The Cyclitol \(L-\)\((+)-\)Bornesitol as an Active Marker for the Cardiovascular Activity of the Brazilian Medicinal Plant *Hancornia speciosa*

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The cyclitol bornesitol is the main constituent of the leaves from the antihypertensive medicinal plant *Hancornia speciosa*. This study aimed to investigate the ability of bornesitol to reduce blood pressure and its mechanism of action. Normotensive Wistar rats were divided into control group and bornesitol groups treated intravenously with bornesitol (0.1, 1.0 and 3.0 mg/kg). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded in non-anesthetized awake animals. Nitric oxide (NO) and angiotensin-converting enzyme (ACE) were measured in plasma by using colorimetric methods. Vascular reactivity study was performed in rat aorta rings and the involvement of nitric oxide synthase (NOS), calcium-calmodulin complex and phosphatidylinositol-3-kinase (PI3K)/Akt pathway in the vasodilator effect was investigated. Administration of bornesitol significantly reduced the SBP, increased the plasmatic level of nitrite, and decreased ACE activity in normotensive rats. In the rat aorta, bornesitol induced endothelium-dependent vasodilatation, which was abolished by NOS blockade. While calcium-calmodulin complex inhibition decreased the vasodilator effect of bornesitol, the inhibition of PI3K/Akt pathway did not alter it. Bornesitol reduced the blood pressure by a mechanism involving an increased production or bioavailability of NO, inhibition of ACE, and by an endothelium- and NO-dependent vasodilator effect. The present results support the use of bornesitol as an active marker for the cardiovascular activity of *Hancornia speciosa*.

Key words cyclitol; blood pressure; endothelium; nitric oxide

INTRODUCTION

Cardiovascular diseases (CVD) causes 17 million deaths annually, more than 50% of them are associated with systemic arterial hypertension (SAH). SAH is the main risk factor for the development of CVD, with 1 billion cases diagnosed in 2008.1 SAH is considered a public health problem due to the high morbimortality rates and costs of treatment.2-3 The therapeutical approach for SAH management takes into account the risk factors associated with the patient and the effects on the cardiovascular system.4 The low adherence to the treatment results from side effects, as well as to failure in restoring the blood pressure and to difficulties in defining the appropriate drugs and/or doses, demanding the search for alternative anti-hypertensive agents.4

The increasing interest in functional foods and medicinal plants has driven the search for new sources of bioactive inositols, due to their potential in preventing cardiovascular diseases.5 \(L-\)\((+)-\)Bornesitol is a methyl myo-inositol found in several plant species.6 It has been identified by gas chromatography in decaffeinated and non-decaffeinated coffee beverages,7 chickpeas, lentils,8 and legumes such as carob beans9 and grass peas.10 Bornesitol has also been reported as responsible for the sweet taste of *Drypetes floribunda* stem and *Sapindus rarak* pericarp.11-13

Bornesitol is an abundant constituent of the leaves from the medicinal plant *Hancornia speciosa* Gomes (Apocynaceae), popularly known as mangaba or mangabeira in Brazil. This species is traditionally used in the country to reduce high blood pressure, among other uses. Its potent antihypertensive effect has been addressed by us in a series of *in vitro* and *in vivo* experiments.12-18 Bornesitol elicits a potent angiotensin-converting enzyme (ACE) inhibition *in vitro* and molecular docking studies showed that this and other cyclitols bind to the same region of the human ACE, which is a tunnel directed towards the active site, distinct from the binding site of the classical ACE-inhibitors like captopril.19 Interestingly, bornesitol is the major constituent (7.75 ± 0.78% (w/w)) of a refined dry extract from *H. speciosa* leaves, standardised according to EMA guideline,20 which induced a dose-dependent, long-lasting reduction in the systolic blood pressure in conscious doxycorticosterone acetate (DOCA)-salt hypertensive mice.21

The regulation of herbal medicinal products (HMP) in Brazil is in charge of the Brazilian Health Regulatory Agency (ANVISA), which establishes two types of markers for the quality control of HMP. The active marker is related to the therapeutic activity of the product, whereas for the analytical marker this relationship has not been demonstrated.21 The aforementioned results led us to raise the hypothesis that bornesitol may be used as an active marker for the biological activity of *H. speciosa* in the cardiovascular system. Therefore, the present work evaluated if bornesitol has the same ability to reduce the blood pressure in normotensive rats, as
observed in studies with *H. speciosa* extracts, and investigated its mechanism of action.

**MATERIALS AND METHODS**

**Extract Preparation and Isolation of Bornesitol** Bornesitol was isolated from the leaves of *H. speciosa*, which were purchased from Indústria Farmacêutica Catedral Ltda., Belo Horizonte, Brazil, on July 2010. The plant material was authenticated by botanists from the Institute of Biological Sciences, UFMG, and the testimonial material was deposited at BHCB Herbarium (register number 165.298). After drying at 40°C for 72 h, the plant material was powdered (100 g) and percolated with 96% EtOH to afford a dark green residue (28.9 g) after solvent elimination under reduced pressure. A portion of the extract (5 g) was fractionated by column chromatography over silica gel to give bornesitol as previously described.22) Bornesitol was further purified by successive crystallization from methanol to afford white needle crystals (39.4 mg). Its chemical structure was confirmed by comparison with an authentic sample in the chromatographic analysis, as well as by spectroscopic techniques including electrospray ionization (ESI)-MS, mono-(1H-) and 13C-NMR, distortional quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC) NMR experiments. NMR spectra were obtained using deuterated dimethyl sulfoxide (DMSO-*d_6*) as a solvent and tetramethylsilane (TMS) as an internal standard, on a Bruker Advance DRX400 spectrometer (Billerica, MA, U.S.A.) operating at 400MHz for 1H and 100MHz for 1^3^C.

**Ultra Performance Liquid Chromatography (UPLC)-ESI-MS Analysis** Further identification of bornesitol isolated from the extract of *H. speciosa* leaves was carried out by UPLC-ESI-MS. An Acquity UPLC system (Waters, Milford, MA, U.S.A.) with a Photodiode Array (PDA) detector was used, interfaced to a triple quadrupole mass spectrometer (TQD) (Waters Micromass, Manchester, U.K.). The analyses were carried out on an amide column (Acquity UPLC BEH, Waters, Ireland; 100 × 2.1 mm i.d., 1.7 μm) in combination with a guard column (Acquity UPLC BEH VanGuard pre-column, Waters; 2.1 × 5 mm i.d., 1.7 μm), using isocratic elution of aqueous ammonium acetate solution (25% (w/v)) and acetonitrile (75%), at a flow rate of 0.3 mL/min, and temperature of 30°C. The isolated bornesitol was dissolved in methanol (2 mg/mL) in an ultrasound bath for 10 min, the solution was filtered through a 0.22-μm polyvinylidene difluoride (PVDF) membrane and aliquots (3 μL) were injected onto the equipment. The ESI negative mode of analysis was employed, with the following inlet conditions: capillary voltage 3.54 kV; gradient cone voltage 10–70 V; source temperature 120°C; desolvation temperature 300°C; desolvation gas flow 840 L/h; cone gas flow 90 L/h. The mass range was set to m/z 90–1000.

**Animals and Ethical Statements** Experimental protocols followed international guidelines and were previously approved by the local ethics committee (protocol 332/2016, UFMG). Male Wistar rats (320–350 g) were kept with free access to a standard diet (Nuvilab, Brazil) and tap water was supplied *ad libitum*, at a constant temperature (23 ± 2°C), with a 12:12 h dark/light cycle. All experiments were carried out using at least five animals per group.

**Measurement of Blood Pressure and Blood Collection** Rats were anesthetized (xylazine 10 mg/kg and ketamine 50 mg/kg; intraperitoneal (i.p.)), and a polyethylene catheter was inserted into the femoral artery by dissection to monitor the blood pressure. A second catheter was implanted in the femoral vein to infuse control solution (0.9% sterile saline) and bornesitol (0.1, 1.0 and 3.0 mg/kg dissolved in saline), as well as for blood collection, before and 30 min after the intravenous administration of bornesitol. Twenty-four hours after the surgery for cannula implantation, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded in non-anesthetized awake animals. Pulsatile arterial pressure (PAP) was recorded by a signal sent to a pressure transducer connected to a data acquisition system (Biopac System, model MP100, U.S.A.). The PAP signal was obtained by a cannula inserted into the femoral artery until reaching the abdominal aorta. The PAP was continuously registered with a sampling frequency of 2000–4000Hz, employing software Acknowledge v.3.5.7 (Biopac System, U.S.A.). SBP values were calculated from PAP and recorded by the system. The parameters were registered during the complete experiment interval, i.e., first record (control) 30 min post-infusion of saline and second record 30 min post-infusion of bornesitol. Venous blood was collected (300 μL) 30 min after either saline or bornesitol infusion. The blood was centrifuged at 9184 ×g for 10 min at 4°C for plasma separation, and it was employed for the determination of nitrite concentration and ACE activity, described below.

**Serum Nitrite Determination** The nitrite (NO_2^-) concentration was measured using the Griess reaction.23) An aliquot (100 μL) of plasma was added to a microtiter plate and the enzymatic treatment was started by adding 100 μL of Griess reagent [0.1% (w/v) N-(1-naphthyl)ethylenediamine and 1.0% (w/v) sulphanilamide in 2.5% (v/v) phosphoric acid] for 10 min at room temperature and sheltered from light. The absorbance of each sample was determined at 545 nm, and total nitrite concentrations were calculated from the slope of the standard curves established using known concentrations of nitrite. Saline was used as a blank solution, and the experiments were performed in triplicate.

**Determination of ACE Plasmatic Activity** ACE activity was measured in plasma, employing a previously reported method.24) Aliquots of plasma (40 μL) were added to a 96-well microtiter plate, and the enzymatic reaction was started by adding 50 μL of N-(2-hydroxyethyl)piperazine-N’′-(2-ethanesulfonic acid) (HEPES) buffer pH 8.15 (mM composition: 50 HEPES, 300 NaCl, 400 Na_2SO_4, pH 8.15) and 30 μL of 100mM hippuryl-glycyl–glycine (Sigma, U.S.A.). After homogenization, the mixture was incubated for 45 min at 37°C. The enzymatic reaction was stopped by adding 50 μL of 0.3 M Na_2WO_4 and 50 μL of 0.32 M H_2SO_4. The solution was homogenized and left to rest for 10 min; following 50 μL of HEPES buffer (pH 8.15) and 3 μL of 6.92 mM 2,4,6-trinitrobenzenesulfonic acid (Sigma, U.S.A.) solution were added. After 20 min, the absorbance of the plate was read at 415 nm against a blank solution prepared in the same way with plasma without bornesitol. Captopril (10 mg/kg, intravenously (i.v.)) was used as positive control, and the experiments were performed in triplicate.

**Vascular Reactivity Study** Male Wistar rats (200–250 g) were euthanized by decapitation. The descending thoracic
aorta was excised, free of fat and connective tissue, cut into rings about 4–5 mm in length and set up in gassed (95% O₂ and 5% CO₂) Krebs–Henseleit solution of the following composition (mM): NaCl 110.8, KCl 5.9, NaHCO₃ 25.0, MgSO₄ 1.07, CaCl₂ 2.49, NaH₂PO₄ 2.33 and glucose 11.51. When necessary, the endothelium was removed by friction with a stainless-steel wire. The tissues were maintained at 37°C under a tension of 1 g and equilibrated for a period of 1 h before initiating experimental protocols. Acetylcholine was used to confirm the presence of functional endothelium, and vessels pre-contracted with phenylephrine (0.3 µM) having more than 60% of relaxation with acetylcholine (1 µM) were considered as having a functional endothelium. Arteries without relaxation with acetylcholine were considered without a functional endothelium. Records were made by a force transducer (World Precision Instruments—WPI, U.S.A.) and amplifier-recorder (TBM-4 model, WPI) and analyzed using WinDaq Data Acquisition software (Dataq Instruments, U.S.A.).

**Fig. 1.** Total Ion Current (TIC) Chromatograms Obtained by UPLC-ESI-MS in the Negative Mode of Analysis for the Ethanol Extract from *H. speciosa* Leaves (A) and Bornesitol Isolated Thereof (B), along with MS Data Recorded for Bornesitol (C).

The employed chromatographic conditions and mass spectrometric parameters are described in Materials and Methods.
Concentration–response curves to bornesitol (0.1 nM to 100 µM) were performed in aortic rings with or without functional endothelium pre-contracted to the same tension (approximately 1.0 g) with submaximal concentrations of phenylephrine (0.3 or 0.1 µM, respectively). The involvement of nitric oxide synthase (NOS) in the vascular relaxation induced by bornesitol was assessed with NG nitro-L-arginine-methyl-ester (L-NAME; 300 µM). The participation of the calcium-calmodulin complex was evaluated by the use of calmidazolium (50 µM). Wortmannin (0.3 µM) and LY294002 (20 µM) were used to investigate the participation of phosphatidylinositol-3-kinase (PI3K)/Akt pathway. All inhibitors were added to the bath 20 min prior to the addition of phenylephrine.

Statistical Analyses
Statistical analyses were performed using GraphPad Prism 6.01 software (GraphPad Software, Inc., U.S.A.). Vascular reactivity data were represented as the percentage of reduction in the sustained contraction induced by phenylephrine (0.3 µM). Two-way ANOVA, followed by Fisher’s LSD post-test, was used to analyze the cumulative concentration-response curves and direct measurement of systolic blood pressure. One-way ANOVA followed by Fisher’s LSD post-test was used for the statistical analysis of nitrite dosage and ACE plasmatic activity. All results were expressed as mean ± standard error of the mean (S.E.M.) and found to be significant if \( p < 0.05 \).

RESULTS AND DISCUSSION

Bornesitol was isolated from an ethanol extract of *H. speciosa* leaves, employing a chromatographic procedure, as previously described.\(^{22}\) Its final purification was accomplished by successive recrystallization from methanol. The presence of bornesitol in the crude extract of *H. speciosa* was initially evaluated by UPLC-ESI-MS in the negative mode of analysis. The chromatograms recorded for the extract (Fig. 1A) and compound isolated thereof (Fig. 1B) show a peak with a retention time of 2.96 min, ascribed to bornesitol. The cyclitol was identified based on the deprotonated ion at \( m/z \) 193.13 Da [M+H]+, as well as by the adducts at \( m/z \) 253.13 Da \([M+CH_3COO]^-\) and \( m/z \) 387.28 Da \([2M−H]^-\) (Fig. 1C). One dimensional (1D) and 2D-NMR spectra obtained for the isolated compound and comparison with data recorded for an authentic sample allowed to confirm the identity of bornesitol (Supplementary Fig. 1), as well as to check its purity (>98%). In addition, the solvent (methanol) used for solubilization of the samples did not appear to interfere (Supplementary Fig. 2).

Wistar rats presented baseline values for SBP and DBP of 110 ± 20 mmHg and 79.6 ± 3.4 mmHg, respectively. The intravenous administration of bornesitol (0.1, 1.0, and 3.0 mg/kg) significantly reduced the SBP at all tested doses (Figs. 2A–C). No significant effect was observed on the DBP (Figs. 2D–F). Previously, we have demonstrated that the ethanol extract of *H. speciosa* leaves reduced the SBP in normotensive mice, with a fast beginning and long duration.\(^{16}\) There is no other study in the literature demonstrating the direct effect of bornesitol on the blood pressure. The present results are in line with the hypotensive effect observed with *H. speciosa* and strongly suggest that bornesitol contributes to this effect. These in vivo results also support bornesitol as chemical and active marker to monitor technological transformation pro-

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![Fig. 2. Effect of Bornesitol (0.1, 1.0 and 3.0 mg/kg), Administered Intravenously, on the Systolic Blood Pressure (SBP, A–C) and the Diastolic Blood Pressure (DBP; D–F) of Normotensive Wistar Rats](image-url)

The results represent the mean ± S.E.M. of the difference (Δ) between the blood pressure before and after the administration of bornesitol for at least five experiments. *\( p < 0.05 \); **\( p < 0.01 \), and ***\( p < 0.001 \) versus saline.
cesses during herbal product manufacture, from the raw vegetable drug to the final product.

Nitric oxide (NO) production was evaluated indirectly by the Griess method. A significant increase in nitrite concentration was observed in rats treated with 1.0 and 3.0 mg/kg of bornesitol (Fig. 3). Consistent with the present findings, we have previously reported an increase of plasmatic nitrite concentration after oral administration of *H. speciosa* extract to normotensive mice, and the effect was abolished by pretreatment with l-NAME, a non-selective inhibitor of NOS. NO plays a key role in the cardiovascular system. It modulates vascular tonus, decreases platelet adhesion and aggregation, and inhibits the growth of vascular smooth muscle cells. Reduction of NO production is a feature associated with endothelial dysfunction in several cardiovascular diseases, including arterial hypertension. Accordingly, we have shown that a NO-dependent reduction in peripheral resistance is the underlying mechanism of the antihypertensive effect of a standardized fraction from *H. speciosa* leaves, enriched in bornesitol and rutin.

Taking into account the above considerations and that bornesitol is the major constituent of the ethanol extract of *H. speciosa* leaves, it is reasonable to assume that the decrease in blood pressure induced by bornesitol is, at least in part, mediated by an increase in NO production. A major source of circulating NO is the NOS of the vascular endothelium. NOS-derived NO is the main endothelium-derived relaxant factor involved in the vasodilatation induced by acetylcholine in the rat aorta. In the present study, bornesitol induced a concentration-dependent vasodilator effect in the rat aorta containing a functional endothelium. However, this vasodilator effect was blunted by endothelium removal (Fig. 4A) and by l-NAME (Fig. 4B). These results show that bornesitol increases the production of NO through the activation of NOS. The calcium-dependent mechanism is activated by the calcium–calmodulin complex, which is inhibited by calmidazolium, a selective antagonist of calmodulin. In the present study, calmidazolium significantly inhibited the maximal effect of the concentration–response curve of bornesitol (Fig. 4C). The calcium-independent activation mechanism of NOS involves the phosphorylation of this enzyme by the PI3K/Akt pathway. Wortmannin and LY294002 are, respectively, non-selective and selective inhibitors of PI3Ks able to inhibit the PI3K/Akt pathway and the calcium-independent mechanism of activation of NOS in the vascular endothelium.

![Fig. 3. Effect of Bornesitol (0.1, 1.0 and 3.0 mg/kg), Administered Intravenously, in the Plasmatic Concentration of Nitrite of Normotensive Wistar Rats](image)

The results represent the mean ± S.E.M. of eight experiments. **p < 0.01 and ***p < 0.001 versus saline.

![Fig. 4. Concentration–Response Curve to Bornesitol in Rat Aorta in the Presence and the Absence of a Functional Endothelium (A), l-NAME (B), Wortmannin and LY294002 (C), and Calmidazolium (D)](image)

The experiments in B, C, and D were performed in the presence of a functional endothelium. All results are expressed as mean ± S.E.M. of five experiments. *p < 0.05, **p < 0.01, and ***p < 0.001 versus control.
tation–response curve to bornesitol was not modified in the presence of wortmannin or LY294002 (Fig. 4D), suggesting that the PI3K/Akt pathway is not involved in its mechanism of action. Therefore, the mechanism involved in the activation of NO production induced by bornesitol seems to be dependent on the activation of the calcium–calmodulin complex.

As pointed out above, bornesitol increased circulating NO at doses of 1.0 and 3.0 mg/kg, but other effects of bornesitol may also explain its hypotensive effect. A previous report from our research group\(^1\) investigated in vitro the ACE inhibitory activity of bornesitol and other structure-related cyclitols and sugars. Bornesitol emerged as the most active compound (IC\(_{50}\) of 41.4 ± 9.6 \(\mu\)M), and some structural features required for the activity were identified. Docking studies between bornesitol and human ACE showed that it binds to a tunnel directed towards the active site of the enzyme, resulting in the decrease of solvation energy and consequent increase in the biological activity of bornesitol.\(^1\) ACE inhibitors generally bind to homologous catalytic sites, i.e., terminal N-ACE (amine-terminal ACE) and terminal C-ACE (carboxy-terminal ACE), containing specific regions for Zn\(^{2+}\) binding, which facilitates nucleophilic attack of a water molecule to the carbonyl group of the substrate at the terminal C-ACE site.\(^2\)

Hence, in order to investigate if the observed hypotensive effect of bornesitol in Wistar rats is related to ACE inhibition, we measured the plasmatic activity of ACE after its intravenous administration at 0.1, 1.0, and 3.0 mg/kg. In this in vivo experimental condition, bornesitol induced a dose-dependent inhibition of ACE (Fig. 5), and the highest assayed dose (3.0 mg/kg) prompted a similar inhibition to that produced by captopril at 10 mg/kg.

As far as we know, this is the first report on the cardiovascular effects of bornesitol. Chemically, bornesitol is classified as inositol, a subclass of cyclitols, namely the isomers of 1,2,3,4,5,6-cyclohexanhexols.\(^3\) Cyclitols, abundantly present in different types of edible and medicinal plants, exert a plethora of physiological and pharmacological effects, regulating a multitude of cellular process, especially lipid metabolism.\(^4\) Clinical data obtained for the combined therapy of myo-inositol plus n-chiro-inositol disclosed an improvement in the metabolic profile of women with the polycystic ovarian syndrome, thus reducing the cardiovascular risk.\(^5\) Therefore, cyclitols with chemical structures similar to bornesitol elicit an indirect effect on the cardiovascular system, by regulating lipid metabolism. The direct effect of bornesitol in the cardiovascular system is a relevant observation for the understanding of the action of medicinal plants and functional foods with beneficial cardiovascular effects, as well as for the development of new phytomedicines and food complements aiming the prevention of comorbidities associated with cardiovascular diseases. However, it is worth mentioning that reduction in the blood pressure can also constitute an undesirable effect for medicinal or food products containing this cyclitol.

CONCLUSION

In conclusion, bornesitol reduces the blood pressure at low doses when administered intravenously in rats. The mechanism of action involves the systemic inhibition of ACE and enhanced production of NO. These findings add new value to the content of bornesitol in medicinal plants and functional foods with beneficial effects on the cardiovascular system. These results also demonstrate that bornesitol is an active marker for the biological activity of \textit{H. speciosa} in the cardiovascular system.

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Conflict of Interest The authors declare no conflict of interest.

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REFERENCES

1) WHO. A global brief on hypertension. \textit{A Glob. Br. Hypertens.}, 1, 1–40 (2013).
2) Malachias MVB, Souza WKS, Plavnik FL, Rodrigues CIS. 7a Diretriz brasileira de hipertensão arterial. \textit{Arq. Bras. Cardiol.}, 107, 1–103 (2016).
3) Lachi-Silva L, Sy SKB, Voelkner A, De Sousa JPB, Lopes JLC, Silva DB, Lopes NP, Kimura E, Derendorf H, Diniz A. Simultaneous characterization of intravenous and oral pharmacokinetics of lycnropholide in rats by transit compartment model. \textit{Planta Med.}, 81, 1121–1127 (2015).
4) Wong D, Tsai PNW, Ip KY, Irwin MG. New antihypertensive medications and clinical implications. \textit{Best Pract. Res. Clin. Anaesth.}, 32, 223–235 (2018).
5) Mulero J, Abellán J, Zafirra P, Amores D, Hernández Sánchez P. Bioactive substances with preventive effect in cardiovascular diseases. \textit{Natr. Hosp.}, 32, 1462–1467 (2015).
6) Loewus FA, Murthy PPN. \textit{myo-Inositol metabolism in plants}. \textit{Plant Sci.}, 150, 1–19 (2000).
7) Ruiz-matute AI, Montilla A, del Castillo MD, Martinez-castro I, Sanz ML. A GC method for simultaneous analysis of bornesitol, other polyalcohols and sugars in coffee and its substitutes. \textit{J. Syp. Sci.}, 30, 557–562 (2007).
8) Moussou N, Corzo-Martinez M, Sanz ML, Zaidi F, Montilla A, Vil-
9) Baumgartner S, Gennert-Ritzmann R, Haas J, Amado R, Neukom H. Isolation and identification of cyclitols in carob pods (Ceratonia siliqua L.). J. Agric. Food Chem., 34, 827–829 (1986).

10) Ruiz-aceituno L, Rodr S, Ruiz-Matute AI, Ramos L, Soria AC, Sanz ML. Optimisation of a biotechnological procedure for selective fractionation of bioactive inositols. J. Sci. Food Agric., 93, 2797–2803 (2013).

11) Chung M, Kim N, Long L, Shammon L, Ahmed W, Sagrero-Nieves L, Kardonos LBS, Kennelly EJ, Pezzuto JM, Soejarto DD, Kinghorn AD. Dereplication of saccharide and polyol constituents of candidate sweet-tasting plants: isolation of the sesquiterpene glycoside mukuroyoside IIb as a sweet principle of Sapindus rarak. Phytochem. Anal., 8, 49–54 (1997).

12) Soejarto Braga F, Wagner H, Lombardi JA, Braga De Oliveira A. Screening the brazilian flora for antihypertensive plant species for in vitro angiotensin-I-converting enzyme inhibiting activity. PhytoMedicine, 7, 245–250 (2000).

13) Ferreira HC, Serra CP, Endringer DC, Lemos VS, Braga FC, Cortes SF. Endothelium-dependent vasodilation induced by Hancornia speciosa in rat superior mesenteric artery. Phyto medicine, 14, 473–478 (2007).

14) Ferreira HC, Serra CP, Lemos VS, Braga FC, Cortes SF. Nitric oxide-dependent vasodilatation by ethanolic extract of Hancornia speciosa via phosphoryl-inositol 3-kinase. J. Ethnopharmacol., 109, 161–164 (2007).

15) Serra CP, Cortes SF, Lombardi JA, Braga De Oliveira A, Braga FC. Validation of a colorimetric assay for the in vitro screening of inhibitors of angiotensin-converting enzyme (ACE) from plant extracts. Phyto Medicine, 12, 424–432 (2005).

16) Silva GC, Braga FC, Lima MP, Pesquero JL, Lemos VS, Cortes SF. Hancornia speciosa Gomes induces hypotensive effect through inhibition of ACE and increase on NO. J. Ethnopharmacol., 137, 709–713 (2011).

17) Silva GC, Braga FC, Lemos VS, Cortes SF. Potent antihypertensive effect of Hancornia speciosa leaves extract. Phyto Medicine, 23, 214–219 (2016).

18) Alves e Silva M, Pacheco C, Madeira M, Saraiva A, de Freitas E, Valverde T, Gomes J de Pâdua R, Kitten G, Alves S, Braga F, da Silva T. Effect of the extract and constituents from Hancornia speciosa fruits in osteoclasts. Planta Medica Int. Open, 6, 67–71 (2019).

19) Endringer D, Oliveira O, Braga F. In vitro and in silico inhibition of angiotensin-converting enzyme by carbohydrates and cyclitols. Chem. Phys. Lett., 68, 37–43 (2014).

20) European Medicines Agency. Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/ traditional herbal medicinal products. Eur. Med. Agency, 2010, 1–18 (2010).

21) Mazzari ALDA, Prieto JM. Monitoramento de interações farmacocinéticas entre plantas medicinais e fitoréplicos e os medicamentos convencionais pelo sistema de farmacovigilância brasileiro. In. Farm., 26, 193 (2014).

22) Endringer DC, Pezzuto JM, Braga FC. NF-κB inhibitory activity of cyclitols isolated from Hancornia speciosa. Phytomedicine, 16, 1064–1069 (2009).

23) Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal. Biochem., 126, 131–138 (1982).

24) Lucas-Filho MD, Silva GC, Cortes SF, Mares-Guia TR, Perpétua Ferraz V, Serra CP, Braga FC. ACE inhibition by astilbin isolated from Erythroxylum gonocladium (Mart.) O.E. Schulz. PhytoMedicine, 17, 383–387 (2010).

25) Dimmelre S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature, 399, 601–605 (1999).

26) Omar SA, Webb AJ. Nitrite reduction and cardiovascular protection. J. Mol. Cell. Cardiol., 73, 57–69 (2014).

27) Moncada S, Palmer RM, Higgs EA. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol. Rev., 43, 109–142 (1991).

28) Brandes RP. Endothelial dysfunction and hypertension. Hypertension, 64, 924–928 (2014).

29) Wang X, Tame-Santos JE, Reiter CD, Dejam A, Shiva S, Smith R.D, Hogg N, Gladwin MT. Biological activity of nitric oxide in the plasmatic compartment. Proc. Natl. Acad. Sci. U.S.A., 101, 11477–11482 (2004).

30) Chen G, Suzuki H, Weston AH. Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br. J. Pharmacol., 95, 1165–1174 (1988).

31) Förstermann U, Pollock JS, Schmidt HH, Heller M, Murad F. Calmodulin-dependent endothelium-derived relaxing factor/nitric oxide synthase activity is present in the particulate and cytosolic fractions of bovine aortic endothelial cells. Proc. Natl. Acad. Sci. U.S.A., 88, 1788–1792 (1991).

32) Tzakos AG, Galanis AS, Spyroulia G, Cordopatis P, Manessi-Zoupa E, Gerothannissis IP. Structure–function discrimination of the N- and C-catalytic domains of human angiotensin-converting enzyme: implications for Cl-activation and peptide hydrolysis mechanisms. Protein Eng., 16, 993–1003 (2003).

33) Ducheck J, Adams DR, Hadlicky T. Chemoenzymatic synthesis of inositol, conduritol, and cyclitol analogues. Chem. Rev., 111, 4223–4258 (2011).

34) Benjamin DI, Louie SM, Mulvihill MM, Kohnz RA, Li DS, Chan LG, Sorrentino A, Bandyopadhyay S, Cozzo A, Ohiri A, Goga A, Ng SW,Nomura DK, Inositol phosphate recycling regulates glycolytic and lipid metabolism that drives cancer aggressiveness. ACS Chem. Biol., 9, 1340–1350 (2014).

35) Minozzi M, Nordio M, Pajalich R. The combined therapy myo-inositol plus α-chiro-inositol, in a physiological ratio, reduces the cardiovascular risk by improving the lipid profile in PCOS patients. Eur. Rev. Med. Pharmacol. Sci., 17, 537–540 (2013).