

Antibacterial activity of *Solanum dolichosepalum* leaves (Bitter) alcohol extracts

Actividad antibacteriana de extractos alcohólicos de hojas de *Solanum dolichosepalum* (Bitter)

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Resumen

Las plantas pertenecientes al género *Solanum* son conocidas por su amplio espectro de actividad biológica. Por esto, el objetivo de este trabajo fue evaluar la acción antibacteriana de extractos etanólicos y metanólicos de *Solanum dolichosepalum* sobre las cepas bacterianas *Staphylococcus aureus*, *Salmonella* spp., *Pseudomonas aeruginosa* y *Aeromonas hydrophila*. Los extractos fueron obtenidos por extracción sólido-líquido en equipos Soxhlet, con posterior concentración por evaporación rotatoria. Para determinar la actividad antibacteriana se usó el método de difusión en disco, empleando agar Mueller Hinton, Cloranfenicol (sensidiscos de 30 mg) como control positivo, y los solventes de extracción como controles negativos. Los extractos metanólicos y etanólicos de *S. dolichosepalum* mostraron un leve efecto inhibitorio contra *S. aureus*, *Salmonella* spp., *A. hydrophila* y *P. aeruginosa*, pero no fue suficiente para considerarse significativo mostrando resistencia a los mismos. Para los dos tipos de extractos usados, el etanólico fue el más activo sobre *S. aureus*, *Salmonella* spp., *A. hydrophila*, y el metanólico frente a *P. aeruginosa*.

Palabras clave: actividad antibacteriana; *Aeromonas hydrophila*; *Pseudomonas aeruginosa*; *Salmonella* spp; *Staphylococcus aureus*; *Solanum dolichosepalum*.

Abstract

The plants belonging to the *Solanum* genus are known for their broad spectrum of biological activity. Therefore, the objective of this work was to evaluate the antibacterial activity of ethanolic and methanolic extracts of *S. dolichosepalum* against bacterial strains *S. aureus*, *Salmonella* spp, *P. aeruginosa*, and *A. hydrophila*. The methanolic and ethanolic extracts were obtained by solid-liquid extraction in soxhlet equipment, with subsequent concentration by rotary evaporation. To determine the antibacterial activity, the disc diffusion method was used, using Mueller Hinton agar, Chloramphenicol (30 mg sensitives) as a positive control, and extraction

solvents as negative controls. The methanolic and ethanolic extracts of *S. dolichosepalum* showed a slight inhibitory effect against *S. aureus*, *Salmonella* spp, *A. hydrophila*, and *P. aeruginosa*, but it was not enough to be considered significant showing resistance to them. The two types of extracts used, ethanolic was the most active against *S. aureus*, *Salmonella* spp, *A. hydrophila*, and methanolic against *P. aeruginosa*.

Keywords: antibacterial activity; *Aeromonas hydrophila*; *Pseudomonas aeruginosa*; *Salmonella* spp.; *Staphylococcus aureus*; *Solanum dolichosepalum*.

Introduction

The indiscriminate use of antibiotics in animals to treat diseases has increased their resistance among pathogenic bacteria (Belém-Costa; Possebón-Cyrino, 2006). Resistance is the mechanism by which microorganisms can decrease the action of antimicrobial agents (Rivera-Calderón; Motta-Delgado; Cerón-Urbano; Chimonja-Coy, 2012), generated from the use of antibiotics in animals and the subsequent transfer of resistance genes in bacteria between animals, animal products and the environment (McEwen; Fedorka-Cray, 2002; Phillips *et al.*, 2004).

Among the mechanisms described for antimicrobial resistance are the expulsion of the antibiotic by efflux pumps, alteration of permeability, modification of the therapeutic target, and/or inactivation of the antibiotic (Arenas; Moreno-Melo, 2018). Regarding an effective alternative to treat bacterial diseases, the use of plants that have a range of biological activity and are a big source of secondary metabolites have been used (Koduru; Grierson; Afolayan, 2006).

Relative to *A. hydrophila*, this is a Gram-negative bacillus that produces a variety of diseases in some animal species, from aquatic to terrestrial, and even to humans (Ji *et al.*, 2015); It is also the cause of gastroenteritis and skin and respiratory infections (Zepeda-Velázquez, 2015), eye infections, peritonitis, bacteremia, meningitis, hemolytic uremic syndrome, and necrotizing fasciitis in humans (Citterio; Biavasco, 2015); Furthermore, it is considered an opportunistic pathogen that causes high mortality in fish causing sepsis (Harikrishnan; Balasundaram, 2005).

S. aureus is a Gram-positive cocoon, which produces a wide variety of suppurative infections in animal wounds (abscesses), mastitis, endometritis, cystitis, osteomyelitis. As for *Salmonella* spp., It is a Gram-negative, aerobic and facultative anaerobic bacillus, mobile and non-spore-forming (Pérez-Rubiano; Cardozo-Torres, 2014; Quinn; Markey; Leonard; FitzPatrick; Fanning, 2015), causes gastroenteritis, abortions in cattle and horses; diarrhea, septicemia in different animal species (Rivera-Calderón *et al.*, 2012).

In reference to *P. aeruginosa*, it is a Gram negative bacillus that produces otitis and suppurative infections in domestic animals, generally with association and burns, corneal damage and wounds (García-Urquijo; Rodríguez-Rodríguez; Rodríguez-Pérez; Lorenzo-Manzanas; Hernández-González, 2014; Quinn *et al.*, 2015).

On the other hand, the genus *Solanum* belonging to the *Solanaceae* family has more than 2300 species worldwide (Sheeba, 2010), which are known to present various types of biological activity, thanks to the compounds that have this potential. *Solanum dolichosepalum* (strawberry) is low montane and high montane moist forest plant located in the Central Cordillera of Colombia (Cárdenas; Isaza; Pérez, 2013; Marin-Ocampo; López-Zuluaga; Pérez-Cardenas; IsazaMejia, 2006). Besides, it is a native and scarce shrub; present in open sites and highly eroded soils (CárdenasBurgos; Pacheco-Maldonado; Vanzela, 2016; González-M; López-Camacho, 2012).

Regarding its use, the leaves and fruits are used to help heal, eliminate lice and treat kidney diseases; likewise, it is used as an immunostimulant, anti-inflammatory, antibacterial and antifungal (Cárdenas *et al.*,

2013; Marin-Ocampo *et al.*, 2006; Ramírez-Cárdenas; Isaza-Mejía; Pérez-Cárdenas; Martínez-Garzón, 2017; Martín-G. ; Cárdenas; Pacheco; Cárdenas, Gómez, 2016). However, *S. dolichosepalum* is a poorly studied species as an antimicrobial agent.

Arango *et al.* (2004) reported antimicrobial effects of *S. dolichosepalum*, where ethanolic extracts inhibited, at a concentration of 250 µg / mL, the growth of *S. aureus*, *Shigella* spp., *Vibrio cholera* and the fungi *Aspergillus flavus* and *Candida albicans*. In turn, Martín-G. *et al.*, (2016) evaluated the effectiveness of acetone and chloroform extracts on two *Fusarium oxysporum* strains, finding that the two types of extracts were active against the studied strains. For their part, Ramírez-Cárdenas *et al.*, (2017) carried out a phytochemical study and a preliminary characterization and antibacterial activity of 4 fractions of the ethanolic extract of fruits, finding metabolites such as flavonoids, alkaloids, steroids and/or free terpenoids, saponins, tannins, and cardiotonic glycosides; and activity against *S. aureus* and *Escherichia coli*, and absence of activity against *P. aeruginosa*.

Thus, the objective of this work is to evaluate the antibacterial action of ethanolic and methanolic extracts of *S. dolichosepalum* on bacterial strains *S. aureus*, *Salmonella* spp., *P. aeruginosa*, and *A. hydrophila*, to deepen the knowledge and biological activity of this species, against microorganisms of livestock interest.

Materials and methods

Vegetal material

The leaves of the plant were collected in the municipality of Tinjacá, Boyacá, Colombia. These were washed by immersion in a 1 % sodium hypochlorite solution, dried at room temperature (20 °C), performing size homogenization with an IKA A11 analytical mill.

Obtaining extracts

The extracts were prepared as reported by Martín *et al.*, (2016) with some modifications. Liquid solid extraction was performed in Soxhlet equipment, using ethanol (J.T. Baker, 99.5 %), and methanol (J.T. Baker, 99.8 %) as solvents; equipment with a capacity of 500 mL of solvent was used, adding an excess of 100 mL to avoid the effects of reducing the extractive agent; the total extraction time was 5 hours, with 5 extraction cycles per hour. The extracts were concentrated using rotary evaporation at low pressure, in an IKA model RV 10 equipment. The average extraction yield was between 11 and 17 %. The extracts were stored in amber jars at -18 °C for further analysis.

Microorganisms used in the study

Four bacterial strains, one Gram-positive (*S. aureus*, ATCC 25923) and three Gram-negative, were used in the study: *Salmonella* spp. (ATCC 700623), *A. hydrophila* (ATCC 35654), and *P. aeruginosa* (ATCC 27853). These strains were purchased from the Pontificia Universidad Javeriana cepario.

Conservation of bacterial strains

The strains of *Salmonella* spp., *P. aeruginosa*, *A. hydrophila*, and *S. aureus* were grown in Brain Heart Infusion (ICC) broth and incubated for 24 hours at 37 °C. The culture was mixed with the same volume of 10 % glycerol (v / v), the homogeneous mixture was dispensed into Eppendorf tubes at a rate of 1 mL/tube, and kept at -20 °C until use.

Antibacterial activity of extracts

Antibacterial activity was evaluated with the disk diffusion method through the Kirby-Bauer technique (Bauer; Kirby; Sherris; Turck, 1966; Bernal; Guzmán, 1984) using Mueller Hinton agar. The bacteria inoculum was brought to a turbidity of 0.5 according to the McFarland scale, which corresponds approximately to 1.5×10^8 CFU / mL. Chloramphenicol (Oxoid, 30 mg) was used as positive control and extraction solvents as negative. The dry extracts (without solvent) were redissolved in their respective extraction solvents (ethanol and methanol) until obtaining an initial concentration of 0.84 g/mL (determined by preliminary studies). The filter paper discs (5 mm diameter Whatman No. 2) were impregnated by immersion with solvents and extracts. The samples were incubated at 37 °C for 24 hours. Later, halos of inhibition were measured after the correction of negative controls, calculating the percentage of inhibition (Equation 1), compared with the value of chloramphenicol. All tests were performed in triplicate.

$$\% IH = \frac{B}{E} \times 100 \quad (1)$$

Where B (mm) is the halo produced by the extract, E (mm) is the halo produced by the positive control.

Minimum Inhibitory Concentration (MIC) (Martin et al., 2016)

To determine the lowest concentration that inhibits bacterial growth, successive dilutions of the extracts were made in the respective extraction solvents, starting from 0.84 g / mL, the inoculation was carried out in Petri dishes. It was taken as MIC at the lowest concentration of extract that produced inhibition of the microorganisms after incubating for 24 hours at 37 °C.

Statistical analysis

A completely randomized design was used, with halos, percentages of inhibition, and MIC as response variables. The independent variables were the strain and the type of extract. ANOVA analysis of variances and Duncan's tests were performed when necessary; both tests were evaluated at 5 % ($p > 0.05$). Analyses were performed in SPSS software (SPSS Inc., Chicago, IL, USA- SPSS version 17).

Results and Discussion

Inhibition halo

All the extracts showed inhibitory activity against the four strains analyzed (see Table 1), the inhibition halos were in a range of 1-4 mm. The data differed statistically according to the ANOVA analysis ($p = 0.006$), which showed that the ethanolic extract was more effective than the methanolic extract. The most sensitive strains were *S. aureus* and *Salmonella* spp., Against the ethanolic extract, and *P. aeruginosa* and *S. aureus* against the methanolic. In another case, Sridhar; Josthna; Naidu (2011) found contrary results, when they studied the effect of methanolic and ethanolic extracts of *S. nigrum* on *S. aureus*. These authors reported larger inhibition halos for the methanolic extract of the leaf, with values of 8.0 mm, while the ethanolic generated zones of 7.0 mm; Also, in the case of the seed, values of 12 mm for the ethanolic extract and 15 mm for the methanolic were evident.

That is, if the values found for *S. dolichosepalum* are compared, they are low and may be due to the low sensitivity of the microorganisms analyzed against the extracts studied. This low response can be attributed to the fact that metabolites present in this plant (alkaloids, flavonoids, saponins, and steroids) reported by

Ramírez-Cárdenas *et al.*, (2017) do not have a high antibacterial potential against the bacteria analyzed in this research.

Table 1.
Halos or zones of inhibition (mm) of the analyzed extracts, against the study strains

	<i>S. aureus</i>	<i>Salmonella spp.</i>	<i>A. hydrophila</i>	<i>P. aeruginosa</i>
EE	3,7 ± 0,6 ^b	3,3 ± 0,6 ^b	1,7 ± 1,1 ^a	1,7 ± 0,6 ^a
EM	1,7 ± 0,6 ^a	1,3 ± 0,6 ^a	1,3 ± 0,6 ^a	2,0 ± 1,0 ^a
ET	2,1 ± 0,6	1,7 ± 0,6	2,1 ± 0,6	3,2 ± 0,0
Me	1,9 ± 0,6	2,3 ± 0,0	2,4 ± 0,6	2,2 ± 0,6

EE: ethanolic extract. MS: methanolic extract. Et: ethanol. Me: methanol

Note: Data is shown as mean ± standard deviation. Different letters in the same column or same row differ statistically according to the Duncan test, evaluated at 5%

Source: self-made

Concerning other reports of the use of plants of the same genus on the analyzed microorganisms, higher values than those evidenced in this study are found in the literature. Pereira *et al.*, (2008), analyzed the effect of methanolic extracts of *Solanum palinacanthum* leaves (10 mg/mL) on *A. hydrophila*, *P. aeruginosa* and *S. aureus*, where zones of inhibition of 12.5 were found, 0.0 and 9.5 mm respectively. Likewise, Latha and Kannabiran (2006), found higher values when analyzing the effect of methanolic extracts of stem (11 mm) and flowers (9 mm) of the species *Solanum trilobatum* Linn. In the same sense, Sheeba (2010), found halos of 13 mm, 4 mm, and 15 mm for *S. aureus*, *P. aeruginosa*, and *Salmonella typhi* respectively, using ethanolic extracts of leaves of *Solanum surattense* Burm in a concentration of 50 mg/mL. For his part, Xavier; *Auxilia; lSelvi* (2013), analyzed methanolic extracts of fresh leaves of *S. erianthum* with inhibition halos of 9.6, 9.5 and 9.0 mm for *S. aureus*, *S. typhi*, and *P. aeruginosa* respectively.

Similarly, Zubair *et al.*, (2011) reported another species with activity on *S. aureus*, *S. nigrum*; its methanolic extracts showed 24.5 mm halos against this strain.

On the other hand, De Britto; Herin; Gracelin; Benjamin; Rathna (2011) used methanolic extracts from leaves (0.1 mg/mL) of different nightshades to inhibit *A. hydrophila*; determining values of 12.00, 14.66, 14.33, 18.00 and 6.33 mm for *S. nigrum*, *S. toroum*, *S. trilobatum*, *S. surattense*, and *S. melongena*, respectively.

Sivapriya; Dinesha; Harsha; Gowda; Srinivas (2011) used values of 15, 16, 14, and 15 mm for *S. aureus*, *Salmonella cibrium*, *Salmonella typhimurium*, and *Pseudomonas spp.* using ethanolic extracts from the *S. toroum epicarp*. Furthermore, aqueous methanol extracts (70% v/v) from *S. americanum* mill leaves were reported as inactive against *S. aureus* and *P. aeruginosa* (Usman; Victor; Waziri, 2018).

With the aforementioned studies on the biological activity of species of the same genus on the microorganisms analyzed, whose data showed that the *S. dolichosepalum* species has low antibacterial activity, it can be understood that the compounds of these extracts (alkaloids, flavonoids, saponins, and steroids) have a mild inhibitory behavior against the tested strains. With the results found for the inhibition halos, it was determined that the analyzed strains are resistant to the extracts, since, according to García *et al.*, (2000) a strain is resistant to an antibacterial when the inhibition halos are less than 12 mm (HI ≤12 mm).

Inhibition percentages

The inhibition percentages generated by the ethanolic and methanolic extracts of *S. dolichosepalum* were less than 25 %, statistically significant (p = 0.001) (see Table 2). Therefore, the resistance of the microorganisms was confirmed versus the evaluated extracts. Cruz-Carrillo *et al.*, (2010) mentioned that an extract has a high

antibacterial action when its relative inhibition percentage is greater than 70 %, intermediate between 50 and 70 %, low when it is between 25 and 50 % and resistant if it is less at 25 %.

Table 2.

Inhibition percentages of the extracts analyzed, against the study strains

	<i>S. aureus</i>	<i>Salmonella spp.</i>	<i>A. hydrophila</i>	<i>P. aeruginosa</i>
EE	23,04 ± 3,63 ^b	21,47 ± 3,41 ^b	8,07 ± 5,59 ^a	5,32 ± 1,84 ^a
EM	10,47 ± 3,63 ^a	8,47 ± 3,67 ^a	6,45 ± 2,79 ^a	9,66 ± 5,68 ^a
Clo	15,9 ± 0,6	15,8 ± 0,5	20,7 ± 1,0	31,3 ± 1,2

EE: ethanolic extract. MS: methanolic extract. Chlo: inhibition (mm) of chloramphenicol

Note: Data is shown as mean ± standard deviation. Different letters in the same column or same row differ statistically according to the Duncan test, evaluated at 5%

Source: self-made

According to the above, some studies were found where plants of the same genus were used and the percentages of inhibition were compared. Thus, in a study with *S. nigrum* (Linn), 66.67 and 58.33 % inhibition were reported for methanolic and ethanolic extracts of leaves against *S. aureus* (Sridhar *et al.*, 2011). Similarly, Sivapriya *et al.*, (2011) found that ethanolic extracts obtained from the *S. torvum* epicarp showed values of 83.33, 100.00, 87.5, and 88.23 % for *S. aureus*, *S. cibrum*, *S. typhimurium*, and *Pseudomonas spp.*

Minimum Inhibitory Concentration (MIC)

Regarding the MIC, values were found in a range of 200.6 to 835.8 mg / mL, these results were not statistically significant ($p = 0.069$). Therefore, it was confirmed that the ethanolic extract was the most effective and the most sensitive strain was *S. aureus* (see Figure 1). These results confirmed that the analyzed strains were resistant to the effect of the extracts used since the extracts showed values higher than 100 mg/mL (Avellaneda-Saucedo; Rojas-Hernández; CuéllarCuellar; Fonseca-Juárez, 2005).

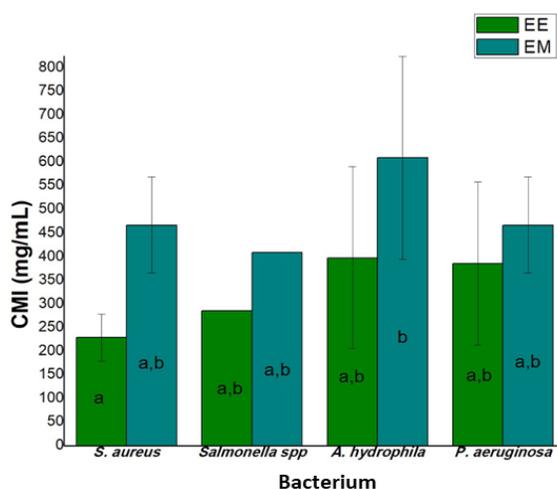


Figure 1. MIC of the methanolic (EM) and ethanolic (EE) extracts against the different bacteria analyzed. Note: The columns show the mean ± standard deviation. Statistically different letters according to the Duncan test, evaluated at 5 %.

Source: self-made

On the other hand, Arango *et al.*, (2004) reported the use of ethanolic extracts from leaves of *S. dolichosepalum* and found that only *S. aureus* was inhibited by the extract, with a MIC of 0.25 mg/mL, lower data to that found in this study (229.3 and 467.9 mg/mL for ethanolic and methanolic extract respectively); regarding *P. aeruginosa* and *S. typhimurium* they did not show activity. However, the data found in this study showed contrary results, since the ethanolic and methanolic extracts yielded average values of 386.0 and 468.0 mg/mL against *P. aeruginosa*, and 286.6 and 468.0 mg/mL for *Salmonella* spp.

Ramírez-Cárdenas *et al.*, (2017), analyzed the antibacterial effect of dried fruits of *S. dolichosepalum* regarding *S. aureus* and *P. aeruginosa*; where they used fractions obtained from the ethanolic extract of the plant. Of the four fractions obtained from this extract, F1 (petroleum ether) and F2 (ethyl acetate-water) presented activity against *S. aureus*, with MIC values of 500.00 and 31.25 mg/mL and in the case *P. aeruginosa* did not obtain activity.

Other investigations report MIC data of other species of the genus *Solanum*, against the analyzed strains, where the methanolic extracts of *S. aculeastrum* leaf showed values of 4.4 and 8 mg / mL for *S. aureus*, *P. aeruginosa* and *Salmonella* Pooni respectively (Koduru *et al.*, 2006), meanwhile, Aliero and Afolayan (2006) studied methanolic extracts of *S. tomentosum* against the same strains, finding a MIC of 5, inactive and 5 mg/mL.

In general, terms, even though the bacteria analyzed were resistant to the effect of the extracts, it was evident that the Gram-positive strain (*S. aureus*) showed a more inhibitory effect than the Gram-negative ones (*Salmonella* spp., *A. hydrophila* and *P. aeruginosa*), especially to the ethanolic extract. This behavior can be explained thanks to the fact that Gram-negative bacteria have amphipathic compounds (phospholipids and porins) that can operate as expulsion pumps for different compounds, reducing the antibacterial effect by suppressing these molecules (Domingo; López-Brea, 2003). Another reason may be that these compounds are not capable of efficiently breaking down the external membrane phospholipids present in Gram-negative and absent in Gram-positive (De Britto *et al.*, 2011), added to this, Gram-positive bacteria are more sensitive to the attack of antimicrobials, since their cell wall is more accessible (Domingo; López-Brea, 2003), since it is composed mainly of peptidoglycan.

Likewise, other investigations also reported higher Gram-positive sensitivity, with the study of the effect of extracts of species of the *Solanum* genus on different strains. Sivapriya *et al.*, (2011) found higher sensitivity of *S. aureus*, *Bacillus subtilis*, and *Streptococcus*, compared to *S. typhimurium*, *Vibrio cholerae*, and *E. coli* when using extracts from *S. torvum*. Ethanolic extracts of *S. nigrum* also showed a greater effect on *S. aureus* and *B. subtilis* compared to *E. coli* and *Pasteurella multocida* (Zubair *et al.*, 2011). Ramírez-Cárdenas *et al.*, (2017) reported that ethanolic extracts of dried fruits of *S. dolichosepalum* affect *S. aureus*, but not against *P. aeruginosa*.

Conclusions

The methanolic and ethanolic extracts of *S. dolichosepalum* showed a slight inhibitory effect against *S. aureus*, *Salmonella* spp., *A. hydrophila*, and *P. aeruginosa*, but it was not enough to be considered significant, cataloging these strains as resistant against the compounds that they are present in the analyzed extracts. Despite this, *S. aureus* was the microorganism that exhibited the greatest inhibitory effect, compared to Gram-negative bacteria. Of the two types of extracts used, ethanolic was the most active on *S. aureus*, *Salmonella* spp., *A. hydrophila*, and methanolic against *P. aeruginosa*.

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