



Phytochemical and *In-vitro* Antioxidants Potential of Aristolochia Bracteolata Root Extract

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Authors' contributions

This work was carried out in collaboration between both authors. Author HMG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MSN managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The study investigated the phytochemical and in-vitro antioxidants potential of *Aristolochia bracteolata* root extracts. Qualitative and quantitative phytochemical analysis of the plant extract and fractions were carried out. The total antioxidant and free radical scavenging activity of extract and fractions were measured by 1, 1-diphenyl-2-picryl-hydrazyl, FRAP and TBARS methods. Results obtained showed that total saponins (10.10 ± 0.05^d) as well as antioxidant activity were found to be significantly ($p < 0.05$) higher in the methanol extract compared to the fractions. From the findings above, *Aristolochia bracteolata* root contains bioactive phytochemicals that might be helpful in preventing or slowing the progress of various oxidative stresses.

Keywords: *Aristolochia bracteolata*; antioxidant; phytochemical; methanol crude extract; fractions i and ii.

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1. INTRODUCTION

Over the centuries, humans use different types of therapeutic plants to treat or cure diseases. Such therapeutic plants are very important and play a key role in a better and happy life, for health at every stage [1].

Experimental and epidemiological evidences ascribe a wide array of positive health effects to the Phytochemicals present in plants [2]. These effects have prompted worldwide interest in investigations into the pharmaceutical properties of plants and in the analyses of their Phytochemicals [3]; such studies are intended to promote healthcare, and the studied plants have been used as conventional or complementary medicines because they present lower toxicity and side effects than do synthetic drugs [3].

In Nigeria, the use of traditional medicine plays an important role in public health care and many medicinal plants have been used to treat several ailments.

(*Aristolochia bracteolata* is an herbaceous perennial medicinal plant with cordate leaves and dark purple colour tubular flowers belonging to the family Aristolochiaceae. The plant commonly called as worm killer in English, Judan kasa in Hausa and Aadutheendapalai in Tamil, due to supposed anthelmintic activity and trapanocidal effect [4]. It has been reported to be distributed throughout the South India, Bengal, Upper Gangetic Plain, Ceylon and Tropical Africa. Traditionally, *Aristolochia bracteolata* has been reported to be used for inflammatory diseases, fever and insect bites.) The whole plant was used as purgative, anthelmintic, antipyretic, anti-inflammatory agents. The plant contain Aristolochic acid, has many medicinal properties in various disease conditions [4]. This study was aimed at determining the Phytochemical and in-vitro antioxidant potential of the root extracts of *Aristolochia bracteolata*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The root bark of *Aristolochia bracteolata* was collected from Girei Local Government Area (9°22'N 12°33'E and 9.37°N 12.550°E) of Adamawa State, Nigeria. The plant was identified and authenticated in the Department of Plant Science, Modibbo Adama University of Technology Yola. (Code: MAU. PS= AB00001/HMG)

2.2 Preparation of Plant Extracts

The ground powdered plant material of *Aristolochia bracteolata* (50 g) was weighed and extraction was carried out on it using maceration technique. The solvents hexane, methanol and ethyl acetate was used for the extraction. The powdered plant was soaked in methanol for 24 hours at room temperature, after which it was filtered using muslin cloth and Whatmann filter paper to obtain the methanol extract, The residue from the methanol extraction was then dried and soaked in hexane for 24 hours after which it was filtered using muslin cloth and Whatmann filter paper to obtain the hexane extract. The residue from the hexane extraction was then dried and soaked in ethyl acetate for 24 hours after which it was filtered using muslin cloth and Whatmann filter paper to obtain the ethyl acetate extract. The filtrate was then concentrated using rotary evaporator at 40 – 50°C and the extract kept in a well tight sterile bottle/container under refrigerated conditions until use [5].

2.3 Phytochemicals Screening

Phytochemical screening for the presence of alkaloids, cardiac glycosides, Terpenoids, triterpenes, flavonoids, saponins, and tannins was carried out using the methods described by Harborne [6] Sofowora [7]; Trease and Evans, [8].

2.3.1 Test for alkaloids

Forty milligram of the crude extract and fractions was stirred with 8 ml of 1% HCl, the mixture was warmed and filtered [6]. Two milliliter of filtrate was treated separately; (a) with three drops of potassium mercuric iodide (Mayer's reagent) and (b) potassium bismuth (Dragendroff's reagent) Precipitation with either of these reagents was taken as evidence for existence of alkaloids.

2.3.2 Test for saponins

Twenty milligram of the crude extract and fractions was boiled in 20 ml of distilled water in a water bath for five minutes and filtered. Ten milliliter of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation.

2.3.3 Test for triterpenes

Presence of triterpenoids in the crude extract and fractions was carried out by taking 5 ml (1 mg/ml) of the extract and mixing it with 2 ml of chloroform, followed by 3 ml of concentrated

H₂SO₄. A reddish brown coloration of the interface indicates the presence of triterpenoids [6].

2.3.4 Test for cardiac glycosides

Five millilitre (10 mg/ml in methanol) of the crude extract and fractions was mixed with 2 ml of glacial acetic acid having one drop of FeCl₃ solution. To the mixture obtained 1 ml of concentrated H₂SO₄ was added to form a layer. The presence of brown ring of the interface indicated deoxy sugar characteristic of cardiac glycosides [8].

2.3.5 Test for flavonoids

To (50 mg) of the crude extract and fractions, 100 ml of distilled water was added, stirred and then filtered to get the filtrate. 5ml of dilute ammonia solution was added to 10 ml of the filtrate followed by few drops of concentrated H₂SO₄. Yellow coloration indicates the presence of flavonoid [7].

2.3.6 Test for tannins

To (0.5 g) of the crude extract and fractions 10mls of distilled water was added, stirred and then filtered. Few drops of 1 % ferric chloride solution were then added to 2 ml of the filtrate. Production of a blue –black color indicated the presence of tannins [8].

2.3.7 Test for terpenoids

To test for terpenoids, (0.5 g) of the crude extract and fractions was mixed with 2 ml chloroform and 3 ml H₂SO₄ was carefully added to form a layer. A reddish brown colouration of the interface was an indication of terpenoids.

2.4 Determination of Antioxidant Activity

The antioxidant activity was determined by ammonium thiocyanate assay [9]. 500 µL of the extract, 200 µL of diluted linoleic acid (25 mg/mL 99 ethanol) and 400 µL of 50 mM phosphate buffer (pH 7.4) was mixed and incubated at 40°C for 15min. Aliquot (100 µL) from the reaction mixture was mixed with reaction solution containing 3 mL of 70% ethanol, 100 µL of ammonium thiocyanate (300 mg/mL distilled water) and 100 µL of ferrous chloride (2.45 mg/mL in 3.5% hydrochloric acid). Final reaction solution was mixed and incubated at room temperature for 3 min. Absorbance was measured at 500 nm. Linoleic acid emulsion

without extract served as control. Inhibition of linoleic acid oxidation was calculated by using the following formula:

$$\% \text{ Inhibition} = \left[\frac{\text{control OD} - \text{sample OD}}{\text{control OD}} \right] \times 100.$$

2.5 Statistical Analysis

The results were represented as Mean ± standard error of mean (SEM). Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 25.0 (SPSS, Incorporation Chicago Illinois, USA). Differences between and within the group means were analyzed using One-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (MRT) for the post-hoc treatment. The results were considered statistically significant at p<0.05.

3. RESULTS

3.1 Phytochemical Screening

Table 1 shows the result of Phytochemical screening of *Aristolochia bracteolata* methanolic crude extract and fractions. From the result, cardiac glycosides and steroids were not detected in both the crude extract and fractions, while tannins and terpenoids were absent in fraction 1 and alkaloids was absent in fraction 2. However, both the crude extract and fractions contain flavonoids, phenols, and saponins.

Table 2 also shows the results for the total alkaloids, saponins, tannins, phenols, terpenoids and flavonoid content of *Aristolochia bracteolata* methanolic crude extract and fractions in percentage (%). Results obtained revealed that methanolic crude extract contained alkaloids saponins, tannins phenols, terpenoids and flavonoids (5.90 ±0.16), while fraction 1 contain alkaloids, saponins (4.38±0.01), phenols and flavonoids (2.15 ± 0.01), and fraction 2 contains saponins (3.74 ± 0.01), tannins, phenols, terpenoids and flavonoids (2.8 ± 0.06). Saponins and flavonoids were found to be significantly higher (p<0.05) in methanolic crude extract compared to the fractions.

4. DISCUSSION

Plants are considered as biosynthetic laboratory for a multitude of compounds that exert physiological effects [10]. The presence of significant amount of alkaloids, flavonoids,

Table 1. Qualitative phytochemical composition of *aristolochia bracteolata*, methanol root extract and fractions

Phytochemicals	Methanol Extract	Fraction 1	Fraction 2
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	-	+
Alkaloids	+	+	-
Phenols	+	+	+
Terpenoids	+	-	+
Steroids	-	-	-
Cardiac glycosides	-	-	-

Key: + = Presence of Phytochemical constituents
 - = Absence of Phytochemical constituents

Table 2. Quantitative phytochemical determination of *Aristolochia bracteolata* root extract and fractions in percentage (%)

Phytochemicals	Methanol Extract	Fraction 1	Fraction 2
Alkaloids	3.45 ± 0.01 ^a	1.06 ± 0.01 ^b	0.13 ± 0.01 ^c
Terpenoids	0.43 ± 0.01 ^b		
Flavonoids	5.90 ± 0.16 ^c	2.15 ± 0.01 ^a	2.8 ± 0.06 ^b
Phenols	5.71 ± 0.06 ^c	1.64 ± 0.01 ^c	3.03 ± 0.01 ^d
Saponins	10.10 ± 0.05 ^d	4.38 ± 0.01 ^d	3.74 ± 0.01 ^d
Tannins	3.92 ± 0.02 ^a	-	1.86 ± 0.01 ^c

Values are expressed as Mean ± SEM (n = 3). Letter(s) with different superscript along the same column and the same row shows there is significant difference at P < 0.05

Table 3. DPPH radical scavenging activity of *Aristolochia bracteolata* root extract and fractions in percentage (%)

Concentration (mg/ml)	(L-Ascorbic acid) %Inhibition	M.E %Inhibition	F1%Inhibition	F2%Inhibition
20	13.59 ± 0.08	56.40 ± 1.19 ^d	49.54 ± 1.70 ^a	57.55 ± 0.28 ^b
40	29.14 ± 0.07	65.11 ± 1.07 ^a	60.66 ± 0.73 ^b	59.81 ± 0.30 ^b
60	60.11 ± 9.03	66.79 ± 1.15 ^a	63.56 ± 0.16 ^c	62.04 ± 0.33 ^c
80	67.89 ± 0.20	69.32 ± 0.33 ^b	65.07 ± 0.26 ^c	65.77 ± 0.45 ^d
100 IC50 (mg/ml)	92.71 ± 0.20	74.76 ± 0.65 ^c	67.06 ± 0.30 ^c	67.89 ± 0.20 ^e
	19.20	20.50	33.30	34.68

Values are expressed as Mean ± SEM (n = 3). Letter(s) with different superscript along the same column shows there is significant difference at P < 0.05

Table 4. Ferric reducing antioxidant power of *Aristolochia bracteolata* root extract and fractions in percentage (%)

Concentration (mg/ml)	(L-Ascorbic acid) %Inhibition	M.E %Inhibition	F1 %Inhibition	F2 %Inhibition
20	90.53 ± 0.04	76.02 ± 0.19 ^a	95.06 ± 0.00 ^a	94.54 ± 0.01 ^a
40	92.87 ± 0.029	94.18 ± 0.02 ^b	95.20 ± 0.01 ^b	94.87 ± 0.01 ^b
60	93.09 ± 0.01	94.37 ± 0.25 ^b	95.47 ± 0.01 ^c	95.09 ± 0.01 ^c
80	94.52 ± 0.01	96.92 ± 0.96 ^c	95.62 ± 0.00 ^d	95.84 ± 0.01 ^d
100 IC50 (mg/ml)	95.24 ± 0.02	96.29 ± 0.05 ^c	95.80 ± 0.06 ^e	97.04 ± 0.01 ^e
	39.05	13.20	47.22	14.66

Values are expressed as Mean ± SEM (n = 3). Letter(s) with different superscript along the same column shows there is significant difference at P < 0.05

IC₅₀=Half maximal inhibitory concentration; is the concentration causing 50% inhibition of the desired activity. The IC₅₀ was obtained by linear regression equation

Table 5. Thiobarbituric Acid Reactive Substances (TBARS) antioxidant activity of *Aristolochia bracteolata* root extract and fractions in Percentage (%)

Concentration (mg/ml)	(L-Ascorbic acid) %Inhibition	M.E %Inhibition	F1 %Inhibition	F2 %Inhibition
20	90.53 ± 0.04	60.57 ± 0.61 ^a	50.40 ± 1.17	55.23 ± 0.12 ^a
40	92.87 ± 0.029	65.76 ± 2.13 ^b	56.91 ± 0.30 ^b	62.27 ± 0.43 ^b
60	93.09 ± 0.01	68.78 ± 0.42 ^d	64.38 ± 0.57 ^c	65.55 ± 1.92 ^c
80	94.52 ± 0.01	72.73 ± 0.14 ^c	68.11 ± 0.13 ^d	68.90 ± 0.36 ^d
100	95.24 ± 0.02	74.91 ± 0.49 ^c	73.77 ± 0.49 ^e	72.06 ± 0.29 ^e
IC ₅₀ (mg/ml)	70.13	44.05	16.11	13.47

Values are expressed as Mean ± SEM (n = 3). Letter(s) with different superscript along the same column shows there is significant difference at P < 0.05

IC₅₀=Half maximal inhibitory concentration; is the concentration causing 50% inhibition of the desired activity. The IC₅₀ was obtained by linear regression equation

saponin, tannins, and phenols in the plant extracts confirmed its use as medicinal plant as reported by Periyasamy et al. [11].

A previous study had revealed the phytochemical constituents of *Aristolochia bracteolata* to include; alkaloids, triterpenoids, steroids and sterols, flavonoids, tannins and phenol compounds and cardio glycosides [12]. However, in this study the Phytochemical screening of the methanol root extract and fractions showed the plant had different varieties of Phytochemicals in the methanolic crude extract which correlates with the earlier report by Idayat et al. [13].

The result of the fraction 1 showed the presence of saponins, tannins, flavonoids, alkaloids, phenols, while the fraction 2 displayed the presence of flavonoids, saponins and phenols. Reports from other studies already confirmed that, saponins, flavonoids and tannins are responsible for several therapeutic activities such as hypoglycemic effects [10].

(Quantitatively, total saponins, total flavonoids, total phenols, total tannins, total alkaloids and total terpenoids were found in varying concentrations in the methanolic crude extract, and fractions of *Aristolochia bracteolata*. This variation may be however, attributed to the difference in polarity of the solvents and molecular size of compounds present in the plant extracts [14].

Antioxidants are substances that protect living cells from the damages caused by unstable molecules known as free radicals. The Antioxidative effect is mainly due to its phenolic components, such as phenolic acids, phenolic diterpenes, polyphenols, tannins, saponins, alkaloids and flavonoids. The antioxidant activity

of phenolic compounds is mainly due to their redox properties, which plays an important role in absorbing and neutralizing free radicals, quenching singlet and triple oxygen, or decomposing peroxides [12]. The use of DPPH radical provides an easy, rapid and convenient method to evaluate antioxidant and radical scavenging potentials of plants products [15].

The antioxidant activity of medicinal plant is mainly related to their bioactive compounds such as phenols and flavonoids. In this study, the methanol crude extract, and fractions of the plant were able to show free radical scavenging activities. DPPH radical was used as a stable free radical to determine the antioxidant property of natural compounds and the scavenging of the stable radical (DPPH) was considered a valid and easy assay to evaluate the scavenging activity of antioxidants [16]. The results of this study indicated that antioxidant activity were found to be significantly (p < 0.05) higher in the methanol crude extract when compared to the fractions.

The increased formation of free radicals was associated with the increase in lipid peroxidation. One of the important roles of antioxidants is to inhibit the chain reaction of lipid peroxidation [17].

In FRAP assay, the total antioxidant activity exhibited by the methanol extract and fractions indicate that both possess antioxidant activities. The low IC₅₀ value (13.20 mg/ml) of the methanol crude extract suggests it has better reducing antioxidant power compared to the fraction 1, fraction 2 and ascorbic acid which is similar to the work by Mallaiah et al. [18].

The antioxidant activity has been attributed to various mechanisms such as prevention of chain

initiations, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen attraction, the reductive capacity of radical scavenging and the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [19,20]. Our findings are in consonance with earlier reports by Lagnika et al. [21] on the use of plants as strong antioxidant agents.

The result of TBARS assay was expressed in terms of percentage inhibition and half maximum inhibitory concentration (IC₅₀). The IC₅₀ of the methanol crude extract, fractions 1, fraction 2 and ascorbic acid were found to be (44.05mg/ml, 16.11mg/ml, 13.47mg/ml and 70.13mg/ml) respectively. The low IC₅₀ value (13.47mg/ml) of the fraction 2 suggests it has a better reactive antioxidant activity compared to the methanol crude extract, fraction 1 and ascorbic acid. However, among the extracts, fraction 1 suggests a better reactive antioxidant activity with the lowest IC₅₀ value (13.47mg/ml).

This revealed that the extract has reactive antioxidant activity against free radicals. The finding was in agreement with the report of Silva et al. [22]. The antioxidant effects revealed by DPPH, TBARS and FRAP could be attributed to the presence of some Phytochemicals in the root extracts of *Aristolochia bracteolata*.

5. CONCLUSION

The methanol extract and fractions of *Aristolochia bracteolata* showed that the plant root contains almost all important types of phytochemical constituents and possess a good source of antioxidant potential at various concentrations. This finding might be helpful in preventing or slowing the progress of various oxidative stresses.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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