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ALPHA ANTIADRENERGIC EFFECT OF *Achyrocline bogotensis* EXTRACT ("Vira Vira") IN ISOLATED RAT AORTIC RING

EFECTO ANTIADRENÉRGICO ALFA DEL EXTRACTO DE *Achyrocline bogotensis* ("Vira Vira") EN ANILLOS AISLADOS DE AORTA DE RATA

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ABSTRACT

Background: The treatment of symptoms of prostatic hyperplasia is among the traditional uses of *Achyrocline bogotensis* (Kunth) [N.V. *"Vira Vira", Compositae*] in Colombia. Pharmacological therapy for this disorder depends mainly on alpha-1 antiadrenergic agents, and the mechanism has not been studied previously using *A. bogotensis*. **Objectives**: To assess the alpha-1 antiadrenergic effect of the extract obtained from the aerial parts of *A. bogotensis* in isolated aortic rings from Wistar rats. **Methods:** The study compared the effects of the ethanol extract of *A. bogotensis*, prazosin (reference) and DMSO (control) in rings stimulated with phenylephrine (PE) or KCl. The capacity to reduce the PE pressor effect by the ethanol extract (pD_2' value) was determined. To quantify the *A. bogotensis* relaxant potency, increasing concentrations of the ethanol extract (0.1 μg/mL-0.1 mg/mL), were added cumulatively to isolated aortic rings pre-contracted with PE (0.1 μM) or KCl (80 mM). To explore the possible participation of nitric oxide (NO), L-NAME (100 μM) was administered to aortic rings exposed to cumulatively increasing concentrations of PE in isolated aortic rings in the presence of the extract (10 μg/mL). Aqueous, butanol and dichloromethane fractions (10 μg/mL) obtained from the ethanol extract were assayed. Phytochemical screening was also performed. **Results**: Prazosin and *A. bogotensis* extract notably reduced the contraction induced by PE whereas their inhibitory effect in rings contracted with KCl were lower. *A. bogotensis* ethanol extract showed a high capacity for reducing the PE pressor response (pD'_2: 5.51) as well as total efficacy for relaxing rings previously precontracted with PE. The relaxant efficacy and potency of *A. bogotensis* extract against rings previously contracted with KCl were notably lower. L-NAME partly reverted the inhibitory effect of *A. bogotensis*. Aqueous, butanol and dichloromethane fractions gave inhibitory responses lower than that obtained with the ethanol extract. Phytochemical screening of *A. bogotensis* extract revealed the significant presence of flavonoid and triterpene metabolites. **Conclusions**: These results suggest that *A. bogotensis* elicits a smooth muscle relaxant effect related to the alpha-1 antiadrenergic mechanism. This response is partially NO dependent and seems to be due to interactions among active metabolites likely to be of flavonoid and/or terpenoid nature.

Keywords: *Achyroclines;* antiadrenergics; Male lower urinary tract symptoms (LUTS); Phenylephrine; Aorta; Flavonoids; Nitric oxide.

RESUMEN

Antecedentes: Uno de los usos tradicionales de la especie *Achyrocline bogotensis* (Kunth) [N.V. *"Vira Vira", Compositae*] en Colombia es el tratamiento de los síntomas de la hiperplasia prostática benigna. La

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terapia farmacológica de este trastorno se basa principalmente en el uso de agentes que ejercen efecto anti-adrenérgico alfa-1, mecanismo no estudiado previamente en esta especie. **Objetivos**: Evaluar el efecto anti-adrenérgico alfa-1 del extracto de la especie *Achyrocline bogotensis* (Kunth) en anillos aislados de aorta de ratas Wistar. **Métodos:** Se comparó el efecto del extracto etanólico de *A. bogotensis*, prazosin (patrón) y DMSO (control) en anillos aislados de aorta de ratas Wistar estimulados con fenilefrina (PE) o KCl. Se determinó la capacidad del extracto etanólico de *A. bogotensis* para reducir el efecto contráctil inducido por PE (pD'_2). Se cuantificó la potencia relajante del extracto etanólico de *A. bogotensis* (0.1 μg/mL - 0.1 mg/mL) en anillos de aorta previamente contraídos con PE (0.1 μM) o KCl (80 mM). Se exploró la posible participación del óxido nítrico (NO), administrando L-NAME (100 μM) en anillos de aorta expuestos a concentraciones acumulativas de PE en presencia del extracto etanólico (10 μg/mL). También se comparó el efecto de las fracciones acuosa, butanólica y diclometanólica (10 μg/mL), obtenidas del extracto etanólico, en anillos estimulados con PE. Además, se efectuó un tamizado fitoquímico del extracto. **Resultados**: Prazosin y el extracto de *A. bogotensis* redujeron notablemente el efecto de PE mientras su efecto inhibitorio sobre la contracción inducida por KCl fue menor. El extracto etanólico mostró una ostensible capacidad para reducir el efecto contráctil inducido por PE (pD_2': 5.51) así como una eficacia total para relajar anillos previamente contraídos con PE. La potencia y eficacia de relajación del extracto de *A. bogotensis* frente a anillos previamente contraídos con KCl fue notablemente menor. L-NAME revirtió parcialmente el efecto inhibitorio del extracto de *A. bogotensis*. Las fracciones acuosa, butanólica y diclorometanólica arrojaron respuestas inhibitorias menores que las inducidas por el extracto etanólico. El tamizado fitoquímico del extracto de *A. bogotensis* mostró la presencia de metabolitos de naturaleza flavonoide y terponoide. **Conclusiones**: Estos resultados muestran que la especie *A. bogotensis* ejerce efectos relajantes sobre el músculo liso vinculados con mecanismos de tipo antiadrenérgico alfa-1. Esta respuesta depende en parte de la presencia de NO y parece deberse a la interacción de metabolitos de naturaleza flavonoide y/o terpenoide.

Palabras clave: *Achyroclines;* antiadrenérgicos; síntomas del sistema urinario inferior; fenilefrina; aorta; flavonoides; óxido nítrico.

INTRODUCTION

Benign prostatic hypertrophy (BPH), the non-malignant enlargement of the prostate due to cellular hyperplasia, is a very common urological problem present in at least one third of men older than 50 years that leads to lower urinary tract symptoms (LUTS), diminished quality of life and increased risk of urological cancer (1). LUTS secondary to BPH are related to smooth-muscle tension in the prostate tissue, a process that is mediated by alpha-1-adrenergic receptors. Therefore, alpha-1–receptor blockade is the mainstay pharmacological therapy for the treatment of moderate-to-severe BPH (2). Tamsulosin and silodosin are uroselective agents for this disorder due to their high relative affinity for the alpha-1a receptor subtype, but alpha-1 antagonists like doxazosin, terazosin, alfuzosin and prazosin are equally effective (2). Other type of agents used for this disorder, depending on the severity and associated complications include the use of 5-alpha-reductase inhibitors and phosphodiesterase-5 enzyme inhibitors (3).

Products from natural sources are very commonly used for LUTS secondary to BPH. *Serenoa repens* fruit ("Saw palmetto"), *Hypoxis rooperi* roots ("South African star grass"), *Pygeum africanum* bark ("African plum tree"), *Urtica dioica* roots ("Stinging nettle"), *Secale cereale* pollen ("Rye") and *Cucurbita pepo* seeds ("Pumpkin") are among the most popular species consumed for this purpose. Suggested active compounds include: phytosterols, fatty acids, lectins, flavonoids, plant oils and polysaccharides. Possible effects ascribed are of varying types: anti-androgenic, antiestrogenic, antiedematous, anti-inflammatory, with mechanisms related to inhibition of 5-alpha-reductase, blockage of alpha-receptors, inhibition of prostatic cell proliferation, inhibition of prostaglandins and strengthening of detrusor muscle. However, more research is required to determine their efficacy in LUTS of BPH (2, 4, 5).

Achyrocline bogotensis (Kunth) [N.V. *"Vira Vira", Compositae*] is a Colombian medicinal plant used traditionally in decoction for inflammatory and infectious disorders of the skin, respiratory tract, urinary tract and prostate (LUTS), among others (6-8). Flavone and cyclobutane dimer compounds are among its metabolites, which have shown *in vitro* antiviral, antineoplastic and immunomodula-

tory actions (9-12). *Achyrocline* species subjected to chemical and biological studies include *A. satureioides* (13-16), *A. alata* (17-19), *A. tomentosa*, (20, 21) and *A. flaccida* (22, 23). The profile of their biological activities include anti-inflammatory, anti-infective and antioxidant effects related, at least in part, to their polyphenol components. *A. satureioides* and flavonoid compounds isolated from this species have shown relaxant effects on the smooth muscle of guinea pig corpus cavernosum (24).

In spite of popular use of *A. bogotensis* for LUTS, there are no studies regarding its effect on smooth muscle sensitive to alpha receptor stimulation. This work aimed to assess the smooth muscle relaxant effect of *A. bogotensis* extract related to its alpha-1 antiadrenergic properties using aortic rings isolated from Wistar rats in a tissue bath.

MATERIALS AND METHODS

Extraction and Fractionation

The aerial parts from plant material used in this study were collected from "*Páramo La Esperanza*", Sutamarchán region (Boyacá, Colombia). A voucher specimen (Col 522900) was deposited in the Colombian National Herbarium of the Institute of Natural Sciences, Bogotá, (Botanist Edgar Linares). The Stems and leaves of *A. bogotensis* were dried in a forced air oven at 40°C and milled. The powder obtained (1128 g) was macerated with 96% ethanol, filtered and concentrated under reduced pressure. The ethanol extract obtained (217.5 g; 19.3% p/p) was partitioned with distilled water, butanol and dichloromethane to yield three respective fractions. The ethanol extract and the obtained fractions were subjected to phytochemical screening according to the methods described by Sanabria (1983). For the *in vitro* experiments, the extract and fractions were dissolved in dimethylsulfoxide (DMSO, 0.01%).

Experimental protocol

The study was performed in a tissue bath containing isolated aortic rings obtained from male Wistar rats provided by Animalarium of Pharmacy Department, School of Sciences, *Universidad Nacional de Colombia*. The animals were maintained at a controlled temperature (22 ± 1°C), with 12 h light/dark cycles and water and food consumption *ad libitum*, except for the test day when rats were fasted for12 hours. Rats (250–400 g) were anesthetized with ether and sacrificed by cervical dislocation. The descending thoracic aorta was dissected and placed in an oxygenated Krebs solution with the following composition (in mM): NaCl, 118.0; KCl, 4,75; $CaCl_2$, 1,8; $MgSO_4$, 1,2; KH_2PO_4, 1,2; $NaHCO_3$, 25; glucose, 11 and ascorbic acid 0,1. Rings of thoracic aorta (4-5 mm in length) were carefully excised and submerged in bath organ chambers containing 10 ml of Krebs solution of bathing medium maintained at 37°C and bubbled with 95% O_2 and 5% CO_2 gas mixture (pH 7.4). The rings were mounted by means of two parallel L-shaped stainless-steel holders inserted into the lumen. One holder served as an anchor while the other was connected to a force-displacement transducer (*Harvard UF1*) that measured the isometric contractile force and recorded it via a computer system (*Videograph CODAS CI*). A basal tension of 2 g was applied. Each preparation was allowed to equilibrate for 60–90 min in Krebs solution prior to the initiation of experimental procedures, and during this period the incubation media was changed every 15 min.

In the screening assay, after equilibration, the aortic rings incubated in Krebs solution were exposed to the ethanol extract of *A. bogotensis* (10 μg/mL), prazosin (1 μM, as reference standard) and (DMSO, 0.1%, as control). Fifteen minutes later, the rings were stimulated with cumulative increasing doses of the alpha-1 agonist phenylephrine (PE, from 0.1 nM to 50 uM) or the calcium channel voltage dependent activator KCl (from 10 μM to 140 mM). Then, according to these results, the capacity of the *A. bogotensis* ethanol extract to reduce the PE pressor effect (pD_2' value) was quantified by assaying 1, 5 and 10 μg/mL concentrations.

In another set of experiments, the *A. bogotensis* relaxant potency was determined in aortic rings stimulated with KCl (80 mM) or PE (1 μM) until the contractile response reached a steady tension. After that, cumulative concentrations of the ethanol extract (0.1 μg/mL - 0.1 mg/mL), were added to isolated aortic rings pre-contracted with PE (0.1 μM) or KCl (80 mM).

To explore the possible participation of nitric oxide (NO), L-NAME (100 μM, NO synthase inhibitor) was administered to aortic rings before adding cumulative concentrations of PE to isolated aortic rings in the presence of the extract (10 μg/mL).

To assess the response obtained due to extract fractioning, the aqueous, butanol and dichloromethane fractions (10 μg/mL) were assayed in rings stimulated cumulatively with PE.

All provisions concerning the protection of animals for experiments stipulated by Resolution 008430/1993 by the *Ministerio de la Protección Social* of Colombia were applied. This work applied experimental protocols endorsed by the Ethics Committee of the Faculty of Sciences of the National University of Colombia (Act 07-2016).

Data analysis

The response of the aortic rings is expressed as a percentage of the maximal plateau contraction (E_{max}) induced by KCl or PE. Concentration-response curves were analyzed to give the logarithm of KCl or PE concentrations producing a 50% of E_{max} (EC_{50}: effective concentration 50) by sigmoidal curve-fitting analysis. In the same way, concentration-response curves to give the logarithm of the extract concentration required to relax isolated aortic rings previously stimulated with PE or KCl to 50% were obtained (IC_{50}). Values of pEC_{50} and pIC_{50} are expressed as $-logCE_{50}$ and $-logCE_{50,}$ respectively. pD_2' corresponds to -log of the extract concentration that reduces the agonist effect to 50% of E_{max} and was obtained by scathcard plot analysis (26, 27).

All results are expressed as means ± the standard error of the mean (SEM). Differences in aorta concentration-response curves were analyzed by one way analysis of variance (ANOVA) followed by Dunnett post hoc tests, with a criterion set for statistical significance at $p<0.05$. *Excel*® and *GraphPad Prism*® software was used for data analysis.

RESULTS

Control rings stimulated cumulatively with PE and KCl attained concentration - response contractions at a plateau (E_{max}) of 2401 ± 328 and 2207 ± 156 mg, respectively. Prazosin (1 μM) and *A. bogotensis* ethanol extract (10 μg/mL) notably reduced the contraction values induced by PE on E_{max}, CE_{50} and pEC_{50}, (Table 1, Figure 1), and their inhibitory effect in rings contracted with KCl is shown in Table 2 and Figure 2.

Table 1. % E_{max}, pEC_{50}, and EC_{50} values with fiducial limits generated by PE (from 0.1 nM to 50 uM) in the absence (control) and presence of *A. bogotensis* ethanol extract (10 μg/mL) or prazosin (1 μM) in isolated aortic rings from Wistar rats.

PE contraction	% E_{max}	pEC_{50}	EC_{50} (M)
Control	99.72 ± 0.85	7.12 ± 0.05	7.54 [5.79 – 9.83]x10^{-8}
A. bogotensis	16.62 ± 1.60	2.32 ± 0.56	4.80 [2.65 – 8.71]x10^{-3}
Prazosin	41.62 ± 2.85	4.23 ± 0.16	5.84 [2.57 – 13.25]x10^{-5}

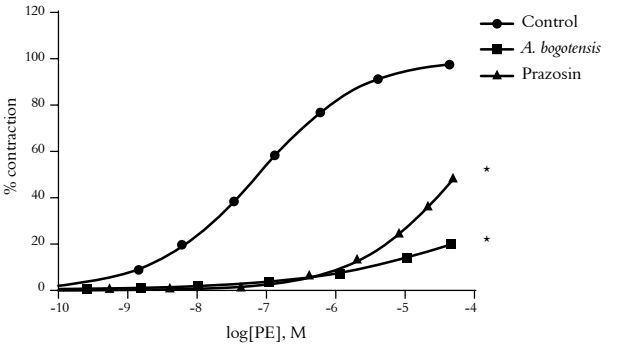

Figure 1. Percentage of contraction of isolated aortic rings from Wistar rats stimulated with cumulatively increasing concentrations of PE in absence (control) and presence of *A. bogotensis* ethanol extract (10 μg/mL) or prazosin (1 μM). Each point represents the mean ± S.E.M. $n \geq 8$, (★$p \leq 0.05$ vs. control).

Table 2. % E_{max}, pEC_{50}, and EC_{50} values with fiducial limits generated by KCl (from 10 μM to 140 mM) in the absence (control) and presence of *A. bogotensis* ethanol extract (10 μg/mL) or prazosin (1 μM) in isolated aortic rings from Wistar rats.

KCl contraction	% E_{max}	pEC_{50}	EC_{50} (M)
Control	99.53 ± 1.52	1.70 ± 0.03	1.98 [1.72 – 2.26]x10^{-2}
A. bogotensis	67.52 ± 2.43	1.27 ± 0.03	5.44 [4.65 – 6.35]x10^{-2}
Prazosin	94.79 ± 2.44	1.52 ± 0.02	3.02 [2.78 – 3.29]x10^{-2}

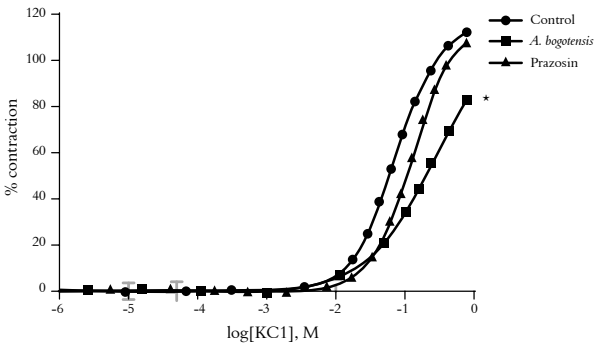

Figure 2. Percentage of contraction of isolated aortic rings from Wistar rats stimulated with cumulatively increasing concentrations of KCl in the absence (control) and presence of *A. bogotensis* ethanol extract (10 μg/mL) or prazosin (1 μM). Each point represents the mean ± S.E.M. $n \geq 8$, (★$p \leq 0.05$ vs. control).

A. bogotensis ethanol extract showed a high capacity for reducing the PE pressor response in a concentration dependent manner (1,5 and 10 μg/mL), giving a pD'_2 value of 5.51 ± 1.70, (Figure 3).

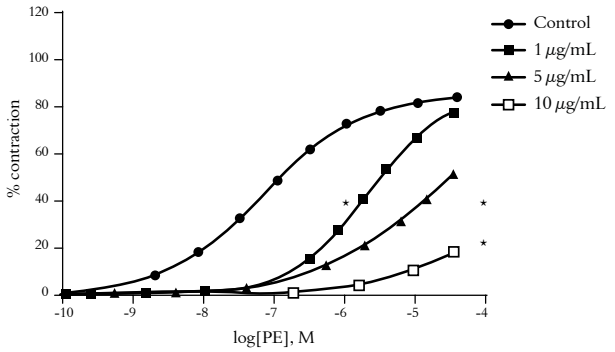

Figure 3. Percentage of contraction of isolated aortic rings from Wistar rats stimulated with cumulatively increasing concentrations of PE in the absence (control) and presence of *A. bogotensis* ethanol extract (1, 5 and 10 μg/mL). Each point represents the mean ± S.E.M. $n \geq 8$, (⋆p≤0.05 vs. control).

A. bogotensis ethanol extract showed complete efficacy in relaxing rings previously precontracted with PE. The relaxant efficacy and potency of *A. bogotensis* extract against rings previously contracted with KCl were notably lower (Table 3, Figure 4).

Table 3. % *Relaxation*, pIC_{50}, and IC_{50} values with fiducial limits generated by cumulative addition of *A. bogotensis* ethanol extract (1 – 100 μg/mL) in isolated rat aortic rings previously contracted with PE (1 μM) or KCl (80 mM).

Stimulant agent	*% Relaxation*	pIC_{50}	IC_{50} *(M)*
PE (1 μM)	98.99 ± 1.01	4.76 ± 0.07	1.75 [0.92 – 3.34]x10^{-5}
KCl (80 mM)	15.69 ± 2.66	3.08 ± 0.35	8.24 [0.24 – 27.69]x10^{-4}

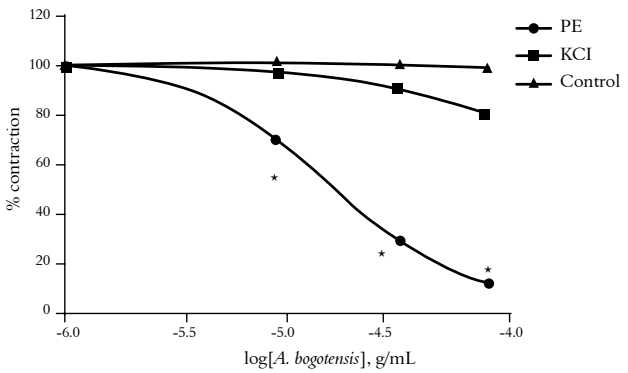

Figure 4. Effect of cumulative addition of *A. bogotensis* ethanol extract (1 – 100 μg/mL) in isolated aortic rings from Wistar rats previously contracted with PE (1 μM) or KCl (80 mM), (⋆p<0.05 versus control: DMSO: 0.1%).

L-NAME significantly reduced the inhibitory effect of *A. bogotensis* extract in rings stimulated with PE. Aqueous, butanol and dichloromethane fractions (10 μg/mL) gave inhibitory responses lower than that obtained with the ethanol extract (Table 4, Fig. 5).

Table 4. % E_{max}, pEC_{50}, and EC_{50} values with fiducial limits generated by PE (from 0.1 nM to 50 uM) in the absence (control) and presence of *A. bogotensis* ethanol extract (10 μg/mL) plus L-NAME (100 μM) and the *A. bogotensis* fractions: aqueous, butanol and dichloromethane, in isolated aortic rings from Wistar rats.

PE contraction	*% E_{max}*	*pEC_{50}*	*EC_{50} (M)*
Ethanol + L-NAME	102.42 ± 3.35	6.09 ± 0.04	8.08 [6.61 – 9.87]x10^{-7}
Aqueous	71.14 ± 3.06	6.22 ± 0.09	6.10 [3.72 – 9.99]x10^{-7}
Butanol	61.24 ± 1.68	5.67 ± 0.15	2.13 [9.36 – 48.23]x10^{-6}
Dichloromethane	66.81 ± 0.52	5.60 ± 0.10	2.51 [1.48 – 4.27]x10^{-6}

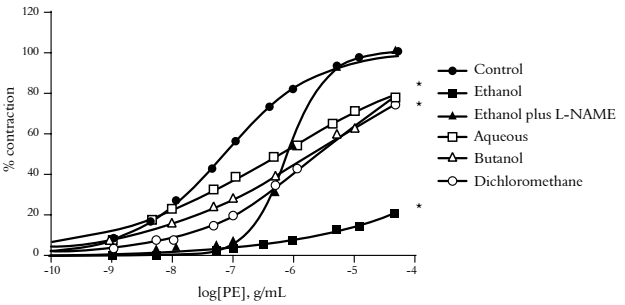

Figure 5. Percentage of contraction of isolated aortic rings from Wistar rats stimulated with cumulatively increasing concentrations of PE in the absence (control) and presence of *A. bogotensis* ethanol extract (10 μg/mL) plus L-NAME (100 μM), or in the presence of the *A. bogotensis* fractions: aqueous, butanol and dichloromethane. Each point represents the mean ± S.E.M. $n \geq 8$, (⋆p≤0.05 vs. control).

Phytochemical screening of the *A. bogotensis* ethanol extract showed significant presence of flavonoid metabolites according to positive results obtained in the *Shinoda test*, revealed with reactive NP-PEG. Triterpene and steroid metabolites were also present according to TLC using the *Liebermann-Burchard* test (25). Alkaloids and saponins were not detected whereas coumarin, tannin and anthraquinone metabolites were present in trace amounts.

Flash column chromatography studies were carried out using glass column with silica gel 60 (0.063-0.200 mm) as stationary phase. The mobile phase used were mix of CH2Cl2/MeOH in increasing polarity. Size-exclusion chromatography were carried out using glass column with

sephadex LH-20 (25-100 mm), as stationary phase. The effluent from the columns was collected into test tubes, fractions with the same Rf (thin layer chromatography) were combined, concentrated and dried under reduced pressure. In thin layer chromatography, 0.25 mm thick pre-fabricated aluminum sheets were coated with silica gel with UV254 fluorescent indicator.

DISCUSSION

These results show that the ethanol extract from the aerial parts of *A. bogotensis* displays a smooth muscle relaxant effect against PE whereas its effect against KCl is lower. This suggest that *A. bogotensis* displays some kind of direct or indirect alpha-1 inhibitory properties while it has less effective inhibitory actions on calcium voltage dependent channels. Because A. *bogotensis* could be exerting modulatory effects in conditions where there is overexpression of alpha-1 mediating receptors, and keeping in mind that its effect is comparable to a synthetic compound like prazosin, it could be proposed that *A. bogotensis* would be useful in the treatment of LUTS related to BHP. Until now, possible antiadrenergic properties of *A. bogotensis* have not been established.

The activity of *A. bogotensis* seems to be dependent, at least in part, on the presence of NO, because L-NAME, an inhibitor of NO synthesis, significantly reduces the inhibitory effect on isolated aortic rings stimulated with PE. In addition, given that the fractions obtained from the ethanol extract gave inhibitory responses lower than those obtained with it, it is possible to propose that more than one active metabolite is present in *A. bogotensis*. If that was not the case, fractionation of the extract should lead to increased inhibitory responses. Because there was a strong presence of flavonoid compounds found in the phytochemical screening, these kind of metabolites could be the main compounds responsible for the antiadrenergic properties of *A. bogotensis*.

Previous works have led to the isolation of 3,5-dihydroxy-6,7,8-trimethoxy flavone from *A. bogotensis*, a compound that possess *in vitro* antineoplasic activity against several tumorigenic cell lines (9, 12, 28). This flavone could also play a pivotal role in the smooth muscle relaxant effects of *A. bogotensis*. It fulfills the criteria proposed for displaying relaxant effects according to structure-activity relationships studied for flavonoid compounds, particularly due to hydroxyl substitution of the B phenolic ring and the absence of these substitutions on the A phenolic ring (29, 30). This conformation would belong to the group of flavone compounds with the 2-phenyl-1,4-benzopyrone backbone.

As for several other flavones, this compound could improve endothelial function by increasing the bioavailability of NO by enhancing its synthesis and/or decreasing its superoxide-mediated breakdown. Therefore, it could help to protect against the development and progression of cardiovascular disease (31). Antioxidant actions would be at the root of its biological activity but other mechanisms could also play an important role (32, 33). Thus, the resulting alpha-1 inhibitory properties of. *A. bogotensis* would be attributable only in part to the presence of nitric oxide, and/or to its antioxidant profile. Additionally, other active compounds could play a role, including those of non-flavonoid nature, such as cyclobutane dimers (achyrodimers), among others (10).

The rat isolated aortic preparation is a suitable model for screening alpha 1-adrenoceptor antagonists [34] and hence, for assessing new agents potentially useful for the treatment of LUTS related to BPH. Tamsulosin, for example, an alpha-1a subtype selective antagonist, show greater relaxant potency in isolated rat aortic rings than in isolated rat vas deferens, an experimental model also routinely used to assess this kind of receptor (35). It is interesting, as found in the results of this work, that *A. bogotensis* displays comparable activity to that seen and described with the reference agent, prazosin (34).

Given that the smooth muscle of the urinary tract is rich in alpha-1a subtype receptors, selective agents like tamsulosin and silodosin are extensively used for LUTS due to BHP. However, adverse events related to alpha-1a interactions, such as floppy iris syndrome, are matters of concern (36). Therefore, non-selective alpha-1 antagonists remain a good alternative for the treatment of LUTS (37).

Future works with the bioactive principles of *A. bogotensis* will have to establish whether they elicit selective actions on alpha-1 receptor subtypes, or display additive or synergistic actions thus exerting a greater activity than the whole extract. In addition to functional studies, alpha-1 binding assays will be necessary (38). In any case, different mechanisms of alpha-1 interaction will have to be considered.

In conclusion, these results suggest that an extract from the aerial parts of the plant species *A. bogotensis* elicits smooth muscle relaxant effects related to the alpha-1 antiadrenergic mechanism. This response is partially NO dependent and seems to be due to interactions among active metabolites in the extract, which would be of flavonoid and/or terpenoid nature. These results give support to the traditional use of this species for LUTS due to BPH.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest in this research.

AUTHORS´ CONTRIBUTIONS

Nidya Lara (MSc) performed the experiments of this work, Javier Rincon (PhD) directed the phytochemical studies and Mario F. Guerrero (PhD) directed the pharmacological procedures.

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