Vasorelaxant activity and acute toxicity of the ethanolic extract of *Zanthoxylum rhoifolium* Lam leaves

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The study evaluated the vasorelaxant effect induced by the ethanolic extract of the leaves of *Zanthoxylum rhoifolium* Lam (EEtOH-Zr/leaves). Wistar rats were treated with the leaf extract containing a single dose of 2,000 mg / kg, v.o. After 14 days, the animals were anesthetized for blood collection and subsequent analysis of the biochemical parameters; they were then euthanized (sodium pentobarbital-100 mg/kg, i.p.) for the removal and morphological analysis of the heart, lung, liver and kidney. The vasorelaxation activity the and vascular reactivity of EEtOH-Zr/leaves were evaluated on artery mesenteric rings isolated from rats. The extract showed no signs of toxicity and no significant difference in the values of the biochemical parameters between the control group and the group of treated animals. In the evaluation of pharmacological activity in the smooth muscle, the EEtOH-Zr/leaves caused vasorelaxant effect on the tonic contraction induced by phenylephrine in mesenteric artery preparations in the presence (pD2=2.17±0.05 µg/mL; Emax=99.8±5.2%) and absence (pD2=2.14±0.05 µg/mL; Emax=95.3±6.4%) of the vascular endothelium. Oral administration of EEtOH-Zr/leaves reduced the contraction induced by the cumulative addition of PHE. It is concluded that the EEtOH-Zr/leaves promote vasorelaxation and reduce vascular reactivity of adrenergic alpha-1 agonist in the mesenteric artery. The results did not show toxic effects of the extract.

**Keywords:** Mesenteric arteries/ drug effects. Vasorelaxant. *Zanthoxylum rhoifolium*/ toxicity. Rats, Wistar. Blood Vessels/ drug effects.

**INTRODUCTION**

Cardiovascular disorders are the leading cause of morbidity and mortality worldwide (Hoyert, Xu, 2012). 25% of adults suffer from this disease and this number will reach 29% by 2025 (Kearney *et al.*, 2005). Lowering blood pressure greatly reduces the risks of developing heart failure, coronary diseases, renal damages and cerebral vascular diseases (Ezzati *et al.*, 2002). Most of these disorders are untreatable and the current pharmacological strategies only aim at the disease control (Canto, Kiefe, Greenland, 2012). Various biochemical compounds, especially those used in the treatment of arrhythmia and heart failure, have serious adverse events. Therefore, there is a growing trend towards using medicinal plants in health care in general medicine, particularly in cardiovascular medicine (Imanshahidi, Hosseinzadeh, 2008). The reputation of plants in saving human beings has a long history, cutting across different cultures in the world (Hosseinzadeh *et al.*, 2015). In the contemporary world of conventional medicine, the practice of herbal medicine has drawn more attention and is becoming accepted globally (Khan, Yadava, 2010). In Brazil, the use of medicinal plants by the population in order to treat diseases was always expressive, mainly due to the extensive and diverse flora (Pasa, 2011). Secondary metabolites extracted from these plants raise great interest due to their pharmacological activities and healing potential (Pereira, Cardoso, 2012).

The species *Zanthoxylum rhoifolium* Lam is a tree belonging to the Rutaceae Juss family (Costa *et al.*, 2014).
Its botanical synonym is *Fagara rhoifolia* (Lam) (Weber, 2005) and it is included in the Rutales order. Popularly known as “maminta-de-porca”, it is found in the Cerrado areas (Moreira, 1996; Salgado et al., 1998), Atlantic forest and the Amazon (Pirani, 2005). In Northeastern Brazil, it is found in the states of Piauí and Ceará (Melo, Zickel, 2004). Some researches point out the medicinal potential of this species. Phytochemical screening of the ethanolic extract of *Zanthoxylum rhoifolium* stem barks suggested the presence of isoprene substances (pentacyclic triterpenes and steroids) and alkaloids (Freitas et al., 2011). The presence of triterpenes in the species was confirmed by the isolation and identification of lupeol (Cameло et al., 2007; Pereira et al., 2010). The phytochemical profile of the leaves was not determined in this study. However, terpenoid constituents such as lupeol can also probably be found.

The bark extract has also demonstrated analgesic activity, popularly mentioned for toothache and earache, as well as fungicidal activity (Pereira, et al., 2010; Carotenuto, et al., 2015). Furthermore, Jullian et al. (2006) were able to prove the antimalarial potential of the shell. Silva, Figueiredo, Yano (2007a, b) evaluated the essential oil of the leaves of *Z. rhoifolium* and observed the cytotoxic effect against tumor cells, thus suggesting a possible therapeutic action against such cells. Freitas et al. (2011) demonstrated that the ethanolic extract of the species bears significant gastric protection because it inhibits the formation of gastric lesions using different models. Costa et al. (2008) identified antibacterial and larvicidal effects in the essential oil extracted from the fruit. *Z. rhoifolium* bark extract showed analgesic activity, popularly mentioned for toothache and earache, as well as fungicidal activity (Pereira, et al., 2010; Carotenuto, et al., 2015). The study by Jullian et al. (2006) was able to prove the antimalarial potential of the shell. Silva, Figueiredo, Yano (2007a, b) evaluated the essential oil of the leaves of *Z. rhoifolium* and observed the cytotoxic effect against tumor cells, thus suggesting a possible therapeutic action against such cells. Freitas et al. (2011) demonstrated that the ethanolic extract of the species exhibits significant gastric protection because it inhibits the formation of gastric lesions using different models. Costa et al. (2008) identified antibacterial and larvicidal effects in the essential oil extracted from the fruit. In addition, a previous study showed that the ethanolic extract from the *Z. rhoifolium* stem bark has antihypertensive effect in spontaneously hypertensive rats (SHR) and vasorelaxant effect in the mesenteric artery of normotensive rats (Ferreira-Filho et al., 2013), and how the vascular tone is directly involved in the regulation and maintenance of blood pressure (Sonkusarc et al., 2006). However, no report on the effect of the leaf extract was found, what allows the present study to contribute to the knowledge of the pharmacological actions of the *Z. rhoifolium* Lam species. In order to shed light to its therapeutic potential for the treatment of cardiovascular diseases, this study is to evaluate the effect of the extract of *Z. rhoifolium* leaves on vascular smooth muscle and its possible action toxicity in rats.

**MATERIAL AND METHODS**

**Preparation of plant extracts**

The botanical identification was performed by Prof. Dr. Roseli Farias Melo de Barros. The voucher specimen (TEPB 13870) was deposited at the Graziela Barroso Herbarium of the Federal University of Piauí (UFPI). The leaves from EtOH-Zr were provided by Prof. Dr. Mariana Helena Keys, Department of Chemistry, Natural Sciences Center, Federal University of Piauí. Dried and powdered leaves of *Z. rhoifolium* (1000g) were extracted at room temperature exhaustively with ethanol. The solvent was removed by evaporation under reduced pressure using a Hedolph Rotary Evaporator to yield the EEtOH extract (75.0 g, 7.5%).

**Drugs and reagents**

The composition of Tyrode’s solution used was (mM): NaCl, 158.3; KCl, 4.0; CaCl$_2$, 2.0; MgCl$_2$, 1.05; NaH$_2$PO$_4$, 0.42; NaHCO$_3$, 10.0; and glucose, 5.6 mM (Tanaka et al., 1999), sodium thiopental (Cristália) and sodium salt of heparin (Roche). In order to prepare the stock solutions of the drugs, all substances were dissolved in distilled water and diluted to the appropriate concentrations. The extract was dissolved in Tyrode’s solution for the in vitro protocols and in brine for the in vivo protocols using Cremophor (0.1% w / w) as the eluent. All solutions were stored at 0°C.

**Animal study**

Wistar rats (*Rattus norvegicus*), weighing between 250 and 300 grams, from the Research Center of the Vivarium on Medicinal Plants of the Federal University of Piaui, kept at 24 ± 2°C and under light-dark cycle of 12 hours with water and feed ad libitum. All protocols were approved by the Ethics Committee on Animal Experiment of the Federal University of Piaui (EAEC N°008/12). The animal euthanasia procedure is in accordance with the Resolution No. 1000 from 2012 of the Federal Council of Veterinary Medicine.
Experimental protocols

**In vivo toxicity of EEtOH-Zr/leaves**

The oral toxicity study was performed using the fixed dose method (OECD guideline no. 420). The animals (n=6) were weighed, identified and treated with the ethanolic extract obtained from the leaves of *Zanthoxylum rhoifolium* Lam (EEtOH-Zr/leaves), orally (rigid orogastric tube) with a single dose of 2000 mg/kg, in a volume of 10 mL/kg. During the treatment, each animal was observed for 14 days to check the occurrence of possible indicative signals of a pharmacological and/or toxicity effect (tremors, convulsions, hypoactivity, ataxia, lethargy, and others) (Gazda *et al.*, 2006). Control group animals received isovolumetric doses of saline (0.9% NaCl) via oral route. After 14 days of treatment, the animals were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and submitted to blood collection by puncturing the abdominal artery. The biochemical parameters were determined in a Biopluss 2000® analyzer to test glucose, AST (aspartate aminotransferase), ALT (Alanine aminotransferase), cholesterol, triglycerides, GSH (glutathione) according to Cartágennes (2009), modified.

**Ex vivo protocols**

**Biochemical tests**

The parameters were evaluated with three different methods using commercial kits. The enzymatic methods assessed glucose, urea, creatinine, cholesterol and triglycerides. The kinetic methods assessed AST, ALT, catalase and GSH (data not shown). The animals were euthanized with a lethal dose of thiopental (100 mg/kg, i.p.) and organs (heart, lung, liver and kidneys) were removed, weighed and morphologically and macroscopically examined (Cartágennes, 2009; Sabino *et al.*, 2013).

**In vitro protocols**

**Preparation of rat superior mesenteric artery rings**

The superior mesenteric arteries were quickly removed and cleaned of adherent connective tissues and fat. Mesenteric rings (2–3 mm length) were obtained and suspended by cotton threads in an organ bath containing 10 mL of Tyrode’s solution, maintained at 37 °C and gassed with a 95% O₂+5% CO₂ mixture (pH 7.4). The rings were stabilized with a resting tension of 0.75 g for at least 60 min, with replacement of Tyrode’s solution every 15 min to prevent the accumulation of metabolites that could cause the results to be biased and thus misinterpreted (Altura, Altura, 1970). Isometric tension was recorded by a force-displacement transducer coupled to a data acquisition software (AECAD 1604, AQCAD 2.0.5; AVS Projetos, SP). When necessary, endothelium was removed by gently rubbing the intimal surface of the vessels with a thin stainless wire and endothelial functionality was assessed through the ability of acetylcholine (10 µM) to induce more than 70% of the relaxation associated with the phenylephrine (PE 10 µM) tonus (Furchgott, Zawadzki, 1980). The absence of relaxation following acetylcholine administration was taken as an evidence that the rings were functionally denuded of endothelium.

**Effect of EEtOH-Zr/leaves PE-induced tonic contractions in endothelium-intact and endothelium-denuded rat mesenteric rings**

After the verification of the endothelium integrity and during the tonic component of a second response to the PE (10 µM), EEtOH-Zr/leaves (0.1 – 750 µg/mL) was cumulatively added to the bath in different preparations. The relaxation was expressed as the reversal percentage of the initial contraction elicited by contractile agents, and pD₂ values (Anti-log concentration of a substance that produces 50% of its maximum effect) were obtained with nonlinear regression from EEtOH-Zr/leaves concentration–response curves of rat mesenteric rings with both endothelium-intact and endothelium-denuded mesentery.

**Effects of EEtOH-Zr/leaves on the vascular tone of mesenteric artery rings**

For the assessment of vascular reactivity in a series of experiments, phenylephrine was added to the tank (10⁻⁹-10⁻⁵ M) to obtain a control curve. In other preparations with the arteries of the animals treated for seven days with EEtOH-Zr/leaves (50 mg/kg/7 days) orally, a study for the vascular reactivity to the cumulative addition of phenylephrine took place (10⁻⁹-10⁻⁵ M).

**Statistical analysis**

Values are expressed as mean ± standard error of the mean. We used the “t” test for unpaired samples. The pD₂ (negative logarithmic effect of EC₅₀) values were calculated by nonlinear regression curves drawn from the percentages of the responses obtained by the substances tested for in vitro experiments. Emax refers to the maximum relaxation value. The level of significance was p <0.05 and the GraphPad Prism 6.0 software was used.
RESULTS

Acute toxicity and biochemical parameters of EEtOH-Zr/leaves

The ethanolic extract of the leaves of *Z. rhoifolium* (EEtOH-Zr/leaves) at a dose of 2,000 mg/kg (orally) showed no obvious toxicity signal and did not cause death of the animals within 30 to 240 minutes, 24 hours and up to 14 days after the administration. Also, there were no significant differences between the groups treated for motor activity parameters, respiration, corneal reflexes and amount of dung (Table I). In relation to the body weight of the animals, there was no significant difference between the control groups and EEtOH-Zr/leaves (Figure 1). The analysis of organs (lung, heart, liver, spleen and kidneys) failed to detect changes or gross weight change in any of the control and EEtOH-Zr/leaves groups (Table II). There was also no significant difference between the values of biochemical parameters observed in the serum of the control group (saline) compared with the group treated with EEtOH-Zr/leaves (Figure 5).

Vasorelaxant effect EEtOH-Zr/leaves on rat mesenteric artery

The EEtOH-Zr/leaves induced concentration-dependent vasorelaxant effect on the tonic contraction induced by phenylephrine in the mesenteric artery preparations in the presence \( \left( \text{pD}_2 = 2.17 \pm 0.05 \text{ mg/mL}; \ E_{\text{max}} = 99.8 \pm 5.2 \% \right) \) and absence \( \left( \text{pD}_2 = 2.14 \pm 0.05 \text{ mg/mL}; \ E_{\text{max}} = 95.3 \pm 6.4 \% \right) \) of the vascular endothelium (Figure 2).

**TABLE I - Acute toxicity of EEtOH-Zr/leaves at the oral dose of 2,000 mg/kg:** (0) No effect; (+) diminished effect; (+) increased effect

| Activities                              | Time (min) |
|-----------------------------------------|------------|
|                                         | 30         | 60         | 120        | 180        | 240        |
| STIMULANT                               |            |            |            |            |            |
| Hyperactivity                           | 0          | 0          | 0          | 0          | 0          |
| Agressiveness                           | 0          | 0          | 0          | 0          | 0          |
| Tremors                                 | 0          | 0          | 0          | 0          | 0          |
| Convulsion                              | 0          | 0          | 0          | 0          | 0          |
| Piloeration                             | 0          | 0          | 0          | 0          | 0          |
| DEPRESSOR                               |            |            |            |            |            |
| Eyelid ptosis                           | 0          | 0          | 0          | 0          | 0          |
| Sedation                                | 0          | 0          | 0          | 0          | 0          |
| Anesthesia                              | 0          | 0          | 0          | 0          | 0          |
| Ataxia Reflection of straightening      | 0          | 0          | 0          | 0          | 0          |
| Catatonia                               | 0          | 0          | 0          | 0          | 0          |
| Analgesia                               | 0          | 0          | 0          | 0          | 0          |
| Loss of eyelid reflex                   | 0          | 0          | 0          | 0          | 0          |
| Loss of the atrial reflex               | 0          | 0          | 0          | 0          | 0          |
| Eyelid ptosis                           | 0          | 0          | 0          | 0          | 0          |
| AUTONOMOUS NERVOUS SYSTEM               |            |            |            |            |            |
| Diarrhea                                | 0          | 0          | 0          | 0          | 0          |
| Cold                                    | 0          | 0          | 0          | 0          | 0          |
| Tearing                                 | 0          | 0          | 0          | 0          | 0          |
| Salivation                              | 0          | 0          | 0          | 0          | 0          |
| Cyanosis                                | 0          | 0          | 0          | 0          | 0          |
| OTHER BEHAVIORS                         |            |            |            |            |            |
| Ambulance                               | 0          | 0          | 0          | 0          | 0          |
| Self-cleaning                           | 0          | 0          | 0          | 0          | 0          |
| Rise                                    | 0          | 0          | 0          | 0          | 0          |
| Climb                                   | 0          | 0          | 0          | 0          | 0          |
| Vocalization                            | 0          | 0          | 0          | 0          | 0          |
| Abdominal contortions                   | 0          | 0          | 0          | 0          | 0          |

**DEATH** 0 0 0 0 0

Figure 3 shows the original record of the vasorelaxant effect of the EEtOH-Zr/leaves.

Effect of the prolonged treatment with EEtOH-Zr/leaves on vascular reactivity in rat mesenteric artery rings

In rat mesenteric artery rings, prolonged administration of the EEtOH-Zr/leaves (50mg/kg/7 days)
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**DISCUSSION**

In general, acute toxicity tests are intended to define the scope of the lethal dose of a drug administered in a single dose or in a short-time interleaved dose. These tests are part of the initial pharmacological screening, which is observed during the action of the drug on important parameters and functions. Studies with EEtOH-Zr/leaves showed that the oral dose of the extract did not cause behavioral changes such as stimulant or depressant action, and death within 30 to 240 minutes, 24 hours and 14 days after the administration. No alterations were observed in the biochemical parameters analyzed (Figure 5).

### TABLE II - Effect of EEtOH-Zr/leaves (2,000 mg/kg, v.o.) on mean relative organ weight (%) in male rats.

| Organs (g) | Saline       | EEtOH-Zr/leaves (2000 mg/kg) |
|------------|--------------|-----------------------------|
| Lungs      | 0.708 ± 0.032| 0.778 ± 0.040               |
| Heart      | 0.324 ± 0.012| 0.332 ± 0.010               |
| liver      | 2.881 ± 0.153| 2.871 ± 0.170               |
| Spleen     | 0.355 ± 0.021| 0.361 ± 0.020               |
| Kidneys    | 0.669 ± 0.027| 0.669 ± 0.010               |

Values are expressed as Mean ± SEM (n = 6)

attenuated the contraction induced by the addition of phenylephrine ($E_{\text{max}} = 0.99 \pm 0.02 \text{ g/ f}^{* *}, p <0.05$) when compared with control animal rings ($E_{\text{max}} = 1.17 \pm 0.03 \text{ g/ f}$), indicating that the EEtOH-Zr/leaves can interfere with the contractile response to phenylephrine (Figure 4).

**FIGURE 2** - Concentration-response curves of the vasorelaxant effect of EEtOH-Zr/leaves on upper mesenteric artery rings isolated from normotensive mice in the $E+$ (■) and non-$E$ (□) vascular endothelium rats. Values were expressed as mean ± SEM of 5 experiments.

**FIGURE 3** - Original tracing of the vasorelaxant effect of EEtOH-Zr/leaves on contractions induced by phenylephrine (10 μM) in presence ($E+$) or absence of vascular endothelium ($E-$).
In the evaluation of pharmacological activity on the vascular smooth muscle, the main observation is that EEtOH-Zr/leaves promoted a vasorelaxant effect dependent on the concentration and independent on the vascular endothelium in mesenteric artery rings pre-contracted with phenylephrine (Figure 3). The contraction and relaxation of the vascular smooth muscle can be regulated by extracellular Ca\textsuperscript{2+} influx via the receptor operative Ca\textsuperscript{2+} channel (ROCC) or the voltage-dependent Ca\textsuperscript{2+}-channel (CaV-L) without endothelial derived factors (Karaki et al., 1997). The contractile activity of the smooth muscle of arteries and arteriole cells is the main determinant of the resistance to blood flow; it consequently influences the regulation of blood pressure (Jackson, 2000).

Natural products with biological activity have been reported in the literature as active in the regulation of the vascular tone. In order to verify the effect of the leaves of *Zanthoxylum rhoifolium* Lam on vascular smooth muscle, phenylephrine, an agonist of the α1-adrenergic receptors that is bound to the Gq/11 protein, was used. By

![Graph](image)

**FIGURE 4** - Vascular Reactivity of EEtOH-Zr/sheets (50 mg/kg/7 days) on contractions induced by cumulative addition of phenylephrine (10\textsuperscript{-9}-10\textsuperscript{-5} M) in absence of the mesenteric vascular endothelium. Values were expressed as mean ± s.e.m. (●) control; (○) EEtOH-Zr (50 mg/kg/7 days); **p<0.05, ***p<0.001 vs. control, n=12 rings.

![Graph](image)

**FIGURE 5** - Biochemistry analysis of the plasma of male rats treated with saline and EEtOH-Zr/leaves, orally, with a single dose of 2,000 mg/kg, in a volume of 10 mL/kg. Values were expressed as mean ± s.e.m. control; (■) and (□) EEtOH-Zr (14 days).
being activated, it induces the formation of inositol-1,4,5-triphosphate (IP$_3$) and diacylglycerol (DAG) through the hydrolysis of the phosphatidylinositol 4,5-biphosphate (PIP$_2$) of the plasmatic membrane. The IP$_3$ binds to its receptor in the sarcoplasmic reticulum (RIP$_3$), which induces the release of calcium, thus generating a process of vascular smooth muscle contraction (Zhang et al., 2010). The contraction induced by phenylephrine is mediated by an increase in the Ca$^{2+}$ influx through the receptor-operated calcium channels and is also sensitive towards the voltage (Lee et al., 2001). The agonists like phenylephrine cause an initial spike in Ca$^{2+}$ followed by small sustained rise in Ca$^{2+}$ above the basal levels, thus increasing the Ca$^{2+}$ sensitivity of MLC phosphorylation and leading to increased contraction (Khalil, 2010).

In the present study, it was observed that in vitro administration of EEtOH-Zr/leaves induced concentration-dependent vasorelaxant effect, despite the presence of the vascular endothelium, which suggests non-involvement of derived relaxing factors from endothelium (EDFR) in that response (Figure 2). In experiments to verify the effect of oral treatment with extended EEtOH-Zr/leaves on the contractions induced by phenylephrine, it was observed that the extract was able to inhibit the contractions induced by phenylephrine with reduction of the maximal effect (Emax) (Figure 4) in endothelium-denuded mesenteric rings. These results suggest that EEtOH-Zr/leaves somehow influence the contractile responses induced by phenylephrine probably by acting on the release of calcium from intracellular stores or inhibiting the calcium influx through the membrane via the ROCC. In a previous study with the bark of the stem of Z. rhoifolium, we demonstrated a vasorelaxation response like that found with the leaves, what leads us to reflect on the bioactive molecules present in the two extracts.

**CONCLUSION**

The EEtOH-Zr/leaves showed no acute toxicity in the animals after 14 days of treatment and alter the contractile response induced by phenylephrine upper mesenteric artery rings isolated from rats. Vasorelaxant activity also showed concentration-dependent and independent on vascular endothelium in the preparations of arteries pre-contracted with phenylephrine.

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**REFERENCES**

Altura BM, Altura BT. Differential effects of substrate depletion on drug-induced contractions of rabbit aorta. Am J Physiol. 1970;219(6):1698-705.

Carotenuto G, Carrieri R, Tarantino P, Alfieri M, Leone A, Tommasi N, et al. Fungistatic activity of Zanthoxylum rhoifolium Lam. bark extracts against fungal plant pathogens and investigation on mechanism of action in Botrytis cinerea. Nat Prod Res. 2015;29(23):2251-5.

Camelo FB, Sousa CMM, Gonzaga WA, Chaves MH. Chemical and pharmacological study of Zanthoxylum rhoifolium. In: XIV Seminary of Scientific Initiation of University Federal of Piaui, Teresina, Brazil, 2007; p.353-4.

Canto JG, Kiefe CI, Greenland P. Coronary heart disease risk factors and mortality—reply. JAMA. 2012;307(11):1137-8.

Cartágenses MSSA. Investigação dos efeitos tóxicos e anti-hipertensivos de Arrabidae chica Verlot (Bignoneacea). João Pessoa. Tese [Doutorado] - Universidade Federal da Paraíba; 2009.

Costa JGM, Rodrigues FFG, Angélico EC, Pereira CK, Souza EO, Caldas, GFR, et al. Composição química e avaliação da atividade antibacteriana e toxicidade do óleo essencial de Croton zehntneri (variedade estragol). Rev Bras Farmacogn. 2008;18(4):583-6.

Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ. Comparative risk assessment collaborating group. Selected major risk factors and global and regional burden of disease. Lancet. 2002;360(9343):1347–60.

Ferreira-Filho ES, Arcanjo DD, Moura LHP, Silva-Filho JC, Paulino ET, Ribeiro E.A., et al. Antihypertensive and vasorelaxant effects of ethanol extract of stem barks from Zanthoxylum rhoifolium Lam. in rats. Indian J Exp Biol. 2013;51(8):661-9.
Freitas FFBP, Fernandes HB, Piauilino CA, Pereira SS, Carvalho KLM, Chaves MH et al. Gastroprotective activity of Zanthoxylum rhoifolium Lam. in animal models. J Ethnopharmacol. 2011;137(1):700-8.

Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980; 288(5789):373-6.

Gazda VE, Gomes-Carneiro MR, Barbi NS, Paumgarten FJR. Toxicological evaluation of an ethanolic extract from Chiococca alba roots. J Ethnopharmacol. 2006;105(1/2):187-95.

Hoyert DL, Xu J. Deaths: preliminary data for 2011. Natl Vital Stat Rep. 2012;61(6):1-51.

Hosseinzadeh S, Jafariukhdan A, Hosseini A, Armand R. The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris. Int J Clin Med. 2015;6:635-42.

Imanshahidi M, Hosseinzadeh H. Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine. Phytother Res. 2008; 22(8):999-1012.

Jackson WF. Ion channels and vascular tone. Hypertension. 2000; 35(1 pt.2): 173-8.

Jullian V, Bourdy G, Georges S, Maurel S, Sauvain M. Validation of use of a traditional antimalarial remedy from French Guiana, Zanthoxylum rhoifolium Lam. J Ethnopharmacol. 2006;106(3):348-52.

Khalil RA. Regulation of vascular smooth muscle function. San Francisco: Morgan & Claypool; 2010.

Karaki H, Ozaki H, Hori M., Mitsui-Saito M, Amano K, Harada K, et al. Calcium movements, distribution, and functions in smooth muscle. Pharmacol Rev. 1997;49(2):157-230.

Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005;365(9455):217-23.

Lee CH, Poburko D, Sahota P, Sandhu J, Ruehlmann DO, Van Breemen C. The mechanism of phenylephrine-mediated [Ca2+]i oscillations under-lying tonic contraction in the rabbit inferior vena cava. J Physiol. 2001;534(pt 3): 641–50.

Melo MFF, Zickel CS. Os gêneros Zanthoxylum L. e Esenbeckia Kunth (Rutaceae) no Estado de Pernambuco, Brasil. Acta Bot Bras. 2004;18(1):73-90.

Moreira F. Plantas que curam: cuide da sua saúde através da natureza. São Paulo: Hermus; 1996.

Pasa MC. Saber local e medicina popular: a etnobotânica em Cuiabá, Mato Grosso, Brasil. Bol Mus Para Emílio Goeldi Cienc Hum. 2011;6(1):179-96.

Pereira RJ, Cardoso MG. Metabólicos secundários vegetais e benefícios antioxidantes. J Biotechnol Biodiversity. 2012;3(4):146-52.

Pereira SS, Lopes LS, Marques RB, Figueiredo KA, Costa DA, Chaves MH. Antinociceptive effect of Zanthoxylum rhoifolium Lam. (Rutaceae) in models of acute pain in rodents. J Ethnopharmacol. 2010;129(2):227-31.

Pirani JR. Flora da Reserva Ducke, Amazônia, Brasil: Rutaceae. Rodriguésia. 2005;56(86):189-204.

Salgado MAS, Resende AV, Sousa-Silva JC, Felfili JM, Franco AC. Crescimento inicial de Zanthoxylum rhoifolium Lam. em diferentes condições de sombreamento. Bol Herbário Ezechias Paulo Heringer. 1998;3:37-45.

Silva SL, Figueiredo PMS, Yano T, Chemotherapeutic potential of the volatile oils from Zanthoxylum rhoifolium Lam leaves. Eur J Pharmacol. 2007a;576(1/3):180-8.

Silva SL, Figueiredo PM, Yano T. Cytotoxic evaluation of essential oil from Zanthoxylum rhoifolium Lam. leaves. Acta Amazonica. 2007b;37(2):281-6.

Sonkusare, S, Palade, PT, Marsh JD, Telemaque S, Petic A, Rusch NJ. Vascular calcium channels and high blood pressure: pathophysiology and therapeutic implications. Vascular Pharmacol. 2006;44(3):131-142.

Tanaka Y, Mochizuki Y, Tanaka H, Shigenobu K. Significant role of neuronal non-N-type calcium channels in the sympathetic neurogenic contraction of rat mesenteric. Br J Pharmacol. 1999;128(7):1602-8.
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Weber AD. Estudo fitoquímico e da atividade biológica de Zanthoxylum rhoifolium. Santa Maria. Dissertação [Mestrado] - Universidade Federal de Santa Maria; 2005.

Zhang Y, Wang QL, Zhan YZ, Duan HJ, Cao YJ, He LC. Role of store operated calcium entry in imperator in-induced vasodilatation of rats small mesenteric artery. Eur J Pharmacol. 2010;647(1/3):126-31.

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