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

Flora Ruth Aigbe, Abdul Sattar Zubaid Munavvar, Hassan Rathore, Olorunfemi Eseyin, Yen Pei Pei, Safia Akhtar, Ashfaq Chohan, Hui Jin, Jooli Khoo, Samual Tan, Mohammed Lazhari, Sheryar Afzar, Fiaz Ahmed, Olufunmilayo Olaide Adeyemi, Edward Johns

A Department of Pharmacology, Therapeutics and Toxicology, College of Medicine, University of Lagos, P.M.B. 12003, Ibi-Araba, Surulere, Lagos, Nigeria
b Renal and Cardiovascular Unit, Department of Physiology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia
c Department of Pharmaceutical and Medicinal Chemistry, University of Uyo, P. O. Box 4274, Uyo, Akwa Ibom State, Nigeria
d Department of Physiology, University College, Cork, Ireland

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A B S T R A C T

Aristolochia ringens Vahl. (Aristolochiaceae (AR); 马兜铃 mǎ dōu líng) is traditionally used 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 cannulation 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 subacue 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-hydroxybenzoyl 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.

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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.4

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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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. 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...
reagent respectively. After 3 min, saturated Na₂CO₃ was added to but modi
calculated in percent using the following formulae:

radical scavenging activity (RSA) was determined at 517 nm after 30 min incubation in the dark (0.0005 
was read using a microplate reader at 725 nm. Gallic acid 
2.8. Diphenyl-1-picryl-hydrazyl (DPPH) assay 
and fractions against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 0.01 ml of 
the mixture, which was made up to 130 ml with distilled water. The 
reaction was kept in the dark for 90 min, after which the absorb-
bance 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:

\[ \text{%RSA} = \left( \frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}} - A_{\text{blank}}} \right) \times 100. \]

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 fluorimetric 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 7th day of the study when a

### Table 1

| 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 | 157.8 ± 4.2 |
| AR | 25 | 165.6 ± 3.0 | 149.5 ± 3.1 | 145.9 ± 2.6 | 137.0 ± 3.9 |
| AR | 50 | 165.1 ± 6.3 | 148.5 ± 4.6 | 140.0 ± 7.3 | 131.6 ± 1.8 |
| Enalapril | 3 | 157.0 ± 7.2 | 133.6 ± 3.9 | 135.0 ± 4.4 | 137.1 ± 8.9 |
| 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 | 105.3 ± 2.6 | 98.1 ± 3.2 |
| AR | 50 | 124.2 ± 3.3 | 104.2 ± 1.9 | 106.6 ± 4.0 | 91.0 ± 3.7 |
| Enalapril | 3 | 118.1 ± 5.3 | 94.3 ± 4.7 | 99.3 ± 3.5 | 86.1 ± 6.7 |
| 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 | 372 ± 7 |
| Enalapril | 3 | 410 ± 20 | 382 ± 17 | 397 ± 17 | 425 ± 27 |

Values are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs. control (Two way analysis of variance followed by Bonferroni’s post hoc test). BPM–beats per minute.
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 minute (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 73.3% respectively (Fig. 2).

In this 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).

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

![Graph](image_url)

**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).

### 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 (mM/l) |             |         |         |         |         |
| Control         | 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         | 10           | 489.40 ± 24.00 | 269.30 ± 25.50 | 337.30 ± 122.20 | 395.00 ± 33.50 |
| AR              | 25           | 393.30 ± 24.90 | 250.00 ± 57.40 | 282.30 ± 22.30 | 318.80 ± 25.10 |
| AR              | 50           | 387.70 ± 59.40 | 311.00 ± 35.40 | 249.30 ± 62.10 | 276.80 ± 31.10 |

Values are mean ± SEM. P < 0.05 (Two way analysis of variance followed by Bonferroni’s post hoc test).
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 sinapic 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.
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*.

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.

**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.

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. 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. 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). 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 and quercetin have been reported. Duarte et al. 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.
Fig. 5. HPLC chromatogram of reference standard, 4-hydroxybenzoic acid (A) and the aqueous root extract of A. ringens (B).
Fig. 6. HPLC chromatogram of reference standard, quercetin (A) and the aqueous root extract of A. ringens (B).
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 hypoten-
sive effect. Its butanol fraction was the most effective against hy-
pertension in spontaneously hypertensive rat. Phytochemical studies showed that the extract contained relatively high concen-
trations 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 bene
fits. Further activity guided fractionation studies on the butanol fraction of AR geared towards characterization of the active principle(s) and elucidation of the mecha-
nism(s) of action of such constituents are underway.

Conflict of interest

We declare that there is no conflict of interest.

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