



Evaluation of antimicrobial and cytotoxic activity of *Handroanthus ochraceus* crude extracts

Marcos Eduardo Lopes Correia, Maria Gabriella Silva Araujo, Wagner Vicente Silva dos Santos, Andressa Letícia Lopes da Silva, Thaís Honório Lins Bernardo, Regina Célia Sales Santos Veríssimo*, Maria Lysete de Assis Bastos and Patrícia de Albuquerque Sarmiento

Research Laboratory in Treatment of Wounds (LpTF), Federal University of Alagoas, Av Lourival Melo Mota, s / n, Tabuleiro dos Martins, 57072-900, phone number: 558299908-1857, Maceio, Alagoas, Brazil

ABSTRACT

The use of medicinal plants is old in society. Several studies have been conducted to assess the biological potential of plant species. One of the studied families is Bignoniaceae. This research aimed to evaluate the antimicrobial and cytotoxic activity of *Handroanthus ochraceus*. The antibacterial activity was determined by the Minimum Inhibitory Concentration (MIC) and to evaluate the samples cytotoxicity was conducted the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay. As the results, the plant species has no antibacterial activity against bacterial strains tested, about cytotoxicity, extracts of leaves and twigs varied their toxicity according to tested concentrations. Further researches that aimed other biological activities are required.

Keywords: *Handroanthus ochraceus*; Antimicrobial activity; Cytotoxic activity

INTRODUCTION

The use of medicinal plants is older, as the emergence of first civilizations. Healing power of plant species was revealed empirically and for many years was the main therapeutic alternative for treatment of several diseases. However, with science advancement, new therapeutic methods have been developed, for example, industrially produced medicines [1].

Even with technological advances, the use of plant extracts to treat illnesses is effective in development countries, as a part of population are low-income and mostly have no access to industrially produced medicines [2].

The association of modern technology to traditional knowledge provides to Plantae, great contributions to production of new industrial drugs, where 25 % of all modern medications being derived directly or indirectly from medicinal plants [3] and when it comes to drugs with antimicrobial and antitumor activity, this percentage reaches to 60 % [4].

In the last years, many antimicrobial drugs proved to be ineffective due to some microorganism's resistance. Besides, its strong side effects and high cost have generated interest on researchers in finding in medicinal plants an alternative therapy, which can be less aggressive in humans [5].

Handroanthus ochraceus belongs to *Bignoniaceae* family. This family is considered extremely important for present many active constituents and pharmacological activities. In traditional medicine, species belonging to this family are used for treatment of skin diseases, cancers, gastrointestinal and respiratory diseases, among other [6].

Handroanthus ochraceus (Cham.) Mattos is popularly known as Yellow Lapacho, Pau D'arco, Yellow Poui, Yellow Ipe and Pau D'arco Amarelo. Has a wide geographical distribution and it is present at Brazil, in North, Northeast and Midwest regions and in phytogeography areas of Cerrado, Caatinga and Atlantic Forest [7].

It is a tree measuring from 6 to 14 meters, with tortuous stem. Flowering period occurs from the end of July until mid-September in Southern Hemisphere, when it is completely stripped of leaves. Pollination is done by bees and its fruits ripen in September until mid-October [8].

Several studies have been conducted to assess the biological potential of *Bignoniaceae* plant species, which shows activities such as antioxidant [9], antiviral [10], antibacterial [11], antifungal [12], anti-haemorrhagic and toxins neutralizing [13] and antitumor [14].

Plant species inserted in this family have active components such as flavonoids, terpenoids, quinones, especially naphthoquinone [15], that may be associated with several biological activities such as antitumor, anti-inflammatory, antiviral, antifungal, antimicrobial, antimalarials and antiparasitic [16].

Considering already in existence studies about biological activities of some plant species from *Bignoniaceae* family, this research aimed to evaluate the antimicrobial and cytotoxic potential of *Handroanthus ochraceus* species.

EXPERIMENTAL SECTION

This was an experimental *in vitro* research, developed at Federal University of Alagoas, at Research Laboratory in Treatment of Wounds (LpTF), Laboratory of Medicinal Chemistry (LQM) and Laboratory of Pharmacology and Immunology (Lafi).

Extracts Preparation

Handroanthus ochraceus was collected in Arboretum at Federal University of Alagoas, located in Maceio/AL, between June and July 2014. Identification was made by MAC at Institute for the Environment of the state of Alagoas with number 46314.

In this study, leaves and twigs were used, which after drying at room temperature were milled. The crude ethanolic extract was obtained by maceration in ethanol (EtOH) 98 %, and concentrated on a rotary evaporator at a maximum temperature of 40 °C and subsequent drying at room temperature.

Biological *In vitro* assays

Cell viability assay

For the assessment of cytotoxicity of the crude extracts of leaves (F1) and twigs (F2) of *Handroanthus ochraceus* species, were performed MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay, based on cells mitochondrial activity by MTT reduction by cleavage of tetrazolium salt (dark yellow) in formazan crystals (dark blue) by succinate dehydrogenase enzyme, present in active mitochondria. The darker the colour at the end of reaction, the higher cell viability [17]. Resulting optical density of MTT assay was determined by spectrophotometer.

J774 macrophages lineage were placed in 96 well plates at density of 1.5×10^5 cells per well grown in culture medium (Dulbecco's Mem-DMEM supplemented with 10 % fetal bovine serum). Each well received 200 μ L of culture medium with cells.

Cells were treated with extracts at concentrations of 1000 and 100 μ g/mL for 48h and placed in an oven at 5% of CO₂. In the period of 1 hour before adding Metiltetrazolium (MTT), three wells containing cells were lysed by Triton 100X (2 μ L), for comparison of cell death. Control wells consisted of dead cells as a positive control (lysed cells - 3 wells) and cultured cells plus the diluent DMSO 0,1 % as negative control.

After the total period of incubation, 48 hours, supernatant was discarded and in each well was added 100 μ L of a MTT solution (500 μ g/mL) and reincubated for 1 hour in an oven at 37 °C and 5 % of CO₂. After this period, supernatant was discarded and the precipitate resuspended in 100 μ L of DMSO.

To quantify the reduced formazan salt, plates were read with assistance of a microplate reader at a wavelength of 540 nm. This technique has the ability to analyse cell viability and metabolic state of cell, from the reduction of tetrazolium salt (dark yellow colour) to formazan (dark blue colour) and is useful to evaluate *in vitro* cytotoxicity.

Broth Microdilution Method

The crude ethanolic extract of *Handroanthus ochraceus* leaves, was solubilised in saline solution at 0.9% and in Dimethyl sulfoxide solution (DMSO) at 2%. Final extract concentration was 20.000 µg/mL. Solubilised extract was tested against standardized bacteria by American Type Cell Collection -ATCC/Manassas-VA/USA, as: *Shigella flexneri* ATCC 12022, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14990, *Acinetobacter calcoaceticus* ATCC 23055, *Escherichia coli* ATCC 14942 and *Pseudomonas aeruginosa* ATCC 27853.

Antibacterial activity of the extracts was determined by Minimum Inhibitory Concentration (MIC) method, based in adapted protocol from National Committee for Clinical Laboratory Standard [18]. Dilutions of the extracts were prepared in triplicate in microdilution plates of 96 wells, leaving a volume of 100 µL of compound per well. As positive control used was Ciprofloxacin, and negative control used was DMSO 2% and saline at 0,9%.

To determine MIC, bacteria samples were solubilized in a solution of $1,5 \times 10^8$ CFU/mL, with concentration according to standard of 0.5 in McFarland scale and subsequently diluted in 1:10 (v/v) to obtain the standard concentration (10^4 CFU/mL). Each well received 5 µl of bacterial inoculum, resulting in a concentration of 10^4 CFU/mL. After this period, 20 uL of 2,3,5-Triphenyltetrazolium chloride at 5% was added in each well, and plates were again stored in bacteriological oven at 35 °C for 3 hours. The wells, which had red colour, indicated bacterial growth, while the original colour indicated inhibition of bacterial growth.

RESULTS AND DISCUSSION**Cytotoxicity by MTT reduction assay**

Cytotoxicity is one of factors that should be investigated when it comes to production of pharmaceuticals. The difference between therapeutic and toxicological effects is an important information when searching to determine a safe concentration range for herbal uses [19].

Most of herbal medicines and/or drugs that are currently used for self-medication or prescription does not have its well-known toxicity profile [20].

In this study, Methyl Tetrazolium (MTT) assay was utilized. It is an in vitro test, which has the ability to determine if a substance can cause cell death through damage in basic functions of cells [21].

Cytotoxicity of leaves and twigs crude ethanolic extract, was measured and extracts absorbance mean were obtained and compared to negative control DMSO, where it was observed that activity varied with tested concentration (100 µg and 1000 µg).

At 1000 µg and 100 µg concentrations, leaves hexane and ethanolic extract of plant species, presented cytotoxicity compared to positive control, while twig crude ethanolic extract, presented no cytotoxicity at 1000 µg.

Many extracts and isolated compounds from plants, which demonstrate antioxidant activity are able to protect DNA from damage, for example, some volatile components of the purple-ipe (*T. impetiginosa*) [22]. However, it should be considered that natural substances may also be toxic and mutagenic [23], [24], as observed in study with lapachol, a naphthoquinone present over several plants, especially in *Tabebuia* genus [25].

In the results obtained to determine the minimum inhibitory concentration by broth microdilution method, *Handroanthus ochraceus* leaves crude ethanolic extract, did not present antibacterial action according to the parameters used in the test (Table 1).

Table 1. Evaluation of Minimum Inhibitory Concentration (MIC) of crude ethanolic extract front bacterial strains

Leaves crude ethanolic extract of <i>Handroanthus ochraceus</i>	
Microorganisms	Minimum Inhibitory Concentration µg/mL
<i>Shigella flexneri</i>	1250
<i>Staphylococcus aureus</i>	1250
<i>Staphylococcus epidermidis</i>	2500
<i>Pseudomonas aeruginosa</i>	2500
<i>Escherichia coli</i>	10000
<i>Acinetobacter calcoaceticus</i>	5000

Crude ethanolic extract of leaves was tested against six bacterial strains, where the Minimum Inhibitory Concentrations ranged from 10,000 µg to 1,250 µg, which is the lowest value of inhibition against of *S. aureus* and *S. flexneri*.

A study involving plants of the genus has revealed antimicrobial activity of several species. *Tabebuia roseo-alba* species demonstrated antimicrobial activity in four (*S. aureus*, *S. epidermidis*, *E. faecalis* and *K. pneumoniae*) of eight microorganisms evaluated in Agar diffusion test [26].

Evaluation of antibacterial activity of *Z. tuberculosa* stem crude extract, belonging to the same family of *Handroanthus ochraceus*, was active against *S. pyogenes*, *S. aureus* and *S. epidermidis* [27], and roots crude ethanolic extract of *Memora nodosa*, another *Bignoniaceae*, demonstrated antimicrobial activity front *Bacillus subtilis*, *Micrococcus roseus*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Candida albicans* [28].

Leaves, stem and roots extracts from *Arrabidaea brachypoda* (*Bignoniaceae* family), when were evaluated according to its antimicrobial potential, showed that all extracts have ability to inhibit the growth of tested microorganisms, and root extract presented better result against *S. aureus* [29].

In another study, conducted in 2013, using six different plants extracts, including *Tabebuia pentaphylla* (Pink Ipe), in which highlight Ipe, showing sensibility, being the only one that presents activity against the three species of bacteria used in the research.

CONCLUSION

The present study demonstrated that *Handroanthus ochraceus* crude ethanolic extract of leaves did not present antibacterial activity against bacterial strains tested. About cytotoxic activity, leaves and twigs extracts had their toxicity varied according to the tested concentrations, except twig crude ethanolic extract, which did not present cytotoxicity at 1000 µg.

Since it is a crude ethanolic extract, certain chemical properties, present in it, may have inhibited its potential front the tested activities.

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