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NATURAL DRUGS

BIOASSAY-GUIDED FRACTIONATION AND ANTIHYPERTENSIVE PROPERTIES OF FRACTIONS AND CRUDE EXTRACTS OF *PERISTROPHE BICALYCULATA* (RETZ) NEES

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Abstract: Hypertension is an important public health issue in both developed and developing countries due to its high incidence and morbidity. This has motivated researchers especially in developing countries to search for strategies for the treatment using different plant parts. The use of the aqueous decoction of the leaves of *Peristrophe bicalyculata* in the treatment of hypertension has been documented. This study was designed to carry out a bioassay-guided isolation of the antihypertensive components of the leaves of *Peristrophe bicalyculata* in L-NAME hypertensive rats, determine the angiotensin-converting enzyme inhibitory activity of the extracts and fractions obtained and identify the constituent(s) present. From our results, L-NAME hypertensive rats given the cold water extract had significantly ($p < 0.05$) lower mean arterial blood pressure (MABP) with longer duration of action than other extracts. Also, the angiotensin-converting enzyme inhibitory activity of the cold water extract was significantly ($p < 0.05$) higher than that of other extracts. From the GC-MS analysis of the most effective fraction (fraction 4), P,P,P-triphenyl-imino(triphenyl)phosphorane and andrographolide 2(3H)-furanone were identified among others. The present work demonstrates the hypotensive effect of the cold water extract of *Peristrophe bicalyculata* on L-NAME hypertensive rats, which further justifies the folkloric application of extracts of the plant in the management as well as treatment of hypertension.

Keywords: angiotensin-converting enzyme, hypertension, *Peristrophe bicalyculata*

Hypertension is a common cardiovascular disease which has become a worldwide problem of epidemic proportions, affecting 15 to 20% of all adults; with ailments such as arteriosclerosis, stroke, myocardial infarction and end-stage renal disease (1). According to the World Health Organization (WHO), about 57 million deaths occurred in 2008, and cardiovascular diseases being the highest recorded, with over 17 million deaths (2). Studies have demonstrated that nearly 90-95% of cases of hypertension are primary and classified as essential hypertension, while the remaining 5-10% is classified as secondary hypertension (3). Substantial evidence demonstrates endothelial dysfunction caused as a result of low nitric oxide (NO) levels and/or increased reactive oxygen species, as a key early event in the development of primary hypertension (4, 5).

Nitric oxide (NO) regulates smooth muscle cell tone, platelet aggregation and adhesion, cell growth, apoptosis, neurotransmission and injury. It also serves as an endogenous vasodilator, platelet inhibitor, antioxidant, and regulator of vascular endothelium by sustaining its anticoagulant and

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antithrombogenic properties (6). In the cardiovascular system, NO is released from the endothelium in response to a physiologic stimulus to activate guanylate cyclase in vascular smooth muscle cells and increase cGMP levels to mediate relaxation (7). The interruption of NO synthesis with several L-arginine analogues such as N^G-nitro-L-arginine methyl ester (L-NAME) has been shown to induce vasoconstriction and arterial hypertension in experimental animals (8) making it a suitable model for studying the antihypertensive effects of new drugs.

Angiotensin converting enzyme (ACE) is a transmembrane zinc metallopeptidase, involved in the regulation of vascular tone by converting angiotensin I (an inactive decapeptide) to angiotensin II (a potent vasoconstrictor). The enzyme also inactivates the vasodilatory nonapeptide bradykinin (9). The ACE inhibitors are used for the treatment of hypertension and prevention of chronic heart failure (10). The effect of ACE inhibitors on the NO/cGMP pathway is not well established (11-13), even though it is well known that the antihypertensive effect of these drugs is attributed to decreased angiotensin II and increased vasodilatory kinins and NO (6). Due to the high incidence and morbidity of hypertension, various drugs and regimes have been advocated for its control. Most of these drugs do not possess complete curative properties; and some demonstrate better efficacy but possess side-effects (14). Recently, attention has been drawn towards herbal sources which are used traditionally as potential therapeutic agents in the prevention and management of cardiovascular diseases (15, 16).

Peristrophe bicalyculata (Retz) Nees is native to warm tropical regions of Africa, in the Sahel part of the region from Mauritania to Niger and Northern Nigeria, India, Burma and Thailand (17). The leaves of the plant have analgesic, antipyretic, anti-inflammatory activities, and antibacterial, fungistatic and bacteriostatic properties (17, 18). Studies have demonstrated the anticancer activity of oils from *Peristrophe bicalyculata* using MCF-7 (human breast tumor) and MDA-MB-468 (human breast tumor) cells (19). The anticancer activity of crude extracts of the plant against Ehrlich ascites carcinoma (EAC) cell lines (20) and human mouth epidermal carcinoma (KB) cells (21) have also been reported. In South-West Nigeria, the plant is used as green manure as well as in the treatment of hypertension and cardiovascular-related diseases (22). The present study was aimed at partially purifying the antihypertensive extract of *Peristrophe bicalyculata*, determine the ACE inhibitory activities of the fractions and identify the antihypertensive component(s)/constituent(s) present.

MATERIALS AND METHODS

Chemicals and reagents

Hippuryl-histidyl-leucine (HHL), angiotensin converting enzyme (ACE), N^G-nitro-L-arginine methyl ester (L-NAME) and captopril were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

Preparation of plant material

The leaves of *Peristrophe bicalyculata* (Retz) Nees were harvested at maturity in the month of June, 2010 at Ibadan, Oyo State, Nigeria. The plant was identified and authenticated by the botanist in the herbarium of the Department of Biological Science, Ahmadu Bello University (ABU), Zaria, with a voucher number 2863.

The leaves of the plant were washed clean under running water, air-dried in the laboratory and powdered. The powdered plant (500 g) was defatted in n-hexane before extracting with methanol. The methanol extract was dissolved in distilled water and then partitioned in ethyl acetate and n-butanol using separating funnel, to obtain ethyl acetate fraction of methanol extract, butanol fraction of methanol extract and water fraction of methanol extract. The cold and hot water extracts were obtained by stirring (Harmony Hot Plate Stirrer, Japan) in cold and hot water, respectively, sieved using a muslin cloth and then filtered under suction pressure with Whatman's filter paper (No. 1). All extracts were then concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland), lyophilized (Christ Alpha 1-2 LD, Germany) and stored at 4^{o}C until needed.

Bioassay-guided fractionation of cold water extract of *Peristrophe bicalyculata*

The cold water extract of *Peristrophe bicalyculata,* was partially purified by column chromatography after thin layer chromatographic separation. The thin layer chromatography was carried out using ethyl acetate : formic acid : methanol (1 : 1 : 3, v/v/v) solvent which gave five separate constituents, visible under UV-spectrum. Column chromatography using silica gel was then run with ethyl acetate (100%), ethyl acetate : formic acid : methanol (15 : 2 : 0.5, v/v/v), ethyl acetate : methanol (1 : 1, v/v), ethyl acetate : methanol (1 : 3, v/v), ethyl acetate : methanol : formic acid (1 : 3 : 1, v/v/v) and methanol

: formic acid (9 : 1, v/v). Elution and fractionation were controlled by TLC and similar fractions were combined.

Determination of angiotensin-converting enzyme inhibitory activity

The assay for ACE inhibitory activity was determined using the Cushman and Cheung (23) method with some modifications on the assay conditions. Briefly, the inhibitor solution (purified extract) was added to 0.1 M potassium phosphate buffer (pH 8.3), which consists of 5 mM hippuryl-histidyl-leucine (HHL), 0.1 M potassium phosphate and 0.3 M NaCl (pH 8.3). Then, the enzyme, ACE was added to the mixture and incubated at 37^{o}C for 30 min. The reaction was terminated by adding 0.25 mL of 1 M HCl, and then 1.5 mL of ethyl acetate was added to extract the hippuric acid formed by the action of ACE. The ethyl acetate was removed by heat evaporation, residual hippuric acid (HA) dissolved in 1 mL of deionized water, and absorbance of the solution was measured at 228 nm to determine the hippuric acid concentration and ACE inhibitory activity. The concentration of ACE inhibitor required to inhibit 50% of the ACE activity under the above assay conditions was defined as IC_{50}.

Experimental design

All experimental protocols were approved and conducted with strict adherence to guidelines and procedures of the Institutional Animal Care and Use Committee of the Natural Product Research and Development Centre, Faculty of Pharmacy, Chiang Mai University, Thailand. One hundred apparently healthy male Sprague-Dawley (SD) rats of about eight weeks' old, weighing between 180 and 230 g were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhon Pathom, Thailand, and maintained in an air-conditioned animal house of the Faculty of Pharmacy, Chiang Mai University, Thailand. They were fed normal rat chow and allowed access to clean water *ad libitum*. The rats were allowed to acclimatize for two weeks before the commencement of the experiment.

The experiment was carried out in two phases. In the first phase, rats were divided into 4 groups of 6 rats each: Group 1 (control) - rats were intravenously given normal saline, groups 2, 3 and 4 were intravenously injected with a solution of L-NAME (3 mg/kg) until blood pressure reached maximum. Captopril (5 mg/kg), water fraction of methanol extract (10 mg/kg) and cold water extract (10 mg/kg) were intravenously administered to hypertensive rats in groups 2, 3 and 4, respectively. In the second phase of the experiment; rats were divided into 6 groups of 6 rats each: Group 1 rats were intravenously given normal saline, groups 2, 3, 4, 5 and 6 were intravenously injected with a solution of L-NAME (3 mg/kg) until blood pressure reached maximum. Fractions 1, 2, 3, 4 and 5 obtained after partial purification of the cold water extract, were given to rats in second, third, fourth, fifth and sixth groups, respectively.

Acute toxicity studies

Acute oral toxicity studies of extracts of *Peristrophe bicalyculata* were carried out as described by Lorke (24) with oral administration of increasing doses of the extracts from 10 mg/kg to 5000 mg/kg, while animals were observed for behavioral changes, toxicity and mortality for 24 h.

Blood pressure and heart rate measurement

Rats were fixed on a supine position on a dissecting table and anesthetized by intraperitoneal administration of pentobarbital sodium (40 mg/kg). A longitudinal mid-tracheal incision, approximately 2 cm long was made in order to expose the trachea, the right jugular vein and both common carotid arteries. The trachea was cannulated with a polyethylene tube (2.75 mm diameter) to maintain a free airway, while the right jugular vein was cannulated for the administration of extracts and isotonic saline solution. The systemic blood pressure was recorded from the cannulated left carotid artery, connected to a physiological pressure transducer (Statham P23 AC Strain gauge Tansducer, Laboratories, Inc. Hato Rey, Puerto Rico) and displayed on a Grass 7D Polygraph (Grass Instrument Co., Quincy, Mass, USA). The cannulation of the carotid artery was performed in the same manner as that of the jugular vein, and the polyethylene tube (1 mm diameter) filled with heparin sodium in saline solution was used. Blood pressure and heart rate were monitored until steady base-line levels were obtained. L-NAME at 3 mg/kg was administered *via* venous cannula to induce hypertension. Blood pressure was allowed to stabilize for 30 min before administration of extracts and fractions through the jugular vein cannula. Changes in blood pressure were recognized as difference between the steady state values before and the peak readings after injection (25). Mean arterial blood pressure (MABP) was calculated as the diastolic BP plus one-third of the pulse width (systolic BP - diastolic BP).

GC-MS analysis of the partially purified antihypertensive fraction of *P. bicalyculata*

The gas chromatography and mass spectrometry (GC-MS) analysis was conducted using Shimadzu apparaus with chromatographic system (model GC-2010). The mass analysis apparatus (GC-MS QP2010) was connected to the column RTX-5MS (Restek) 30 m, 0.25 mm internal diameter.

Spectra were generated using the in-built software (GC-MS solution version 2.5 SUI). GC-MS real time and GC-MS post-run analyses were evaluated using three libraries: NIST, Wiley intergrated and a domestic library using a CAS number of EMBRAPA Genetic Resources and Biotechnology. Each spectrum was confirmed by two replicated readings to ensure reproducibility. All peaks were further analyzed for compound identification, based on their similarities with structures available in the libraries.

Statistical analysis

Data obtained were expressed as the mean ± standard error of the mean (mean ± SEM) and analyzed using Statistical Package for the Social Sciences (SPSS Inc., Release 17.0, Chicago, IL., USA). The significance among groups was determined by one way analysis of variance and LSD *post-hoc* test was applied for multiple comparisons. Values of $p < 0.05$ were regarded as statistically significant.

RESULTS

Percentage yield of extracts of *Peristrophe bicalyculata*

The percentage yields of extracts of *Peristrophe bicalyculata* are presented in Table 1. The yields from 50 g powdered plant of the cold and hot water extracts were 9.03 ± 0.44 and 7.79 ± 2.24 g, respectively, while extraction of 500 g with methanol yielded 41.44 ± 5.87 g. Further fractionation of the methanol extract with ethyl acetate, butanol and water yielded 12.28 ± 4.37, 8.61 ± 2.66 and 8.49 ± 3.46 g, respectively.

Acute toxicity

In the acute toxicity study, a single oral administration of all extracts of *Peristrophe bicalyculata* at

Table 1. Yield and percentage yield of extracts of *Peristrophe bicalyculata*.

Extract	Yield (g)	Percentage yield (%)
Cold water (50 g)	9.03 ± 0.44	18.06 ± 0.88
Hot water (50 g)	7.79 ± 2.24	15.58 ± 4.49
Hexane (500 g)	4.61 ± 1.17	1.00 ± 0.23
Methanol (500 g)	41.44 ± 5.87	8.29 ± 1.15
Ethyl acetate of methanol	12.28 ± 4.37	2.46 ± 0.88
Butanol of methanol	8.61 ± 2.66	1.72 ± 0.52
Water of methanol	8.49 ± 3.46	1.70 ± 0.69

Values are the mean ± SEM. Ethyl acetate, butanol and water extracts of methanol extract were obtained from methanol extract by fractionating with ethyl acetate, butanol and water, respectively.

Table 2. Effect of *Peristrophe bicalyculata* on L-NAME-induced hypertension in rats.

Extract	MABP change (%)	HR change (%)	Duration of action (min)
Control	- 2.13 ± 0.03^{c}	1.50 ± 0.02^{f}	-
L-NAME	+ 65.00 ± 5.06^{f}	9.15 ± 2.24^{a}	185.00 ± 51.40
Captopril (5 mg/kg)	- 62.70 ± 5.56^{a}	2.45 ± 1.09^{d}	209.00 ± 12.07^{h}
Water fraction of methanol extract (10 mg/kg)	- 15.67 ± 1.02^{b}	2.67 ± 0.84^{d}	33.33 ± 04.01^{g}
Cold water extract (10 mg/kg)	- 15.16 ± 2.09^{b}	4.33 ± 0.71^{e}	150.00 ± 39.50^{e}

Values are the mean ± SEM. MABP = mean arterial blood pressure; HR = heart rate; $^{a-i}$ = Values with different superscripts in the same column are significantly ($p < 0.05$) different; + : increase in blood pressure; - : decrease in blood pressure, L-NAME = N^{G}-nitro-L-arginine methyl ester.

doses from 10 to 5000 mg/kg did not produce any apparent toxic symptom or mortality after the 24 h observation period.

Effect of *P. bicalyculata* on L-NAME-induced hypertensive rats

The effect of *Peristrophe bicalyculata* on L-NAME-induced hypertensive rats is presented in Table 2. The intravenous administration of L-NAME to normal Wistar rats induced a sustained arterial hypertension which lasted over three hours (185 ± 51.40 min) and significantly ($p < 0.05$) increased MABP by 65 ± 5.06% compared to rats in the control group whose MABP decreased (-2.13 ± 0.03%), but remained constant. Heart rate of hypertensive rats increased significantly ($p < 0.05$) by 9.15 ± 2.24% from 1.50 ± 0.02% in control rats. The MABP was reduced significantly ($p < 0.05$) immediately following intravenous administration of captopril (62.70 ± 5.56%), water fraction of methanol extract (15.67 ± 1.02%) and cold water extract (15.16 ± 2.09%). The reduction in MABP and duration of action (209.00 ± 12.07 min) of captopril in hypertensive rats was significantly ($p < 0.05$) higher than in rats, given water fraction of methanol extract and cold water extract. Although there was no significant ($p > 0.05$) difference in MABP of hypertensive rats given the water fraction of methanol extract and cold water extract, the duration of action of the cold water extract (150.00 ± 39.50 min) in hypertensive rats was significantly higher ($p < 0.05$) than in those given water fraction of methanol extract (33.33 ± 04.01 min).

Table 3. Fractions and yields of cold water extract of *Peristrophe bicalyculata* obtained by column chromatographic separation.

Solvents	Fractions	Yield (g)	New fractions
Ethyl acetate (100%)	Fraction 1	0.0062	1
Ethyl acetate : formic acid : methanol (15 : 2 : 0.5)	Fraction 2a	0.0042	(Fractions 1 and 2a)
	Fraction 2b	0.2127	2 (Fraction 2b)
	Fraction 2c	1.3438	3 (Fraction 2c)
Ethyl acetate : methanol (1 : 1)	Fraction 3	0.2043	4
Ethyl acetate : methanol (1 : 3)	Fraction 4	0.0388	(Fractions 3 and 4)
Ethyl acetate : methanol : formic acid (1 : 3: 1) Methanol : formic acid (9 : 1)	Fraction 5a	0.6499	(Fractions 5a, 5b,5c and 6)
	Fraction 5b	0.1936	
	Fraction 5c	0.0516	
	Fraction 6	0.0077	
Total		**2.7128**	

Table 4. Effect of fractions of cold water extract of P. *bicalyculata* on L-NAME hypertensive rats.

Groups	MABP change (%)	HR change (%)	Duration of action (min)
Control	1.21 ± 0.06^{a}	1.67 ± 0.10^{h}	-
L-NAME (3 mg/kg)	$+60.00 \pm 9.02^{b}$	12.15 ± 2.62^{i}	161.02 ± 18.09
Captopril (5 mg/kg)	-40.14 ± 9.08^{c}	3.13 ± 1.06^{m}	$182.00 + 30.21^{s}$
Fraction 1 (0.03 mg/kg)	$+13.17 \pm 2.43^{d}$	$9.17 \pm 0.65^{k,i}$	120.00 ± 80.50^{x}
Fraction 2 (0.71 mg/kg)	-21.00 ± 2.94^{e}	4.67 ± 0.76^{m}	102.83 ± 43.32^{y}
Fraction 3 (4.48 mg/kg)	-12.67 ± 1.60^{f}	$6.00 \pm 2.62^{k,m}$	116.67 ± 37.16^{y}
Fraction 4 (0.81 mg/kg)	-29.33 ± 2.26^{g}	4.33 ± 0.80^{m}	69.17 ± 13.57^{z}
Fraction 5 (3.00 mg/kg)	-25.17 ± 1.11^{e}	2.33 ± 1.11^{m}	65.00 ± 3.16^{w}

Values are the mean ± SEM. MABP = mean arterial pressure; HR = heart rate; $^{a-z}$ = Values with different superscripts in the same column are significantly ($p < 0.05$) different; + : increase in blood pressure; - : decrease in blood pressure
L-NAME = N^{G}-nitro-L-arginine methyl ester.

Table 5. Angiotensin-converting enzyme inhibitory effect of cold water extract of *Peristrophe bicalyculata*.

Samples	IC_{50} (µg/mL)
Captopril	2.38 ± 0.19[a]
Fraction 1	24.98 ± 1.73[b]
Fraction 2	27.23 ± 1.60[b]
Fraction 3	15.86 ± 1.78[c]
Fraction 4	9.40 ± 1.58[d]
Fraction 5	13.85 ± 0.78[c]

Values are the mean ± SEM. [a-e] = Values with different superscript letters are significantly different ($p < 0.05$).

Bioassay-guided fractionation of cold water extract of *Peristrophe bicalyculata*

From the results (Table 3), 10 different fractions were obtained; with the ethyl acetate (100%), ethyl acetate : methanol (1 : 1, v/v), ethyl acetate : methanol (1 : 3, v/v) and methanol : formic acid (9 : 1, v/v) solvents, giving one fraction each (fractions 1, 3, 4 and 6, respectively); while ethyl acetate : formic acid : methanol (15 : 2 : 0.5, v/v/v) and ethyl acetate : methanol : formic acid (1 : 3 : 1, v/v/v) yielded 3 fractions each: fractions 2a, 2b, 2c and 5a, 5b and 5c, respectively. The yield from fraction 2c was the highest (1.3738 g), which was about 50% of the extract used; and the least was fraction 2a (0.0042 g). The fractions were reduced to five after TLC separation, based on their spots color, retention factor and physical characteristics. These fractions were then administered to hypertensive rats to determine the most active.

Effect of fractions of cold water extract of *P. bicalyculata* on hypertensive rats

From the results (Table 4), the percentage MABP of L-NAME-induced hypertensive rats increased significantly ($p < 0.05$) by 60.00 ± 9.02% and persisted for over 2.5 h (161.02 ± 18.09 min), while their heart rates also increased by 12.15 ± 2.62%. Following intravenous administration of captopril, and fractions 2, 3, 4 and 5, the MABP was significantly ($p < 0.05$) reduced by 40.14 ± 9.08%, 21.00 ± 2.94%, 12.67 ± 1.60%, 29.33 ± 2.26% and 25.17 ± 1.11%, respectively. The intravenous administration of captopril in rats significantly ($p < 0.05$) reduced MABP with a longer duration of action (182.00 + 30.21 min) than in those given other fractions. Fraction 4 at 0.81 mg/kg reduced MABP of hypertensive rats by 29.33 ± 2.26% for over one hour, which was significantly ($p < 0.05$) higher than reduction percentages induced by fractions 2 (21.00 ± 2.94%), 3 (12.67 ± 1.60%) and 5 (25.17 ± 1.11%). Conversely, fraction 1 increased blood pressure of hypertensive rats by 13.17 ± 2.43%.

The percentage decrease in heart rate of hypertensive rats given fractions 4 (4.33 ± 0.80%) and 5 (2.33 ± 1.11%) did not differ significantly ($p > 0.05$) from each other.

Angiotensin-converting enzyme inhibitory activity of extracts and fractions of *Peristrophe bicalyculata*

Results of the ACE inhibitory activity of the fractions of *Peristrophe bicalyculata* are presented in Table 5. As shown, captopril inhibited 50% of the ACE activity (IC_{50}) at a concentration (2.38 ± 0.19 µg/mL) significantly lower ($p < 0.05$) than that recorded for any other fraction. The ACE inhibitory activity of fraction 4 (9.40 ± 1.58 µg/mL) was significantly ($p < 0.05$) lower than all other fractions, while, fractions 1 and 2 inhibited the enzyme at the highest concentrations (24.98 ± 1.73 and 27.23 ± 1.60 µg/mL, respectively).

Gas chromatography and mass spectrometry analysis of the partially purified antihypertensive fraction of *Peristrophe bicalyculata*

The results of the GC-MS analysis identified the various compounds present in the partially-purified fraction (Table 6). Figure 1 shows the gas chromatogram with 5 distinct peaks identified by the GC-MS.

The major compound identified by GC-MS in the partially-purified antihypertensive fraction of *Peristrophe bicalyculata* was P,P,P-triphenylimino(triphenyl)phosphorane with retention time (RT) 47.28 min and molecular weight (m.w.) of 278 g. Other compounds present are propanoic acid (RT 3.41 min; m.w. 102 g), 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (RT 11.71 min; m.w. 144 g), 1,1,1,5,7,7,7-heptamethyl-3,3-bis(trimethylsiloxy)-tetrasiloxane (RT 42.79 min; m.w. 444 g) and andrographolide 2(3H)-furanone (RT 58.62 min; m.w. 350 g).

DISCUSSION

The antihypertensive and ACE inhibitory activities of the cold water extract and water fraction of methanol extract were investigated based on the result of previous study (26). In the present study, the effect of the extract on L-NAME-induced hypertensive rats was investigated. This is because it is well established that the alteration of the nitric oxide

Table 6. Components identified in antihypertensive fraction of cold water extract of *Peristrophe bicalyculata* by GC-MS.

Name of compound	Retention time (min)	Molecular formula	Molecular weight (g)
Propanoic acid	3.41	$C_5H_{10}O_2$	102
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	11.71	$C_6H_8O_4$	144
1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	42.79	$C_{13}H_{40}O_5Si_6$	444
Pentadecanal	45.40	$C_{15}H_{30}O$	226
P,P,P-triphenyl-imino(triphenyl)phosphorane	47.28	$C_{18}H_{16}NP$	277
Diazoprogesterone	49.85	$C_{21}H_{30}N_4$	338
5-Ethyl-2-nonanol	51.07	$C_{11}H_{24}O$	172
Arachidonic acid trimethylsilyl ester	56.31	$C_{23}H_{40}O_2Si$	376
Andrographolide 2(3H)-furanone	58.62	$C_{20}H_{30}O_5$	350
Pseduosarsasapogenin-5,20-dien methyl ether	61.40	$C_{28}H_{44}O_3$	428

The compounds presented in the Table are those which matched similar compounds in the NIST, Wiley intergrated and a domestic library using a CAS number of EMBRAPA Genetic Resources and Biotechnology and which contained the molecular ion of the matching compound.

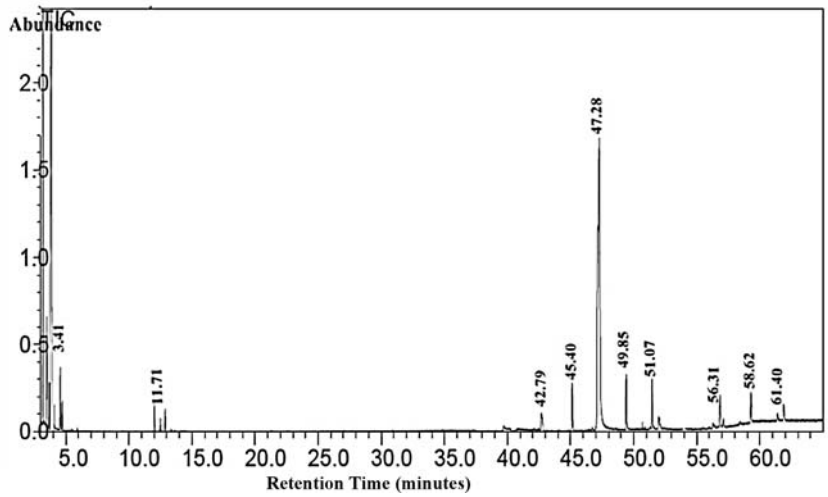

Figure 1. Gas chromatogram of partially-purified antihypertensive fraction of *Peristrophe bicalyculata*

system plays an important role in the development or maintenance of clinical hypertension (27). This model of hypertension also mimics essential hypertension, which affects 90-95% of hypertensive individuals as it is usually caused by endothelial dysfunction due to the inhibition of NO (28).

The cold water extract of *Peristrophe bicalyculata* was found to be the most effective in reducing MABP of hypertensive rats. This significant ($p < 0.05$) reduction in MABP observed in L-NAME hypertensive rats after acute intravenous administration of the cold water extract which lasted over 2 h validates the claim that the aqueous extract of the plant possesses antihypertensive activity (26). Although the percentage reduction of MABP by the cold water extract (15.16 ± 2.09%) and water fraction of methanol extract (15.67 ± 1.02%) was not significantly different ($p > 0.05$), the duration of action of the cold water extract was significantly ($p < 0.05$) higher, hence our choice of further purifying the cold water extract. The consistent and normal MABP in rats within the control group is an evidence that the rats were healthy and not hypertensive, while acute administration of L-NAME to the rats significantly increased MABP. It is well documented that acute intravenous administration of L-NAME results in sustained hypertension attributed to the inhibition of nitric oxide synthase, known to

inhibit the synthesis of nitric oxide from L-arginine, thereby causing severe and progressive arterial hypertension (8, 29).

It has previously been reported that the cold water extract of *Peristrophe bicalyculata* possesses significant ACE inhibitory activity (22) compared to other extracts of the plant. In the present study, partial purification of the cold water extract yielded five fractions; with fraction 4 inhibiting ACE at the lowest concentration compared to other fractions; and thus, considered the most active inhibitor. Fraction 4 also reduced MABP significantly ($p < 0.05$) at a lower concentration (0.81 mg/kg) compared to others (Table 4). Thus, we may suggest that the antihypertensive effect of *Peristrophe bicalyculata* may be due in part to its ability to prevent the conversion of angiotensin I to angiotensin II by inhibiting ACE in the renin-angiotensin system (RAS). This is consistent with studies by Jimenez-Ferrer et al. (30) that antihypertensive plants with good ACE inhibitory activity exert their effect by inhibiting the conversion of angiotensin I to angiotensin II. However, several studies have demonstrated that the generalized vasoconstriction characteristic of chronic nitric oxide synthase (NOS) inhibition model is maintained by an interaction between the sympathetic nervous system and the RAS, (31, 32), whereas, in the acute NOS inhibition model, the RAS may not be involved (33). Thus, it is assumed that ACE inhibitors may alleviate acute hypertension by synthesis of vasodilatory substances such as NO, prostacyclin, and endothelium-dependent hyperpolarizing factor (EDHF) (11, 34, 35). Thus, we may postulate that the mechanism underlying the acute antihypertensive effect of the cold water extract and partially purified fraction (fraction 4) of *Peristrophe bicalyculata*, which in the present study were found to be potent ACE inhibitors, may be linked to the synthesis of vasodilatory substances by bradykinin, as it has been shown that ACE inhibition enhances the vasodilator effects of bradykinin and that blockade of the bradykinin receptor attenuates the hypotensive action of ACE (36).

The potent antioxidant activity of the plant (37) may have played a significant role in alleviating hypertension (30, 38), as phenols have been reported to enhance vascular NO activities by inducing NO production through NOS expression or by protecting NO against destruction, while flavonoids are known to possess cardioprotective and antihypertensive effects by specifically targeting cardiovascular ionic channels and playing important roles in vascular tone regulation (39). Furthermore, flavonoids from different plant sources have been found to inhibit ACE (40, 41). Flavones of Roxb., apigenin and luteolin have demonstrated a dose-dependent enzyme inhibition (10). Thus, the antioxidant effects of the plant may have contributed in reducing blood pressure by synthesizing vasodilatory substances and scavenging free radicals, known to cause oxidative stress.

The significant ($p < 0.05$) reduction in heart rate of hypertensive rats given captopril (Tables 3 and 5) corroborates studies showing that ACE inhibitors such as captopril reduces heart rate (42, 43), while, others (44, 45) have shown that it is not affected by captopril. Captopril is a potent vasodilator, and several vasodilators have been shown to increase heart rate due to increased cardiac function and oxygen consumption. However, it has been reported that captopril decreases blood pressure without increasing heart rate due to its ability to increase vagal tone as a result of angiotensin II inhibition (46, 47). To the best of our knowledge, the effect of *Peristrophe bicalyculata* on heart rate has not been reported, but our results contradict the findings of Zhang et al. (48), demonstrating that *Andrographis paniculata*, a plant with antihypertensive properties, belonging to the same family as *Peristrophe bicalyculata*, has no effect on heart rate. However, more experiments need to be done especially on aortic vasorelaxation to determine the exact mechanism of heart rate reduction.

The cold water extract of *Peristrophe bicalyculata* did not exhibit acute toxicity up to the maximum dose of 5000 mg/kg, suggesting that it is practically non-toxic, and may be a good source of pharmacological material (24).

P,P,P-triphenyl-imino(triphenyl)phosphorane was found to be the most abundant compound present in the partially purified antihypertensive fraction of *Peristrophe bicalyculata,* but may not necessarily be responsible for the antihypertensive activity of the plant, as there is a need to isolate and test all compounds individually before making a logical conclusion. This compound is a derivative of immunophosphoranes, known to be important reagents in synthetic organic chemistry for the synthesis of naturally occurring products, compounds with biological and pharmacological activity (49). It is also possible that the compound may have improved the biological function of the antihypertensive compound as immunophosphoranes are used for the modification of cell surfaces, protein engineering, proteomic studies, labeling of nucleic acids and as tools for bioconjugation (50), or may have improved the antioxidant or antihypertensive activity of the plant (51).

In conclusion, this study demonstrates the acute antihypertensive properties of *Peristrophe bicalyculata* in NO-deficient hypertensive rats. The acute antihypertensive effects might be due to the synthesis of vasodilatory substances by bradykinin as well as the ability of the plant to act as an effective antioxidant. The data obtained also provide useful leads in the development of an effective anti-hypertensive drug from *Peristrophe bicalyculata*. Isolation and purification of the antihypertensive component await further study.

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