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Original Article

Alterations of haemodynamic parameters in spontaneously hypertensive rats by *Aristolochia ringens* Vahl. (Aristolochiaceae)

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ABSTRACT

Aristolochia ringens Vahl. (Aristolochiaceae (AR); *馬兜鈴 mǎ dōu líng)* is used traditionally in Nigeria for the management of various disorders including oedema. Preliminary investigation revealed its modulatory effect on the cardiovascular system. This study was aimed at investigating the effect of the aqueous root extract of *A. ringens* (AR) on haemodynamic parameters of spontaneously hypertensive rats (SHRs). The effect of oral subacute (21 days) and intravenous acute exposure of SHRs to the extract were assessed using tail cuff and carotid artery canulation methods respectively. In the latter, the effect of chloroform, butanol and aqueous fractions of AR were also evaluated. The extract significantly reduced systolic and diastolic blood pressures in SHRs, with peak reductions of 20.3% and 26.7% respectively at 50 mg/kg by the 21st day of oral subacute exposure. Upon intravenous exposure, AR (50 mg/kg) reduced systolic and diastolic blood pressure by as much as 53.4 ± 2.2 and 49.2 ± 2.8 mmHg respectively. A dose-dependent reduction in heart rate, significant at 25 and 50 mg/kg was also observed. Hexamethonium (20 mg/kg) and atropine (1 mg/kg) inhibited the extract's reduction of systolic blood pressure, diastolic blood pressure and heart rate significantly. The extract's butanol fraction produced the greatest systolic and diastolic blood pressures reduction of 67.0 ± 3.8 and 68.4 mmHg respectively at 25 mg/kg and heart rate reduction of 40 ± 7 beats per minute at 50 mg/kg. HPLC analysis revealed the presence of 4-hydroxybenzoic acid and quercetin in AR. The extract's alterations of haemodynamic parameters in this study show that it has hypotensive effect on spontaneously hypertensive rats.

1. Introduction

Hypertension, a condition in which the arteries have persistently elevated blood pressure, is a prevalent risk factor for cardiovascular diseases; affecting over 1 billion people worldwide.[1] A continuous relationship between blood pressure and cardiovascular risks, renal disease and mortality has been reported. This relationship particularly holds more for systolic than for diastolic blood pressure.[2] There is a doubling of the risk of stroke and ischaemic heart disease mortality for every 20/10 mmHg increase in blood pressure over the level 115/75 mmHg.[3] In 2000, it was estimated that 25% of the world's adult population were hypertensive, and predicted that this would rise to 29% by 2025. By the age of 60, more than one-half of adults in most regions of the world will be hypertensive.[3]

Two forms of hypertension, namely primary and secondary hypertension, have been described. The more common form is primary hypertension also known as essential or idiopathic hypertension. It accounts for 90–95% of all cases of hypertension. In

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spite of the immense progress in understanding of its pathophysiology and availability of various efficacious management approaches, essential hypertension is a major risk factor for cardiovascular disorders. Hypertension increases the risk of cardiovascular diseases for millions of people worldwide. In the past decade, age-related incidence of stroke, renal and heart failure has reportedly risen. This observation is partly due to inadequate control of blood pressure in the hypertensive population.[4] It has been reported that less than 25% of treated hypertensive patients achieve target blood pressure.[5] Studies have demonstrated that although antihypertensives are very beneficial therapeutically, they are associated with certain limitations, the outcome of which however is influenced by risk factors such as patient's age and pre-existing co-morbid conditions.[6] The need for more effective and better tolerated antihypertensive therapies cannot be over-emphasized. Medicinal plants are very important sources of potential novel antihypertensives. Indeed, the antihypertensive effects of some of these plants have been validated and others disproved.

Aristolochia ringens is one of the over 500 species of the Aristolochiaceae family of medicinal plants. Historically, *Aristolochia* species of plants have long been in use in traditional medicine practice. They have also been very much featured in numerous chemical and pharmacological research.[7] However, there have been reports of the potential toxicity of plants of the Aristolochiaceae family due to the presence of aristolochic acids, which has resulted in the ban of products containing *Aristolochia* species or aristolochic acids in several countries of the world. Despite this fact, studies in some quarters have shown the immense potential benefits of *Aristolochia* species owing to the presence of other phytochemicals present in them. Kuo et al.[8] reported the neuroprotective, antispasmodic, antiaddictive and antimycobacterial effects of other *Aristolochia* plants' phytochemicals (other than aristolochic acids). Some other classes of phytochemicals isolated from the species include lignans, terpenoids, steroids, coumarins and flavonoids among others.

A. ringens is a commonly used species in Nigeria. It is known as "Akogun" (Yoruba language) in Southwestern Nigeria. It is used for the management of oedema, worm infestation, gastrointestinal and inflammatory disorders as well as several other ailments.[9] Studies have also revealed its antidiarrhoeal,[10] anti-inflammatory[11] and anticancer activities.[12] Although *A. ringens* contains aristolochic acid II, sesquiterpenes and monoterpenes have also been isolated from the plant.[13] Wu et al.[14] reported the presence of specific monoterpenoids (e.g. limonene) and sesquiterpenoids (e.g. caryophyllanes). Its composition of magnesium, phosphorus, calcium, iron, zinc, copper and other elements have also been reported.[15] Clearly, *A. ringens* contains other useful phytochemicals that could be harnessed as lead compounds for drug development in the management of various disorders including primary hypertension. A pilot study carried out in our laboratory revealed that it reduces inotropic and chronotropic effects of an isolated rabbit heart and also induces vasomodulatory activity on isolated rat aortic ring. This study was aimed at investigating some effects of the aqueous root extract of *A. ringens* (AR) on haemodynamic parameters of essential hypertension as modelled by spontaneously hypertensive rats.

2. Materials and methods

2.1. Plant collection and identification

The root of *A. ringens* collected from a local market in Mushin, Lagos, Nigeria was identified and authenticated by Mr. T.K. Odewo, a taxonomist of the Department of Botany and Microbiology, University of Lagos, Nigeria where a herbarium specimen was deposited with voucher number LUH 4061.

2.2. Plant extraction

Air dried root (100 g) was macerated in 1000 ml of distilled water and placed in a refrigerator at 4 °C for 5 days. It was then filtered using cotton wool and filter paper, and the filtrate dried in an oven (Gallenhamp®, England) at 40 °C. The percentage yield of the aqueous root extract of *A. ringens* (AR) obtained was 4.9% (w/w). For some of the experiments, the extract was further subjected to liquid-liquid partitioning to obtain chloroform, butanol and aqueous fractions of the extract.

2.3. Experimental animals

Adult male spontaneously hypertensive rats (180–320 g) were obtained from the Animal Research Unit and Service Centre (ARASC) of Universiti Sains Malaysia, Penang, Malaysia. The animals were allowed to acclimatize for a week, housed under standard environmental conditions in standard plastic cages and maintained under a 12 h light and 12 h dark cycle. They were fed with normal commercial rat chow (Gold Coin Feed Mills, Sdn Bhd, Malaysia) and water *ad libitum*. Animal handling and all procedures on animals were carried out in accordance with the guidelines of the Animal Care and Use Committee of the Universiti Sains Malaysia.

2.4. Chemicals

The chemicals used in this study include dimethyl sulfoxide, acetone, petroleum ether, butanol, chloroform, hexamethonium, atropine, Folin-Ciocalteu reagent, sodium bicarbonate, gallic acid, quercetin, 4 hydroxybenzoic acid, 2,2-diphenyl-1-picrylhydrazyl, Acetic acid (Sigma Aldrich, Malaysia), distilled water (Cardiovascular and Renal Units Laboratory, USM, Malaysia).

2.5. Effect of chronic administration of AR on blood pressure and heart rate

Four groups of 5 spontaneously hypertensive rats (SHRs) each were orally administered AR (25 and 50 mg/kg), enalapril (3 mg/kg) and vehicle (distilled water at 5 ml/kg) for 21 days. Blood pressure and heart rate were measured using the tail cuff method with CODA® non-invasive blood pressure monitor (Kent Scientific Co-operation, USA) on days 0, 7, 14 and 21. On these days, 24-hour urine samples were also obtained by placing each rat singly in metabolic cages for 24 h.[16] The volume of urine collected was measured using measuring cylinder; and the urine Na^+ and K^+ concentrations were determined using a flame photometer (Jenway, Uk).

2.6. Effect of acute exposure to AR on blood pressure and heart rate

This was done using a modification of the methods described by Shah and Gilani.[17] Spontaneously hypertensive rats fasted for 12 h, were anaesthetized with pentobarbitone (60 mg/kg, i.p.). Immediately following the anaesthesia, tracheotomy was performed using an endotracheal cannula (PP 240, Portex Ltd, Kent, UK) to maintain a free flow of air through the trachea. The left jugular vein was then catheterized using PP 50 tubing (Portex Ltd, Kent, UK) to allow the infusion of supplementary anaesthesia, AR and its vehicle and fractions. For the measurement of blood pressure and heart rate, the right carotid artery was cannulated with PP 50 tubing

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(Portex Ltd Kent, UK), which was connected to a pressure transducer (P23 ID Gould, Statham Instruments, UK) linked to a data acquisition system (PowerLab®, ADInstruments, Sydney, Australia) through a Quad Amp (ADInstruments, Australia) using chart (V.6.1.1) software. The temperature of the animal was maintained at 37 °C using an overhead lamp. Animals were allowed to equilibrate for at least 30 min before the administration of any drug. Arterial pressure was allowed to return to the resting level between the administrations. Prior to the administration of AR, its vehicle fractions, they were passed through a 0.45 mm pore syringe filter. The haemodynamic parameters were noted before and after the administration of AR (6.25–50 mg/kg) and corresponding equal volumes of the vehicle. The effect of the chloroform, butanol and aqueous fractions of AR were also determined. In some of the experiments, the effect of the extract at 50 mg/kg was also noted in the presence of intravenously administered atropine (1 mg/kg) or hexamethonium (20 mg/kg).

2.7. Determination of phenolics in AR and fractions

For the determination of the phenolic contents of AR and its petroleum ether, chloroform, butanol and aqueous fractions, the Folin-Ciocalteu method as described by Barros et al.[18] was applied but modified for use with microplates. Twenty three (23) μl of AR and fractions (5 mg/ml) were added to 23 μl of Folin-Ciocalteu reagent respectively. After 3 min, saturated Na_2CO_3 was added to the mixture, which was made up to 130 μl with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read using a microplate reader at 725 nm. Gallic acid (0.0005–0.5 mg/ml) was used to obtain standard calibration curve. Estimation of the phenolic contents was carried out in triplicate and results were expressed as gallic acid equivalents.

2.8. Diphenyl-1-picryl-hydrazyl (DPPH) assay

To determine the radical scavenging activity (RSA) of AR and its fractions against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 0.01 ml of AR (0.0005–0.5 mg/ml), its fractions (0.0005–0.5 mg/ml) or blank was added to 0.29 ml of DPPH 10^{-4} M respectively and the absorbance (A) determined at 517 nm after 30 min incubation in the dark at room temperature. Radical scavenging activity (RSA) was calculated in percent using the following formulae:

$$\%RSA = \left(A_{DPPH} - A_{sample} \Big/ A_{DPPH} - A_{blank}\right) \times 100.$$

2.9. Identification and quantification of phenolics in AR using HPLC

HPLC analysis for phenolic contents of AR was conducted using Shimadzu HPLC apparatus coupled with a flourimetric detector. The phenolic compounds were detected at excitation and emission wavelengths, λex/λem = 226/420 nm. Solvent gradients were formed using the dual pump system by varying the proportion of solvent A [water–acetic acid (97:3)] to solvent B (methanol). The solvent gradient elution programme used was as follows (total run time of 60 min); 100% solvent A/0% solvent B at 0 min; 90% A/10% B at 10 min, 30% A/70% B at 40 min, 100% A/0% B at 44–50 min. Under these conditions, 20 μl of sample (AR 20 mg/ml) or standard phenolics (25–100 μg/ml) were injected. All sample analyses were assayed for in triplicate. The phenolic contents of AR were detected by matching the retention time and their spectral characteristics against those of standards. Quantitation was made according to the calibration curves of respective standard compounds.

2.10. Statistical analyses

Results obtained were expressed as mean ± SEM. Experimental data obtained from the studies were analyzed using one way analysis of variance (ANOVA) followed by Turkey's multiple comparison test or two way ANOVA followed by Bonferroni's post hoc test using Graph Pad Prism 5 statistical package. Results were considered significant at $p < 0.05$.

3. Results

3.1. Effect of 21 days treatment of AR on blood pressure and heart rate of SHRs

AR (25–50 mg/kg; p.o.) administered for 21 days significantly reduced systolic and diastolic blood pressure in SHRs. The hypotensive effect of the extract was evident by the 7th day of administration and continued through the 14th to the 21st day of exposure. The peak reduction of systolic and diastolic blood pressure by AR at 50 mg/kg was observed by the 21st day. This reduction of 165.1 ± 6.3/124.0 ± 3.3 mmHg (blood pressure by day 0) to 131.0 ± 1.8/91.0 ± 3.7 mmHg was comparable to the reduction of 157.0 ± 7.2/118.0 ± 5.3 mmHg to 126.0 ± 8.9/86.0 ± 6.7 mmHg produced by enalapril (3 mg/kg). No significant alteration in heart rate by AR was observed except on the 14th day of the study when a reduction from 401 ± 15 to 337 ± 07 beats per minute was observed in SHRs receiving AR (50 mg/kg) as depicted in Table 1.

Table 1
Effect of 21 days treatment of AR on blood pressure and heart rate of SHRs.

Group	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
Systolic blood pressure (mmHg)					
Control	10 (ml/kg)	161.2 ± 2.9	168.4 ± 0.8	168.7 ± 4.1	157.6 ± 4.2
AR	25	165.6 ± 3.0	149.5 ± 3.1	145.9 ± 2.6[a]	137.0 ± 3.9
AR	50	165.1 ± 6.3	148.5 ± 4.6[a]	140.0 ± 7.3[c]	131.6 ± 1.8[c]
Enalapril	3	157.0 ± 7.2	133.6 ± 3.9[d]	135.0 ± 4.4[d]	137.1 ± 8.9[a]
Diastolic blood pressure (mmHg)					
Control	10 (ml/kg)	117.9 ± 1.9	125.8 ± 1.7	128.7 ± 1.0	115.2 ± 3.5
AR	25	124.0 ± 3.5	108.0 ± 1.0[b]	105.3 ± 2.6[c]	98.1 ± 3.2[a]
AR	50	124.2 ± 3.3	104.2 ± 1.9[c]	106.6 ± 4.0[c]	91.0 ± 3.7[d]
Enalapril	3	118.1 ± 5.3	94.3 ± 4.7[d]	99.3 ± 3.5[d]	86.1 ± 6.7[d]
Heart rate (beats per minute)					
Control	10 (ml/kg)	404 ± 28	367 ± 6	402 ± 24	434 ± 34
AR	25	382 ± 10	358 ± 11	358 ± 7	374 ± 10
AR	50	401 ± 15	391 ± 9	337 ± 7[a]	372 ± 7
Enalapril	3	410 ± 20	382 ± 17	397 ± 17	425 ± 27

Vaules are mean ± SEM. [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, [d]$p < 0.0001$ vs. control (Two way analysis of variance followed by Bonferoni's post hoc test). BPM-beats per minute.

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3.2. Effect of AR on urine volume and electrolytes

In the assay to determine the effect of AR on urine volume and electrolytes, it was observed that AR produced no significant change in 24 h urine output of SHRs on days 7, 14 and 21 of AR exposure. No significant changes in sodium and potassium concentrations of these urine samples were observed (Table 2).

3.3. Effect of acute administration of AR on blood pressure and heart rate of SHRs

In the experiment to determine the effect of acute intravenous exposure of SHRs to AR (6.25–50 mg/kg), significant dose-dependent reductions in systolic and diastolic blood pressures were observed in SHRs. The greatest effect was observed at 50 mg/kg with reductions of 53.4 ± 2.2 and 49.2 ± 2.8 mmHg in systolic and diastolic blood pressures respectively. A dose-dependent reduction in heart rate, significant at 25 and 50 mg/kg was also observed with intravenous exposure of SHRs to AR. The extract (50 mg/kg) reduced heart rate by 14 ± 2.3 beats per minutes (Fig. 1). The reduction of blood pressure and heart rate by AR (50 mg/kg) was significantly inhibited by hexamethonium (20 mg/kg) and atropine (1 mg/kg). Hexamethonium inhibited AR-induced reduction in systolic blood pressure, diastolic blood pressure and heart rate by 61.0%, 51.6% and 69.6% respectively. Atropine also inhibited AR-induced reduction of these parameters by 66.5%, 61.9% and 73.9% respectively (Fig. 2).

In the aspect of the study to evaluate the effect of intravenously administered AR and its fractions, butanol and aqueous fractions significantly reduced blood pressure and heart rate of SHRs. The butanol fraction produced the greatest reduction, with systolic and diastolic blood pressure reductions by 67.0 ± 3.8 and 68.4 mmHg respectively at 25 mg/kg and heart rate reduction by 40 ± 7 beats per minute at 50 mg/kg. The reduction of these haemodynamic parameters observed with the aqueous fraction was comparable to that of the extract. The chloroform fraction on the other hand, produced no significant effect on these parameters (Fig. 3).

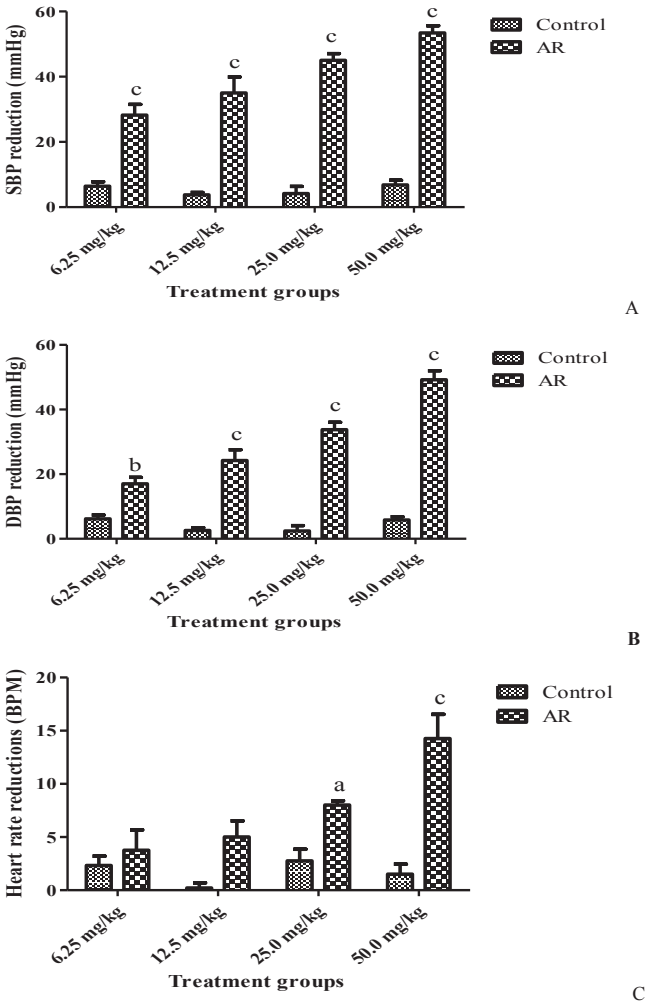

Fig. 1. Effect of acute administration of AR on systolic blood pressure (A), diastolic blood pressure (B) and heart rate (C). Bars represent mean ± SEM. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. control (Two way analysis of variance followed by Bonferroni post test).

3.4. Quantitative determination of total tannins, flavonoids and phenolics in AR and its fractions

In the study to determine the concentration of these components in AR and its fractions, it was observed that the aqueous root extract of AR contains 26.00 ± 0.00 mg/g tannic acid equivalent, 48.00 ± 0.01 mg/g quercetin equivalent and 222.00 ± 0.00 mg/g gallic acid equivalent of tannins, flavonoids and phenolics respectively. The chloroform fraction of AR had the highest content of tannins (130.00 ± 0.03 mg/g tannic acid equivalent), while the butanol fraction contained the most flavonoids (80.00 ± 0.01 mg/g quercetin equivalent) and phenolics (316.00 ± 0.00 mg/g gallic acid equivalent) as shown in Table 3.

3.5. Diphenyl-1-picryl-hydrazyl (DPPH) assay

In this study, AR and its fractions (0.0005–0.5 mg/ml) scavenged free radicals. AR produced 65% inhibition of free radical generation at 0.5 mg/ml. Of the fractions of AR tested, the butanol fraction

Table 2
Effect of AR on urine volume and electrolytes of SHRs.

Group	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
Urine volume (ml)					
Control	10 (ml/kg)	5.88 ± 1.25	8.36 ± 1.68	11.10 ± 4.50	7.12 ± 1.17
AR	25	6.10 ± 0.73	7.20 ± 2.03	8.10 ± 3.04	7.90 ± 2.06
AR	50	5.40 ± 0.80	6.80 ± 1.00	6.70 ± 1.64	5.90 ± 0.60
Urine sodium (nM/l)					
Control (ml/kg)	10	165.50 ± 30.30	129.30 ± 0.70	128.50 ± 13.20	130.80 ± 18.40
AR	25	165.00 ± 40.90	137.80 ± 20.40	134.60 ± 14.30	155.00 ± 5.40
AR	50	155.50 ± 12.60	138.80 ± 07.10	131.50 ± 19.80	121.00 ± 15.10
Urine potassium (mM/l)					
Control (ml/kg)	10	489.40 ± 24.00	269.30 ± 25.50	337.30 ± 122.20	395.00 ± 33.50
AR	25	393.30 ± 29.40	250.00 ± 57.40	282.30 ± 22.30	318.80 ± 25.10
AR	50	387.70 ± 59.40	331.00 ± 35.40	249.30 ± 62.10	276.80 ± 31.10

Vaules are mean ± SEM. P < 0.05 (Two way analysis of variance followed by Bonferoni's post hoc test).

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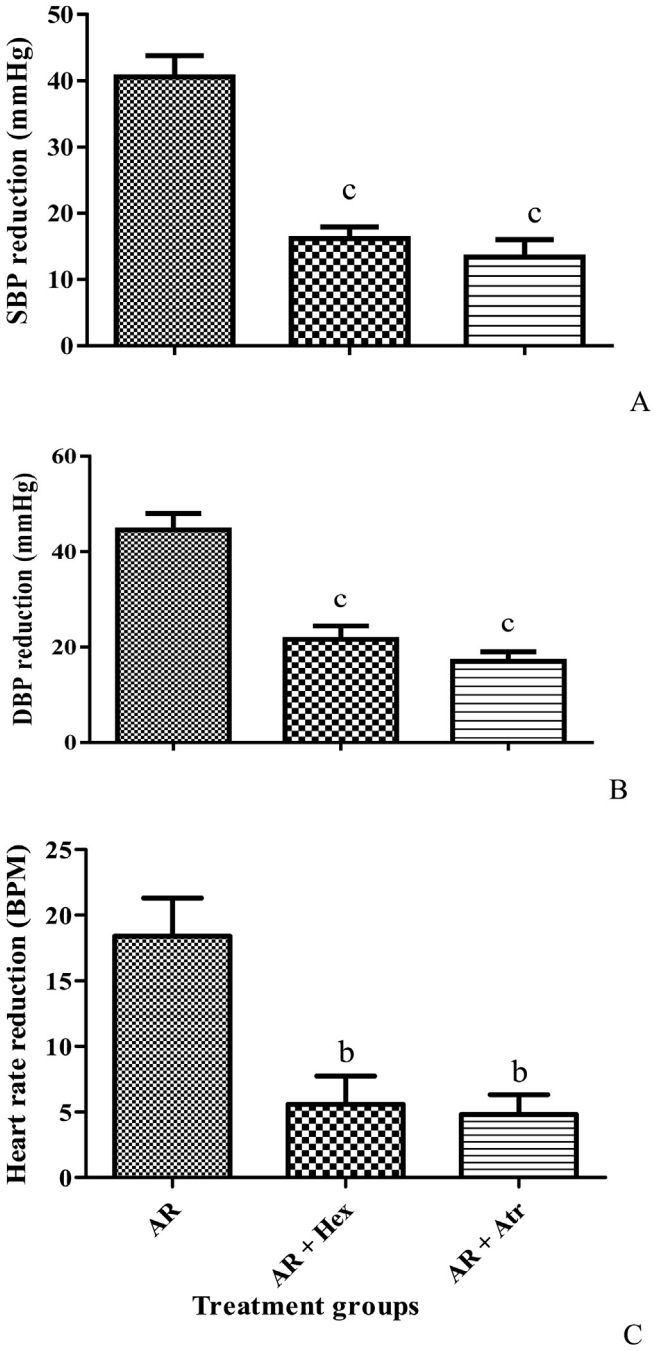

Fig. 2. Effect of AR on systolic blood pressure (A), diastolic blood pressure (B) and heart rate (C) in the absence and presence of 20 mg/kg hexamethonium (Hex) and 1 mg/kg atropine (Atr). Bars represent mean ± SEM. $^{b}p < 0.01$, $^{c}p < 0.001$ vs. control (One way analysis of variance followed by Tukey's multiple comparison test).

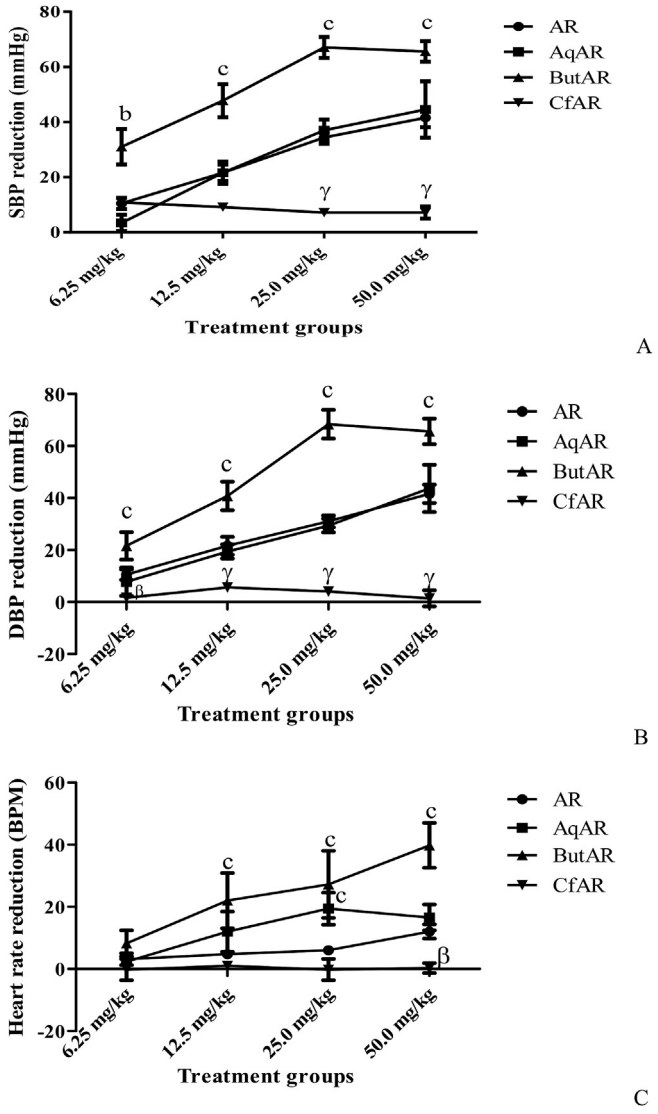

Fig. 3. The effect of AR and fractions on systolic blood pressure (A), diastolic blood pressure (B) and heart rate (C). Lines represent mean ± S.E.M. $^{b}p < 0.01$, $^{c}p < 0.001$ vs. control; $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ vs. AR (Two way ANOVA followed by Tukey's multiple comparison test). AR-aqueous root extract of *A. ringens*, AqAR-aqueous fraction of AR, ButAR-butanol fraction of AR, CfAR-chloroform fraction of AR.

showed the greatest inhibition (77% inhibition) at 0.5 mg/ml; this action was greater than that of the reference flavonoid, quercetin (67% inhibition), at the same concentration (Fig. 4).

3.6. Identification and quantification of phenolics in AR using HPLC

In the HPLC analysis to identify and quantify specific phenolics in AR, 4-hydroxybenzoic acid, caffeic acid, catechin hydrate, coumaric acid, chlorogenic acid, ferulic acid, quercetin and sinnapic acid were assayed for. Of these, 4-hydroxybenzoic acid and quercetin were detected and found to be 0.469 ± 0.09 mg and 2.290 ± 0.33 mg 4-hydroxybenzoic acid and quercetin equivalents per g of AR respectively. Figs. 5 and 6 show the chromatograms of AR aligned with those of 4-hydroxybenzoic acid and quercetin respectively.

4. Discussion

Daily administration of AR via the oral route for 21 days, caused significant reduction of systolic and diastolic blood pressure in conscious spontaneously hypertensive rats. This effect of the extract, which was observable by the 7th day of exposure was found to be comparable to the hypotensive effect of enalapril.

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Table 3
Quantitative analysis of tannin, flavonoid and phenolic contents of AR and its fractions.

AR/fraction	Tannins (mg tannic acid equivalent per g of dried extract)	Flavonoids (mg quercetin equivalent per g of dried extract)	Phenolics (mg gallic acid equivalent per g of dried extract)
AR	26.00 ± 0.00	48.00 ± 0.01	222.00 ± 0.00
PeAR	26.00 ± 0.02	34.00 ± 0.01	100.00 ± 0.03
CfAR	130.00 ± 0.03	50.00 ± 0.02	198.00 ± 0.04
ButAR	20.00 ± 0.01	80.00 ± 0.01	316.00 ± 0.00
AqAR	26.00 ± 0.00	34.00 ± 0.01	174.00 ± 0.05

Values are mean ± S.E.M. AR-aqueous root extract of *A. ringens*, PeAR-petroleum ether fraction of AR, CfAR-chloroform fraction of AR, ButAR-butanol fraction of AR, AqAR-aqueous fraction of AR.

Change in heart rate was only observed by the 14th day of exposure to AR at 50 mg/kg. Such nearly insignificant effect on cardiac rate has been reported previously of potentially hypotensive medicinal plants such as *Lepidium sativum* and *Fraxinus excelsior*.[19] Diuresis is one of the approaches employed in the management of hypertension. In the aspect of the study to investigate the effect of AR on urine volume and electrolyte, it was observed that AR showed no diuretic effect; as urine volume, sodium and potassium were unaffected throughout the chronic exposure study period. This shows that the blood pressure lowering action of AR is not mediated via diuretic mechanism.

The results of the present study also showed that AR, on intravenous administration, induces a dose-dependent acute hypotensive effect on anaesthetized SHRs; reducing significantly, systolic and diastolic blood pressure as well as heart rate, the reduction of which was significant at 25 and 50 mg/kg. Consistent with the results in the 21 days oral exposure, AR produced greater reductions in the systolic blood pressure compared to diastolic blood pressure. Compared to oral administration, intravenous administration resulted in greater reduction of blood pressure, which shows that oral administration of AR may reduce the pharmacological effect of the extract, perhaps due to hepatic metabolism of the orally administered extract, thus reducing its bioavailability.

To examine the possible mechanism involved in this response, the extract's effect on the haemodynamic parameters studied were determined in the presence of atropine (a non-selective muscarinic antagonist) and hexamethonium (an autonomic ganglion blocker). Pre-treatment of rats with hexamethonium and atropine significantly inhibited the hypotensive effect of the plant extract; reducing significantly, the extent of systolic and diastolic blood pressure reductions as well as heart rate reduction. This suggests that the extract's mechanism of hypotensive action may involve interferences with transmissions at the autonomic ganglia and muscarinic receptors.

In the study to investigate the action of the fractions of AR; the effect of its chloroform, butanol and aqueous fractions were compared to that of its crude form. Its butanol fraction was found to be the most active. The effects of the butanol fraction of AR on blood pressure and heart rate were significantly greater than those of AR, while the effects of its aqueous fraction were comparable to that of AR. The effect observed with butanol fraction may be due to its relatively high content of phenolics and flavonoids; as it was shown to possess the highest concentration of these components in the study. Compared to AR, the chloroform fraction produced no significant effect on blood pressure and heart rate, suggesting that it does not contain principles with activity in this regard. In fact, the chloroform fraction demonstrated a significant reversal of the extract's effects on systolic and diastolic blood pressure as well as heart rate of the rats.

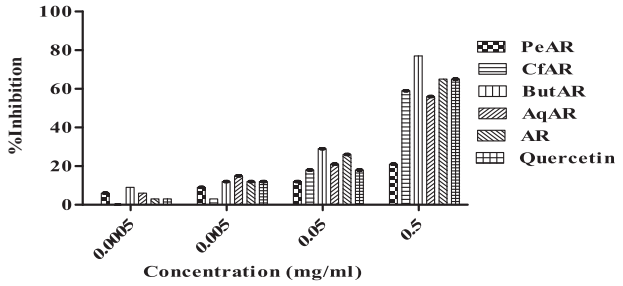

Fig. 4. Effect of AR and its fractions in the 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) assay. Bars represent mean ± S.E.M. PeAR-petroleum ether fraction of AR, CfAR-chloroform fraction of AR, ButAR-butanol fraction of AR, AqAR-aqueous fraction of AR.

Tannins, flavonoids and other phenolics were found to be present in both the crude extract and fractions of AR using quantitative methods of analyses. From this aspect of the study, AR was found to contain as much as 26 mg tannic acid equivalent per g of AR. Its petroleum ether, chloroform, butanol, and aqueous fractions were also found to contain tannic acid ranging from 20 to 130 mg tannic acid equivalent per g of AR. This appreciable concentration of total tannins in AR and its fractions reveal that indeed some of the actions of AR can be accounted for by its tannin content. Phenolics and flavonoids have been reported to possess many beneficial effects including antihypertensive actions.[20] The antihypertensive effect of tannins have been reported by Turgut et al.[21]

Phenolics are reported to be highly ubiquitous in nature. The total flavonoids and phenolics assay showed that AR and its fractions contain very appreciable concentrations. Phenolics, which include flavonoids and tannins are known to be highly effective in free radical scavenging and are therefore used to manage ailments in which oxidative stress are implicated.[22] The presence of these phytochemicals in *A. ringens* and its fractions is therefore highly significant in this study. Of the fractions tested, the butanol fraction of AR, was shown to possess the highest concentration of flavonoids and phenolics.

DPPH assay is an antioxidant test procedure that employs 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), which has an unpaired electron and exhibits a stable violet colour in methanol solution. It is commonly used for evaluation of the free radical scavenging activity of antioxidants.[23] The assay is based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant, resulting in the formation of the non-radical form, (DPPH-H).[24,25] In this assay, the butanol fraction of AR, which showed the highest concentration of phenolics and flavonoids, produced the most antioxidant activity.

More specifically, HPLC analysis revealed the presence of phenolic, 4-hydroxybenzoic acid, and flavonoid, quercetin, in AR, the medicinal properties of which can be largely attributable to the inhibition of free radical activity. Indeed, the antioxidant activity of 4-hydroxybenzoic acid[26] and quercetin[27] have been reported. Duarte et al.[28] reported the antihypertensive effect of quercetin in spontaneously hypertensive rats. It is therefore clear that the presence of these components in AR contribute to the effects observed in this study.

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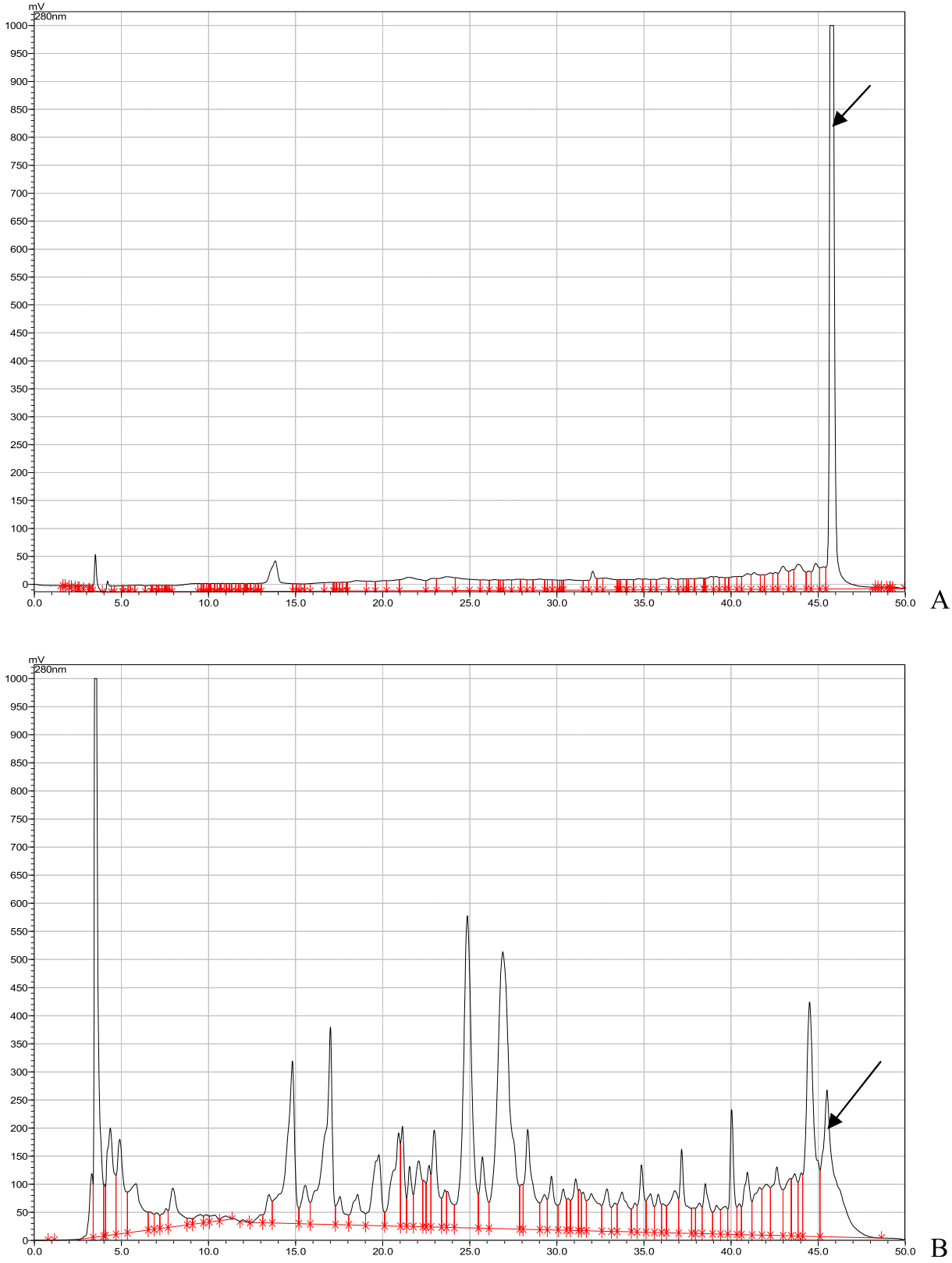

Fig. 5. HPLC chromatogram of reference standard, 4-hydroxybenzoic acid (A) and the aqueous root extract of *A. ringens* (B).

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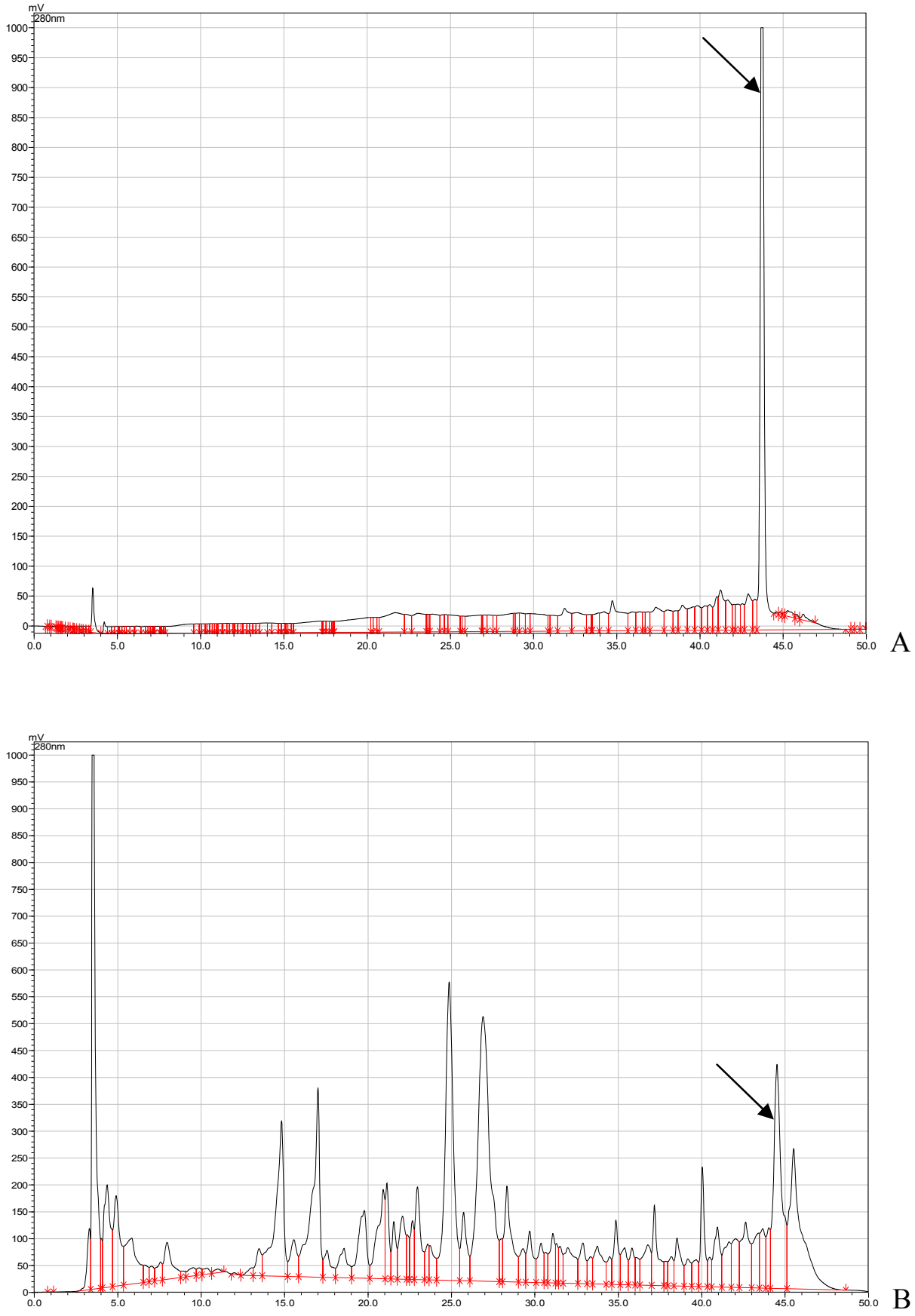

Fig. 6. HPLC chromatogram of reference standard, quercetin (A) and the aqueous root extract of *A. ringens* (B).

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5. Conclusion

It can be concluded that the extract possessed hypotensive activity in spontaneously hypertensive rats and induced bradycardia in them. These effects were shown to be mediated via its interaction at the autonomic ganglia and muscarinic receptors. The study also showed that diuresis is not involved in the extract's hypotensive effect. Its butanol fraction was the most effective against hypertension in spontaneously hypertensive rat. Phytochemical studies showed that the extract contained relatively high concentrations of various phenolics, of which 4-hydroxybenzoic acid and quercetin were identified. These findings reveal that the aqueous extract of *A. ringens* contains principles that could be harnessed for their therapeutic benefits. Further activity guided fractionation studies on the butanol fraction of AR geared towards characterization of the active principle(s) and elucidation of the mechanism(s) of action of such constituents are underway.

Conflict of interest

We declare that there is no conflict of interest.

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