through inhibition of SET oncoprotein present in cancer progression. This result comes from the docking study results that revealed amphiceramides A-B and acetamidoglucosyl ceramide showed the highest energy binding affinities and interaction in the binding site of SET protein [16].

Furthermore, other natural compounds analyzed with the computational method were three Sabal species cultivated in Egypt, *S. causiarum*, *S. palmetto,* and *S. yapa*. In this research, the cytotoxic potential of such products was evidenced through software based on neural networks using the method of prediction of activity spectra for substances—PASS. This algorithm relies on structural similarity between the query compounds and other reported inhibitors of a wide range of biological targets [17].

In the Sabal survey, resulting activity scores (Pa) of 0.5 or more indicate a high probability of experimental activity. Among the compounds isolated from Sabal species, tangeretin was the only one that presented itself as a cytotoxic agent in the A172 group (Pa: 0.824), due to its high probability of inhibiting RAF kinase, which induces glioblastoma in humans. Likewise, the compounds vitexin and diosmetin were considered cytotoxic in the PC-3 group (Pa: 0.737 and 0.851, respectively), in addition to presenting a high probability of kinase inhibition (Pa: 0.76 and 0.922, respectively). Thus, in silico predictions showed that there is a high cytotoxic potential of Sabal extracts against A172 and PC-3 cell lines [17].

Furthermore, it is worth emphasizing the importance of in silico studies on the oral use of essential oils and their toxicity through chemical composition analysis [18]. One of them demonstrated that there is a high cytotoxic relevance in essential oils derived from *Bignonia nocturna*. In this research, docking simulations were performed to investigate whether benzaldehyde, a plant-derived compound, can interact with the protein acetylcholinesterase (AChE) and it was found that there is a high affinity between the compound and the enzyme [19]. This fact is relevant, as this protein has a strong importance in the functioning of the cholinergic synapses of the central and peripheral nervous system, which makes it an attractive target for the development of new drugs [20]. Regarding the consumption of essential oils, it was observed that toxicity depends on the amount consumed and that to define whether it will affect the consumer, it is necessary to have a deep knowledge of its chemical structure [18].

In this way, it is clear that the advancement of digital technology is proportional to the advancement of scientific techniques. Therefore, when there is a combination of software and methods, the in silico activity and the research itself are improved, as there is an increase in the speed and ease of the processes. Another advantage of computational analysis is the existence of a standard database, which is the in silico fragmentation database (Universal Database of Natural Products—ISDB-UNPD), which enables an integration of information and facilitates scientific research in the area of natural products [21].

4. Conclusions

In silico analysis is an important scientific tool to expand the study of the cytotoxicity of natural products, because it allows the realization of studies without the need for a physical laboratory. In this context, natural compounds together with the in silico method are widely used for drug bases, therapies and treatments, such as in various types of cancer, which characterizes computational analysis as a source of knowledge about the structures of natural products and their pharmacological implications.

Thus, the use of algorithms is very broad due to the different methods and the ability to adapt to any type of research. In relation to natural substances, the computational analysis reflects a large percentage of reality, so much so that some sources have compared the veracity of the in vivo method to in silico. Therefore, it is clear that in silico methodologies have become essential in the process of analyzing the cytotoxicity of natural products and discovering new drugs, obtaining great potential in the health area.

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