

Full Length Research Paper

Antibiotic potential of a crude ethanol extract from *Asplenium serratum* L. fern leaves

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Asplenium serratum L. (Aspleniaceae) is a terrestrial, epiphytic or humicole fern used as traditional medicine in different countries to treat infection. As such, this study aimed to assess the antibacterial potential of a crude ethanol extract from *A. serratum* leaves against multidrug-resistant bacteria from a hospital setting. Fern leaves were dried and ground using 96° Gay Lussac ethanol, with the extract concentrated in a rotary evaporator. Eleven bacterial strains were tested, including gram-positive (*Enterococcus faecalis* 1, *Staphylococcus aureus*, methicillin-sensitive *S. aureus*, *S. aureus* 8380, *S. aureus* ATCC 25923, *Staphylococcus epidermidis*) and gram-negative bacteria (*Proteus mirabilis* 464, *Pseudomonas aeruginosa* 36408, *Enterobacter cloacae* 406, *Klebsiella pneumoniae* 6891, *Escherichia coli* 635). The disk agar diffusion test was used to assess the antimicrobial activity of the extracts. Disks of filter paper (5 mm wide) were prepared, each containing 5 µl of different plant extract concentrations (5, 10, 15, 20, 30, 40 and 50 mg.ml⁻¹, using dimethyl sulfoxide - DMSO - as solvent). The antibiotic oxacillin (1 mg) was used as a positive control and disks containing DMSO only as negative control. The Petri dishes and disks were incubated for 24 h (36.5-37°C), after which the formation of zones of inhibition was measured. The tests were performed in duplicates. After incubation, all the positive control (oxacillin) disks contained zones of inhibition of bacterial growth, while none were observed for negative controls or the disks containing different concentrations of *A. serratum* extract. Ethanol extracts of *A. serratum* leaves showed no antibiotic activity against the microorganisms tested, precluding confirming the popular belief of anti-infective properties for this fern species for multidrug-resistant bacteria.

Key words: Aspleniaceae, multidrug-resistant bacteria, ethnobotany, medicinal plants, ferns.

INTRODUCTION

Many lycophytes and ferns (lumped together as pteridophytes in old classifications) (PPG I, 2016) are used as aromatic, ornamental, art, cosmetic, edible,

ritualistic, veterinary, building material and medicinal plants (May, 1978; Keller and Prance, 2015). Important reviews on secondary metabolites in lycophytes and ferns were

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conducted by Hegnauer (1962), Cooper-Driver and Haufler (1983), Soeder (1985), Gottlieb et al. (1990) and Vetter (2018). Santos et al. (2010) published a review of phytochemical studies on lycophytes and ferns that grow in Brazil and Cao et al. (2017) investigated the potential of phytochemicals from ferns in medicinal applications. These reviews described the presence of phenolic acids, flavonoids, monoterpenes, sesquiterpenes, steroids, alkaloids, cyanogenic substances and fatty acids.

Pharmacological and ethnopharmacological studies have shown that lycophytes and ferns exhibit a range of biological activities, such as analgesic, anti-inflammatory, antioxidant, antibiotic, antitumor, diuretic, sedative and anticonvulsant properties, as well as hepatoprotective, antihyperglycemic, leishmanicidal, trypanocidal and immunomodulatory activity and chemopreventive effects (Santos et al., 2010; Cao et al., 2017).

Several fern species exhibit antimicrobial activity (Maruzzella, 1961; Banerjee and Sen, 1980; Peres et al., 2009; Santos et al., 2010; Souza et al., 2012; Morais-Braga et al., 2013; Chai et al., 2013; Bahadori et al., 2015; Cao et al., 2017; Longtine and Tejedor, 2017), including activity against multidrug-resistant bacteria (Soare and Sutan, 2018). However, many authors have reported that, when compared to angiosperms, research on the pharmacology of phytochemicals of ferns and lycophytes is scarce, including antimicrobial activity (Santos et al., 2010; Cao et al., 2017). The genus *Asplenium* belongs to the family Aspleniaceae and contains around 700 species worldwide (PPG I, 2016). Antibiotic activity has been recorded in *A. dalhousiae* Hook., *A. nidus* L. (Banerjee and Sen, 1980), *Asplenium scolopendrium* L. and *Asplenium adiantum-nigrum* L. (Bahadori et al., 2015).

Levy and Marshall (2004) highlight that clinically important bacteria are characterized by both single drug resistance and multiple antibiotic resistances. According to the authors, multidrug-resistant bacteria are considered a serious health problem, and as such, new substances developed should target these strains.

The *A. serratum* L. is a fern typically epiphyte, but also grows on the ground or on humus-covered rocks (Figure 1). It occurs from the Southern United States (Florida) to Paraguay and Argentina, including islands in the Caribbean Sea (The Antilles, Trinidad and Tobago) and Pacific Ocean (Galapagos) (Sylvestre, 2001).

DeFilippis et al. (2004) reported its use in Guyana, Suriname and French Guiana to treat rashes and as an appetite stimulant. In Guatemala, it is known as “Pájaro Salvaje” (wild bird in English) and the leaves are ritualistically used for malaise, body aches and headaches and vaginal infections (Rodriguez, 2008), while in Peru it is called “lengua de perro” (dog’s tongue) and used for stomach pain and ovarian inflammation (Luziatell et al., 2010). In Panama, it is recommended in the treatment of burns (Gupta et al., 2005) and in Brazil, where it is known as “rabo-de-aranata” (aranata’s tail), “feto-macho-do-Pará” (Pará’s male fern) or “pena-de-

Xangô” (Xango’s feather), the fern is used in rituals (Arjona et al., 2007) and to fight infection (Barros and Andrade, 1997).

Antimicrobial activity of *A. serratum* was reported for four American Type Culture Collection (ATCC) strains, including gram-positive and gram-negative bacteria (Timóteo, 2015), and no multidrug-resistant bacteria were targeted. Thus, in light of traditionally-held belief in its anti-infective properties (Barros and Andrade, 1997; DeFilippis et al., 2004; Luziatell et al., 2010; Rodriguez, 2008), and its activity on ATCC strains, this study aimed to investigate the antibacterial potential of a crude ethanol extract from *A. serratum* leaves on multidrug-resistant bacteria from a hospital setting.

MATERIALS AND METHODS

Plant material

Leaves (fertile and sterile) from 10 *A. serratum* plants were collected in January 2012 from Serra da Tiririca State Park in Rio de Janeiro state, Brazil, under a scientific research license (no. 053/2011) issued by the Instituto Estadual do Meio Ambiente - INEA (State Environmental Institute) of Rio de Janeiro state. The voucher material was deposited in the Herbarium of the Faculdade de Formação de Professores da Universidade do Estado do Rio de Janeiro (RFFP 19636).

Preparing the plant extract

The *A. serratum* leaves were dried in an oven at 40°C, ground and weighed. The plant material was macerated in 96° Gay Lussac GL ethanol at a proportion of 1 L of solvent to 100 g of macerated plant over 45 days, at 26±2°C, with daily agitation. The extracts were concentrated in a rotary evaporator and then freeze-dried.

Antimicrobial activity

Antimicrobial assays were conducted using clinically relevant bacterial strains collected from patients attending Universidade Federal Fluminense’s Antônio Pedro University Hospital. The strains were identified using traditional microbiological and biochemical methods, except *Staphylococcus aureus* 25923, which is a reference strain from the American Type Culture Collection (ATCC). Eleven strains were tested, including gram-positive (*Enterococcus faecalis* 1, *S. aureus*, oxacillin-sensitive *S. aureus*, *S. aureus* 8380, *S. aureus* ATCC 25923, *S. epidermidis*) and gram-negative bacteria (*Proteus mirabilis* 464, *Pseudomonas aeruginosa* 36408, *Enterobacter cloacae* 406, *Klebsiella pneumoniae* 6891, *Escherichia coli* 635).

Initially, the bacteria (one colony) were seeded in broth containing Mueller-Hinton (MH) agar and incubated in an oven (36.5- 37°C) for approximately 5 h. Next, inocula of the bacterial strains were transferred to Petri dishes containing solid MH agar using a cotton swab. A total of 22 Petri dishes with MH agar were used, two for each strain.

The disk agar diffusion test was used to assess the antimicrobial activity of the extracts. Disks of filter paper (5 mm wide) were prepared, each containing 5 µl of the different plant extract concentrations. Solutions were prepared from the crude extract, in the following concentrations: 5, 10, 15, 20, 30, 40 and 50 mg.mL⁻¹, using dimethyl sulfoxide (DMSO) as solvent. The antibiotic oxacillin



Figure 1. *Asplenium serratum* L.

using dimethyl sulfoxide (DMSO) as solvent. The antibiotic oxacillin (1 mg) was used as a positive control and disks containing pure DMSO as negative control, in the centre of the dish (Figure 2).

Next, the Petri dishes containing bacterial strains and disks with different concentrations of *A. serratum* extract, DMSO and oxacillin were placed on the inoculated growth medium. The Petri dishes and disks were incubated for 24 h (36.5-37°C), after which the formation of zones of inhibition was measured. The inhibition halos were considered as the diameter of the area without growth formed around the disk. The tests were performed in duplicate.

RESULTS

After incubation, all the positive control (oxacillin) disks contained zones of inhibition of bacterial growth, while none were observed for negative controls or the disks containing different concentrations of *A. serratum* extract. The ethanol extracts of *A. serratum* leaves showed no antibiotic activity against the microorganisms tested. An example of the results found is shown in Figure 2.

DISCUSSION

Timóteo (2015) reported antimicrobial activity for methanol extracts of *A. serratum* leaves. However, the author only analyzed four strains of ATCC bacteria, whereas the present study used 10 strains of multidrug-resistant bacteria from a hospital setting and only one ATCC. Multidrug-resistant bacteria are considered a serious health problem, especially in developed countries (Levy and Marshall, 2004) and as such, new substances developed should target these strains.

Falkenberg et al. (1999) reported that almost all the constituents of interest in phytochemical analysis exhibit some degree of solubility in ethanol or methanol mixtures, with both solvents exhibiting similar polarity and the potential to extract the same substances. Banerjee and Sen (1980) used extracts with water, methanol, ethanol, acetone and ether. They observed positive results with the methanol extract for *Asplenium dalhousiae* Hook. and for *Asplenium nidus* L. with the ethanol extract.

Banerjee and Sen (1980) conducted a comprehensive study on the antibiotic activity (gram-positive and negative bacteria and fungi) of ferns and lycophytes using the disk diffusion agar method and testing 114 species. Of these, 72 displayed some degree of inhibition of the microorganisms tested. Among them seven *Asplenium* species analyzed, only *A. dalhousiae* and *A. nidus* exhibited antibiotic activity. Bahadori et al. (2015) analyzed methanol extracts of eight fern species using the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) tests, with *A. scolopendrium* L. and *A. adiantum-nigrum* L. displaying antibacterial activity against *Staphylococcus aureus*.

According to Banerjee and Sen (1980), the distribution and concentration of antimicrobial substances in different fern organs can vary significantly. In most cases, plants with fertile leaves obtained more positive results. In the tests performed with *A. serratum*, the extracts were prepared using a sample composed of fertile and sterile leaves. Bahadori et al. (2015) found that rhizome extracts of *A. scolopendrium* and *A. adiantum-nigrum* showed greater antibiotic activity than that of leaf extracts. Other factors that may interfere in the production of substances with antimicrobial activity are seasonality, altitude and



Figure 2. The disk diffusion test in a Petri dish with oxacillin-sensitive *Staphylococcus aureus*. Crude extract concentrations: S5 = 5 mg.ml⁻¹ of derivative; S10 = 10 mg.ml⁻¹ of derivative; S15 = 15 mg.ml⁻¹ of derivative; + = positive control (oxacillin); 3 = negative control (DMSO).

plant habit (Banerjee and Sen 1980). The *A. serratum* plants collected to prepare the extracts were growing on rocks covered in humus.

According to Elisabetsky (2003), failure to observe a pharmacological effect in experimental models using a crude extract may lead researchers to doubt the beliefs of traditional medicine and classify the plant as a placebo. Thus, the fact that the ethanol extract of *A. serratum* leaves showed no activity against the *in vitro* tested multidrug-resistant bacteria from a hospital setting does not rule out the possibility that the plant may have other properties indicated in traditional medicine.

Conclusion

Based on *in vitro* test results, the ethanol extract of *A. serratum* leaves has no antibacterial properties for the six gram-positive and five gram-negative strains analyzed here. Thus, we were unable to confirm the popular belief of anti-infective properties for these fern species against multidrug-resistant bacteria from a hospital setting.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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