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Research Article

# *PAVONIA ALNIFOLIA* A. ST. HIL.: *IN VIVO* HYPOTENSIVE EFFECT AND *IN VITRO* ACE INHIBITORY ACTIVITY

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**ABSTRACT**

*Pavonia alnifolia* A. St. Hil (Malvaceae) is a vulnerable Brazilian plant species. There is no data about its chemical composition or its biological effects. Therefore, the aim of this study was to evaluate the chemical composition and the in vitro and in vivo hypotensive activity of *P. alnifolia*. A hydroethanolic extract of stems of *P. alnifolia* (EPA) was prepared and the chemical fingerprint composition was evaluated using the HPLC-isocratic method (MeOH:$H_2O$, 95:05). The in vitro angiotensin converting enzyme (ACE) inhibition was assessed by a colorimetric method and the in vivo acute hypotensive effect was quantified using male spontaneously hypertensive rats (SHR) and their normotensive controls, Wistar-Kyoto (WKY) rats. The HPLC chemical fingerprint of the EPA revealed a simple profile, with the predominance of peaks of polar compounds. Rutin was identified as one of the compounds, and a quantification of the flavonoids yielded the presence of 1.28 ± 0.17% as calculated by rutin. EPA showed 59.6 ± 4.7 % ACE *in vitro* inhibitory activity at a concentration of 100 µg/ml. EPA elicits a dose-dependent hypotensive effect in normo- and hypertensive animals with no statistic difference at all doses administered (4, 40, 80, 160, 600 mg/Kg). In conclusion, the results indicate a promising role of this specie as anti-hypertensive plant, as a dose-dependent hypotensive effect and the inhibition of ACE were observed. The rutin might be one of the active compounds.

**Keywords:** *Pavonia alnifolia,* Angiotensin converting enzyme, Rutin, Endangered species, Hypotensive effect, Flavonoids.

## INTRODUCTION

Brazil is known for its biodiversity, mainly due to the high biodiversity found in the Atlantic Forest [1]. However, in this biome has substantially increased the number of species threatened with extinction by historical factors, such as disorderly occupation of land, demand for timber and agricultural frontier expansion [1]. In a survey conducted by the State Institute of Environment and Water Resources of Espírito Santo (IEMA) it was stated, that 753 plant species are threatened by extinction in different levels [2]. The chemical composition and the biological proprieties of many of those species remain unknown [3,4].

The specie *Pavonia alnifolia* A. St. Hil. (Malvaceae) is endangered and regarded as vulnerable in the official list of endangered flora of Espírito Santo, Brazil [2]. In the database consulted, no data about the chemical constituents and the biological activity of this specie could be found. However, few other species of the genus have been studied, mainly by evaluating their antimicrobial effect [5,6]. The methanolic extract of leaves of *P. zeylanica* has been reported as larvicidal [5], and also an antibacterial effect has been proven [6]. The chemical constitution, however, has been poorly described for these species. The saponin pavophylline was isolated from stem of *P. zeylanica* [6].

There are some species of the Malvaceae family showing a substantial antihypertensive effect such as *Hibiscus sabdariffa* [7,8,9]. The aqueous extract of calyx of *H. sabdariffa,* standardized on 9.6 mg of total anthocyanins, showed an antihypertensive effect similar to captopril 50 mg/day in a clinical trial [7]. The aqueous extract of calyx of *H. sabdariffa* was evaluated in two types of experimental hypertension: salt-induced and Nω-L-arginine methyl ester (L-NAME)-induced hypertension, and in normotensive controls [8]. In this study, the aqueous extract of calyx of *H. sabdariffa* at the dose range 1–125 mg/Kg elicited a dose-dependent antihypertensive, hypotensive and negative chronotropic effects [8]. Recently, there was demonstrated that the aqueous extract of *H. sabdariffa* showed an in vitro angiotensin converting enzyme (ACE) inhibitory activity [9]. The two most abundant anthocyanins, delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside were identified as the active compounds [8].

ACE (E.C. 3.4.15.1), a component of the renin-angiotensin system (RAS), plays a substantial role in regulating the homeostatic mechanism of mammals by modulating the RAS [10,11]. ACE is a dimeric dipeptidylcarboxypeptidase which catalyzes the conversion of an inactive form of decapeptide, angiotensin I, to a potent vasoconstrictor, octapeptide angiotensin II, and inactivates bradykinin, acting as depressor [10,11]. The deregulation of RAS causes an increase of ACE resulting in hypertension [10]. Therefore, in vitro inhibition of ACE is considered an effective screening method in the research activities for new antihypertensive agents [12,13].

The lack of chemical and biological information about *P. alnifolia* along with its ecological impact has encouraged the present research with threatened species. Thus, the aim of this study is to generate preliminary knowledge about the in vitro ACE inhibition, the in vivo acute hypotensive effect of the hydroethanolic extract of stems of *P. alnifolia* and its chemical fingerprints.

## MATERIALS AND METHODS

### Plant material and extraction

Stems of *P. alnifolia* were collected in August 2009, at *Parque Estadual Paulo Cesar Vinha* (Protocol permission number 629/09, IEMA). A voucher specimen has been deposited at the Herbarium of the Federal University of Espírito Santo (VIES 17697). After drying at 45°C for 72h, the plant material was grained (200.0 g) and percolated with hydroethanolic solution (80% v/v) at room temperature. The solvent was removed under reduced pressure furnishing 69 g of a brown residue. The hydroethanolic extract of stems of *P. alnifolia* (EPA) was kept in desiccator under vacuum for at least 48 h for complete removal of the solvent. Aliquots of the extract were dissolved in saline, to be used for the ACE inhibition assay and the analysis of the hypotensive effect.

### Chromatographic characterization (HPLC-RP)

A Waters 1515 system (USA) is composed of a binary pump, UV/VIS detector (model 2489), and manual a sampler and Breeze software were used for data processing. The analyses were performed on a XBridge™ C-18 column (150 x 4.6 mm i.d., 3.5 µm, Waters) in combination with XBridge™ C-18 guard column (20 x 4.6 mm i.d.,

3.5 μm, Waters), at room temperature and flow rate of 0.80 mL.min$^{-1}$. UV detection was performed at 254 nm and 365 nm. An isocratic elution of MeOH: $H_2O$ (95:0.5, 1% phosphoric acid, pH 4.0) was employed. Solvents used were of HPLC grade (Merck, Germany), water was ultrapure (ELGA 18.2 Ω) and degassed by sonication before use. Standards and samples were dissolved in MeOH to final concentrations of 2 and 10 mg/ml, respectively, for standards (rutin, epigalocathequin, pyrogallic acid) and EPA. After centrifugation at 8.400*g* for 5 min, the sample solutions (20 μL) were manually injected into the apparatus. Standard stock solution of rutin was prepared by dissolving 10 mg of rutin in methanol, yielding 10 ml of a concentration 1.00 mg/ml. Series of dilutions were prepared to yield 10 ml of standard solutions containing 1.95, 3.90, 7.80, 15.6, 31.3, 62.5, 125.0 and 250 mg/ml of rutin, respectively. Epigalocathequin and pyrogallic acid were not identified in the EPA.

**Angiotensin converting enzyme inhibition in vitro assay**

The effect of EPA angiotensin converting enzyme in vitro was determined by measuring Gly-Gly (glycil-glycine) cleavage product of Hip-Gly-Gly by ACE. The assay was performed as previously described [12].

**Acute hypotensive effect evaluation**

The animal experiments were performed according to the recommendations of the Brazilian Council for Animal Care and were approved by the Ethics Committee of the University Centre of Vila Velha. Male spontaneously hypertensive rats (SHR) and their normotensive controls, the Wistar-Kyoto (WKY) rats, were used. The animals were three months old with a body weight ranging between 280-300g. They were housed at 22 ± 3 ◦C under a 12 h light/12 h dark cycle and had free access to standard pellet diet (ration Probiotério, Windmill Primor SA) and tap water.

A polyethylene catheter (PE 50 - Clay Adans ®, USA) was connected to the femoral artery and vein. Under anesthesia by sodium pentobarbital (50 mg/Kg, intra-peritoneal Hypnol ®, Crystal, Brazil), an incision in the inguinal region was carried out, with a subsequent isolation of the vascular-nerve plexus which enabled catheterization of the aorta via the femoral artery and the femoral vein. The termination of the catheter was kept open filled with saline (0.9%) and occluded with stainless steel pins. Catheters were placed into the femoral artery for recording arterial blood pressure and into the femoral vein for administration of EPA.

The femoral arterial catheter was connected to pressure transducers (Spectramed - Statham ®, P23XL, USA) through a flexible catheter. The registry values of mean arterial pressure (MAP) were obtained through a computerized system (Pentium MMX 233 MHz) and a program for biological data acquisition (Biopac ® - Biopac Systems, Inc., Santa Barbara, California, USA, mod. 100A/serie 94,111,065 MP).

The animals were connected to a pressure transducer, followed by stabilization. Thereafter, levels of MAP at baseline were recorded for 15 minutes. Thereafter increasing doses of EPA (4, 40, 80, 160, 600 mg/Kg) were administered randomly, as well the vehicle (isotonic saline 1 ml/Kg). For each dose the MAP was measured before and after (maximum decrease) its administration. The results were expressed using the change in MAP (percentage of decrease) produced by the administration of each of them. The values of MAP were first left to return to baseline prior to administration of a next dose.

**Statistical analysis**

Data of the biological evaluation of the EPA were expressed as mean values ± standard error of the mean (S.E.M.). Values of the baseline MAP as well the changes in MAP produced by EPA were subjected to one-way analysis of variance (ANOVA). The *post hoc* test used for each case was the Fisher's *t*-test for multiple comparisons. Differences were considered as statistically significant when $p < 0.05$. Results from ACE analysis were expressed as mean ± S.E.M. The statistical significance for the inhibition ACE was determined by Student's t-test. Differences were considered significant at $P < 0.05$.

**RESULTS AND DISCUSSION**

EPA elicited a moderate ACE *in vitro* inhibitory activity (59.6 ± 4.7 %, 100 μg/ ml) when compared to some other plant extracts, such as species of *Senecio* and *Salvia elegans* [13,14]. The EtOAc fraction of aerial parts of *Senecio* species showed an inhibitory activity of ACE, $IC_{50}$ = 219.4 ± 1.4 μg/ml and $IC_{50}$ = 192 ± 1.8 μg/ml, respectively for *S. ambiguous* subsp *ambiguous* and *S. inaequidens* [13,14]. Whereas, the ethanolic extract of *Salvia elegans* and its *n*-BuOH fraction elicited respectively 50.3 ± 5.1 % and 78.4 ± 2.2 % of ACE inhibition, when assayed at the concentration of 2.7 mg/ml. The inhibition activity of those species was attributed to the flavonoids contents [13,14].

The HPLC fingerprint of the assayed EPA showed a simple profile, with the predominance of peaks of polar compounds. The standards solutions of rutin, epigalocathequin and pyrogallic acid injected in the same chromatographic conditions, did not indicate the presence of pyrogallic, epigalocathequin, but produced a peak with identical retention time of rutin. The chromatographic profiles registered for EPA and the flavonoids quantification showed the presence of 1.28 ± 0.17 % w/w of flavonoids, measured by rutin, in EPA. Several studies reported about the antihypertensive and ACE inhibitor activity of flavonoids and procyanidins [13, 14,15]. Flavonoids are generally regarded as moderate ACE inhibitors, yielding $IC_{50}$ values from 158.9 a 708.8 μM [13, 16]. The synergism between the components present in extracts were discussed, indicating that administration of flavonoid-enriched extracts provide greater therapeutic benefit [13,14]. Some studies have demonstrated that the flavonoid rutin itself has an antihypertensive effect [17, 18]. Hence, some flavonoids, such as rutin, were suggested to show *in vitro* activity *via* the generation of chelate complexes within the active center of ACE [14-18]. The flavonoids present in EPA might be involved in the ACE in vitro inhibition activity.

As expected, SHR showed an elevated MAP (160±5 mm Hg) as compared to control normotensive animals (99±4 mm Hg; $p<0.01$). The acute effect of EPA on the MAP in normotensive (WKY) and hypertensive (SHR) animals are depicted in *Figure 1*.

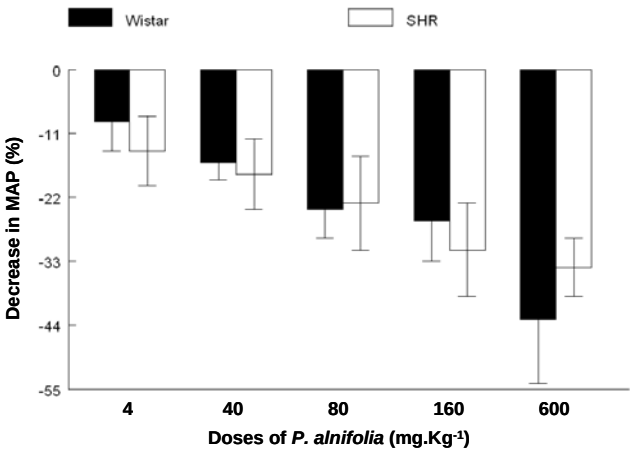


**Fig. 1: Dose-dependent hypotensive effect of the hydroalcoholic *P. alnifolia* (EPA) in normotensive (WKY) and hypertensive (SHR) animals. Values are expressed as mean ± S.E.M.**

EPA elicits an acute dose-dependent hypotensive effect in animals (*Figure 1*). The present study shows for the very first time that the endangered specie *P. alnifolia* posses an in vivo hypotensive effect together with an in vitro ACE inhibitory activity. This work is the first one to report about the ability of *P. alnifolia* to reduce blood pressure in both, normotensive and hypertensive animals (using the SHR model of hypertension), and the presence of rutin as one of the chemical constituents, the data of this study suggested that hypotensive effect of EPA could be related to the in vitro ACE inhibition [18].

In conclusion, the hydroethanolic extract of stems of *P.alnifolia* showed an acute in vivo dose-dependent hypotensive effect and the inhibition of ACE could be one of the pathways for this effect and the rutin one of the active compound. However, further investigation remains to be conducted to indicate the main compound and the pathway of a chronic hypotensive effect.

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