



# Hypotensive activity of essential oil extracted from the resin of *Protium heptaphyllum* (Aubl.) Marchand: possible involvement of adrenoceptors and muscarinic receptor

Atividade hipotensora do óleo essencial de *Protium heptaphyllum* (Aubl.) Marchand: possível envolvimento de adrenorreceptores e receptores muscarínicos

M. B. Mendes<sup>1</sup>; L. R. Nunes<sup>1</sup>; E. P. Sena<sup>1,2</sup>; A. F. Rêgo<sup>1,2</sup>; R. P. Souza<sup>1,2</sup>;  
M. S. Rocha<sup>3</sup>; S. G. Lima<sup>3</sup>; A. P. Oliveira<sup>1,2,4\*</sup>

<sup>1</sup>Cardiovascular Pharmacology Laboratory – NPPM/UFPI

<sup>2</sup>Postgraduate Program in Pharmacology, Center for Health Sciences/CCS, UFPI, 64048-395, Teresina - PI, Brazil

<sup>3</sup>Department of Chemistry, Federal University of Piauí/UFPI, 64049-550, Teresina - PI, Brazil

<sup>4</sup>Medicinal Plants Research Center, Center for Health Sciences/CCS, 64048-395, Teresina - PI, Brazil

\*aldeidiaoliveira@ufpi.edu.br

(Recebido em 29 de maio de 2025; aceito em 31 de junho de 2026)

*Protium heptaphyllum* (Aubl.) Marchand produces an oily resin used in folk medicine as an analgesic, cicatrizing and expectorant. This study evaluated the cardiovascular effect of normotensive nonanesthetized rats. The essential oil of *Protium heptaphyllum* - PhOE at doses (3.125, 6.25 and 12.5 mg/kg iv) induced hypotension ( $-18.66 \pm 1.39^*$ ,  $-30.42 \pm 2.16^{***}$ ,  $-35.62 \pm 0.67^{***}$  mmHg, respectively,  $p < 0.05$  vs. PhOE,  $*** p < 0.001$  vs PhOE) and reduced heart rate with the highest doses (6.25 and 12.5 mg/kg ( $-36.59 \pm 9.05^{***}$ ,  $-28.95 \pm 9.99^{***}$  bpm, respectively,  $*** p < 0.001$  vs PhOE). It was found that in the presence of atropine, the hypotensive response of PhOE was attenuated, whereas blockade with hexamethonium inhibited hypotension at the lowest dose (3.125 mg/kg i.v.). However, in the presence of yohimbine, inhibition of PhOE-induced hypotension was observed at doses (3.125 and 6.25 mg/kg i.v.). Meanwhile in the presence of propranolol and prosozine, inhibition of PhOE-induced hypotension was observed at all doses. In conclusion, we can affirm that PhOE has hypotensive and bradycardic action involving the action of the muscarinic and adrenergic receptors probably ( $\alpha$  and  $\beta$ ).

Keywords: hypotension, essential oil, *Protium*.

*Protium heptaphyllum* (Aubl.) Marchand produz uma resina oleosa utilizada na medicina popular como analgésico, cicatrizante e expectorante. Este estudo avaliou o efeito cardiovascular em ratos normotensos não anestesiados. O óleo essencial de *Protium heptaphyllum* – PhOE, nas doses de 3,125; 6,25 e 12,5 mg/kg (via intravenosa), induziu hipotensão ( $-18,66 \pm 1,39^*$ ,  $-30,42 \pm 2,16^{***}$ ,  $-35,62 \pm 0,67^{***}$  mmHg, respectivamente,  $p < 0,05$  vs. PhOE,  $*** p < 0,001$  vs PhOE) e redução da frequência cardíaca nas doses mais altas (6,25 e 12,5 mg/kg:  $-36,59 \pm 9,05^{***}$ ,  $-28,95 \pm 9,99^{***}$  bpm, respectivamente,  $***p < 0,001$  vs. PhOE). Verificou-se que, na presença de atropina, a resposta hipotensora do PhOE foi atenuada, enquanto o bloqueio com hexametônio inibiu a hipotensão na dose mais baixa (3,125 mg/kg, i.v.). No entanto, na presença de ioimbina, observou-se inibição da hipotensão induzida por PhOE nas doses de 3,125 e 6,25 mg/kg (i.v.). Já na presença de propranolol e prazosina, houve inibição da hipotensão induzida por PhOE em todas as doses. Conclui-se que o PhOE possui ação hipotensora e bradicárdica, envolvendo provavelmente a atuação sobre receptores muscarínicos e adrenérgicos ( $\alpha$  e  $\beta$ ).

Palavras-chave: hipotensão, óleo essencial, *Protium*.

## 1. INTRODUCTION

Blood pressure (BP) is characterized by the pressure that blood exerts on the walls of our blood vessels, which depends on many factors such as: cardiac output (DC), vascular resistance and total blood volume [1]. Natural products have enormous chemical diversity and structure which are sources of research that inspire new discoveries in chemistry, biology and medicine, being evolutionarily optimized as drug-like molecules and remain the best source of discovery of new drugs [2].

Many plants have volatile oils that contain a wide variety of pharmacological activities, which may cause vasorelaxation and / or decrease the heart rate by exerting hypotensive activities [3].

Despite the enormous advances in research, development and use of natural products as a therapeutic agent, the detailed understanding of the mechanisms of action is increasing [4, 5]. On the other hand, the evaluation of the pharmacological effects and the clarification of the pathways and mechanism of action of the natural compounds can be used as a logical research strategy to search for new drugs [6, 7].

The plant species *Protium heptaphyllum* (Aubl.) Marchand, Burseraceae, commonly known as "almécega", "white pitch" and "musk", almécega of Brazil, lemon gum, found in South America including the Amazon region, produces an oily resin which can yield from 2.1 to 20%, composed of a mixture of triterpenes, monoterpenes and sesquiterpenes [8, 9]. In folk medicine this plant is used as an analgesic, cicatrizant and expectorant and some authors indicate the presence of antimicrobial, antihyperglycemic and hypolipidemic action [10-13]. And some PhOE constituents such as  $\alpha$  and  $\beta$  amirine have antidepressant and anxiolytic effects  $\alpha$ -pinene has gastroprotective activity, limonene has antinociceptive action and linalool and  $\alpha$ -terpineol have hypotensive and vasorelaxant activity [14-18].

Considering the absence of the use PhOE in the cardiovascular system, our study aimed to evaluate the pharmaco-biological potential of *Protium heptaphyllum* in the cardiovascular system of normotensive rats as well as to investigate the possible mechanisms of action underlying this pharmacological activity.

## 2. MATERIALS AND METHODS

### 2.1 General experimentation procedures

#### 2.1.1 Plant material

The resin from which the essential oil was extracted was obtained from the Laboratory of Organic Geochemistry – LAGO/UFPI in February 2016 kept in refrigeration until the moment of extraction. The identification of the species *Protium heptaphyllum* (Aubl.) Marchand occurred in the Graziela Barroso Herbarium at the Federal University of Piauí - Brazil and the voucher model was registered under the number 28730 [18].

#### 2.1.2 Extraction of essential oil

The extraction of the essential oil from the resin was performed according to the method described by Mobin et al. (2017) [18], the resin, obtained from the leaves of *P. heptaphyllum*, was submitted to hydrodistillation in a modified Clevenger type apparatus, and the fraction was collected shortly after 3 hours of extraction. The extracted oil was accommodated in a glass container sealed with a lid wrapped in foil and kept under refrigeration at a temperature of less than 5 °C.

#### 2.1.3 Analysis by Gas Chromatography-Mass Spectrometry

Analysis was performed by Gas Chromatography (ThermoScientific, TSQ Quantum XLS Ultra, Triple Quadrupole), equipped with an AS3000 Automatic Sampler (Thermo Fisher Scientific Inc., Waltham, MA, USA). Chromatography Components consisted of an EquityTM-1 column with a length of 30 m and 0.25 mm internal diameter and 0.10 mm film thickness. He was used as a carrier gas with a flow rate of 1.0 mL/min, injector temperature 220 °C. The column was programmed at an initial temperature of 50 °C (3.0 min) followed by an increase of 3 °C/min to 180 °C (for 2 min), then 6 °C/min to 260 °C (for 2 min) and detector temperature 230 °C. The sample was dissolved in dichloromethane (1.0  $\mu$ L) was injected in split mode (1:10). The mass detector was operated in the electron impact ionization (EI) mode with ionization energy of 70 eV. The identification of the respective compounds was made by comparing the internal library mass spectra (NIST 11) as well as by experimental retention index, calculated from a series of *n*-alkanes (C8 to C20 - Sigma Aldrich) and by comparison with literal Adams (2007) [19] data and also through websites such as <http://www.webbook.nist.gov> and <http://www.pherobase.com>.

The constituents of the essential oil from *Protium heptaphyllum* (Aubl.) March (PhOE), investigated in this study was described by Mobin et al. (2017) [18], analyzed by GC-MS consisted of: Limonene (34.51%); Eucalyptol (20.64%); *p*-cymene (17.04%);  $\alpha$ -terpineol (9.75%); 3-carene (5.11%); Carvone (1.63%); *p*-ciment-8-ol (1.5%);  $\alpha$ -phellandrene (1.42%);  $\beta$ -sinensal (1.29%); *trans*-carveol (1.21%); 4-terpineol (1.12%);  $\beta$ -linalool (0.83%); Cryptone (0.8%); Piperitone (0.73%);  $\alpha$ -pinene (0.71); Sabinene (0.48%); Cuminal (0.4%); Limonene Oxide (0.35%);  $\beta$ -pinene (0.29%) and  $\alpha$ -tujene (0.19%). Among these constituents, several monoterpenes have been reported to exert biological activities on the cardiovascular system, including *p*-cymen-8-ol,  $\beta$ -limonene, *p*-cymene,  $\beta$ -pinene, terpinen-4-ol, and eucalyptol [20, 21].

#### 2.1.4 Animals

Male Wistar rats (200-320 g) were obtained from the animal care facilities of the Medicinal Plants Research Center of the Federal University of Piauí, Brazil. The rats were kept in a large cage under controlled conditions of temperature and illumination (lights on: 0600-1800 h). The rats received food and water ad libitum. All procedures were approved by the Animal Experiment Ethics Committee of the Federal University of Piauí, Brazil (Protocol number 069/2014) and were in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication 85-23, revised 1996).

#### 2.1.5 Drugs and Reagents

The drugs used include: Sodium nitroprusside, yohimbine, propranolol, hexamethonium, prazosin, N $\omega$ -nitro-L-arginine methyl ester (L-NAME), atropine, (all from SIGMA, St. Louis, MO, USA), thiopental sodium (CRISTALIA, São Paulo, Brazil), heparin (ARISTON, São Paulo, Brazil), Xylazine (CEVA, São Paulo, Brazil), Ketamine (CEVA, São Paulo, Brazil). In the preparation of the stock solutions, PhOE was diluted in demineralized water / Tween 80 solution (0.5% v / v). Other drugs were diluted only in saline solutions. All stock solutions were kept at 0 ° C and diluted to the desired concentration with deionized water or saline solution when necessary. The concentrations of Tween 80 (Dynamics, São Paulo, Brazil) used, showed no effect in control experiments.

#### 2.1.6 Experimental groups

The animals were divided into 6 experimental groups with 6 animals per group: Control group – saline; atropine group; hexamethonium group; yohimbine group; prazosin group; propranolol group.

#### 2.1.7 Blood Pressure in Normotensive Rats

The rats were anesthetized by intraperitoneal injection of xylazine and ketamine (mg/kg PC). The femoral artery may be filled with polyethylene sulfide 50 (Clay-Adams PE-50), filled with 100 IU of heparin/mL connected to a pressurized pressure causing arterial blood pressure. The blood pressure signal was amplified and converted into digital signal by a bridge amplified with AQCAD (AVS-PROJETOS, SP-BRAZIL). Measurements of mean arterial pressure and heart rate were performed as described by Mendes et al. (2014) [22]. Sodium nitroprusside (10  $\mu$ g/kg; i.v.) was administered to verify the efficacy of venous catheter insertion. After the stabilization period, mean arterial pressure and heart rate were recorded in conscious and free-moving mice before (baseline) and after intravenous administration of bolus PhOE (3.125, 6.25 and 12.5 mg/kg) to obtain of dose-response curves. Successive injections of PhOE were administered in separate sufficient time intervals to allow complete recovery of the baseline parameter (30 min). In order to elucidate the possible mechanisms underlying the hypotensive response, PhOE was administered to animals pretreated with one of the following drugs: a ganglionic blocking agent (hexamethonium, 20 mg/kg), a  $\beta$ -adrenergic blocking agent (propranolol, 2mg/kg), an alpha 2-adrenergic antagonist (yohimbine, 2 mg/kg), a non-selective muscarinic

receptor antagonist (atropine 2 mg/kg), and an alpha 1-adrenergic antagonist (prazosin 1 mg/kg). After protocols, all animals were euthanized with thiopental sodium (100 mg/kg, i.p.).

## 2.2 Statistical analysis

The results are expressed as the mean  $\pm$  S.E.M. (standard error of the media) of six animals. Statistical evaluation was performed using ANOVA post Tukey test. In all analysis, the value of  $p < 0.05$  was considered as statistically significant. All procedures were performed using Graph Pad Prism 6.0 TM. (Graph Pad Software, Inc., San Diego, CA, USA).

## 3. RESULTS AND DISCUSSION

Several studies have reported the effects of essential oils on the cardiovascular system of rats [23-25]. However, no report on the hypotensive effect of PhOE has been found in the literature, but studies have shown that some chemical components of PhOE contribute to its pharmacological activity such as  $\alpha$ -terpineol and linalool, which have a hypotensive and a vasorelaxant effect) [14, 17].

In this study, the biological effects of PhOE was evaluated on the cardiovascular system. Baseline values for mean arterial pressure (MAP) and heart rate (HR) were  $109.67 \pm 3.51$  mmHg and  $348.67 \pm 38.69$  bpm, respectively. Venous administration of doses (3.125, 6.25 and 12.5 mg/kg, i.v.) of PhOE in rats induced hypotension ( $-18.66 \pm 1.39^*$ ,  $-30.42 \pm 2.16^{**}$  ( $\pm 0.25 \pm 0.67^{***}$  mmHg, respectively) without altering the HR at the lowest dose 3.125 mg/kg i.v. ( $-1.69 \pm 17.39$  bpm). Bradycardia occurred with the use of other doses ( $-36.59 \pm 9.05^{***}$ ;  $-28.95 \pm 9.99^{***}$  bpm,  $* p < 0.05$  vs PhOE;  $*** p < 0.001$  vs PhOE) (Figure 1).

Intravenous administration of PhOE in rats induced hypotension without altering heart rate at the lowest dose (3.125 mg/kg); at the other doses, bradycardia followed by hypotension occurred. Stimulation of the cardiac muscarinic receptors of the sinoatrial nodule by vagal action induces intense bradycardia, which may be followed by hypotension due to a decrease in cardiac output [26].

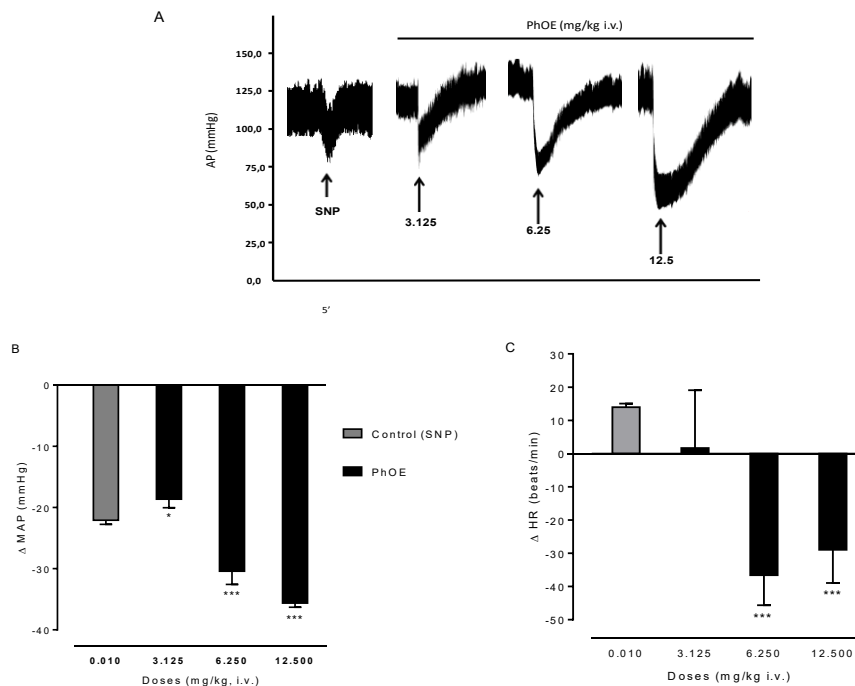


Figure 1. Original record showing the PhOE effect on BP (A). (B) Shows the values the of PhOE effect on MAP (mmHg) and (C) Shows the average of the values of Heart rate (bpm) in normotensive mice. Values expressed as mean  $\pm$  SEM.  $* p < 0.05$ ,  $*** p < 0.001$  vs. PhOE. (ANOVA Tukey post test).

One of the possibilities of the hypotensive effect of PhOE could be related to its action in the parasympathetic nervous system, which is of great importance to the cardiovascular system playing a fundamental role in the regulation of BP and producing responses that counterbalance those associated with the sympathetic nervous system as described by Harvey (2012) [27]. In the presence of atropine (2 mg/kg) a non-selective muscarinic receptor antagonist as described by Blum et al. (2013) [28], it was observed that the PhOE induced inhibition of the hypotensive effect ( $-13.44 \pm 0.066$ ) at the lower dose (3.125 mg/kg) and the highest dose (12.5 mg/kg) ( $-27.29 \pm 0.99$ ). In relation to bradycardia, a reversal occurred at the lowest dose ( $4.14 \pm 3.4$  bpm) and at the highest dose studied. Meanwhile the intermediate dose (6.25 mg/kg) did not change the MAP significantly (Figure 2).

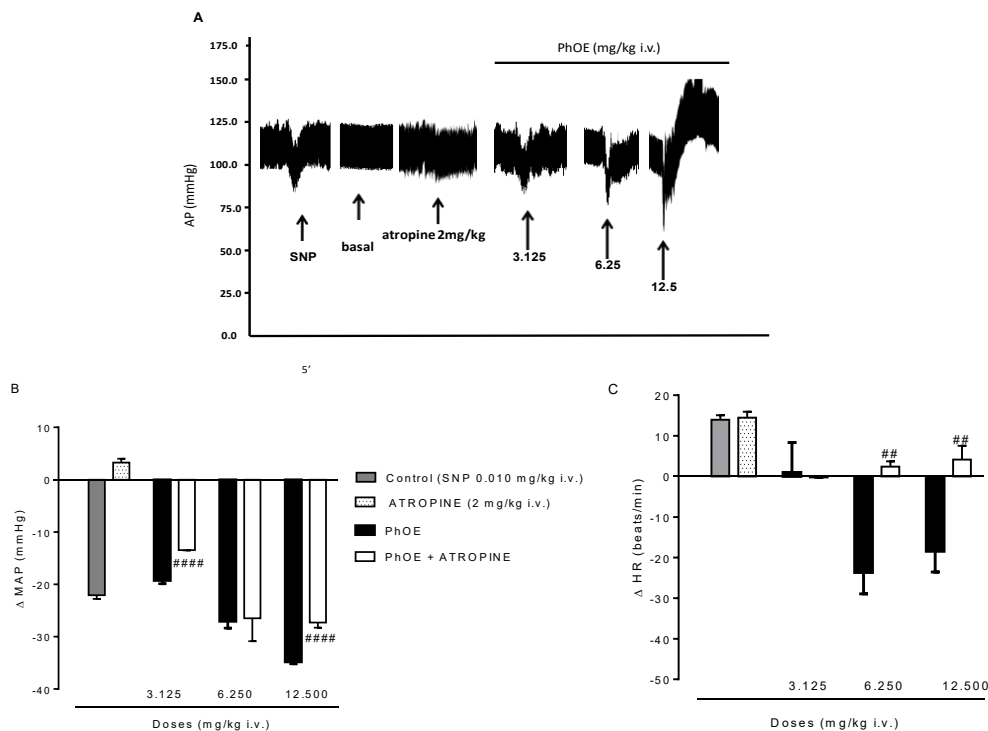


Figure 2. (A) Original record showing the PhOE response on BP after muscarinic blockade (B) shows the MAP values (mmHg) (C) shows the mean values of HR (bpm) in normotensive mice after acute inhibition of muscarinic receptors (atropine 2 mg / kg iv). Values expressed as mean  $\pm$  SEM. Values expressed as mean  $\pm$  SEM.  $p < 0.01$ ,  $p < 0.0001$  vs. PhOE. (ANOVA Tukey post test).

Atropine blocks the effect of the vagus nerve in both the sinoatrial node and the atrioventricular node, increasing the rate of sinoatrial node firing and facilitating atrioventricular conduction which may justify the reversal of the PhOE-mediated bradycardia, indicating the participation of muscarinic receptors in the hypotensive effect of PhOE [29, 30]. However, the biphasic effect (U-shaped curve) may be related to the saturation of receptors other than muscarinic receptors that provoke a hypotensive effect, such as nicotinic and adrenergic receptors [31].

The bradycardia action of certain substances can occur by mechanisms that increase parasympathetic activity or decrease sympathetic activity directly in the heart or by action on neuronal regions involved in autonomic regulation, having direct influence on nitric oxide donors present in vague nerve endings [32].

In order to evaluate potential mechanisms causing hypotension and bradycardia that may be related to the alteration of the parasympathetic tone efferent to the heart, a possible indirect effect of PhOE via the vagus nerve was performed and the animals were pre-treated with hexamethonium (20 mg/kg i.v.), a ganglionic nicotinic receptor blocker [33]. At lower dose of PhOE (3.125 mg/kg), hypotension inhibition ( $-9.5 \pm 0.15$  mmHg) was observed without HR

alteration ( $0.08 \pm 0.02$  bpm). This is an indicative of the involvement of nicotinic ganglionic receptors. An increase in the hypotensive effect occurred with the intermediate dose ( $-36.08 \pm 0.27$  \* mmHg) which also inhibited bradycardia ( $0.36 \pm 0.04$  bpm), that may be related to central and peripheral sympatholytic effects of  $\alpha_2$ -adrenergic agonists that may increase ganglionic blockade, as described by McCallum et al. (1998) [34], which was observed in the experiment. At the highest dose (12.5 mg/kg) MAP ( $-33.55 \pm 0.76$  mmHg) did not change and bradycardia were inhibited, ( $9.59 \pm 1.69$  bpm). \*  $p < 0.05$  vs PhOE,  $p < 0.001$  vs PhOE,  $p < 0.0001$  vs. PhOE (Figure 3), due to the loss of cardiovascular reflexes caused by ganglion blockade in response to hexamethonium chloride.

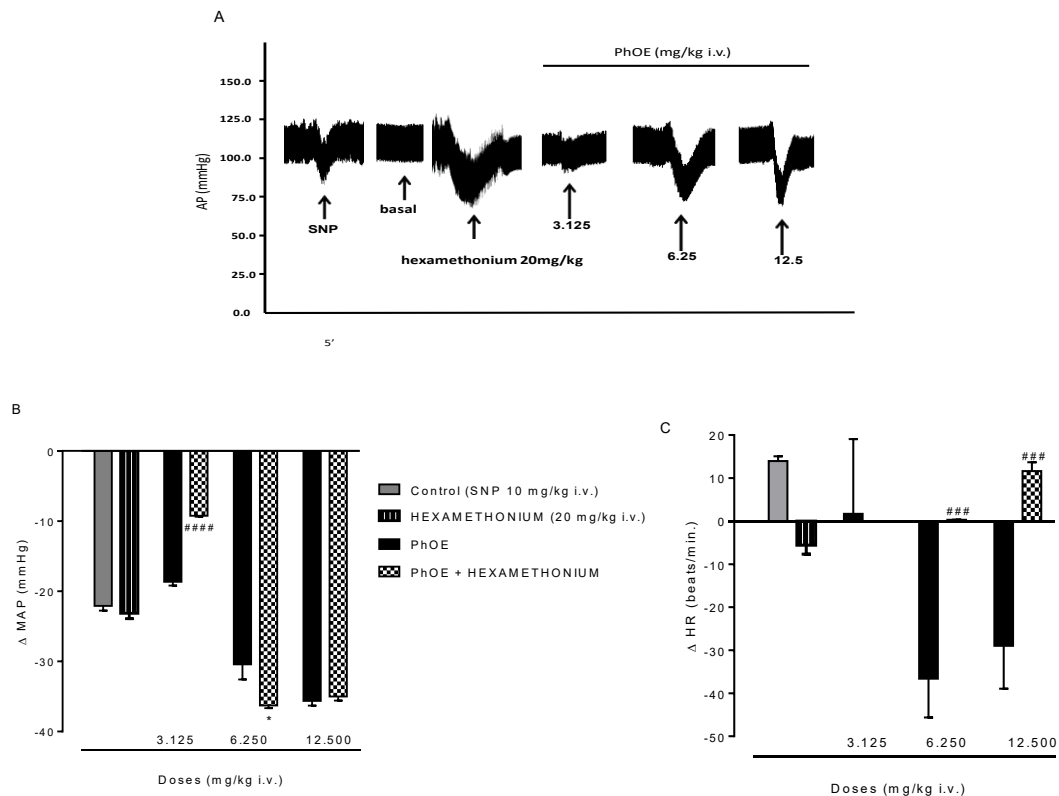


Figure 3. (A) Original record showing PhOE response on BP after acute inhibition of ganglionic receptors. (B) shows the mean of the effects of PhOE on MAP (mmHg) and (C) shows the mean HR values (bpm) in normotensive mice, after acute inhibition of ganglionic receptors (hexamethonium 20 mg / kg i.v.). Values expressed as mean  $\pm$  SEM. Values expressed as mean  $\pm$  SEM. \*  $p < 0.05$ ,  $p < 0.001$ ,  $p < 0.0001$  vs PhOE (ANOVA Tukey post test).

The  $\alpha_2$ -Adrenergic agonists stimulate central and peripheral nervous system receptors, causing inhibition of norepinephrine release by nerve terminals, decreasing sympathetic outflow to the periphery. Consequently, this reduces vascular peripheral resistance and induces changes in cardiac output and bradycardia, resulting in hypotension [35]. In order to verify whether the decrease in MAP induced by PhOE involved the activation of  $\alpha_2$ -adrenergic receptors of the CNS, we used yohimbine, a selective antagonist of these receptors [36]. The  $\alpha_2$ -Adrenergic agonists stimulate  $\alpha_2$ -adrenergic receptors in the CNS and peripheral nerves and may also stimulate the activation of presynaptic  $\alpha_2$ -adrenoceptors at the peripheral nerve endings, inhibiting noradrenaline exocytosis which partially explains the hypotensive and bradycardic effect of the agonists of these receptors [35]. However, the activation of postsynaptic  $\alpha_2$  receptors in the smooth muscle of arterial and venous blood vessels produces vasoconstriction. In the CNS, the activation of  $\alpha_2$  receptors in the vasomotor center decreases sympathetic efflux, decreasing circulating catecholamines and potentializing parasympathetic nervous activity leading to a

reduction in blood pressure [37]. Administration of yohimbine (2 mg/kg iv)  $\alpha_2$ -adrenergic receptor antagonist abolished PhOE-induced hypotension ( $0.00 \pm 0.22$  mmHg and  $1.39 \pm 0.84$  mmHg) at doses of 3.125 mg/kg and dose 6,25 mg/kg, respectively. HR at the lowest dose continued at baseline ( $-0.10 \pm 0.10$  bpm), but the intermediate dose decreased bradycardia ( $-2.52 \pm 0.67$  bpm) when compared to PhOE without blocking  $\alpha_2$ -adrenergic receptors. At 12.5 mg / kg, there was no significant change in MAP ( $-33.03 \pm 0.97$  mmHg) and HR suffered a more pronounced decrease ( $-68.59 \pm 1.21$  \* bpm), \*  $p < 0.05$  vs PhOE,  $p < 0.01$  vs PhOE,  $p < 0.001$  vs PhOE,  $p < 0.0001$  vs. PhOE (Figure 4). This implies that PhOE is acting at the  $\alpha_2$ -adrenergic receptors.

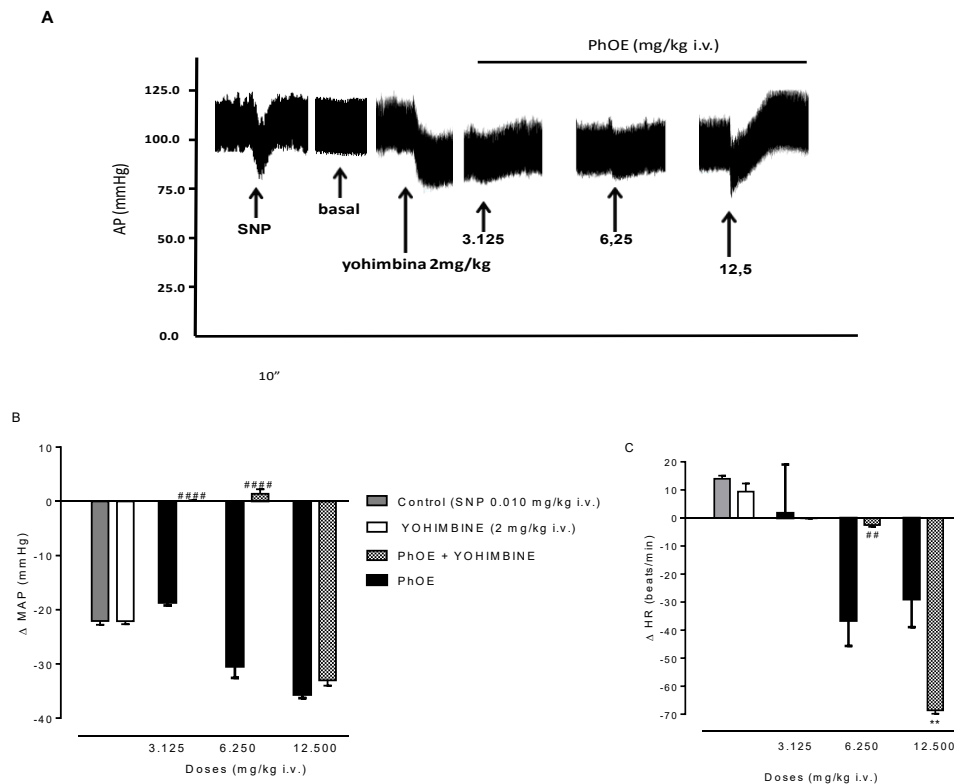


Figure 4. (A) Original record showing the PhOE response on BP after acute inhibition of  $\alpha_2$ -adrenergic receptors. (B) shows the mean values of HR (bpm) in normotensive rats after acute inhibition of  $\alpha_2$ -adrenergic receptors (yohimbine 2 mg / kg iv). Values expressed as mean  $\pm$  SEM. Values expressed as mean  $\pm$  SEM. \*\* or  $p < 0.01$ , \*\*\*\*  $p < 0.0001$  vs. PhOE (ANOVA Tukey post test).

Another possibility of inducing the hypotensive effect would be the antagonism at the  $\alpha_1$ -adrenoceptors, which results in relaxation of the smooth muscle and decrease of the total peripheral resistance, consequently the reduction of blood pressure [38]. Activation of  $\alpha_1$ -adrenergic receptors may induce positive inotropic or negative inotropic responses by altering  $K^+$  and  $Ca^{2+}$  currents, intracellular pH and  $Ca^{2+}$  sensitivity of myofilaments [39]. In order to verify a possible action on  $\alpha_1$ -adrenergic receptors, prazosin, a specific antagonist of these receptors was used [39]. Hypotension and bradycardia induced by PhOE (6.25 mg/kg i.v.) were inhibited ( $-17.93 \pm 0.52$  mmHg and  $-3.72 \pm 1.09$  bpm, respectively) in the presence of prazosin. At doses of (3.125 and 12.5 mg/kg i.v.), there was no change in MAP ( $-14.94 \pm 0.25$  mmHg,  $-28.49 \pm 0.76$  mmHg). Concerning HR, bradycardia occurred ( $-22.31 \pm 1.00$  \* bpm) at the lowest dose (3.125 mg/kg) after blockade of  $\alpha_1$ -adrenergic receptors. At the highest dose (12.5 mg/kg) there was no significant change in HR ( $-18.68 \pm 0.48$  bpm) when compared PhOE without blockade (Figure 5).

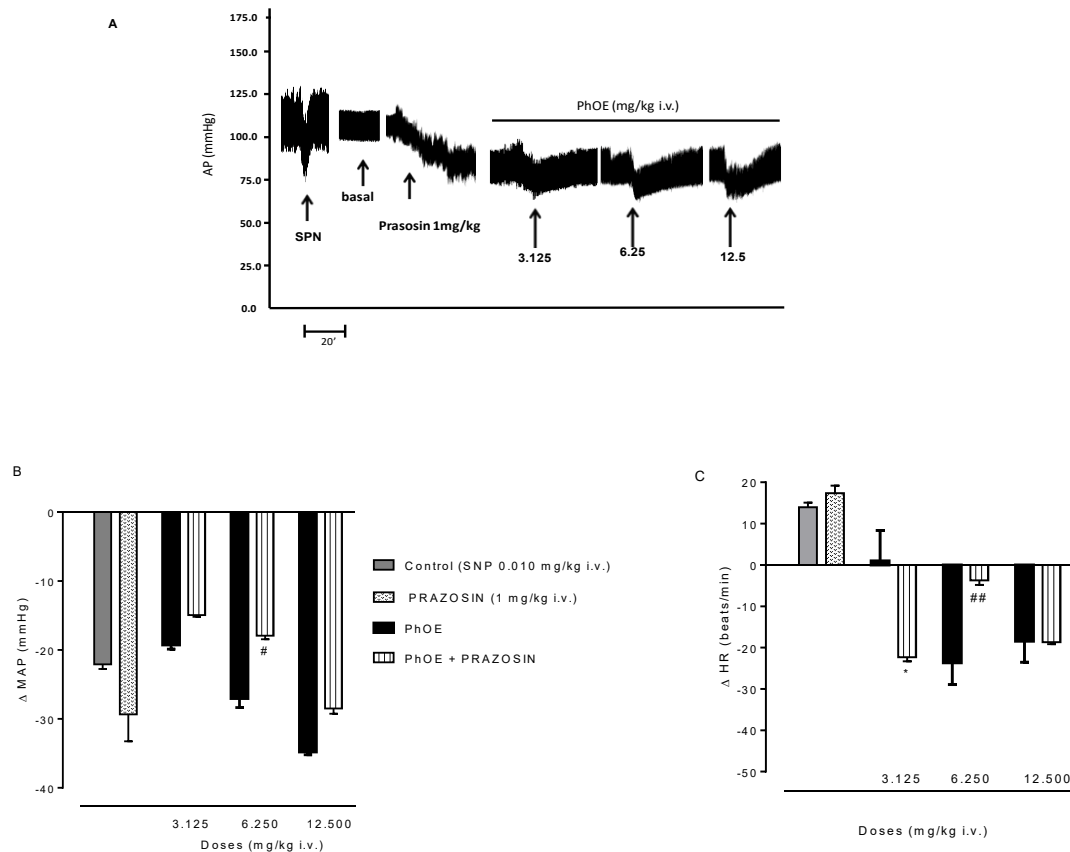


Figure 5. (A) Original record showing PhOE response on BP after acute inhibition of  $\alpha_1$ -adrenergic receptors. (A) shows the mean of the effects of PhOE on MAP (mmHg) and B) shows the mean HR values (bpm) in normotensive mice following acute inhibition of  $\alpha_1$ -adrenergic receptors (prazosin 1 mg / kg iv.). Values expressed as mean  $\pm$  SEM. or \*  $p < 0.05$  and  $p < 0.001$  vs. PhOE. (ANOVA Tukey post test).

The major constituent of PhOE inducing hypotensive and bradycardic effect is eucalyptol and limonene that has a vasorelaxant effect [25, 40]. Another constituent of PhOE is  $\alpha$ -terpineol, which induces hypotension and tachycardia leading to vasorelaxation, mediated partly by the endothelium, more probably through the release of NO and activation of the NO-cGMP pathway [41]. Due to the performance of different constituents of PhOE with different effects on chronotropism, bradycardia may be related to eucalyptol and limonene and its inhibition may be related to the effect of  $\alpha$ -terpineol, which could be inhibiting bradycardia when dose increases to 6.25 mg/kg indicating that PhOE is acting at the  $\alpha_1$ -adrenergic receptors.

For this reason, we decided to verify whether PhOE would be acting at levels of  $\beta$ -adrenergic receptors, which are essential regulators of cardiovascular homeostasis. In addition to its prominent role in the heart,  $\beta$ -adrenergic receptors are located in vascular smooth muscle cells, which mediate the vasodilatory effects of endogenous catecholamines [42].  $\beta$ -adrenergic receptors, members of the G-protein-coupled receptor superfamily, provide an important regulatory mechanism for the cardiovascular system, intervening in the effects of catecholamines on the sympathetic nervous system [43].

In order to verify the influence of PhOE on the adrenergic receptors mainly the  $\beta_1$  adrenoreceptors, propranolol was used as a non-selective  $\beta$ -adrenergic receptor blocker [44]. In the presence of propranolol, hypotension ( $-0.88 \pm 0.15$ ;  $-0.38 \pm 0.11$  and  $-7.92 \pm 3.1$  mmHg) induced by PhOE was inhibited in all doses and bradycardia ( $1.12 \pm 0.36$  bpm) was also inhibited at the dose of 6.25 mg/kg. At doses 3.125 and 12.5 mg/kg, there was no significant change in HR ( $1.86 \pm 0.99$  and  $-11.31 \pm 5.7$  bpm, respectively) when compared to the control. This implies that PhOE can also act on  $\beta$ -adrenergic receptors, adding to the effect on blood pressure (Figure 6).

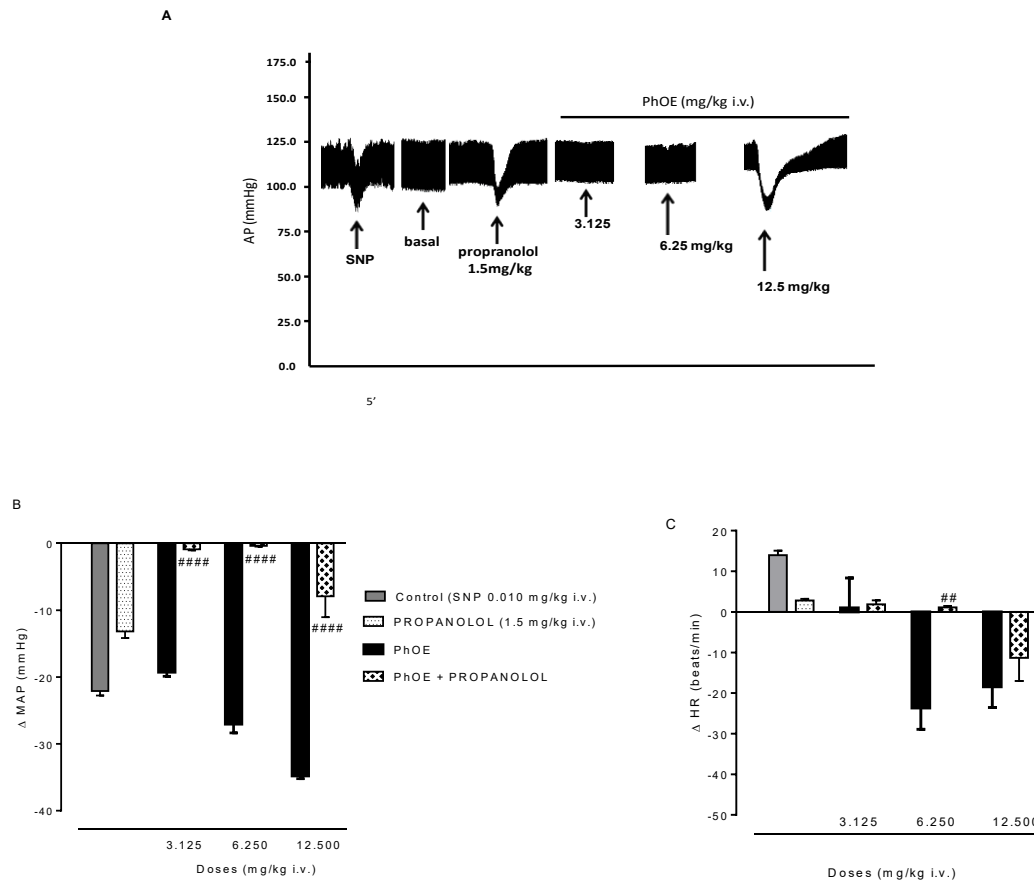


Figure 6. (A) Original record showing PhOE response on BP after acute inhibition of  $\beta$ -adrenergic receptors (B) shows the mean of the MAP values (mmHg) and (C) shows the mean HR values (bpm) in normotensive mice following acute inhibition of  $\beta$ -adrenergic receptors (propranolol 1.5 mg / kg iv). Values expressed as mean  $\pm$  SEM.  $p < 0.05$  and  $p < 0.001$  vs. PhOE. (ANOVA Tukey post test).

#### 4. CONCLUSION

The essential oil extracted from the resin derived from the leaves of *Protium heptaphyllum* (PhOE) induces hypotension and bradycardia in rats. These effects appear to involve the activation of muscarinic receptors and the modulation of  $\alpha$ - and  $\beta$ -adrenergic pathways.

#### 5. ACKNOWLEDGMENTS

The authors thank CNPq, PPSUS, CAPES and FAPEPI for financial support and Boris Timah for grammatical revision.

#### 6. REFERENCES

1. Kamal I, Hamdy R, Nourhan M. Kidneys: The victim of hypertension: Review. J Nephrol Ther. 2016 Jan;6(1):1-4. doi: 10.4172/2161-0959.1000231
2. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 2012 Mar;75(3):311-35. doi: 10.1021/np200906s
3. Saljoughian S, Roohinejad S, Bekhit AEDA, Greiner R, Omidzadeh A, Nikmaram N, et al. The effects of food essential oils on cardiovascular diseases: A review. Crit Rev Food Sci Nutr. 2018 Jul;58(10):1688-705. doi: 10.1080/10408398.2017.1279121

4. Biala A, Finckenberg P, Korpi A, Loytainen M, Martonen E, Levijoki J, et al. Cardiovascular effects of the combination of levosimendan and valsartan in hypertensive Dahl/Rapp rats. *J Physiol Pharmacol*. 2011 Jun;62(3):275-85.
5. Del Rio D, Costa LG, Lean MEJ, Crozier A. Polyphenols and health: What compounds are involved? *Nutr Metab Cardiovasc Dis*. 2010 Jan;20(1):1-6. doi: 10.1016/j.numecd.2009.05.015
6. Hajhashemi V, Ghannadi A, Pezeshkian SK. Antinociceptive and anti-inflammatory effects of *Satureja hortensis* L. extracts and essential oil. *J Ethnopharmacol*. 2002 Oct;82(2-3):83-7. doi: 10.1016/s0378-8741(02)00137-x
7. Jakala P, Pere E, Lehtinen R, Turpeinen A, Korpela R, Vapaatalo H. Cardiovascular activity of milk casein-derived tripeptides and plant sterols in spontaneously hypertensive rats. *J Physiol Pharmacol*. 2009 Dec;60(4):11-20.
8. Albino C, Oliveira PC, Prosdocimi F, Silva, Bizzo HR, Gama PE, et al. Oxidation of monoterpenes in *Protium heptaphyllum* oleoresins. *Phytochemistry*. 2017;136:141-6. doi: 10.1016/j.phytochem.2017.01.013
9. Lima EM, Nascimento AM, Lenz D, Scherer R, Meyrelles SS, Boëchat GAP, et al. Triterpenes from the *Protium heptaphyllum* resin - chemical composition and cytotoxicity. *Rev Bras Farmacogn*. 2014;(24):399-407. doi: 10.1016/j.bjp.2014.06.003
10. Bernadi WA, Zanotelli JC, Lima EM, Souza TD, Edringer DC, Souza VRC. Effects of topical application of essential oil from resin of almesca (*Protium heptaphyllum* (Aubl.) Marchand) in experimentally induced skin wounds in rats. *Rev Bras Ciênc Vet*. 2015;22(1):10-5. doi: 10.4322/RBCV.2015.311
11. Maia RM, Barbosa PR, Cruz FG, Roque NF, Fascio M. Triterpenos da resina de *Protium heptaphyllum* March (Burseraceae): caracterização em misturas binárias. *Quim Nova*. 2000;23(5):623-26. doi: 10.1590/S0100-40422000000500010
12. Pinto FE, Heringer OA, Silva MA, Andrade TU, Ribeiro JS, Lenz D, et al. Stability and disinfecting proprieties of the toothbrush rinse of the essential oil of *Protium heptaphyllum*. *Afr J Pharm. Pharmacol*. 2015;9(6):173-81. doi: 10.5897/AJPP2014.4224
13. Santos FA, Frota JT, Arruda BR, de Melo TS, da Silva AACA, Brito GAC, et al. Antihyperglycemic and hypolipidemic effects of  $\alpha$ ,  $\beta$ -amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. *Lipids Health Dis*. 2012;11(1):98. doi: 10.1186/1476-511X-11-98
14. Aragão GF, Carneiro LMV, Frota-Junior AP, Vieira LC, Bandeira PN, Lemos TLG, et al. A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. *Pharmacol Biochem Behav*. 2006;85(4):827-34. doi: 10.1016/j.pbb.2006.11.019
15. Pinheiro MA, Magalhães R, Torres D, Cavalcante R, Mota FX, Coelho EMAO, et al. Gastroprotective effect of alpha-pinene and its correlation with antiulcerogenic activity of essential oils obtained from Hyptis species. *Pharmacogn Mag*. 2015;11(41):123-30. doi: 10.4103/0973-1296.149725
16. Anjos PJC, Lima AO, Cunha PS, de Sousa DP, Onofre ASC, Ribeiro TP, et al. Cardiovascular effects induced by linalool in normotensive and hypertensive rats. *Z Naturforsch C J Biosci*. 2013;68(5-6):181-90. doi: 10.5560/ZNC.2013.68c0181
17. Amaral JF, Silva MIG, Aquino Neto MR, Teixeira Neto PF, Moura BA, Melo CTV, et al. Antinociceptive effect of the monoterpene R-(+)-limonene in mice. *Biol Pharm Bull*. 2007;30(7):1217-20. doi: 10.1248/bpb.30.1217
18. Mobin M, Lima SG, Almeida LTG, Silva Filho JC, Rocha MS, Oliveira AP, et al. Gas chromatography-triple quadrupole mass spectrometry analysis and vasorelaxant effect of essential oil from *Protium heptaphyllum* (Aubl.) March. *Biomed Res Int*. 2017(2017):1928171. doi: 10.1155/2017/1928171
19. Adams RP. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. 4.1. ed. Carol Stream (IL, USA): Allured Publishing Corporation; 2007.
20. Santos MRV, Moreira FV, Fraga BP, de Sousa DP, Bonjardim LR, Quintans LJ. Cardiovascular effects of monoterpenes: A Review. *Rev Bras Farmacogn*. 2011(21):764-71. doi: 10.1590/S0102-695X2011005000119
21. Soares MC, Damiani CE, Moreira CM, Stefanon I, Vassallo DV. Eucalyptol, an essential oil, reduces contractile activity in rat cardiac muscle. *Braz J Med Biol Res*. 2005 Mar;38(3):453-61. doi: 10.1590/s0100-879x2005000300017
22. Mendes MB, Silva-Filho JC, Sabino CKB, Arcanjo DDR, Sousa CMM, Costa ICG, et al. Pharmacological Evidence of  $\alpha_2$ -Adrenergic Receptors in the Hypotensive Effect of *Platonia insignis* Mart. *J Med Food*. 2014;17(10):1079-85. doi: 10.1089/jmf.2013.0151
23. Alves-Santos TR, de Siqueira RJB, Duarte GP, Lahlou S. Cardiovascular effects of the essential oil of *Croton argyrophyllloides* in normotensive rats: Role of the autonomic nervous system. *Evid Based Complement Alternat Med*. 2016(2016):4106502. doi: 10.1155/2016/4106502

24. Esmacili H, Sharifi M, Esmailidehaj M, Rezvani ME, Hafizibarjin Z. Vasodilatory effect of asafoetida essential oil on rat aorta rings: The role of nitric oxide, prostacyclin, and calcium channels. *Phytomedicine*. 2017;1(36):88-94. doi: 10.1016/j.phymed.2017.10.002
25. Lahlou S, Figueiredo AF, Magalhães PJC, Leal-Cardoso JH. Cardiovascular effects of 1,8-cineole, a terpenoid oxide present in many plant essential oils, in normotensive rats. *Can J Physiol Pharmacol*. 2002;80(12):1125-31. doi: 10.1139/y02-142
26. Moreira FV, Bastos JFA, Blank AF, Alves PB, Santos MRV. Chemical composition and cardiovascular effects induced by the essential oil of *Cymbopogon citratus* DC. Stapf, Poaceae, in rats. *Rev Bras Farmacogn*. 2010;20(6):904-9. doi: 10.1590/S0102-695X2010005000012
27. Harvey RD. Muscarinic receptor agonists and antagonists: Effects on cardiovascular function. *Handb Exp Pharmacol*. 2012;(208):299-316. doi: 10.1007/978-3-642-23274-9\_13
28. Blum J, Carreiro S, Hack J. Difficulty creating an anticholinergic rat model with atropine. *Clin Toxicol (Phila)*. 2013;51(9):910. doi: 10.3109/15563650.2013.841914
29. Flôres FN, Moraes A, Oleskovicz N, Oliveira FA. Hemodynamic and hemogasometric in the atropine administration in dogs anesthetized with chlorpromazine and dexmedetomidine and isoflurane. *Ciência Rural*. 2008;38(4):1024-9. doi: 10.1590/S0103-84782008000400018
30. Stengel PW, Gomeza J, Wess J, Cohen ML. M(2) and M(4) receptor knockout mice: muscarinic receptor function in cardiac and smooth muscle in vitro. *J Pharmacol Exp Ther*. 2000;292(3):877-85. doi: 10.1016/S0022-3565(24)35366-2
31. Bukhari IA, Dar A. Behavioral profile of *Hypericum perforatum* (St. John's Wort) extract. A comparison with standard antidepressants in animal models of depression. *Eur Rev Med Pharmacol Sci*. 2013;17(8):1082-9.
32. Wang L, Henrich M, Buckler KJ, McMenamin M, Mee CJ, Sattelle DB, et al. Neuronal nitric oxide synthase gene transfer decreases  $[Ca^{2+}]$  in cardiac sympathetic neurons. *J Mol Cell Cardiol*. 2007;43(6):717-25. doi: 10.1016/j.yjmcc.2007.09.005
33. Berg T. Voltage-Sensitive  $K^+$  Channels inhibit parasympathetic ganglion transmission and vagal control of heart rate in hypertensive rats. *Front Neurol*. 2015;6(260):1-13. doi: 10.3389/fneur.2015.00260
34. McCallum JB, Boban N, Hogan Q, Schmeling WT, Kampine JP, Bosnjak ZJ. The mechanism of alpha2-adrenergic inhibition of sympathetic ganglionic transmission. *Anesth Analg*. 1998;87(3):503-10. doi: 10.1097/00005539-199809000-00001
35. Flacke JW. Alpha2-adrenergic agonists in cardiovascular anesthesia. *J Cardiothorac Vasc Anesth*. 1992;6(3):344-59. doi: 10.1016/1053-0770(92)90156-2
36. Chen YW, Fiscella KA, Bacharach SZ, Tanda G, Shaham Y, Calu DJ. Effect of yohimbine on reinstatement of operant responding in rats is dependent on cue contingency but not food reward history. *Addict Biol*. 2014;20(4):690-700. doi: 10.1111/adb.12164
37. Flascik MT, Perez DM. Alpha1-adrenergic receptors: new insights and directions. *J Pharmacol Exp Ther*. 2001;298(2):403-10. doi: 10.1016/S0022-3565(24)29397-6
38. Zhao M, Wu J, Gao Y. The Specific  $\alpha 1$ -adrenergic receptor antagonist prazosin influences the urine proteome. *PLoS One*. 2016;11(10):e0164796. doi: 10.1371/journal.pone.0164796
39. O'Connell TD, Jensen BC, Baker AJ, Simpson PC. Cardiac alpha1-adrenergic receptors: Novel aspects of expression, signaling mechanisms, physiologic function, and clinical importance. *Pharmacol Rev*. 2013;66(1):308-33. doi: 10.1124/pr.112.007203
40. Barbosa-Filho JM, Cunha RM, Dias CS, Athayde-Filho PF, Silva MS, da-Cunha EVL, et al. GC-MS analysis and cardiovascular activity of the essential oil of *Ocotea duckei*. *Rev Bras Farmacogn*. 2008;18(1):37-41. doi: 10.1590/S0102-695X2008000100009
41. Ribeiro TP, Porto DL, Menezes CP, Antunes AA, Silva DF, Sousa DP, et al. Unraveling the cardiovascular effects induced by  $\alpha$ -terpineol: a role for the NO-cGMP pathway. *Clin Exp Pharmacol Physiol*. 2010;37(8):811-6. doi: 10.1111/j.1440-1681.2010.05383.x
42. Chruscinski A, Brede ME, Meinel L, Lohse MJ, Kobilka BK, Hein L. Differential distribution of  $\beta$ -Adrenergic receptor subtypes in blood vessels of knockout mice lacking  $\beta 1$ - or  $\beta 2$ -Adrenergic receptors. *Mol Pharmacol*. 2001;60(5):955-62. doi: 10.1124/mol.60.5.955
43. Xiao RP, Cheng H, Zhou YY, Kuschel M, Lakatta EG. Recent advances in cardiac beta(2)-adrenergic signal transduction. *Circ Res*. 1999;85(11):1092-100. doi: 10.1161/01.res.85.11.1092
44. Cheung SYA, Rodgers T, Aarons L, Gueorguieva I, Dickinson GL, Murby S, et al. Whole body physiologically based modelling of  $\beta$ -blockers in the rat: events in tissues and plasma following an i.v. bolus dose. *Br J Pharmacol*. 2018 Jan;175(1):67-83. doi: 10.1111/bph.14071