

In vitro antibacterial activity of dried extract from *Anredera vesicaria* rhizomes

Abstract

Anredera vesicaria is known by the common name of “yucahiedra”. The ethanolic maceration of their rhizomes is used as anti-inflammatory in traditional medicine and contains mucilages, saponins, phenolic compounds and flavonoids. There are no reports of possible antibacterial activity. To evaluate the antibacterial activity *in vitro* of the ethanolic extract from *A. vesicaria* rhizomes, plant rhizomes were collected and disinfected, dried and pulverized. The biomass was treated with 70% ethanol under ultrasound. The extract obtained was concentrated to dryness and subjected to an *in vitro* antibacterial study by the Bauer-Kirby and broth micro-dilution methods.

The ethanolic extract of *A. vesicaria* rhizomes showed antibacterial activity *in vitro* against *P. aeruginosa*, *S. aureus*, *S. typhimurium* and *E. coli*. In all cases inhibition halos were obtained above 9mm. The best results were obtained against *S. typhimurium* and *S. aureus* with minimal inhibitory concentrations of 46.87 and 46.93µg/mL respectively.

Keywords: *Anredera vesicaria*, ultrasound assisted extraction, phenolic compounds, antibacterial test

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Abbreviations: ATCC, american type culture collection; DMSO, dimethylsulfoxide; MIC, minimum inhibitory concentration; ID, diameter of the inhibition

Introduction

After the pharmaceutical industry was devoted exclusively to the manufacture of synthetic drugs leaving behind the old medicines based on plant extracts, today there is a qualitative change in the industrial programs dedicated to the search for new medicines of herbal origin.¹ Plants are one of the largest reservoirs of medicinal wealth in nature and the benefits to health, as well as their economic value have motivated a growing attention in recent decades. *Anredera vesicaria*, known by the common name yucahiedra is a perennial herbaceous plant belonging to the Basellaceae family. The maceration in ethanol of its rhizomes is used in eastern Cuba for the treatment of wounds, blows, fractures and bruises.² Polyphenols are related to the anti microbial and antioxidant activities of many plants,³⁻⁵ and the rhizomes of *A. vesicaria* have a high content of phenolic compounds and flavonoids,⁶ however, no reports of the antibacterial activity of this plant have been observed. In this work we describe a preliminary study of the antibacterial activity of the ethanolic extracts obtained from rhizomes of *A. vesicaria*.

Materials and methods

The plant material was collected on June 18, 2017 in Palmas Altas, community belonging to the municipality of Manzanillo, Granma province, Cuba (20° 19'54.588 "N -77° 4'19.128" E) at 8:10 a.m., at a temperature of 31.4 °C with a relative humidity of 73%. The experimental work was developed in the Natural Products Laboratory of the Center for Applied Chemistry Studies of the University of Granma. A sample of rhizomes of *A. vesicaria* was dried for a week in the shade on perforated cardboard sheets, stirring the material twice

a day; this action was completed in a stove with air circulation at 40°C for 4h.⁷ It was then pulverized until obtaining a particle size of 0.5mm in diameter, and it was subjected to an ultrasound extraction process using 70% ethanol as Solvent.⁸ The extract obtained was concentrated in a rotary evaporator. The Phytochemical analysis of extract was carried out by using following standard methods.⁹⁻¹¹ The reagents and solvents used were of “pure” or “analytical” quality from Merck. The antibacterial activity of the extract was evaluated by the method of diffusion method in agar by Bauer-Kirby¹² surface disc dissemination and by the method of micro dilution in broth¹³ against a battery composed of strains of *Pseudomonas aeruginosa* (wild), *Staphylococcus aureus* (wild), *Escherichia coli* (wild) and *Salmonella typhimurium* (ATCC 14028). 4 treatments were applied with 3 replications in each applied method, using as a negative control dimethyl sulfoxide (DMSO) that also served as solvent for the dry extract, and as a positive control disks of the commercial antibiotic chloramphenicol of 30µg/disc (Sensi-Disc™, France).¹⁴ The variables measured were:

- Diameter of the inhibition halo (ID) (mm) whose results were expressed as the average diameter of the inhibition halos in each treatment; and
- Minimum inhibitory concentration (MIC), which was expressed as the minimum concentration of the extract that completely inhibited bacterial growth in each treatment with the naked eye in a range of concentration of the extract between 2-1500µg/mL.

Results and discussion

Considering that the population uses the ethanolic extract of rhizomes of *A. Vesicaria* (Figure 1) for medicinal purposes, an extract was obtained in 70% ethanol that was treated until obtaining a dry extract which was preserved for the microbiological study.



Figure 1 Foliage and rhizomes from *A. vesicaria*.

Phytochemical analysis

A phytochemical analysis was performed from the ethanolic extract,⁹⁻¹¹ the results are shown in Table 1. The chemical composition of the ethanolic extract of rhizomes, matches with those reported by de la Cruz et al.⁶ The presence of alkaloids and abundance of phenols and flavonoids give the plant antibacterial potential activity.¹⁵ These results agree with those reported for other species from *Anredera* genus.^{16,17}

Anti bacterial activity in vitro

Four bacterial strains of pathogenic and investigative interest were evaluated. The results obtained are shown in Table 2.

Minimum inhibitory concentration

Table 3 shows the MIC values obtained in the evaluation of the dry extract of the rhizomes of *A. Vesicaria* against a gram-positive strain (*S. aureus*) and 3 gram-negative strains (*E. coli*, *P. aeruginosa* and *S. typhimurium*).

The zone of inhibition in the experiment was conceived as the area formed around the antibiotic discs, here bacterial growth was completely inhibited (Figure 1). The result of each trial (Table 1) was reported as a halo of inhibition. The ethanolic extract of rhizomes

showed anti bacterial action against Gram-positive and Gram-negative strains. In all cases, halos of inhibition greater than 9mm were observed, highlighting the effect against *S. aureus* and *P. Aeruginosa* with halos of inhibition of 15 and 18 mm respectively. These results are encouraging because it is not common to observe halos of inhibitions pronounced in plant extracts.^{18,19} The MIC values for *S. aureus* and *S. Typhimurium* were very close to each other, however, in the *E. Coli* test a higher concentration of the extract (187.5) was needed to inhibit bacterial growth (Table 3). This could be due to the fact that *E. coli* is a gram-negative bacterium whose cell wall has two membranes in the form of lipoprotein matrices with intermediate inclusions of peptidoglycans that can hinder the diffusion of certain molecules.²⁰ A highly acylated lipopolysaccharide located in the external leaflet has been detected of the outer membrane of gram-negative bacteria at the essential to maintain the barrier function that prevents the passive diffusion of hydrophobic solutes such as antibiotics and detergents inside the cell.²¹ The strain of *E. Coli* used is wild, therefore, it is more resistant to the action of antibacterials.²² The result so obtained constitutes the first report of *in vitro* antibacterial activity of *A. vesicaria* and may contribute to justify the medicinal properties attributed to this plant.

Table 1 Phytochemical analysis of rhizomes of *Anredera vesicaria*

Metabolite	Ethanolic extract
Carbohydrates	+
Phenols	++
Flavonoids	++
Alkaloids (Mayer)	+
Alkaloids (Wagner)	+
Triterpenes and steroids	+
Saponins	++
Mucilages	++

Legend: (-) absent, (+) present, (++) abundant

Table 2 Antibacterial activity of the dry extract of *A. vesicaria* rhizomes

Product	Halos of inhibition (\bar{X} , mm)			
	<i>S. typhimurium</i> (ATCC 14028)	<i>E. coli</i> (Salvaje)	<i>S. aureus</i> (Salvaje)	<i>P. Aeruginosa</i> (Salvaje)
Ethanolic extract of rhizomes	10	11	15	18
DMSO	-	-	-	-
Chloramphenicol	16	20	21	17

Table 3 Minimum inhibitory concentration of dry extract of *A. vesicaria* rhizomes

Extract	Minimum inhibitory concentration (\bar{X} , µg/mL)			
	<i>S. typhimurium</i> (ATCC 14028)	<i>E. coli</i> (Wild)	<i>S. aureus</i> (Wild)	<i>P. aeruginosa</i> (Wild)
Ethanolic (rhizomes)	46.87	187.5	46.93	93.75

Conclusion

The ethanolic extract of *A. vesicaria* rhizomes presented remarkable *in vitro* antibacterial activity against gram-positive and gram-negative strains. The metabolites responsible for the antibacterial activity could be the phenolic compounds, flavonoids and alkaloids.

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Conflict of interest

This research was completely financed by the University of Granma and there are not conflicts of interests among the involved parts.

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