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Cytotoxicity of plants from the Brazilian semi-arid region: A comparison of different selection approaches

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ABSTRACT

This study aimed to answer the following questions: (1) Are plants that are selected using the ethnobotanical or chemical-ecological approach more cytotoxic than those selected using a random approach? (2) Are trees and shrubs more cytotoxic than herbs? (3) For a given ethnobotanical approach, are the cytotoxicity results in cancer cell lines using medicinal plants commonly selected for cancer treatment different from the results in the same cell lines using plants selected using the syndromic importance value (SIV) method? We selected 18 plant species using the ethnobotanical approach, 20 using the random approach and 20 using the chemical-ecological approach. After acquiring 50 μg/mL of hexanic and methanolic extracts, the cytotoxic activity of the samples was tested in HEp-2 (larynx cancer) and NCI-H292 (lung cancer) cell lines using the MTT method (3-[4.5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). No significant differences were observed between the ethnobotanical, chemical-ecological and random selection approaches. Additionally, no significant differences were observed when the cytotoxicity of trees was compared with that of the shrubs and herbs. Although the percentage of active plant species used for cancer treatment was higher than those selected using the SIV, 50% and 16.67%, respectively, these differences were not significant. Neither approach provided meaningful insight into the search for cytotoxic agents in the local flora.

1. Introduction

Cancer is a disease that arises from biological malfunction at the cellular level and is characterized by uncontrolled proliferation and tissue invasion by malignant cells (Suresh, 2007). In 2002, there were 10.9 million new cases, 6.7 million deaths and 24.6 million cases worldwide related some type of cancer, considering only 26 types (Parkin et al., 2005).

In this context, secondary plant metabolites have become vital components in the development of new chemotherapeutic drugs. For example, the alkaloid isolates of *Catharanthus roseus* (L.) G. Don, vincristine and vinblastine have been used in the treatment of breast cancer and sarcomas, and taxol, isolated from *Taxus brevifolia* Nutt., has been used to treat advanced breast cancer (Cragg and Newman, 2005). However, difficulties such as resistance, low specificity and toxicity (Mesquita et al., 2009) arise when using these substances for treatment, prompting a demand for new bioactive molecules.

Although plants are considered to be promising sources of bioactive compounds, the extent of the herbal diversity makes the search for new active substances a challenge and strategies for successful searches invaluable. Three approaches are generally used by researchers: the random approach, the chemical-ecological approach and the ethnobotanic/ethnopharmacological approach (Brito, 1996; Donaldson and Cates, 2004; Albuquerque and Hanazaki, 2006; Albuquerque et al., 2014). In the random approach, collections are often made without specific criteria governing the plant selection (Brito, 1996). The benefit of this approach is that species excluded by other approaches may be selected for study. In addition to the random approach being costly, the probability of finding a species with a desired pharmacological activity is low (Farnsworth and Kaas, 1981).

The chemical-ecological approach has been used in previously conducted ethnobotanical investigations (Albuquerque and Lucena, 2005; Almeida et al., 2005; Alencar et al., 2009; Albuquerque, 2010), and we applied the apparency hypothesis in the present study. The apparency hypothesis classifies plants into two basic groups: apparent plants, which are dominant in an ecosystem (typically arboreal perennial plants such as trees and shrubs), and non-apparent plants such as herbs. The apparency hypothesis suggests that apparent plants tend to

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produce quantitative compounds such as tannins to repel herbivores by acting as digestion inhibitors. Nonetheless, non-apparent plants produce predominantly qualitative compounds with high toxicity, such as alkaloids.

When applying this hypothesis in bioprospective research, the success of finding new bioactive molecules against cancer is greater in non-apparent plants than in apparent plants. However, in arid ecosystems, extracts of apparent plants have exhibited better *in vitro* activity against cancer cells than non-apparent plant extracts (Donaldson and Cates, 2004). Donaldson and Cates (2004) demonstrated that the probability of finding active extracts is higher in long-lived plants (perennials) than in short-lived plants (annuals). This phenomenon can be explained by the fact that annuals invest in faster growth and reproduction and consequently have relatively low levels of effective chemical defenses. This is in contrast to perennials, which have developed effective chemical defenses in the form of a mixture of toxic compounds and digestibility reducers to resist the constant pressure of natural enemies (Donaldson and Cates, 2004).

By employing the ethnobotanic/ethnopharmacological approach, information regarding medicinal plant use has been collected from communities that practice traditional medicine and combined with the findings of chemical/pharmacological studies conducted in the laboratory (Elisabetsky and Souza, 2004). Plants commonly used for treating cancer, tumors and warts have been prioritized for pharmacological assessment (Mans et al., 2000) relative to plants with other popular uses, although other categories have been and are being studied. Spjut and Perdue (1976) demonstrated that plants that have been shown to be popular anthelmintics, as well as those reported to be poisonous, exhibited higher active extracts with antitumor activity than plants that were randomly collected.

The present study aimed to answer the following questions: (1) Are plants that are selected using the ethnobotanical or chemical-ecological approaches more cytotoxic than those selected using a random approach? (2) Are trees and shrubs more cytotoxic than herbs? (3) For a given ethnobotanical approach, are the results using medicinal plants commonly used for cancer treatment different from the results obtained from plants selected based on their syndromic importance value (SIV)? We propose the following hypotheses: (1) plants selected using ethnobotanical and chemical-ecological approaches will exhibit predominantly higher cytotoxicity and higher active extract levels than those selected using the random approach; (2) trees and shrubs will be more cytotoxic and harbor higher active extract levels than herbs because the shrub-arboreal habitat of medicinal plants from the semi-arid region exhibits a greater chemical diversity than that of herbs (Almeida et al., 2005; Alencar et al., 2009, 2010); and (3) the results of cytotoxicity for signs and symptoms associated with the types of strains available are equivalent to the results of plants directly reported for the treatment of cancer.

2. Materials and methods

2.1. Characterization of the study area

We conducted an ethnobotanical and a floristic survey of a semi-arid area of northeastern Brazil, specifically the Caatinga vegetation (seasonal dry forest), in the rural community of Carão and its surroundings (08°35′13.5″S × 36°05′34.6″W) located in the municipality of Altinho, state of Pernambuco. The Caatinga is a vegetation type that covers most of the territory north of Minas Gerais and of northeast Brazil and has endured a steady degradation of the native vegetation, similar to other Brazilian ecosystems (Sampaio et al., 2002). Although it is considered by many to be an ecosystem with poor biodiversity, several studies have shown that this region harbors a wide variety of physiognomic types, with more than 300 endemic plant species (Sampaio et al., 2002). In general, the populations that are embedded in this environment, such as the Indigenous, the Quilombolas (who are descendants of African slaves with a distinct history and culture and live in relatively isolated parts of Brazil) and farmers who are dedicated to agriculture and/or subsistence livestock, broadly apply the plant resources, including in a medicinal manner (Albuquerque et al., 2007; Araújo et al., 2007).

Altinho is a city/municipality located in a Caatinga environment (seasonal dry forest) with an area of 454,486 km^2 and at a distance of 163.1 km from the state capital, Recife. The climate is hot and semi-arid with an annual average temperature of 23.1 °C (State Agency of Planning and Research of Pernambuco CONDEPE/FIDEM, 2010). The Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística - IBGE) estimates that in 2009 there were approximately 22,427 inhabitants (IBGE, 2010). The community of Carão is located 16 km from the city center of Altinho. In 2007, Carão was home to 189 inhabitants, of which 112 (59.26%) were over 18 years of age. The community provides basic services such as electricity, a health center, basic education up to the 4th grade, public transportation to the city schools for students in primary and secondary school and a public telephone. The local economy relies on agriculture and livestock.

2.2. Selection of plants for random and chemical-ecological approaches

A floristic survey was performed in the region, which identified 48 taxa of shrub-arboreal stratum and 119 species of herbaceous plants. To select species using the random approach, we listed all herbs, shrubs and trees in alphabetical order in a Microsoft Excel spreadsheet and conducted a random-draw using BioEstat 5.0. Twenty species were randomly selected from the worksheet according to their row numbers. For the chemical-ecological approach, we included only the tree and shrub species in the list.

Leaves were collected from the trees and shrubs, and aerial portions were collected from the herbs. We collected the leaves, but not the bark or the roots. This technique is relatively less damaging, and we believe that the leaves exhibit the strongest chemical defenses against herbivores because they are the site of photosynthesis and are available only in the rainy season. We collected both mature and young leaves with no signs of herbivory. Due to the small size and low plant biomass yields, we also collected the aerial parts of herbaceous species, from which dozens of individuals needed to be collected for some species.

2.3. Selection of plants by the ethnobotanical approach

The ethnobotanical approach was divided into two parts. First, we recorded the medicinal plants known and/or used by 101 residents aged 18 years or older, along with their respective indications and part(s) of the plant(s) used. Second, we selected 12 informants who cited a greater number of medicinal plants and use. A new interview was conducted with these local experts on medicinal plants, and they were asked whether they knew of or had personal experience with any plants used for the treatment of cancer. Eight plants used in cancer treatment were selected based on verbal references. More details about the techniques used in the ethnobotanical survey, local people, ethical aspects and characteristics of the study site can be found in Araújo et al. (2008), Santos et al. (2009), Alencar et al. (2010), Lins-Neto et al. (2010), Sieber et al. (2011) and Albuquerque et al. (2012). To select plants that have been observed to treat symptoms related to lung and larynx cancer, we used the syndromic importance value (SIV), modified from Leduc et al. (2006) and Araújo et al. (2008), which yielded the following formula:

$$SIV = \frac{(\Sigma p \times s) \pm (\Sigma p \times f)/F}{2}$$

p the weight of indications
s the mentioned symptoms that are related to the signs and symptoms