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ORIGINAL RESEARCH

**Investigation of The Phytochemical Contents, Mineral Contents, Free Radical Scavenging, and Alpha-Amylase Inhibitory Activities of *Aristolochia ringens* (Vahl.) Root****Seide M. Akoro, Oyinlade C. Ogundare, Mutiat A. Omotayo¹, Dorcas Durosimi, Deborah O. Awofeso***Department of Chemical Sciences, College of Basic Sciences, Lagos State University of Science and Technology, Nigeria.***Abstract:****Introduction:** *Aristolochia ringens* is a medicinal plant that has been used traditionally in the management of several diseases.**Aims:** This study focused on investigating the phytochemical contents, mineral contents, free radical scavenging, and alpha-amylase inhibitory activities of *Aristolochia ringens* (Vahl.) root.**Materials and Methods:** The plant material was collected, dried, coarsely grounded, and extracted using methanol. The methanol extract was partitioned into n-hexane and ethyl acetate to obtain the respective extracts. The qualitative phytochemical screening of the extracts was carried out using standard methods. Selected elements were determined from the plant material using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The antioxidant assays were carried out using the reducing power and 2,2-Diphenyl-1-picrylhydrazyl assay methods. The alpha-amylase inhibitory activities were determined preliminarily using the starch-iodide assay.**Results:** The extraction gave the methanol extract (ArMe) which on partitioning gave the n-Hexane (ArnH), ethyl acetate (ArEa), and the residual methanol extracts (ArRMe). Qualitative phytochemical screening showed the presence of flavonoids, cardiac glycosides, terpenoids, steroids, phlobatannins, and phenols in all the extracts; alkaloids, tannins, and reducing sugar in ArMe, ArnH, and ArRMe, while saponins were found in ArMe, ArnH, and ArRMe. Elemental analysis showed a significant level of the selected elements Ca, Mg, K, Fe, Zn, Na, Cu, Co, and Se in ppm. Antioxidant assay results showed that the extracts exhibited dose-dependent reducing properties and an increased DPPH scavenging activity.**Conclusion:** These results confirmed some of the traditional uses of *A. ringens* in the management of high blood pressure, diabetes, and inflammatory conditions.**Keywords:** *Aristolochia ringens*, phytocontents, elemental contents, antioxidant activities, alpha-amylase inhibitory**Correspondence**

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1. INTRODUCTION

In the history of humans, plants have played a significant role in food and medicine. The medicinal properties of plants have been connected to their phytochemical contents [1]. To understand fully and utilize the health benefits of phytochemicals, there is a need to study the several thousands of medicinal plants yet to be explored [2]. New drug development has its origin from the explored medicinal plants which are reported to constitute about 25% of conventional medications still in use today [2-4]. Some medicinal plants are used in their crude forms for the treatment of diseases [2].

Aristolochia ringens (Aristolochiaceae) is commonly known as “*Akoogun*” or “*Awo igba arun Oyo*” in the southern-eastern part of Nigeria. It is reported to be used traditionally as analgesics, anti-cancer agents, anti-inflammatory agents, sedatives muscle relaxants, antihistaminics, antiallergics, antimicrobials, and antimalarials [5, 6].

A. ringens has been reported for short-time management of disorders like worm infestation, gastrointestinal, oedema, and inflammatory disorders [7]. Previous works also reported the antidiabetic properties of *A. ringens* [8]. The aqueous root extract of *A. ringens* has been used to manage and treat inflammation [9]. Studies have shown that *A. ringens* displayed antioxidant activities as it has proven folkloric uses in the treating and managing of some skin ailments such as eczema, psoriasis, and heat rashes because it contains tannins and flavonoids which are examples of naturally occurring antioxidants in plants [10].

The earth's crust is well endowed with heavy metals, which are highly beneficial to Man but required in trace quantities because of the possibility of becoming toxic at high concentrations [11-13]. These metals gain entry into the body system through food, drinking water, and air as trace metals [11-13]. Common examples are selenium, copper, and zinc which are useful for the maintenance of the metabolism of the human body. Magnesium, calcium, and potassium, are useful for muscular function and blood pressure control [11-13]. However, they are required in minute quantities as they may become toxic at high concentrations [13]. This work is reporting the phytochemical, mineral contents, free radical scavenging, and alpha-amylase inhibitory activities of *Aristolochia ringens* (Vahl.) root extracts.

2. MATERIAL AND METHODS

2.1 General instrumentation and materials

All chemicals used in this work are of analytical grade. Ultraviolet Spectroscopy data were obtained using Spectrum Lab 752s. Elemental analysis was carried out using Agilent 5800 ICP-OES.

2.2 Collection and extraction of plant materials

The plant material, *Aristolochia ringens* root was purchased at the Mushin market, Lagos, Nigeria. It was then authenticated at the University of Lagos Herbarium (LUTH 5997). The plant material was air-dried and grounded into a coarse form using an electrical blender. The grounded plant materials (219.15 g) were extracted by macerating for 72 h in 80% methanol. The extract was filtered and concentrated using a rotary evaporator and further dried in an air oven at 40°C to obtain a completely dried sample (ArMe, solid, 6.99 g).

2.3 Partitioning of the crude methanol extract

The crude methanol extract (ArMe, 6.99 g) was dissolved in methanol and water (3:1). The extract was partitioned in n-Hexane (50 mL × 3): The solvent, n-Hexane (50 mL) was added to the crude methanol extract in the separating funnel, and the mixture was homogenized and left to stand for a while to give a clear separation. The fraction was separated and dried to give a solid (ArnH, green solid, 0.44 %). The residual methanol extract was further partitioned with ethyl acetate (50 mL × 3) and the fraction was separated and dried to give a solid (ArEa, greenish-brown solid, 8.54 %) and the residual methanol extract also a solid (ArRMe, greenish-brown solid, 29.11%). The extracts were kept in the refrigerator until when required for further use.

2.4 Qualitative phytochemical screening

The extract was screened to detect the presence of secondary metabolites: alkaloids, flavonoids, saponins, tannin, phlobatannins, cardiac glycoside, terpenoids, steroids, reducing sugar and phenol using the standard methods described by Sofowora [14].

2.4.1 Test for alkaloid

2.4.1.1 Wagners Test

A few drops of Wagner's reagents were added to the plant extracts. The formation of a reddish-brown precipitate indicates the presence of alkaloids.

2.4.1.2 Dangendorff Test

The Dragendorff's reagent (a few drops) was added to 1 mL of the filtrate obtained by boiling 0.01 g of the extract which was dissolved in 1% aqueous hydrochloric acid. The presence of a reddish-brown colouration indicates the presence of alkaloids.

2.4.2 Test for flavonoids

To the plant extract (about 0.01 g) was added dilute ammonia solution (1.0M, 5 cm³), followed by the addition of 5 cm³ of concentrated hydrogen tetraoxosulphate (VI) acid. The formation of a yellow colouration which disappeared on standing shows the presence of flavonoids

2.4.3 Test for Saponins

Frothing Test: A small amount of distilled water was added to the plant extract (about 0.01 g) and shaken. The appearance of froth indicates the presence of saponins.

2.4.4 Test for Tannin

Ferric Chloride Test: To the plant extract (about 0.2 g) was added 10 mL of distilled water and shaken for some minutes before filtering. FeCl₃ (15%) was added to the filtrate. Tannins are indicated by the formation of deep blue colour.

2.4.5 Test for Phlobatannins

Each of the extracts was boiled with hydrochloric acid (1%, 5 cm³), and the deposit of a red precipitate shows a positive test.

2.4.6 Test for Glycoside

2.4.6.1 Keller kelliiani Test

About 0.01 g of the plant extract was treated with chloroform and evaporated to dryness. 0.4 mL of glacial acetic acid containing a trace amount of ferric chloride was added, followed by the careful addition of 0.5 mL of concentrated H₂SO₄. The presence of blue colour in the acetic layer indicates the presence of glycosides.

2.4.6.2 Legal Test

The extract was dissolved in pyridine and five drops of 2% sodium nitroprusside together with four drops of 20% of NaOH were added. A deep colour indicates the presence of glycoside.

2.4.7 Test for Terpenoids

To each of the plant extracts (0.01 g), a mixture of chloroform (2 cm³) and concentrated hydrogen tetraoxosulphate (VI) acid (3 cm³) was added to form a layer. The presence of a reddish-brown colouration at the interface shows a positive result for the presence of terpenoids.

2.4.8. Test for Steroid

Salkowskis test: 0.01 g of the extract was dissolved in 2 mL of chloroform. Concentrated hydrogen tetraoxosulphate (VI) acid was added carefully to form a lower layer. A reddish-brown colouration at the interface indicates the presence of a steroidal ring (aglycone portion of the cardiac glycoside).

2.4.9 Test for Reducing Sugar

To the extract solution (5 mL), an equal volume of Fehling A and B solutions was added and the mixture was warmed. The formation of a brick-red precipitate at the bottom of the test tube indicates reducing sugar.

2.4.10 Test for Phenols

The extract solution was treated with four drops of FeCl₃ solution, the formation of bluish-black colour indicates the presence of phenols.

2.5 Ashing and trace elements analysis

Air-dried and coarsely grounded *A. ringens* (5 g) were weighed into the crucible and were ashed in a muffle furnace at 550°C for 4h. The ashed sample was dissolved with 10 mL of aqua regia. This was transferred into a 100 mL volumetric flask to make a

100 mL solution. The trace elements were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) [15]. The experiment was carried out in duplicate [16].

2.6 Antioxidant assay

Antioxidant assays were carried out on each of the plant extracts using the reducing power and DPPH free radical scavenging assay methods.

2.6.1 Reducing power assay of plant extracts

The reducing power of each of the plant extracts was determined as described by Oyiazu [17]. The extracts were mixed with 2.5 mL of phosphate buffer (0.2M, pH 6.6) and potassium ferricyanide (1%, 2.5 mL). The mixture was incubated at 50°C for 20 mins after which a portion (1 mL) of 10% trichloroacetic acid was added to stop the reaction. The mixture was centrifuged for 10 mins at 3000 rpm. The upper layer of the solution (1.5 mL) was mixed with distilled water (1.5 mL) and FeCl₃ (0.1 mL, 0.1%), and the content was incubated for 10 mins and the absorbance was measured at 700 nm. An increase in reducing power was indicated by an increase in absorbance as the concentration of extracts in the reaction mixture increased.

2.6.2 DPPH Scavenging activity of plant extracts

The DPPH solution (5 mL) was added to 1 mL of the plant extract solution at different concentrations. The reaction mixture was allowed to stand after shaking at room temperature for 30 mins. The absorbance of the solution formed was measured spectrophotometrically at 517 nm. For this experiment, Ascorbic acid was used as standard with the control prepared without the plant extract at different concentrations. The scavenging activity was expressed as the percentage inhibition and calculated using the formula below:

$$\% \text{ Scavenging activity} = \frac{\text{Control Absorbance (Ac)} - \text{Sample Absorbance (As)}}{\text{Control Absorbance (Ac)}} \times 100$$

Where Ac = Absorbance of control at 517 nm and As = Absorbance of the sample.

2.7 Alpha-amylase inhibitory assay

The alpha-amylase inhibitory assay was carried out using the starch iodide assay as described by Akoro *et al.* [18].

2.7.1 Starch-Iodine Assay

To 250 µL of the plant extract in DMSO (concentration range 0, 1, 10, 100, 1000 µg/mL), was added to 250 µL enzyme solution (250 µg/mL pancreatic alpha-amylase enzyme) dissolved in 0.02M Sodium phosphate buffer with 0.006 M sodium chloride, the solution was incubated for 10 min at 37°C. Soluble starch (potato starch) (1%; 250 µL) was then added to all the test tubes and incubated again for 10 min 37°C. This was followed by the addition of 250 µL 1M HCl to terminate the enzymatic reaction and then the addition of 100 µL of iodine reagent. The colour changes were recorded.

2.8 Statistical analysis

Statistical analysis was carried out using MS Excel 2010. Results were expressed as mean ± standard deviation (SD). The student's t-test was used where appropriate to compare the significance in mean values at different concentrations at a probability level of < 0.05.

3. RESULTS AND DISCUSSION

3.1 Extraction of plant material and phytochemical screening.

The plant material (219.15 g) was extracted with methanol by maceration to yield 6.32 g (2.88 %) of the methanol extract (ArMe). The methanol extract (6.32 g) was partitioned in n-hexane and ethyl acetate to yield the respective extracts (ArnH and ArEa) and the residual methanol extract (ArRMe). The results are summarised in Table 1.

Table 1. Yield, percentage yield, and colour of *A. ringens* extracts

Extracts	Mass of plants material (g)	Mass of extract obtained (g)	Percentage Yield	Colour
ArMe	219.15	6.32	2.88	Greenish brown
ArnH	6.32	0.66	10.44	Green
ArEa	6.32	0.54	8.54	Greenish brown
ArRMe	6.32	1.84	29.11	Greenish brown

Key: ArMe- crude methanol extract of A. ringens; ArnH- n-hexane extract; ArEa- ethyl acetate extract; ArRMe- residual methanol extract from the partitioning

Table 2. Phytochemical contents of the *A. ringens* extracts

Phytochemicals	ArMe	ArnH	ArEa	ArRMe
Alkaloids	+	+	-	-
Flavonoid	+	+	+	+
Saponins	+	+	-	+
Tannins	+	+	-	-
Phlobatannins	+	+	+	+
Cardiac glycoside	+	+	+	+
Terpenoids	+	+	+	+
Steroid	+	+	+	+
Reducing sugar	+	+	-	-
Phenol	+	+	+	+

Key: + = Detected; - = Not Detected; ArMe- crude methanol extract of A. ringens; ArnH- n-hexane extract; ArEa- ethyl acetate extract; ArRMe-residual methanol extract from the partitioning.

3.2 Elemental analysis

The elemental analysis of the plant for some selected heavy metals and trace elements - Ca, Na, Mg, Zn, K, Fe, Cu, Se, and Co - (Table 3) was carried out using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

Table 3. Mean percentage ash and mean amount of some selected elements detected in *A. ringens* in ppm

Mean % Ash	Element	Amount (ppm)
10.91±0.23	Ca	64.38±17.77
	Mg	21.46±5.93
	K	13.41±3.70
	Na	0.65±0.18
	Fe	3.25±0.90
	Zn	0.92±0.25
	Cu	0.43±0.12
	Se	0.12±0.03
	Co	0.18±0.05

** Result is mean of two experiments*

3.3 Antioxidant activities of the plant extracts

The antioxidant activities of each of the plant extracts were determined from the reducing power and DPPH radical scavenging assays (Figures 1 and 2).

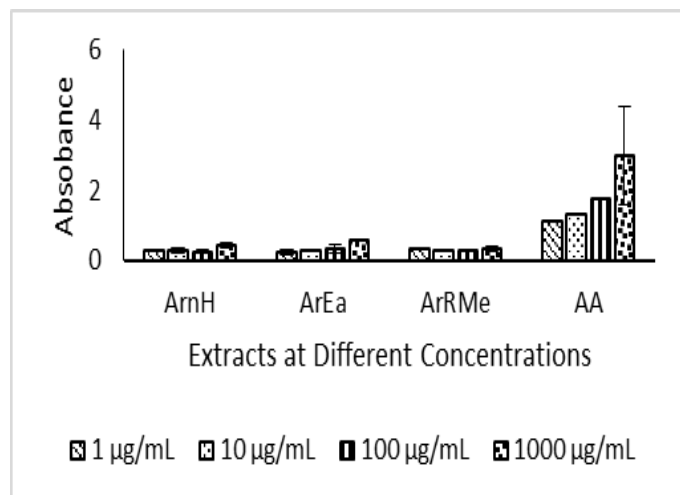


Fig. 1. Graph of concentration against absorbance indicating reducing property

ArnH- n-hexane extract; ArEa- ethyl acetate extract; ArRMe- residual methanol extract after partitioning.

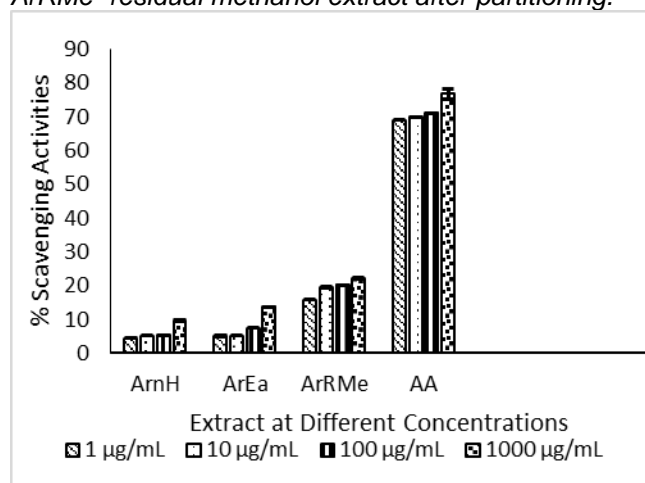


Fig. 2. DPPH scavenging activity of each extract

ArnH- n-hexane extract; ArEa- ethyl acetate extract; ArRMe- residual methanol after partitioning.

3.4 Alpha-amylase assay of *A. ringens* Extracts

The colour changes observed in the preliminary alpha-amylase inhibitory assay using the starch iodide method are shown in Table 4.

Table 4: Alpha-amylase Inhibitory assay-

Colour changes were observed in each extract.

Concentration (µg/mL)	Colour Observed		
	ArnH	ArEa	ArRMe
0	Orange	Orange	Orange
1	Orange	Orange	Blue-black
10	Deep orange	Blue-black	Blue-black
100	Deep orange with a trace of blue-black	Blue-black	Blue-black
1000	Deep orange with a trace of blue-black	Blue-black	Blue-black
Conclusion	Dose-dependent Partial Inhibition	Dose-dependent inhibition	Inhibition at all the concentration

Key: ArnH: *A. ringens* n-Hexane Extract; ArEa: *A. ringens* Ethyl acetate Extract; ArRMe: *A. ringens* residual methanol Extract; Orange= No inhibition (starch is completely digested); Deep orange = Partial inhibition (starch partially digested); blue-black= Inhibition – Starch is not digested

Preliminary phytochemical screening of the *A. ringens* extracts indicated the presence of flavonoids, cardiac glycosides, terpenoids, steroids, phlobatannins, and phenols in all the extracts; alkaloids, tannins, and reducing sugar were detected in only the crude methanol extract and n-hexane extract while saponins were detected in all the extracts except the ethyl acetate extract. (Table 2). The presence of these secondary metabolites may explain the various medicinal properties attributed to this plant [5,6].

The elemental analysis of the plant for some selected heavy metals and trace elements - Ca, Na, Mg, Zn, K, Fe, Cu, Se, and Co - (Table 3) was carried out using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), which is used for elemental analysis in samples at trace level [21]. ICP-OES is an elemental analysis technique that uses the emission spectra of a sample to identify and quantify the elements present. Samples are introduced into the plasma in a process that desolvates, ionises, and excites them. The results showed the following trend in the amount of elements: Ca > Mg > K > Fe > Zn > Na > Cu > Co > Se (Table 3). Generally, metals are essential for animal body functions [13]; calcium, which is the most abundant element detected in *A. ringens* in

this work is known to give good support to the body framework [13]; magnesium and potassium are useful in the control of blood glucose and pressure [18]. Potassium is also useful in maintaining normal fluid levels in the body cells while sodium maintains the fluid level outside the body cells [11-12, 19]. Cobalt is a major component of vitamin B12 [20] while iron is useful in the transportation of oxygen by the blood [21], manganese and zinc act as enzymes and they are useful elements that help in the proper functioning of the liver [22].

The antioxidant activities of each extract were determined from the reducing power and DPPH radical scavenging assays (Figures 1 and 2). The results of the reducing power assay indicate activity in the n-hexane and ethyl acetate extracts. For the n-hexane and ethyl acetate extract, the reducing activity was not significantly different ($p < 0.05$) at the concentrations studied (1, 10, 100, 1000 $\mu\text{g/mL}$). Also, in the residual methanol extract, the reducing activity was not significantly different ($p < 0.05$) from those observed in the n-hexane and ethyl acetate at the lower concentrations indicating a lower reducing power than the n-hexane and ethyl acetate extracts. The results of the DPPH scavenging assay indicated activity in all extracts studied especially in the residual methanol extract (ArRMe). For the n-hexane and ethyl acetate extract, the DPPH scavenging activity was not significantly different ($p < 0.05$) at the lower concentrations (1-100 $\mu\text{g/mL}$) but was pronounced and significant at 1000 $\mu\text{g/mL}$. However, the residual methanol extract showed higher reducing activity than all other extracts. However, the DPPH scavenging activity was not significantly different ($p < 0.05$) at all the concentrations studied. In the two antioxidant assay methods used, the ascorbic acid standard showed relatively higher activity than all the extracts. These results can be concluded to indicate a moderate antioxidant activity in support of the folkloric use of *A. ringens* to mop up free radicals. [5, 6].

The preliminary alpha-amylase inhibitory activities of each of the extracts were determined using the starch iodide assay. A dose-dependent inhibitory activity was observed in the n-hexane (ArnH) and ethyl acetate (ArEa) extracts based on the trend in the colour change from 10 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$, with partial inhibition in ArnH and a move from partial inhibition to complete inhibition in ArEa (Table 4). However, in the methanol extract (ArRMe), there was complete inhibition at all concentrations (Table 4). The ability of the extract to inhibit alpha-amylase enzyme activity was indicated by the presence of starch (blue-black colour in iodine); partial inhibition was indicated by orange or brownish blue colouration while yellow colour indicated complete digestion of the starch [18]. The measurement of post-prandial blood glucose is one of the parameters monitored in the management of type-2 Diabetes mellitus [18, 23]. The inhibition of the activities of digestive enzymes like alpha-amylase, and alpha-glucosidase plays a major role in the

reduction of post-prandial glucose levels in Diabetes [23].

4. CONCLUSION

The results of this study support and further explain the folkloric uses of *A. ringens* in the management of several diseases including high blood pressure, diabetes, and inflammatory conditions associated with free radical accumulation.

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COMPETING INTERESTS

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AUTHORS' CONTRIBUTIONS

The work was designed by author SMA. However, all authors contributed directly to the analysis and compilation of this work. All authors read and approved the final manuscript.

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